JCI The Journal of Clinical Investigation

# Metreleptin improves insulin sensitivity independent of food intake in humans with lipodystrophy

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*J Clin Invest.* 2018. https://doi.org/10.1172/JCI95476.

Clinical Medicine In-Press Preview Endocrinology Metabolism

**BACKGROUND.** Recombinant leptin (metreleptin) ameliorates hyperphagia and metabolic abnormalities in leptin-deficient humans with lipodystrophy. We aimed to determine whether metreleptin improves glucose and lipid metabolism in humans when food intake is held constant.

**METHODS.** Patients with lipodystrophy were hospitalized for 19 days with food intake held constant by controlled diet in an inpatient metabolic ward. In a non-randomized cross-over design, previously metreleptin-treated patients ($n$ = 8) were continued on-metreleptin for five days, and off-metreleptin for the next 14 days (withdrawal cohort). This order was reversed in metreleptin-naïve patients ($n$ = 14), who were restudied after six months of metreleptin treatment on an ad libitum diet (initiation cohort). Outcomes included insulin sensitivity by hyperinsulinemic-euglycemic clamp, fasting glucose and triglycerides, lipolysis measured using isotopic tracers, and liver fat by magnetic resonance spectroscopy.

**RESULTS.** With food intake constant, peripheral insulin sensitivity decreased by 41% after stopping metreleptin for 14 days (withdrawal cohort) and increased by 32% after starting metreleptin for 14 days (initiation cohort). In the initiation cohort only, metreleptin decreased fasting glucose by 11%, triglycerides by 41%, and increased hepatic insulin sensitivity. Liver fat decreased from 21.8% to 18.7%. In the initiation cohort, […]



# Metreleptin improves insulin sensitivity independent of food intake in humans with lipodystrophy

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**Conflict of Interest:** The authors have declared that no conflict of interest exists.

**Role of the funding source**: This study was supported by the intramural research program of the NIDDK. Metreleptin for this study was donated by Aegerion Pharmaceuticals, which had no role in the design, conduct, analysis, interpretation, or decision to publish the study.

## Abstract

**Background.** Recombinant leptin (metreleptin) ameliorates hyperphagia and metabolic abnormalities in leptin-deficient humans with lipodystrophy. We aimed to determine whether metreleptin improves glucose and lipid metabolism in humans when food intake is held constant.

**Methods.** Patients with lipodystrophy were hospitalized for 19 days with food intake held constant by controlled diet in an inpatient metabolic ward. In a non-randomized cross-over design, previously metreleptin-treated patients (N=8) were continued on-metreleptin for five days, and off-metreleptin for the next 14 days (withdrawal cohort). This order was reversed in metreleptin-naïve patients (N=14), who were restudied after six months of metreleptin treatment on an *ad libitum* diet (initiation cohort). Outcomes included insulin sensitivity by hyperinsulinemic-euglycemic clamp, fasting glucose and triglycerides, lipolysis measured using isotopic tracers, and liver fat by magnetic resonance spectroscopy.

**Results.** With food intake constant, peripheral insulin sensitivity decreased by 41% after stopping metreleptin for 14 days (withdrawal cohort) and increased by 32% after starting metreleptin for 14 days (initiation cohort). In the initiation cohort only, metreleptin decreased fasting glucose by 11%, triglycerides by 41%, and increased hepatic insulin sensitivity. Liver fat decreased from 21.8% to 18.7%. In the initiation cohort, lipolysis did not change independent of food intake, but decreased after six months on metreleptin on an *ad libitum* diet by 30% (palmitate turnover) to 35% (glycerol turnover).

**Conclusion.** Using lipodystrophy as a human model of leptin deficiency and replacement, we showed that metreleptin improves insulin sensitivity, and decreases hepatic and circulating triglycerides, independent of its effects on food intake.

**Trial registration.** ClinicalTrials.gov, NCT01778556

**Funding.** This research was supported by the intramural research program of the National Institute of Diabetes and Digestive and Kidney Diseases.

## Introduction

Leptin is an adipocyte-derived hormone that signals overall body energy sufficiency (1) and acute energy balance (2). Leptin deficient states, such as starvation or mutations in the leptin gene, lead to hyperphagia. An additional leptin deficient state is lipodystrophy, in which a deficiency of adipose tissue results in hypoleptinemia, which induces hyperphagia, with energy intake ~40% higher than predicted (3). The excess caloric intake is stored as ectopic fat in liver and muscle, causing severe insulin resistance and diabetes, along with hypertriglyceridemia, low levels of high-density lipoprotein cholesterol (HDL-C), and non-alcoholic fatty liver disease (NAFLD) (4, 5). Therefore, patients with lipodystrophy can serve both as a model of leptin-deficiency and replacement, as well as metabolic disease that is analogous to, albeit more severe than, that seen in patients with obesity-associated metabolic syndrome.

Treatment with metreleptin, a recombinant analog of human leptin, in humans with lipodystrophy ameliorates hyperphagia, ectopic lipid storage, hypertriglyceridemia, insulin resistance, and reproductive dysfunction (4, 6-9). The reduction in food intake is likely responsible for part of the observed improvements in glucose and lipid metabolism. Rodent studies in leptin-treated ob/ob mice and n-SREBP-1c lipodystrophic mice showed an additional reduction in glucose and insulin levels compared to pair-fed controls, suggesting that leptin has a hypoglycemic effect independent of its effects on food intake (10, 11). Whether leptin has these energy intake independent effects in humans has not previously been determined.

Using lipodystrophy as a human model of leptin deficiency and replacement, we conducted a non-randomized crossover study to determine the energy intake independent effects of leptin on glucose and lipid metabolism. We hypothesized that, during constant food intake, patients with lipodystrophy would have greater insulin sensitivity and reduced lipolysis during the period of leptin replacement versus the leptin-deficient state. Patients with no prior exposure to metreleptin constituted the initiation cohort, and patients already undergoing metreleptin treatment prior to our study constituted the withdrawal cohort. All patients were hospitalized for 19 days with energy and macronutrient intake held constant by controlled diet in an inpatient metabolic ward during metreleptin treated and untreated periods. The withdrawal cohort was on-metreleptin for five days, and off-metreleptin for the next 14 days. This order was reversed in the initiation cohort, who were restudied after six months of metreleptin on an *ad libitum* diet (Figure 1).

## Results

*Study participants.* Twenty-five patients with lipodystrophy were enrolled. The flow of participants in this non-randomized crossover study is shown in Figure 2. Of the fifteen initiation subjects, one did not complete study procedures for the short-term, controlled food-intake portion of the study but completed six-month follow-up, and one completed the short-term study but was excluded from analysis of the six-month data due to non-compliance with the study drug. In the withdrawal cohort, eight subjects completed the study and were included in the analysis.

Baseline characteristics of subjects are shown in Table 1. Of the 15 subjects in the initiation group, three had generalized lipodystrophy and 12 had partial lipodystrophy. Nine were non-Hispanic White, four were Hispanic White, one was Asian, and one was Other race. In the withdrawal cohort, all eight patients had generalized lipodystrophy. Four were non-Hispanic White, two were African-American, and two were Hispanic White. The majority were female (~70%) in both groups. At baseline, the initiation cohort had an endogenous leptin level of 9.5 ± 10.2 ng/dL. Seventy-one percent were taking insulin with a mean insulin dose among insulin users of 225 ± 136 units per day. In contrast, the withdrawal cohort had a lower endogenous leptin level prior to metreleptin therapy of 1.2 ± 0.5 ng/dL, reflecting greater leptin insufficiency in patients with generalized lipodystrophy, and had an average of 7.7 ± 4.7 (range 0.9-14.5) years of prior metreleptin treatment. None were taking insulin. The expected relationship between fat mass and the log of endogenous leptin was observed in the combined cohorts ($R^2 = 0.69$, $p<0.0001$), with no difference by sex, cohort (initiation versus withdrawal), or lipodystrophy type (generalized versus partial).

***Short-term effects of metreleptin independent of food intake***

*Food intake, diet, and body composition.* During the 19-day inpatient stay, patients were required to consume all study-provided food and forbidden to consume any outside food. Any uneaten portions of the study diet were weighed, and uneaten nutrients were replaced at the next meal when possible. Energy intake and macronutrient content were successfully held constant in the off- versus on-metreleptin periods in both groups (Table 2). Furthermore, multivariate analyses showed that the effects of metreleptin on outcome measures of interest were not significantly influenced by actual caloric intake during the off and on metreleptin periods in either cohort (Supplemental Table 1).

In the initiation cohort, body weight and fat mass significantly decreased by 0.7 kg and 0.3 kg, respectively, after two weeks on metreleptin. There was no change in body weight or fat mass in the withdrawal cohort, and no change in lean mass or percent body fat in either group.

