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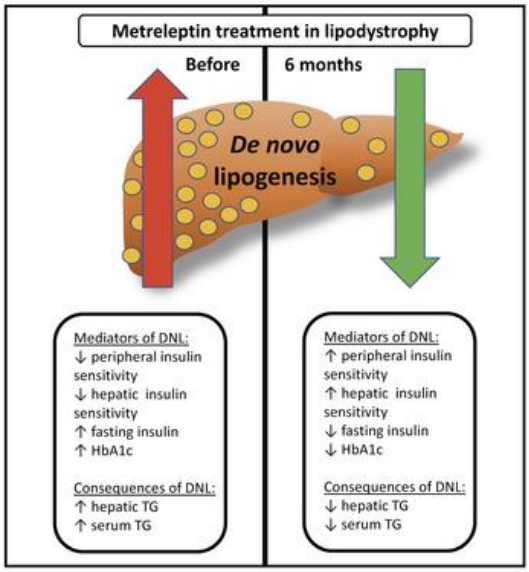
Graphical abstract



Leptin Decreases De Novo Lipogenesis in Patients with Lipodystrophy

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Abstract

De novo lipogenesis (DNL) plays a role in the development of hepatic steatosis. In humans with lipodystrophy, reduced adipose tissue causes lower plasma leptin, insulin resistance, dyslipidemia and ectopic triglyceride (TG) accumulation. We hypothesized that recombinant leptin (metreleptin) for 6 months in 11 patients with lipodystrophy would reduce DNL by decreasing insulin resistance and glycemia, thus reducing circulating and hepatic-TG.

The percentage of TG-rich lipoprotein particle (TRLP)-TG derived from DNL (%DNL) was measured by deuterium incorporation from body water into palmitate. At baseline, DNL was elevated, similar to levels previously shown in obesity-associated nonalcoholic fatty liver disease (NAFLD). After metreleptin, DNL decreased into the normal range. Similarly, absolute DNL (TRLP-TG x % DNL) decreased by 88% to near-normal levels. Metreleptin improved peripheral insulin sensitivity (hyperinsulinemic-euglycemic clamp) and lowered HbA1c and hepatic-TG. Both before and after metreleptin, DNL positively correlated with insulin resistance, insulin doses, and hepatic-TG, supporting the hypothesis that hyperinsulinemia stimulates DNL and that elevated DNL is integral to the pathogenesis of lipodystrophy-associated NAFLD.

These data suggest that leptin-mediated improvement in insulin sensitivity increases clearance of blood glucose by peripheral tissues, reduces hepatic carbohydrate flux, and lowers insulinemia, resulting in DNL reductions, and improvements in hepatic steatosis and dyslipidemia.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the U.S. (1), and NAFLD progressing to nonalcoholic steatohepatitis is estimated to overtake hepatitis C as the primary cause of liver transplantation in the US (2). One source of hepatic-triglyceride (TG) is de novo lipogenesis (DNL), the synthesis of fatty acids from non-lipid, primarily carbohydrate, precursors (3). Leptin replacement in leptin-deficient rodents reduces hepatic steatosis by decreasing DNL (4); by contrast, rodent studies suggest that high leptin in obesity might contribute to hepatic fibrosis (5, 6). In humans, NAFLD is observed in both the hyperleptinemic state of obesity and the leptin-deficient state of lipodystrophy (7, 8). Therefore, the role of leptin in mediating DNL and NAFLD is unclear.