*Peripheral insulin sensitivity was greater on metreleptin therapy independent of food intake in both initiation and withdrawal cohorts. Short-term metreleptin therapy increased hepatic insulin sensitivity independent of food intake in the initiation cohort only.* In the initiation cohort, peripheral insulin sensitivity measured by hyperinsulinemic-euglycemic clamp increased from 4.4 ± 2.3 mg/kg$_{FFM}$/min at the end of Period 1 pre-metreleptin, to 5.8 ± 2.2 mg/kg$_{FFM}$/min at the end of Period 2 on-metreleptin (p=0.001) (Figure 3). Similarly, in the withdrawal cohort, peripheral insulin sensitivity decreased from 10.9 ± 4.1 mg/kg$_{FFM}$/min at the end of Period 1 on-metreleptin to 6.4 ± 1.8 mg/kg$_{FFM}$/min (p=0.01) at the end of Period 2 after metreleptin withdrawal (Figure 3). The magnitude of the increase in insulin sensitivity in the on- versus off-metreleptin condition was

greater in the leptin withdrawal cohort. In the withdrawal cohort, there was a correlation between the reduction in peripheral insulin sensitivity after metreleptin withdrawal, and increases in fasting glucose ($p=0.014$) and c-peptide ($p=0.006$).

In the initiation cohort, hepatic insulin sensitivity measured as insulin-mediated suppression of hepatic glucose production (HGP) increased from 61 ± 23% at the end of Period 1 to 75 ± 33% ($p=0.008$) at the end of Period 2 (Figure 3). Suppression of HGP did not change in the withdrawal cohort (Figure 3).

Changes in hepatic triglyceride content significantly predicted changes in both peripheral and hepatic insulin sensitivity with metreleptin in the initiation cohort, only (Supplemental Tables 2 and 3). Moreover, changes in peripheral and hepatic insulin sensitivity with metreleptin in the initiation cohort were no longer statistically significant after adjustment for changes in hepatic triglyceride content. By contrast, intramyocellular triglyceride content was not a significant predictor of either peripheral or hepatic insulin sensitivity in most models, and improvement in peripheral and hepatic insulin sensitivity with metreleptin remained statistically significant after adjustment for intramyocellular triglyceride content (Supplemental Tables 2 and 4). Changes in body composition did not predict changes in insulin sensitivity with metreleptin, and improvements in insulin sensitivity remained statistically significant after adjustment for body composition.

*Short-term metreleptin therapy decreased fasting glucose and glucosuria independent of food intake in the initiation cohort.* In the initiation cohort, fasting glucose decreased from 152 ± 42

mg/dL at the end of Period 1 pre-metreleptin to 136 ± 34 mg/dL (p=0.003) at the end of Period 2 on-metreleptin (Figure 3). In addition, 24-hour urine glucose excretion decreased from 2.0 [0.2,10.3] g/24h at the end of Period 1 pre-metreleptin to 1.2 [0.2,7.2] g/24h (p=0.049) at the end of Period 2 on-metreleptin (Table 3). HbA1c decreased from 8.7 ± 2.0% at the end of Period 1 pre-metreleptin to 8.0 ± 1.3% (p=0.002) at the end of Period 2 on-metreleptin. However, because the initial HbA1c reflected glycemic control for the three months prior to the study, this change cannot be considered as independent of food intake. Relative to hospital admission when patients were on an *ad libitum* diet, mean insulin dose in these patients decreased by 95 ± 126 units per day at the end of Period 2 on metreleptin (p=0.04); however, there was no significant change in insulin dose or insulin secretion (measured as fasting C-peptide) independent of food intake. Fasting glucose, HbA1c, C-peptide, and urine glucose excretion did not change in the withdrawal cohort.

*Short-term metreleptin therapy decreased triglycerides and total cholesterol independent of food intake in the initiation cohort, but did not change HDL-C, free fatty acids, or LDL-C.* In the initiation cohort, triglycerides decreased from 556 [224,1144] (geometric mean [$25^{th}$,$75^{th}$ percentile]) mg/dL at the end of Period 1 pre-metreleptin to 335 [162,611] mg/dL at the end of Period 2 on-metreleptin (p=0.01) (Figure 4). Total cholesterol also decreased from 241 ± 116 mg/dL at the end of Period 1 to 171 ± 48 mg/dL at the end of Period 2 (p=0.002) (Table 3). In the withdrawal cohort, triglycerides and total cholesterol did not change. The magnitude of the decrease in total cholesterol in the on- versus off-metreleptin condition was greater in the leptin initiation cohort. Free fatty acids, HDL-C, and LDL-C did not change in either the initiation or withdrawal cohort (Table 3).

*Short-term metreleptin therapy did not change lipolysis independent of food intake.* Lipolysis was quantified by infusing $D_5$-glycerol and $^{13}C_{16}$-palmitate to measure turnover through isotope dilution studies. In the initiation and withdrawal cohorts, short-term metreleptin with food intake held constant did not change the endogenous rate of appearance (Ra) of glycerol or palmitate (Figure 4).

*Short-term metreleptin therapy decreased hepatic triglyceride content independent of food intake in the initiation cohort.* In the initiation cohort, there was a reduction in liver fat from 21.8 ± 10.9% at the end of Period 1 pre-metreleptin to 18.7 ± 12.5% (p=0.03) at the end of Period 2 on-metreleptin (Figure 4). Liver fat did not change in the withdrawal cohort independent of food intake. Short-term metreleptin did not change either extramyocellular (EMCL) or intramyocellular lipid (IMCL) content independent of food intake in either the initiation or withdrawal cohorts (Table 3).

*Short-term metreleptin therapy decreased total and resting energy expenditure independent of food intake in the initiation cohort*. In the initiation cohort, total energy expenditure decreased from 2463 ± 362 kcal/day at the end of Period 1 pre-metreleptin to 2319 ± 400 kcal/day at the end of Period 2 on-metreleptin (p=0.001). Resting energy expenditure also decreased in this cohort from 1855 ± 289 kcal/day to 1736 ± 308 kcal/day (p=0.01); this change was no longer statistically significant after adjusting for changes in lean and fat mass. Non-resting energy expenditure (total minus resting) did not change in the initiation cohort. Total, resting, and non-resting energy expenditure did not change in the withdrawal cohort independent of food intake.

***Long-term effects of metreleptin while on an ad libitum diet***

To study the long-term effects of metreleptin, the initiation cohort returned for a follow-up visit after 6.8 ± 1.0 months of metreleptin therapy. At this visit and during the six months prior, patients were on an *ad libitum* diet, thus any observed effects of metreleptin were not independent of food intake.

*Long-term metreleptin therapy decreased body weight, fat mass, lean mass, and percent body fat in the initiation cohort.* At the six-month follow-up visit for the initiation cohort while on an *ad libitum* diet, body weight decreased from 73.8 ± 16.0 kg pre-metreleptin to 70.8 ± 16.8 kg (p=0.005), fat mass decreased from 18.3 ± 10.6 kg to 15.5 ± 10.0 kg (p=0.028), lean mass decreased from 53.1 ± 9.2 kg to 51.5 ± 9.4 kg (p=0.002), and percent body fat decreased from 24.3 ± 10.8% to 21.3 ± 10.6% (p=0.02).

*Long-term metreleptin therapy maintained improvements in peripheral and hepatic insulin sensitivity.* At the six-month follow-up visit for the initiation cohort while on an *ad libitum* diet, peripheral insulin sensitivity improvement was maintained at 8.0 ± 4.0 mg/kg$_{FFM}$/min (p=0.01 vs Period 1). There was no further increase in peripheral insulin sensitivity at six-month follow-up relative to Period 2 in the unadjusted analysis (p=0.09, Figure 3), although this difference was significant after adjustment for covariates (p=0.048, Supplemental Table 1). Similarly, hepatic insulin sensitivity improvement was maintained at the six-month follow up visit at 86 ± 18% suppression of HGP (p=0.02 vs Period 1), but there was no further increase in hepatic insulin sensitivity relative to Period 2 (Figure 3).

Changes in hepatic triglyceride content significantly predicted long-term changes in peripheral insulin sensitivity with metreleptin (Period 1 versus six-month follow-up, and Period 2 versus 6-month follow-up, Supplemental Tables 2 and 3). Hepatic triglyceride content also significantly predicted change in hepatic insulin sensitivity from Period 2 to six-month follow-up (Supplemental Tables 2 and 3). Changes in both peripheral and hepatic insulin sensitivity (Period 1 versus 6-month follow-up) were no longer statistically significant after adjustment for changes hepatic triglyceride content (Supplemental Table 3). Intramyocellular triglyceride content was not a significant predictor of long-term change in peripheral or hepatic insulin sensitivity in most models (Supplemental Tables 2 and 4).

*Long-term metreleptin therapy maintained improvements in fasting glucose and HbA1c.* At the six-month follow-up for the initiation cohort while on an *ad libitum* diet, the reduction in fasting glucose was maintained at 126 ± 26 mg/dL (p=0.02 vs Period 1), and HbA1c reduction was also maintained at 6.9 ± 1.4% (p=0.01 vs Period 1), but there were no further decreases relative to Period 2 (Figure 3). Reductions in glycemia in the initiation cohort were observed despite decreases in insulin doses in nine of 10 insulin-treated subjects. Relative to hospital admission, mean insulin dose in these patients decreased by 112 ± 109 units per day (a 50% reduction) at six-month follow-up (p=0.01). Two subjects discontinued insulin use by their six-month follow-up. The mean number of diabetes medications (insulin + other agents) did not change after six months. There were no significant changes in C-peptide during the study.