Lipodystrophy syndromes are characterized by adipose tissue deficiency with metabolic manifestations similar to obesity-associated metabolic syndrome (8). Lipodystrophy syndromes are associated with low circulating leptin due to low adipose mass, thus these syndromes serve as a model to understand effects of leptin deficiency and replacement on metabolic disease. Leptin treatment with recombinant human methionyl leptin (metreleptin) in lipodystrophic patients improves hepatic steatosis and hypertriglyceridemia (9), though the exact mechanisms by which metreleptin mediates these responses have yet to be elucidated. In an earlier report of three patients with partial lipodystrophy, DNL was elevated, suggesting that increased DNL may play a role in lipodystrophy-associated NAFLD (10). We hypothesized that metreleptin treatment in patients with lipodystrophy would decrease DNL by lowering hepatic insulin exposure and carbohydrate flux, and that reductions in DNL would be associated with reductions in circulating- and hepatic-TG. Effects of metreleptin on DNL were assessed by labeling of body water with deuterium and measuring its incorporation into TG-rich lipoproteins (TRLP) using mass isotopomer analysis in 11 patients with lipodystrophy before and after 6 months of metreleptin administration. Potential mediators of changes in DNL were investigated including measures of glucose disposal (hyperinsulinemic euglycemic clamp) or insulin exposure (exogenous insulin use) and glycemia. Consequences of changes in DNL were investigated including serum-TG and hepatic-TG content by

magnetic resonance spectroscopy (MRS).

Results

Baseline characteristics and metreleptin treatment

Eleven patients (3 men, 8 women), four with congenital generalized lipodystrophy and seven with familial partial lipodystrophy, aged 34±17 years, were treated with metreleptin for 7.0±0.8 months at a dose of 8.1±2.7 mg/day. Metreleptin increased serum leptin concentrations from a baseline of 9.5±11.0 to 155.0±71.5 ng/dl (P=0.002; **Table 1**).

Metreleptin decreased fasting de novo lipogenesis

After 6 months of metreleptin, the TG content of TRLP (TRLP-TG) decreased by 38±40%, from 160 to 98 mg/dl (P=0.02; **Figure 1A**). The percentage of TRLP-TG that was derived from DNL (%DNL) decreased from a baseline of 20.9% [18.0,29.7] to 7.3% [5.8,11.6] (P<0.001; **Figure 1B**). Absolute DNL also decreased by 88±7%, from 54.2±32.1 mg/dl to 8.6±6.5 mg/dl (P=0.003; **Figure 1C**).

Metreleptin improved insulin sensitivity

As previously reported in an overlapping cohort of subjects (11), metreleptin treatment for 6 months improved multiple measures of insulin sensitivity (**Table 1**). Peripheral insulin sensitivity, assessed as glucose disposal during a hyperinsulinemic-euglycemic clamp, increased by 101±128% (P=0.034). Similarly, hepatic insulin sensitivity, assessed as suppression of hepatic glucose production during the clamp, increased by 48±49% (P=0.012). Fasting insulin and C-peptide decreased by 29±40% (P=0.049) and 37±28% (P=0.006), respectively. Insulin total daily dose among insulin users decreased non-significantly by 36±52% (P=0.15). One subject with generalized lipodystrophy was able to completely discontinue insulin treatment after 6 months of metreleptin.

Metreleptin reduced carbon sources for de novo lipogenesis (glucose and branched-chain amino acids)

As previously reported in an overlapping cohort of subjects (11), metreleptin treatment for 6

months lowered hemoglobin A1c by 15±21% (absolute reduction 1.5%, *P*=0.037), and led to non-significant reductions in fasting plasma glucose (*P*=0.071). Branched chain amino acids (BCAA), measured using nuclear magnetic resonance (NMR), decreased by 21±18% after metreleptin, (*P*=0.005**;** **Table 1**).

Metreleptin improved serum lipids and hepatic steatosis

As previously reported in an overlapping cohort of subjects (11), metreleptin treatment for 6 months improved hepatic steatosis and dyslipidemia (**Table 1)**. Metreleptin treatment decreased total and LDL cholesterol by 18±23% (*P*=0.032) and 23±15% (*P*=0.028), respectively. Serum-TG trended down by 23±58% (*P*=0.061; **Figure 1D**) and hepatic-TG decreased by 32±51% after metreleptin (*P*=0.016; **Figure 1E**).