*Long-term metreleptin therapy maintained improvements in triglycerides and total cholesterol.* At the six-month follow-up for the initiation cohort while on an *ad libitum* diet, the reduction in

triglycerides was maintained at 304 [122,547] (p=0.24 vs Period 1, Figure 4), and reduction in total cholesterol was maintained at 129 ± 32 (p=0.02 vs Period 1) but there were no further decreases relative to Period 2. Free fatty acids, HDL-C, and LDL-C did not change in either the initiation or withdrawal cohort (Table 3). The mean number of lipid-lowering medications did not change during the study.

*Long-term metreleptin therapy decreased glycerol and palmitate turnover in the initiation cohort.* At six-month follow-up for the initiation cohort while on an *ad libitum* diet, palmitate turnover decreased by 30% from 3.2 ± 1.3 μmol/$kg_{LBM}$/min prior to metreleptin in Period 1 to 2.2 ± 0.7 μmol/$kg_{LBM}$/min (p=0.02), and glycerol turnover decreased by 35% from 4.5 ± 2.3 μmol/$kg_{LBM}$/min prior to metreleptin in Period 1 to 2.9 ± 0.7 μmol/$kg_{LBM}$/min (p=0.02), indicating a decrease in lipolysis (Figure 4).

*Long-term metreleptin therapy maintained reduction in liver fat, and reduced ALT and AST.* At six-month follow-up for the initiation cohort while on an *ad libitum* diet, reduction in liver fat was maintained at 13.6 ± 9.7% (p=0.006 vs Period 1), but there was no further improvement relative to Period 2 (Figure 4). ALT and AST were measured on study entry (prior to the controlled diet) but not at the end of Period 1, thus any changes observed were not independent of food intake. Mean ALT was elevated at study entry (pre-metreleptin) at 64 ± 54 U/L (normal ≤ 41 in males over 18 years, ≤ 33 in females over 18 years, ≤ 30 in children), decreased non-significantly to 43 ± 23 after 2 weeks, and decreased significantly to 26 ± 13 after six months of metreleptin relative to study entry (p=0.004). Mean AST was borderline elevated at study entry at 39 ± 25 U/L (normal ≤ 40 in males over 18 years, ≤ 32 in females over 18 years, ≤ 40 in children), decreased

significantly to 30 ± 19 after 2 weeks, and further decreased to 22 ± 7 after six months of metreleptin (p=0.03 relative to study entry; p=0.04 relative to 2 weeks).

*Long-term metreleptin therapy did not change IMCL, and had variable effects on EMCL.* IMCL did not change in any muscle group after six months of metreleptin, but EMCL decreased in the lateral vastus and tibialis anterior muscles and increased in the soleus muscle (Table 4).

*Long-term metreleptin therapy maintained reduction in total and resting energy expenditure.* At six-month follow-up for the initiation cohort while on an *ad libitum* diet, reductions in total and resting energy expenditure were maintained at 2296 ± 372 kcal/day and 1731 ± 236 kcal/day, respectively (p=0.02 vs Period 1 for both), but there was no further change relative to Period 2.

*Adverse Events*. The following not-serious adverse events occurred in one subject each in the initiation cohort during long-term metreleptin treatment, and were considered at least possibly related to research: decreased appetite, weight loss, hair loss, hypoglycemia (in a subject treated with insulin), injection site reaction, and menorrhagia. Serious adverse events considered not related to research were: abdominal pain of unknown etiology (n=1) and angioedema secondary to angiotensin converting enzyme inhibitor use (n=1). Serious adverse events considered at least possibly related to research were: anemia secondary to menorrhagia (two events in one subject).

## Discussion

In patients with lipodystrophy, metreleptin therapy ameliorates metabolic abnormalities by reducing food intake (3, 12, 13), improving insulin resistance and diabetes (4, 13-15), and reducing ectopic lipid (7). These improvements in glucose and lipid metabolism are likely due in part to the reduction in food intake, but the clinical effects of metreleptin that are independent of changes in food intake have been poorly explored in humans. A single patient with acquired, generalized lipodystrophy was studied while taking metreleptin, and during metreleptin withdrawal, with constant energy intake (13). Upon metreleptin withdrawal, this patient experienced no changes in blood glucose, but a rise in serum insulin and triglycerides within one week (13). Although based on a single subject, these data suggested that leptin affects both insulin resistance and lipid metabolism independent of energy intake in humans. Our study demonstrates that metreleptin has food-intake independent effects in humans with lipodystrophy to increase peripheral and hepatic insulin sensitivity, and decrease fasting glucose, triglycerides, total cholesterol, and percent liver fat. As expected, the magnitude of metreleptin's effects independent of food intake over 2 weeks was smaller than the maximal effects of long-term metreleptin treatment during *ad libitum* food intake shown in prior studies (Figure 5) (4, 7, 15).

Because leptin reduces appetite (13), its effects independent of food intake cannot be studied in a free-living environment with *ad libitum* access to food. In this study, the tightly controlled nature of a metabolic ward permitted meticulous control of dietary intake, and our data confirmed that we successfully held food-intake constant for three weeks. It is likely that many of leptin's biological effects require more than two weeks of treatment initiation or withdrawal to show

maximal changes and thus, our study may underestimate the biological effects of leptin that are independent of food intake. Although it would have been informative to continue the study for a longer duration, three weeks was the practical limit during which we could keep patients hospitalized on a controlled diet.

The most consistent effect of metreleptin independent of food-intake was improvement in peripheral insulin sensitivity, which was 32% greater in the initiation cohort and 41% greater in the withdrawal cohort during the metreleptin treated periods. Hepatic insulin sensitivity was higher during metreleptin treatment in the initiation cohort, only. Our human data are consistent with prior findings in rodents, which showed that leptin improved peripheral and hepatic insulin sensitivity by 12-33% and 32-41%, respectively, independent of food intake (16, 17). Consistent with the improvements in insulin sensitivity, two weeks of metreleptin improved fasting glucose by 11%. Similar ~42-53% reduction in fasting glucose has been observed in pair-fed, leptin-deficient rodent studies (10, 11).

Based on prior data in humans (7, 18), we hypothesized that improved insulin sensitivity with metreleptin would be due to reductions in ectopic triglyceride in liver and myocytes. However, only reductions in hepatic triglyceride were observed. Numerous studies have demonstrated an association between hepatic triglyceride content and peripheral insulin resistance, although the direction of causality in this relationship is unclear (19-22). Although it is possible that lipid laden hepatocytes secrete cytokines or other substances that increase muscle insulin resistance, it is also possible that skeletal muscle or adipose tissue insulin resistance lead to hepatic triglyceride accumulation through mechanisms such as increased free fatty acid delivery from

adipose tissue to liver, or increased de novo lipogenesis stimulated by hyperinsulinemia. In rodents, a liver-targeted mitochondrial uncoupling agent led to decreases in both hepatic and peripheral insulin resistance, supporting the notion of a causal relationship between hepatic triglyceride and peripheral insulin resistance (23). Multivariate analyses in the current study also support a stronger role for intrahepatic triglyceride (versus intramyocellular triglyceride) in mediating both hepatic and peripheral insulin resistance, although we cannot prove a causal relationship. Changes in hepatic triglyceride content, but not intramyocellular triglyceride content, significantly predicted changes in both peripheral and hepatic insulin sensitivity, and changes in insulin sensitivity were no longer statistically significant after adjustment for changes in hepatic triglyceride. This suggests that reductions in hepatic triglyceride content with metreleptin may have mediated the improvements in insulin sensitivity.

Although patients who initiated metreleptin lost a small amount of weight and fat mass during metreleptin treatment with constant food intake, these changes in body composition did not predict changes in insulin sensitivity, and thus the small reductions in weight and body fat observed in the metreleptin-initiation cohort were not likely to have contributed to improved insulin sensitivity. An unexpected finding was the decrease in resting and total energy expenditure in the metreleptin-initiation cohort during constant food intake. Limited data in patients with congenital leptin deficiency or weight loss have suggested that metreleptin either does not change energy expenditure (24), or increases non-resting energy expenditure (25, 26). The biology underlying the reduction in resting energy expenditure in this study remains to be determined, but might include decreased urinary glucose loss, decreased energetic cost of hepatic glucose production (27, 28), decreased patient movement during measurement of energy

366 expenditure after repeated testing (29), and that subjects were in slightly negative energy
367 balance. Regardless of the reason for decreased energy expenditure, it is clear that there was no
368 increase in energy expenditure with metreleptin that contributed to weight loss or improved
369 insulin sensitivity.
370
371 Our study shows that there are food-intake independent effects of metreleptin on lipid metabolism,
372 with a reduction in circulating and hepatic triglycerides and total cholesterol in humans with
373 lipodystrophy. Although rodent studies have not demonstrated clinically relevant changes in lipids
374 independent of food intake, mechanistic studies in rodents have suggested that these effects may
375 be mediated by increased expression of enzymes and transcription factors involved in fatty acid
376 oxidation (e.g. mitochondrial and peroxisomal acyl-coenzyme A oxidase, peroxisomal
377 proliferator-activated receptor-alpha) and decreased expression of those regulating fatty acid
378 synthesis (e.g. stearoyl-CoA desaturase-1) (30-32).
379
380 We found that metreleptin treatment for six-months while on an *ad libitum* diet decreased both
381 glycerol and palmitate turnover in subjects with lipodystrophy, indicating a reduction in lipolysis.
382 This reinforces data from a prior study in three subjects with lipodystrophy, in whom three to five
383 months of metreleptin non-significantly decreased glycerol turnover (7). In contrast, *in vitro* and
384 *in vivo* rodent studies have shown that leptin treatment reduces muscle, liver, and adipose
385 triglyceride content by increasing lipolysis and fatty acid oxidation (33-39). These lipolytic effects
386 of leptin have been shown in obese rodents with mutations in the leptin gene or leptin-receptor,
387 but not in rodents with lipodystrophy, suggesting that the observed lipolytic effects of leptin
388 require normal adipose depots. Contrary to the findings in obese rodent models, long-term

metreleptin had anti-lipolytic effects in subjects with lipodystrophy. Although humans with lipodystrophy have a paucity of adipose tissue, these subjects are known to have elevated rates of lipolysis compared to gender-, age-, and BMI-matched controls prior to metreleptin therapy, presumably reflecting greater lipolysis in their residual fat mass (7). The effects of long-term metreleptin to suppress lipolysis are presumably secondary to improved insulin sensitivity, and hence increased insulin-mediated suppression of lipolysis. Given the hierarchy of physiologic responses to insulin, with suppression of lipolysis being the most sensitive, followed by suppression of hepatic glucose production, followed by glucose uptake in muscle, it is somewhat surprising that short-term metreleptin treatment did suppress hepatic glucose production and increase muscle glucose uptake, but did not decrease lipolysis. We speculate that the null effects of short-term metreleptin on lipolysis may be due to opposing direct lipolytic effects of leptin, versus indirect suppression of lipolysis mediated by improved insulin sensitivity.

A limitation of our study was the small number of participants, but lipodystrophy is a rare disorder. We had limited success in demonstrating biological effects of metreleptin withdrawal independent of food intake. Other than effects on peripheral insulin sensitivity, the withdrawal cohort did not experience the food-intake independent effects of metreleptin therapy that were observed in the initiation cohort. This may have been due to small sample size, as there were few statistical differences for metabolic changes in the on- versus off-metreleptin periods between the withdrawal and initiation cohorts. The lack of changes in the withdrawal cohort may also be due to two biological factors. First, the withdrawal cohort had an average of 7.7 ± 4.7 (range 0.9-14.5) years of prior metreleptin treatment, resulting in euglycemia and normal triglycerides despite their lipodystrophy diagnosis. Second, two weeks of metreleptin withdrawal may have been insufficient

to detect metabolic changes in the withdrawal cohort. By contrast, the initiation cohort had no exposure to metreleptin and worse metabolic profiles at baseline, allowing for metabolic changes that were of greater magnitude. The two groups also differed in types of lipodystrophy. In the withdrawal cohort, all subjects had generalized lipodystrophy, and in the initiation cohort, most subjects had partial lipodystrophy. This difference is not a likely explanation for the lack of effects in the withdrawal cohort because we would have expected greater effects in subjects with generalized lipodystrophy who have lower endogenous leptin levels, but this was not observed.

By using lipodystrophy as a model for leptin-deficiency and replacement, we have successfully demonstrated that metreleptin therapy has food-intake independent effects on glucose and lipid metabolism in humans. In addition to serving as a model for leptin-deficiency, lipodystrophy is also a more severe form of the obesity-associated metabolic syndrome. Although metreleptin treatment has biological effects in states of chronic hypoleptinemia, it has little effect on appetite, body weight, or hormonal axes in leptin replete subjects undergoing either mild, ongoing caloric restriction, or acute, severe energy restriction (72 hour fast), despite the fact that caloric restriction can acutely decrease leptin levels (40-43). This study provides evidence for food-intake independent effects of metreleptin in leptin-deficient humans, but effects of leptin independent of food intake have yet to be explored in leptin-sufficient human models such as the obesity-associated metabolic syndrome.

## Methods

*Study subjects.* This was a non-randomized, crossover group study. Two groups of patients aged 14 to 70 years with lipodystrophy were studied: leptin initiation and leptin withdrawal. Participants were recruited by referral from November 2012 to January 2017. Leptin initiation subjects had no prior exposure to exogenous metreleptin and leptin withdrawal subjects had taken a stable dose of exogenous metreleptin for a minimum of four months prior to study participation. The flow chart of study participants in each cohort is shown in Figure 1. Of the 25 patients enrolled, 15 were in the leptin-initiation cohort and 10 were in the leptin-withdrawal cohort. In the leptin-initiation cohort, one subject did not complete data collection for the short-term study, but continued study drug and completed the long-term study, and another subject completed the short-term study but was excluded from analysis of the long-term study because of non-compliance with metreleptin therapy. In the leptin-withdrawal cohort, one subject withdrew consent and another subject was excluded from analysis due to recurrent hypoglycemia during the short-term study. Therefore, 14 subjects in the leptin-initiation cohort and eight subjects in the leptin-withdrawal cohort were included in final analysis.

*Inclusion/Exclusion criteria.* Eligibility was based on a clinical diagnosis of lipodystrophy, age ≥ 14 years, and one or more metabolic abnormalities including diabetes mellitus defined by the 2007 American Diabetes Association criteria, insulin resistance (fasting insulin ≥ 30 μIU/mL), or hypertriglyceridemia (fasting triglyceride > 200 mg/dL). Patients were also required to have low endogenous serum leptin measured either at NIH or at an outside laboratory prior to metreleptin treatment (< 8 ng/mL in males, < 12 ng/mL in females). Exclusion criteria included HIV-associated lipodystrophy, active inflammatory disease or glucocorticoid use, and changes in

diabetes or lipid-lowering medications within the past six weeks. Because of the risk of worsening metabolic status with metreleptin withdrawal, additional exclusion criteria applied to the leptin-withdrawal cohort, including: age < 18 years, HbA1c ≥ 9%, serum triglycerides > 800 mg/dL, > 1 lifetime episode of acute pancreatitis, or ≥ 1 episode of pancreatitis while on metreleptin, lipase greater than upper limit of normal at study entry, or known presence of neutralizing antibodies to leptin.

*Study design.* The study design is shown in Figure 2. Initiation subjects were studied for the first five days without metreleptin (Period 1), then treated with metreleptin (5 mg subcutaneously q12 hours) for the next 14 days (Period 2). Withdrawal subjects were studied for the first five days on their home dose of metreleptin (Period 1), then withdrawn from metreleptin for the next 14 days (Period 2). Metreleptin was donated by Aegerion Pharmaceuticals (Cambridge, MA). Subjects and investigators were not blinded to the intervention. All subjects were hospitalized on the metabolic unit of the NIH Clinical Center, and consumed a controlled diet provided by the metabolic kitchen. The study diet was controlled for macronutrient content (20±5% protein, 25±5% fat, 55±5% carbohydrate). Research dietitians used the Mifflin St. Jeor equations for males with an activity factor of 1.5 to estimate total caloric requirements (for both male and female participants). Food intake (total kilocalories and macronutrient content) was adjusted for body weight fluctuations to ensure eucaloric feeding during Period 1 and then the energy was clamped for Period 2, in order to assess leptin's effects independent of energy intake. Subjects were instructed in the importance of eating 100% of food given, and not consuming any additional food. However, to determine possible deviations from the study diet, any uneaten food was weighed and the uneaten kilocalories were recorded. At the end of Period 2, metreleptin was restarted in subjects in the withdrawal

cohort at their previous doses. Patients in the initiation cohort continued self-administered metreleptin treatment after discharge, and underwent follow-up evaluation after six months of treatment on an *ad libitum* diet. For patients in the initiation cohort with partial lipodystrophy, metreleptin was continued at a dose of 5 mg q12 hours. For patients in the initiation cohort with generalized lipodystrophy, the metreleptin dose was lowered at the end of Period 2 to prevent excessive weight loss during the six-month follow-up period.

Apart from insulin and sulfonylureas, subjects continued their pre-admission medications throughout the study, including oral hypoglycemic agents, lipid-lowering medications, and other medications either related or unrelated to lipodystrophy and its complications. Initiation subjects taking insulin or sulfonylureas were at risk of hypoglycemia due to improved insulin sensitivity after metreleptin. None of the withdrawal subjects were taking insulin. Glucose monitoring was performed in subjects with diabetes prior to meals and at bedtime. Due to hypoglycemia risk, insulin and sulfonylurea doses were reduced as needed to minimize hypoglycemia.