Effects of metreleptin on other potential mediators of serum- and hepatic-TG

Metreleptin treatment for 6 months of increased plasma β-hydroxybutyrate, a marker of hepatic fatty acid oxidation (*P*=0.009; **Table 1**). Fasting chylomicrons, as measured by plasma apolipoprotein B48, did not decrease after metreleptin treatment (*P*=0.55; **Figure 1F**). As previously reported in an overlapping cohort of subjects (11), there was a trend toward decreased lipolysis after metreleptin, measured by glycerol and palmitate rate of appearance (P=0.058 and P=0.049, respectively; **Table 1**).

Correlations of DNL with metabolic parameters

Lower endogenous leptin at baseline did not correlate with baseline DNL (*P*=0.65), but did correlate with lower DNL after 6 months of metreleptin (r=0.81, *P*=0.02). Both before and after metreleptin, higher peripheral insulin resistance and higher insulin doses were significantly associated with higher levels of DNL (**Table 2**). HbA1c was only associated with higher DNL after metreleptin (*P*=0.604 and *P*=0.019;

before and after metreleptin, respectively; **Table 2**). There was a trend toward positive association between BCAA and DNL both before and after metreleptin (*P*=0.097 and *P*=0.086; **Table 2**).

Correlations of serum- and hepatic-TG with DNL and lipolysis

Both serum- and hepatic-TG correlated positively with DNL before metreleptin (r= 0.79, *P*=0.012, and r= 0.70, *P*=0.035 respectively; **Table 2**). However, DNL did not correlate well with serum- or hepatic-TG after metreleptin (*P*=0.17 and *P*=0.061, respectively; **Table 2**). There were no correlations either before or after metreleptin between lipolysis and serum- or hepatic-TG (**Table 3).**

Discussion

This study demonstrates for the first time elevated fasting DNL in patients with lipodystrophy that decreased after 6 months of metreleptin treatment. In the current study, we found that subjects with lipodystrophy had fasting DNL of 11-35%. In lean, healthy individuals under similar conditions of labeling, DNL contributes to ~5-10% of TRLP-TG in the fasting state, increasing to approximately 10% in the fed state (12, 13). However, increased DNL is thought to play a key role in the pathogenesis of obesity-associated NAFLD, as patients with NAFLD have an increased percentage of hepatic and circulating-TG derived from DNL, to as much as 20-40% in the fasted state (14-16). Prior to metreleptin, subjects with lipodystrophy in this study had %DNL comparable to those with obesity-associated NAFLD. Remarkably, after 6 months of metreleptin, %DNL decreased to the normal range of 5-10%. Furthermore, absolute DNL decreased by 88% to a mean of only ~9 mg/dl (range: 0.4-19.4 mg/dl), which is comparable to the mean level of 4 mg/dl reported in lean, healthy individuals (13).

As carbohydrates and insulin are thought to be the main regulators of DNL (16, 17), we hypothesized that a metreleptin-mediated reduction in DNL would be associated with decreases in hepatic carbohydrate flux and insulin exposure. Carbohydrates, particularly fructose, are sufficient to stimulate DNL; however, insulin signaling is thought to be necessary to drive pathological increases in DNL in