*Primary outcomes.* The aim of this study was to determine the energy intake-independent effects of leptin on glucose and lipid metabolism in lipodystrophic subjects. The pre-specified primary outcome for glucose metabolism was total body insulin sensitivity (measured as glucose disposal rate during a hyperinsulinemic-euglycemic clamp), and for lipid metabolism was the rate of lipolysis (measured using glycerol stable isotope tracers). For leptin initiation and withdrawal cohorts, clinical values were collected at study entry, end of Period 1, and end of Period 2 (Figure 2). Additional clinical values were obtained from the leptin initiation cohort at the six-month follow-up visit while on an *ad libitum* diet.

Additional outcomes included serum leptin levels, anthropometric parameters (body mass index [BMI] and body fat percent), glycemic and lipid variables (fasting glucose, fasting insulin, fasting c-peptide, HbA1c, lipids, urinary glucose excretion, number of anti-diabetic and lipid-lowering medications, insulin use and average daily insulin dose among insulin users), hepatic insulin sensitivity (measured as suppression of endogenous glucose production during a hyperinsulinemic-euglycemic clamp), rates of lipolysis and fatty acid turnover (measured using glycerol and palmitate stable isotope tracers), and lipid content in liver and skeletal muscles (measured using magnetic resonance spectroscopy [MRS]).

*Metabolites and hormones*. Blood samples were obtained following an 8-12 hour fast. Urine was collected over 24-hour periods. Glucose, insulin, C-peptide, HbA1c, total cholesterol, HDL-C, LDL-C, triglycerides, and urinary glucose excretion were analyzed using standard techniques of the NIH Clinical Center laboratory. In the withdrawal cohort, endogenous leptin in fasting serum samples was measured prior to metreleptin initiation by radioimmunoassay (EMD-Millipore, Billerica MA). The intra and inter assay coefficient of variation were 9.3% and 9.6% respectively. Of note, these samples for measurement of endogenous leptin were collected immediately prior to metreleptin initiation, 0.9 to 14.5 years prior to participation in the current study, under other IRB-approved protocols. In both cohorts, leptin was also measured in fasting EDTA-plasma samples at the end of Periods 1 and 2, and again after six months of metreleptin in the initiation cohort, by ELISA (EMD-Millipore, Billerica MA). The intra and inter assay coefficient of variation were 3.9% and 4.8% respectively.

*Body composition.* A DXA scan was obtained to measure fat and lean body mass at the end of Period 1 and Period 2 for both cohorts, and during the six-month follow-up for the initiation cohort only (iDXA, GE Healthcare, Madison, WI).

*Energy expenditure.* Energy expenditure was measured at the end of Period 1 and Period 2 for both cohorts, and during the six-month follow-up for the initiation cohort only. Resting energy expenditure (REE) was measured using indirect calorimetry with a hood calorimeter (ParvoMedics TrueOne2400, Sandy UT) upon awakening after a minimum 8-hour fast, in a resting supine position. Twenty-four-hour total energy expenditure (TEE) was using a whole-room indirect calorimeter (metabolic chamber) (44). Periods of exercise during the 24-hour metabolic chamber stay were assessed using a microwave detection system; these periods were excluded from the analysis of TEE, with data renormalized to a 24-hour period. Non-resting energy expenditure was calculated as the difference between TEE and REE.

*MRI/MRS.* Hepatic triglyceride content was measured using MRS as previously described (45, 46). Intramyocellular and extramyocellular triglyceride content in the vastus lateralis, anterior tibialis, and soleus muscles were measured using MRS as previously described (46).

*Tracer dilution and clamp studies*. Following an overnight fast, stable isotope tracers were used to measure glucose, glycerol, and palmitate turnover using the tracer dilution method. At 0500 hours, one catheter was inserted into the forearm vein to infuse stable isotopically labeled tracers. A second catheter was inserted into a vein in the contralateral hand or arm to obtain blood samples. A primed, continuous infusion of [6,6-$^{2}H_{2}$]glucose (priming dose 28 μmol/kg of body weight;

infusion rate 0.4 µmol/kg of body weight/min for 180 min) was used to measure basal endogenous production (Cambridge Isotope Laboratories). At 0700 hours, a primed, continuous infusion of [$^{2}H_5$]glycerol (priming dose 0.045 µmol/$kg_{BW;}$ infusion rate: 0.18 µmol/$kg_{BW}$/min) and an unprimed infusion of [U-$^{13}C_{16}$]palmitate (infusion rate: 0.006 µmol/$kg_{BW}$/min) were administered for 60 minutes to measure rate of lipolysis (Cambridge Isotope Laboratories).

At 0830 hours, a hyperinsulinemic-euglycemic clamp study began. Regular human insulin was infused at a priming rate of 240 mU/m2/min for eight minutes, followed by a continuous insulin infusion at 120 mU/$m^2$/min for approximately 3 hours. [6,6-$^{2}H_2$]glucose was infused at 25% of the baseline rate (0.1 µmol/$kg_{BW}$/min). Dextrose solution (20%) enriched with 2.5% [6,6-$^{2}H_2$]glucose tracer was infused at a variable rate to maintain blood glucose at 100±5 mg/dL. Due to severe insulin resistance and hyperglycemia, two subjects maintained a steady state glucose of 132±1.3 mg/dL for all visits. Blood samples (0.5 mL) were obtained every five to 10 min for analysis of whole-blood glucose concentration, measured by an automated glucose analyzer (Yellow Springs Instruments Co.). Blood samples for analysis of glucose, insulin, C-peptide, and [6,6-$^{2}H_2$]glucose were collected every 10 minutes during steady-state (the final 30 minutes of the study).

*Liquid chromatography-mass spectrometry*. Isotope enrichment was measured using a Waters Acquity UPLC and a Thermo Scientific Q-Exactive (high resolution – accurate mass). The separation was on a Waters BEH Amide column (1.7 µm 2.1 x 100 mm) using solvent A (30% ACN, 70% $H_2O$, 0.1% $NH_3$) and solvent B (80% ACN, 20% $H_2O$, 0.1% $NH_3$). The Q-Exactive with HESI-II electrospray source negative ion used targeted-SIM mode at 70K resolution for palmitate, 70K full scan for glycerol and targeted-SIM mode at 140K for glucose. Each targeted-

SIM was triggered by an inclusion list of the natural occurring molecule. Glucose was measured at m/z 179.0556, [6,6-$^{2}H_2$]glucose at 181.0684, glycerol at 91.0388, [$^{2}H_5$]glycerol at 96.0700, palmitate at 255.2336 and [U-$^{13}$C]palmitate at 271.2874. Standards of 0 – 16.7 molar percent enrichment (MPE) of [6,6-$^{2}H_2$]glucose, 0 – 13.1 MPE [$^{2}H_5$]glycerol, and 0 – 0.9 MPE [U-$^{13}$C]palmitate were calibrated with $R^2 > 0.99$ (47).

*Calculations.* The rate of appearance of glucose, glycerol, and palmitate per kg of lean body mass was calculated by measuring isotope enrichment using the single pool model (48). Peripheral insulin sensitivity (M value) was calculated as the average glucose infusion rate during 30-minute steady-state of the hyperinsulinemic-euglycemic clamp and corrected for fat-free mass (49). Hepatic glucose production was calculated as the difference between basal glucose rate of appearance and glucose infusion rate during clamp steady-state.

*Power and sample size calculations.* Power analyses were conducted a priori based on data from previous human studies using leptin-deficient and replacement models and indicated that a sample size of 10 subjects in each group (leptin-initiation and leptin-withdrawal) would provide 80% power to detect significant differences between the off versus on metreleptin condition during constant food intake for the following primary and secondary outcomes: peripheral insulin sensitivity, hepatic insulin sensitivity, fasting plasma glucose, rate of lipolysis, and fasting triglycerides. Given the limited pool of subjects with lipodystrophy already taking metreleptin (leptin-withdrawal cohort) who met inclusion/exclusion criteria, we were unable to accrue the target sample size of 10 for this group.

*Statistics.* For all outcomes, normally distributed data were reported as mean ± SD. Non-normally distributed data were reported as geometric mean [$25^{th}$,$75^{th}$ percentiles]. Measurements in each of the primary and secondary outcomes were analyzed to detect differences between Period 1 and Period 2 for each cohort (leptin-initiation and leptin-withdrawal). For the leptin-initiation cohort, secondary analyses were conducted to detect differences between Period 1 and six-month follow-up, and between Period 2 and six-month follow-up using both multiple paired comparisons, as well as linear mixed models with Bonferroni correction for multiple comparisons for pairs of timepoints.

Data analysis for primary and secondary outcomes was done in two ways: without covariate adjustment and with covariate adjustment. Potential covariates included in each model were: baseline (pre-diet) value for the outcome, age, sex, race, type of lipodystrophy (partial versus generalized, initiation cohort only), endogenous leptin level prior to metreleptin treatment, and measured mean caloric intake during Period 1 and Period 2. For total body insulin sensitivity, additional models were conducted including the above covariates plus body weight, fat mass, and lean mass during the metreleptin treated and untreated conditions. For both hepatic and total body insulin sensitivity, additional models were conducted including covariates age, sex, and hepatic and intramyocellular triglyceride content (together and in separate models) during the metreleptin treated and untreated conditions. For total, resting, and non-resting energy expenditure, models included fat mass and lean body mass as covariates.

Unadjusted comparisons for each outcome were conducted using the paired t-test (for normally distributed variables) or Wilcoxon paired test (for skewed variables). For adjusted comparisons

for each outcome, a variable selection for linear mixed model was conducted and then a final linear mixed model with the selected covariates was performed to compare timepoints. With a single exception, noted in the Results, adjustment for covariates did not alter the statistical significance of any primary or secondary outcome. Therefore, only the unadjusted analyses are presented in the Results and Figures. Linear mixed model analyses for covariate-adjusted analysis are presented in Supplemental Tables 1-4. If significant differences were present in Period 1 versus Period 2 for an outcome in either the initiation or withdrawal cohort, we compared the delta between Periods 1 and 2 for the two cohorts using 2-sample t-tests (for normally distributed variables) or Mann-Whitney tests (for skewed variables). Only differences that were statistically significant are mentioned in the Results.

For the two prespecified co-primary outcomes of total body insulin sensitivity and lipolysis (glycerol Ra), a p-value $<0.025$ was considered statistically significant to account for multiple comparisons. No multiplicity corrections were used for secondary outcomes, and a p-value $<0.05$ was considered statistically significant. All reported p-values are two-sided. Data analysis was conducted by using SAS software (version 9.4, Cary, NC) and GraphPad Prism (version 7.00, GraphPad Software, La Jolla California USA, www.graphpad.com).

*Study Approval.* The institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases approved this study. All patients or legal guardians for those under 18 years of age provided written informed consent before participation, and minor subjects provided written assent. This study was registered at www.clinicaltrials.gov (trial ID NCT01778556).

## Author Contributions

RJB initiated the investigation, led the clinical experiments and wrote, reviewed, and edited the manuscript. AV obtained and analyzed the data, and wrote, edited, and reviewed the manuscript. MS obtained data, and wrote, edited, and reviewed the manuscript. EC obtained data, and edited and reviewed the manuscript. AG obtained and interpreted MRS data, and reviewed and edited the manuscript. RJB and KYC obtained and interpreted energy expenditure data, and reviewed and edited the manuscript. PW, HMG, HC, and MW obtained data, and edited and reviewed the manuscript. AC and SB designed and implemented the controlled study diet and reviewed and edited manuscript. AS provided statistical guidance prior to study implementation, conducted statistical analyses, and edited and reviewed the manuscript. PG contributed to the design of the study, and reviewed and edited the manuscript. All authors gave final approval of the version to be published.

**Acknowledgements**

This study was supported by the intramural research program of the NIDDK. We would like to thank the nurses in the Metabolic Unit of the NIH Clinical Center for excellent patient care. Metreleptin for this study was donated by Aegerion Pharmaceuticals. This study would not have been possible without the altruism of our patients and their families, who devoted their time and energy to help us enhance scientific knowledge.

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## Figures and Figure Legends

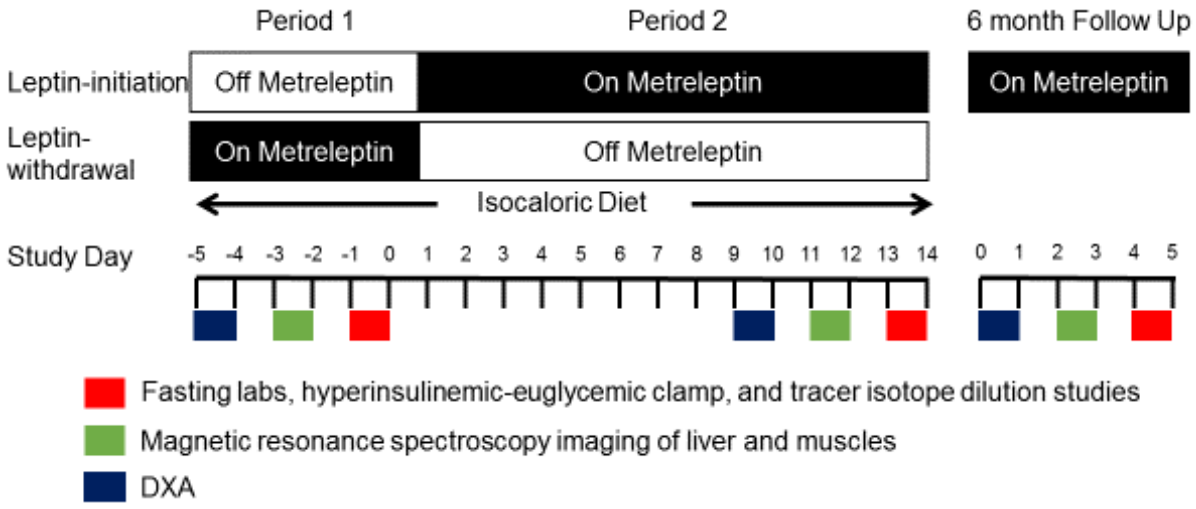


**Figure 1. Study design.** The leptin-initiation cohort was untreated for first five days (Period 1), then metreleptin was given for the following 14 days (Period 2). This order was reversed for leptin-withdrawal cohort. During the short-term study, an isocaloric diet was maintained for both cohorts to permit study of metreleptin's effects during constant energy and macronutrient intake. During both Periods 1 and 2, patients in both cohorts underwent a DXA scan, hyperinsulinemic-euglycemic clamp, and MRS/MRI scan. This was repeated at a six-month follow-up visit in the initiation cohort only during *ad libitum* diet.

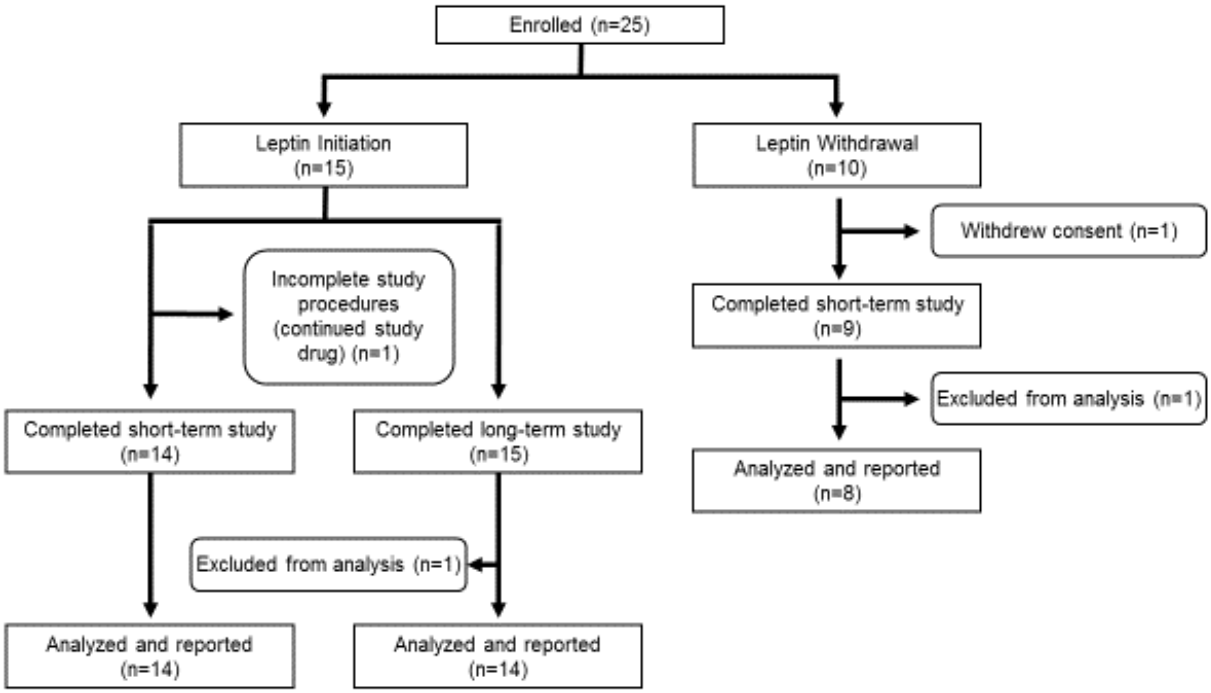


**Figure 2. Study flow chart.** A total of 25 subjects were enrolled in the study, 15 in the initiation and 10 in the withdrawal cohort. In the initiation cohort, one subject did not have complete data collection for the short-term study, but completed the long-term study, and one subject was excluded from final analysis of the long-term study because of non-compliance with metreleptin. In the withdrawal cohort, one subject withdrew and another subject with type 1 diabetes was excluded from the analysis due to recurrent hypoglycemia during the short-term study.