149 insulin-resistant states through increased expression of sterol regulatory element-binding protein-1c, a
150 major lipogenic transcription factor (10, 17). Metreleptin treatment improved insulin sensitivity and
151 glycemia control in our cohort of lipodystrophic patients, consistent with previous studies (9, 11). Both
152 before and after metreleptin, higher peripheral insulin resistance and higher insulin doses were
153 significantly associated with higher levels of DNL**,** supporting the hypothesis that hyperinsulinemia,
154 whether endogenous or exogenous, stimulates DNL. Consistent with this, administration of diazoxide to
155 a patient with partial lipodystrophy suppressed hyperinsulinemia, and lowered VLDL-TG (18).
156 Increased peripheral tissue glucose disposal after metreleptin might also be expected to reduce
157 DNL by reducing carbohydrate availability in the liver. Glycemia, assessed as HbA1c, improved after
158 metreleptin treatment, but was only associated with DNL after metreleptin. These data are consistent
159 with the hypothesis that, in the hyperinsulinemic state prior to metreleptin, insulin is the primary driver of
160 pathologically elevated DNL. Only after insulin resistance and hyperinsulinemia decreased with
161 metreleptin could an association between hyperglycemia and DNL be observed, suggesting that
162 carbohydrate availability is rate limiting for DNL in the more insulin sensitive state. Our findings are
163 consistent with a recent study showing strong relationships between insulin sensitivity, 24 hour integrated
164 glycemia and insulinemia, and DNL (16).
165 Changes in insulin and glucose are not the only mechanisms by which metreleptin might lower
166 DNL. Rodent studies have demonstrated that leptin may also lower DNL via central nervous system
167 signaling by downregulating enzymes involved in de novo fatty acid synthesis (acetyl-coenzyme A-
168 carboxylase, fatty acid synthase, and stearoyl-coenzyme A desaturase-1) (19). A recent study showed
169 that this effect was mediated through vagal signaling to the liver (20). Unfortunately, measures of
170 autonomic nervous system activity in the liver were not available in the current study and are unlikely to
171 be feasible in human studies. In addition to carbohydrates, branched-chain amino acids (BCAA) can be a
172 carbon source for DNL. BCAA decreased with metreleptin therapy and trended toward positive
173 association with DNL before and after metreleptin, suggesting that metreleptin may reduce DNL via

reductions in both BCAA and carbohydrate precursors. However, BCAAs can also be thought of as a measure of positive energy balance that is improved after metreleptin, thus reductions in BCAA after metreleptin may not be causal for reductions in DNL.

Consistent with prior studies (9, 21), metreleptin decreased serum-TG and hepatic-TG. We hypothesized that one way by which metreleptin leads to lower serum- and hepatic-TG is through reductions in DNL. Prior to metreleptin, DNL correlated with both serum- and hepatic-TG, supporting a key pathogenic role of DNL in the development of hepatic steatosis and hypertriglyceridemia not only in obesity-associated NAFLD, but also in lipodystrophy. However, DNL did not correlate well with serum- or hepatic-TG after metreleptin, reflecting its diminished contribution in the insulin sensitive state.

Circulating and hepatic-TG can derive not only from DNL, but also from chylomicrons from dietary fat, reesterification of FFA from adipocyte lipolysis, and spillover of FFA from lipolysis of TRLP (7, 15). We hypothesized that decreases in circulating and hepatic-TG after metreleptin would be only partly mediated through decreased DNL, with the potential for additional metreleptin-mediated reductions in circulating and hepatic-TG resulting from decreased lipolysis (9, 11), decreased chylomicrons (22), and/or increased fatty acid oxidation (23-26). In obesity-associated NAFLD, the majority of fatty acids found in circulating and hepatic-TG are derived from adipocyte lipolysis (15). Consistent with previous studies (9, 11), there was a trend toward decreased lipolysis after metreleptin, suggesting that decreased glycerol and FFA availability to the liver for TG synthesis is a potential mechanism contributing to reductions in circulating and hepatic-TG after metreleptin. However, there was no correlation between lipolysis and serum- or hepatic-TG, suggesting that lower lipolysis after metreleptin is not the major driver of reductions in serum- or hepatic-TG. Metreleptin is known to suppress appetite and food intake in states of leptin deficiency including lipodystrophy (27-31), thus reduction in dietary fat intake is a likely mechanism by which metreleptin lowers serum- and hepatic-TG. Consistent with this, a prior publication from our group showed a reduction in chylomicrons assessed by lipid NMR in patients with lipodystrophy after metreleptin (22). The lack of reduction in chylomicrons measured by apolipoprotein

B48 after metreleptin in the current study is therefore somewhat surprising. This may be due to measurement of chylomicrons in the fasting state, rather than postprandially, differences in methodology, or due to the large variance between subjects. Alternatively, this may suggest that metreleptin has more complex effects on chylomicron uptake or turnover independent of its effects on dietary fat intake (32). Prior rodent studies have shown that leptin upregulates hepatic transcription factors involved in fatty acid oxidation (peroxisome proliferator activated receptor gamma coactivator (PGC-1α), peroxisome proliferator activated receptor alpha (PPARα), carnitine palmitoyltransferase-1A (CPT-1a), and CD36) (23-26). Consistent with this, we observed an increase in plasma β-hydroxybutyrate after metreleptin, suggesting increased hepatic FFA utilization.