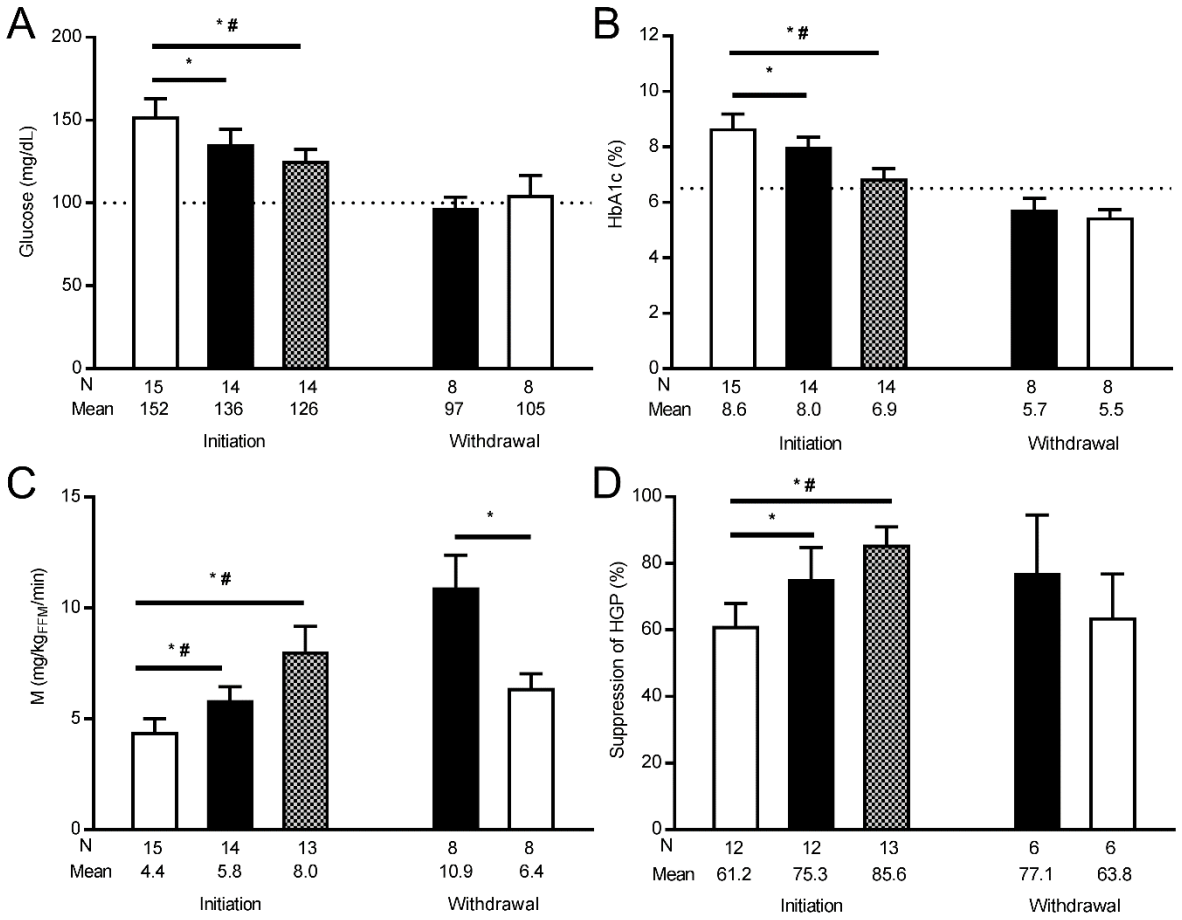


**Figure 3. Glucose control and insulin sensitivity improved in humans with lipodystrophy while on metreleptin independent of food intake.** (A) Fasting glucose levels in leptin-initiation and leptin-withdrawal subjects while off (white bars), on (black bars), and after six months on (gray bars) metreleptin. The dotted gray line indicates of the upper limit of normal (100 mg/dL). (B) Hemoglobin A1c. The dotted gray line indicates the threshold for diagnosis of diabetes (6.5%). (C) Whole-body insulin sensitivity reflected by the M value (hyperinsulinemic-euglycemic clamp). (D) Insulin-mediated suppression of hepatic glucose production (HGP) as an indicator of hepatic insulin sensitivity. Data shown represent the mean ± SEM. The study was powered to detect differences between the off versus on leptin state (black versus white bars) during constant

825 food intake. * indicates $P<0.05$ determined by 2-tailed *t* test or Wilcoxon matched-pairs signed
826 rank test between each pair of time points based on data distribution. # indicates $P<0.05$ by linear
827 mixed model for all three timepoints with post-hoc pairwise Bonferroni correction in the leptin-
828 initiation cohort.

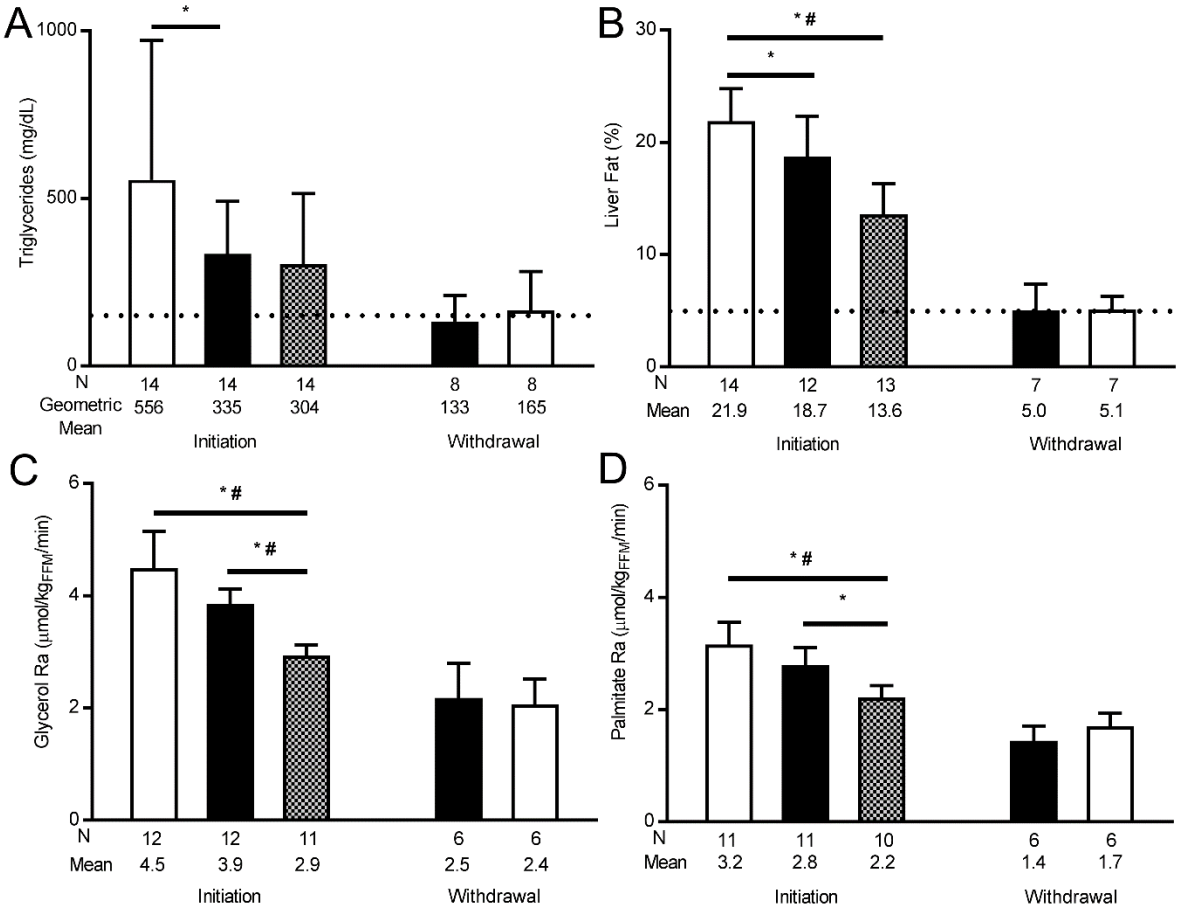


**Figure 4. Triglycerides and liver fat decreased in humans with lipodystrophy while on metreleptin independent of food intake**. (A) Triglycerides of leptin-initiation subjects and leptin-withdrawal subjects while off (white bars), on (black bars), or after six months on (gray bars) metreleptin. The dotted line indicates the upper limit of normal (150 mg/dL). (B) Percent liver fat measured by magnetic resonance spectroscopy. The dotted line indicates upper limit of normal (5%). (C) Glycerol rate of appearance (Ra) in plasma. (D) Palmitate Ra in plasma. Data shown represent the mean ± SEM or geometric mean +/- 95% CI (triglycerides). The study was powered to detect differences between the off versus on leptin state (black versus white bars) during constant food intake. * indicates $P<0.05$ determined by 2-tailed *t* test or Wilcoxon matched-pairs

signed rank test between each pair of time points based on data distribution. # indicates $P<0.05$ by linear mixed model for all three timepoints with post-hoc pairwise Bonferroni correction in the leptin-initiation cohort.

| | Effects of leptin independent of food intake | Maximal effects of leptin |
|---|---|---|
| Peripheral Insulin Sensitivity | ↑ | ↑ |
| Hepatic Insulin Sensitivity | ↑ | ↑ |
| Blood glucose | ↓ | ↓ |
| Lipolysis | ↔ | ↓ |
| Plasma triglycerides | ↓ | ↓ |
| Hepatic triglyceride | ↓ | ↓ |
| Intramyocellular lipid | ↔ | ↓ |

**Figure 5. Effects of leptin in patients with lipodystrophy independent of food intake versus maximal effects of leptin during *ad libitum* food intake.** The current study demonstrated effects of leptin replacement with metreleptin with food intake held constant over 2 weeks. These effects were smaller in magnitude than the maximal effects of metreleptin demonstrated in long-term studies with ad libitum food intake.