Prior studies have shown that metreleptin has greater efficacy to improve metabolic disease in lipodystrophic patients with more severe leptin deficiency (21). Consistent with this, lower endogenous leptin at baseline correlated with lower DNL at 6 months, suggesting greater normalization of DNL in patients who were more leptin deficient. However, metreleptin decreased absolute DNL in all patients, with endogenous leptin ranging from 0.5 to 35.7 ng/mL. Importantly, the metreleptin doses used in this study were pharmacologic rather than hormone replacement, resulting in supraphysiologic plasma leptin concentrations. This suggests that metreleptin at pharmacologic doses might be effective in reducing hepatic steatosis by lowering DNL even in non-lipodystrophic, non-leptin deficient populations with NAFLD. However, the pathophysiology of obesity and lipodystrophy differ not only by endogenous leptin levels, but in other ways such as adipose tissue storage capacity. Therefore, although metreleptin lowered DNL in subjects with lipodystrophy over a wide range of endogenous leptin levels, these findings do not necessarily predict equivalent lowering of DNL in individuals with similar leptin levels without lipodystrophy. In fact, metreleptin has been shown to have only modest effects in its primary action to suppress appetite and cause weight loss in obese subjects with high leptin (33). Thus, additional study is needed to test its effects on DNL and NAFLD in this population. Furthermore, some rodent studies suggest a profibrogenic effect of leptin, suggesting that leptin might be causal for progression of

NAFLD to NASH in the context of obesity (5, 6). However, metreleptin treatment has not been shown to increase hepatic fibrosis in humans with lipodystrophy (34). Therefore, the pro-fibrogenic effect in rodents might be due to model specific pathology.

Limitations

DNL may be underestimated in this study due to dilutional effects of unlabeled TG from chylomicron remnants; however, apolipoprotein B48 did not change after metreleptin, suggesting that the observed decrease in DNL was primarily due to reductions in VLDL. DNL may also be underestimated due to the relatively short duration of deuterium labeling of body water (11 hours) (16) – although the overnight labeling method has been shown to distinguish populations with significantly different levels of DNL (35). Finally, we speculate that metreleptin-mediated improvements in insulin sensitivity and glycemia were causal for decreased DNL, which in turn was causal for decreased circulating and hepatic-TG. However, this study can only demonstrate association, not causality, between these variables, and there is evidence to support that lower hepatic-TG may be causal for lower insulin resistance (36). A demonstration that insulin sensitivity and glycemia improved prior to changes in DNL would help support the causal role of insulin and glucose in mediating metreleptin-induced reductions in DNL. Although our prior publication showed that insulin sensitivity and glucose improved as early as 2 weeks after metreleptin initiation in an overlapping cohort of subjects (11), DNL data were unfortunately not available at that time point.

Conclusions

In conclusion, 6 months of metreleptin treatment in very insulin resistant humans with lipodystrophy led to near normalization of DNL. Improvements in DNL were associated with reductions in glycemia and improved peripheral and hepatic insulin sensitivity, supporting a strong link between metreleptin's effects to lower insulinemia and increase clearance of blood glucose by peripheral tissues

and reduce hepatic carbohydrate flux, and resultant reductions in DNL. This led to lowered hepatic steatosis and dyslipidemia and suggests that treatments targeting multi-organ insulin resistance may improve NAFLD. Importantly, metreleptin-induced improvements in DNL and metabolic disease were observed across all levels of endogenous leptinemia, suggesting that metreleptin may be effective in the broader population with obesity-associated NAFLD, who are not leptin-deficient.

Methods

Study Design

Leptin-naive patients with lipodystrophy participated in an open-label study of metreleptin (donated by Aegerion Pharmaceuticals) at the National Institutes of Health (NIH). This analysis includes a subset (9 of 15) of patients described in the primary results of this study (11) plus two additional patients who enrolled after the previous publication.

Details of the study design have been published (11). Briefly, patients were admitted and studied for 5 days before metreleptin initiation (5 mg s.c. every 12 hours). Metreleptin was continued for 14 days inpatient, then patients were discharged to continue metreleptin as outpatients for 6 months. At discharge, the metreleptin dose was decreased in patients with generalized lipodystrophy to prevent excessive weight loss. In all patients, insulin and sulfonylurea doses were reduced as needed to avoid hypoglycemia due to improved insulin sensitivity after metreleptin initiation. No increases in medications for diabetes or dyslipidemia were permitted.

Study Procedures

Study procedures were performed after an 8-12 hour fast at baseline (prior to metreleptin) and after ~6 months of metreleptin administration. Fasting DNL was measured following oral administration of deuterated water (^2H_2O) in four doses between 2100 and 0300 hours to reach a concentration of 0.3% ^2H_2O of total body water, measured by IRMS as described (37). To isolate the TRLP fraction (density < 1.006 g/ml), serum underwent ultracentrifugation for 20 hours with a Beckman Ti rotor at 39,000 rpm at 4° C and the upper ~1 mL was collected by tube slicing. Lipoprotein-TG were separated by thin layer chromatography and fatty acids transesterified to be analyzed by gas chromatography/mass spectrometry. The fractional contribution of DNL-derived TG palmitate in TRLP was calculated using mass isotopomer analysis as described (15). By convention this fraction was multiplied by the TG concentration to estimate absolute DNL (35).

Concentrations of glucose, insulin, and C-peptide were measured every 10 minutes for 30

minutes prior to the hyperinsulinemic-euglycemic clamp, and the mean of the four measurements reported. Standard methods of NIH Clinical Center laboratory were used to measure glucose, total cholesterol, TG, and HDL-C (Roche Cobas 6000 analyzer), insulin and C-peptide (electrochemiluminescence immunoassay on Roche Cobas e601 analyzer), FFA (colorimetric assay on Roche Cobas C501 analyzer), and HbA1c (high-performance liquid chromatography). LDL-C was calculated using the Friedewald equation if TG was <400 mg/dL. Plasma leptin was measured by ELISA (MilliporeSigma kit #EZHL-80SK). The intra- and inter-assay coefficients of variation were 3.9% and 4.8%, respectively. Plasma BCAA concentration was measured via NMR spectroscopy using the 400-MHz proton Vantera Clinical Analyzer with LP4 deconvolution algorithm as described (22). As previously reported, body composition was measured by dual energy X-ray absorptiometry, and hepatic-TG by MRS (11). Apolipoprotein B48 was measured by ELISA (FUJIFILM kit #637-10641). The intra- and inter-assay coefficients of variation were both 10%.

Glucose, glycerol, and palmitate turnover were measured using the isotope tracer dilution method with [6,6-$^{2}H_2$] glucose, $^{2}H_5$-glycerol, and [U-$^{13}C_{16}$] palmitate (Cambridge Isotope Laboratories) as previously reported (11). Hyperinsulinemic-euglycemic clamp studies were performed to measure hepatic and total body insulin sensitivity as reported (11). Briefly, patients received a primed insulin infusion for 8 min at 240 mU/m^2/min followed by a continuous infusion for approximately 3 hours at 120 mU/m^2/min. The high dose of insulin was chosen to stimulate peripheral glucose uptake with incomplete suppression of hepatic glucose production in this population with severe insulin resistance. Insulin sensitivity (M) was assessed as the mean glucose infusion rate during the final 30 min of the clamp, normalized to fat free mass (mg/kg$_{FFM}$/min). Hepatic insulin sensitivity was determined by percent suppression of endogenous glucose production using [6,6-$^{2}H_2$] glucose.