**Table 1. Baseline characteristics in initiation and withdrawal cohorts**

| Clinical Values | Initiation (n=15) | Withdrawal (n=8) |
|---|---|---|
| Type of lipodystrophy (Generalized/Partial) | (3/12) | (8/0) |
| Sub-type of lipodystrophy | 3 CGL<br>12 FPL | 7 CGL<br>1 AGL |
| Sex (Male/female) | (3/12) | (3/5) |
| Age (years) | 32 ± 17 | 25 ± 6 |
| Race/ethnicity | 9 Caucasian<br>4 Hispanic<br>1 Asian<br>1 Other | 4 Caucasian<br>2 African-American<br>2 Hispanic |
| Endogenous leptin level (ng/dL) | 9.5 ± 10.2[A] | 1.2 ± 0.5[A] |
| Duration of metreleptin treatment prior to study (years) | 0 | 7.7 ± 4.7 |
| Subjects on insulin (%) | 71 | 0 |
| Insulin dose (units per day, insulin users only) | 225 ± 136 | 0 |
| Number of diabetes medications | 1.6 ± 1.2 | 0.4 ± 0.5 |
| Number of lipid medications | 1.8 ± 0.9 | 0.4 ± 0.7 |

Data represent mean ± SD except as noted. CGL: Congenital generalized lipodystrophy; FPL: Familial partial lipodystrophy; AGL: Acquired generalized lipodystrophy. [A]Endogenous leptin levels were measured by ELISA in the initiation cohort, and by RIA in the withdrawal cohort prior to metreleptin initiation.

854 **Table 2. Diet and body composition off- and on-metreleptin treatment.**

| | **Initiation (n=14)** | | | **Withdrawal (n=8)** | | |
|---|---|---|---|---|---|---|
| | **OFF (Period 1)** | **ON (Period 2)** | **P** | **ON (Period 1)** | **OFF (Period 2)** | **P** |
| **Diet Composition** | | | | | | |
| Energy Intake (kcals) | 2416 ± 312 | 2422 ± 370 | 0.85 | 2350 ± 501 | 2425 ± 525 | 0.38 |
| Protein intake (%) | 17.3 ± 1.1 | 17.4 ± 1.8 | 0.61 | 17.6 ± 2.1 | 17.6 ± 2.2 | 0.74 |
| Carbohydrate intake (%) | 52.2 ± 1.8 | 52.2 ± 1.8 | 0.44 | 53.2 ± 1.1 | 52.9 ± 1.3 | 0.26 |
| Fat intake (%) | 30.4 ± 1.0 | 30.0 ± 0.5 | 0.22 | 29.2 ± 1.9 | 29.5 ± 2.1 | 0.13 |
| **Body Composition** | | | | | | |
| Body Weight (kg) | 73.8 ± 16.0 | 73.1 ± 15.8 | 0.04 | 59.3 ± 17.2 | 59.0 ± 16.7 | 0.53 |
| BMI ($kg/m^2$) | 25.5 ± 4.5 | 25.0 ± 4.7 | 0.01 | 19.8 ± 4.2 | 20.0 ± 4.1 | 0.41 |
| Lean mass (kg) | 53.1 ± 9.2 | 52.7 ± 9.3 | 0.32 | 54.3 ± 13.8 | 54.8 ± 13.1 | 0.81 |
| Fat mass (kg) | 18.3 ± 10.6 | 18.1 ± 10.6 | 0.02 | 4.1 ± 1.2 | 4.2 ± 0.9 | 0.76 |
| Percent Fat Mass (%) | 24.3 ± 10.8 | 24.1 ± 10.9 | 0.16 | 7.4 ± 1.6 | 7.6 ± 1.2 | 0.81 |
| **Plasma leptin (ng/dL)[A]** | 9.5 ± 10.2 | 71.0 ± 25.3 | 0.0001 | 62.0 ± 79.4 | 3.7 ± 8.6 | 0.008 |

855 Data represent mean ± SD. [A]The plasma leptin assay measures both endogenous leptin and

856 exogenous metreleptin.

**Table 3. Metabolic characteristics off- and on-metreleptin treatment.**

| | Initiation (n=14) | | | Withdrawal (n=8) | |
|---|---|---|---|---|---|
| | **OFF (Period 1)** | **ON (Period 2)** | **ON (6-month)** | **ON (Period 1)** | **OFF (Period 2)** |
| **Glycemic Parameters** | | | | | |
| Fasting glucose (mg/dL) | 152 ± 42 | 136 ± 34[A] | 126 ± 26[B] | 97 ± 18 | 105 ± 33 |
| Fasting insulin (μU/mL) | 40 [23,57] | 33 [18,63] | 25 [12,66] | 20 [13,28] | 31 [15,51] |
| Fasting c-peptide (ng/mL) | 4.0 ± 1.6 | 4.2 ± 1.9 | 3.4 ± 1.9 | 3.5 ± 1.4 | 5.2 ± 2.2 |
| Urinary glucose excretion (g/24h) | 2.0 [0.2,10.3] | 1.2 [0.2,7.2][A] | 0.4 [0.1,0.6] | 0.2 [0.1,0.7] | 0.3 [0.1,2.4] |
| **Lipid Parameters** | | | | | |
| Triglycerides (mg/dL) | 556 [224,1144] | 326 [162,660][A] | 304 [122,547] | 133 [78,215] | 165 [99,361] |
| Total cholesterol (mg/dL) | 241 ± 116 | 171 ± 48[A] | 171 ± 58[B] | 129 ± 32 | 123 ± 29 |
| LDL-C (mg/dL) | 87 ± 34 | 78 ± 33 | 73 ± 32 | 68 ± 27 | 54 ± 24 |
| HDL-C (mg/dL) | 27 ± 5 | 25 ± 5 | 28 ± 5 | 32 ± 7 | 29 ± 8 |
| FFA (mEq/L) | 0.43 ± 0.17 | 0.42 ± 0.18 | 0.41 ± 0.10 | 0.20 ± 0.09 | 0.23 ± 0.06 |

FFA, free fatty acids. Data represent mean ± SD or geometric mean [25$^{th}$,75$^{th}$ centile] based on distribution of data. [A]Significant difference between Period 1 vs Period 2, [B]Significant difference between Period 1 vs six-month visit. There were no significant differences between Period 2 vs six-month visit in the initiation cohort.

**Table 4. Intramyocellular (IMCL) and extramyocellular (EMCL) lipid content in muscles during off- and on-metreleptin treatment.**

| | Initiation (n=12) | | | Withdrawal (n=6) | |
|---|---|---|---|---|---|
| | **OFF (Period 1)** | **ON (Period 2)** | **ON (6-month)** | **ON (Period 1)** | **OFF (Period 2)** |
| **IMCL (%)** | | | | | |
| Lateral Vastus | 7.7 ± 4.1 | 7.7 ± 3.5 | 7.2 ± 4.9 | 3.9 ± 3.4 | 3.7 ± 3.3 |
| Tibialis Anterior | 8.0 ± 4.7 | 7.9 ± 3.9 | 6.6 ± 3.4 | 4.9 ± 1.8 | 6.3 ± 3.7 |
| Soleus | 11.8 ± 6.3 | 18.0 ± 10.1 | 13.3 ± 7.6 | 7.9 ± 6.0 | 10.0 ± 7.8 |
| **EMCL (%)** | | | | | |
| Lateral Vastus | 19.4 ± 10.5 | 16.5 ± 11.8 | 13.4 ± 9.2[A] | 2.5 ± 2.2 | 4.4 ± 2.9 |
| Tibialis Anterior | 27.5 ± 16.4 | 28.6 ± 22.4 | 18.0 ± 12.8 [A] | 5.0 ± 4.2 | 6.2 ± 3.9 |
| Soleus | 50.8 ± 25.7 | 41.5 ± 23.0 | 54.6 ± 39.1[B] | 5.4 ± 3.6 | 6.3 ± 2.8 |

Data show mean ± SD of all subjects. There were no significant differences between Period 1 vs Period 2, [A]Significant decrease from Period 1 to six-month visit, and [B]Significant increase from Period 2 to six-month visit.