Statistics

Outcomes are reported as mean±SD or median [25th,75th percentile] based on data distribution.

Non-normally distributed data were log transformed prior to analysis. Paired t-tests or Wilcoxon signed-rank tests were used to compare outcomes before versus after metreleptin for normally and non-normally distributed deltas, respectively. Pearson's or Spearman's correlations were performed to test associations between DNL and endogenous leptin levels, potential mediators of DNL, and consequences of changes in DNL. Correlations were conducted at baseline and 6-month follow-up. $P<0.05$ represented statistical significance. All *P*-values are two sided. Analyses were conducted using GraphPad Prism, version 8.1 (GraphPad Software).

Study Approval

This study (NCT01778556) was approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases. Patients or legal guardian(s) provided written informed consent prior to participation; minors provided written assent.

Author Contributions: APB analyzed data and wrote the manuscript. EJP conducted experiments, analyzed data, and contributed to manuscript writing. RS, MMS-A, SC, EC, MS, AMG, RM, PJW, MW, RM, and STC conducted experiments and critically reviewed the manuscript. RJB designed the study, conducted experiments, acquired data, analyzed data, and wrote the manuscript.

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Tables

Table 1. Secondary outcomes before and after 6 months of metreleptin administration

	Baseline	6 months	*P*-value
Plasma leptin (ng/dL)	9.5±11.0[D]	155.0±71.5	0.002[D]
Weight (kg)	70.5±19.8	68.1±21.2	0.028
Insulin dose (U/day)[A]	196±146	134±121	0.15
Peripheral insulin sensitivity (mg/kg$_{LBM}$/min)[B]	4.0 [3.1,7.9]	8.8 [5.4,11.2][D]	0.034[D]
Hepatic insulin sensitivity (%)[C]	61.0 [48.5,69.3]	84.7 [75.2,107.6][D]	0.012[D]
Fasting insulin (μU/mL)	25 [14,84]	14 [9,26][D]	0.049[D]
Fasting C-peptide (ng/mL)	3.2 [2.7,4.4]	2.3 [1.6,3.3][D]	0.006[D]
Hemoglobin A1c (%)	8.6±1.8	7.1±1.4	0.037
Fasting Glucose (mg/dL)	143±56	119±36[D]	0.071[D]
Total Cholesterol (mg/dL)	188±65	148±46	0.032
HDL Cholesterol (mg/dL)	27±6 [D]	27±6	0.98[D]
LDL Cholesterol (mg/dL)	75±14[F]	66±30[E]	0.028[G]
Plasma free fatty acids (mEq/L)	0.34 [0.30,0.55]	0.41 [0.31,0.44]	0.90
R_aGlycerol (μmol/kg$_{LBM}$/min)	4.5 [2.9,5.9]	3.2 [2.7,4.2][D]	0.058[D]
R_aPalmitate (μmol/ kg$_{LBM}$/min)	2.8±1.1	2.1±0.5[D]	0.049[D]
β-hydroxybutyrate (mM)	0.32±0.12	0.44±0.10	0.009
Branched-Chain Amino Acids (mmol/L)	578 [476,712]	425 [382,443]	0.005

Data are mean±SD or median [25th,75th centile], n=11 except as noted. Comparisons were made using paired, 2-tailed Student's *t* test or Wilcoxon signed-rank test for normally and non-normally distributed data, respectively. FFM, fat free mass; LBM, lean body mass; R_a, rate of appearance.
[A]Insulin users only; [B]Glucose infusion rate during hyperinsulinemic-euglycemic clamp; [C]Suppression of hepatic glucose production during hyperinsulinemic-euglycemic clamp; [D]n=10; [E]n=8; [F]n=6; [G]n=5.

Table 2. Correlations with absolute DNL (mg/dL) at baseline and after 6-months of metreleptin administration

	Predictor		Response Variable	
			Pre-leptin absolute DNL	Post-leptin absolute DNL
Potential mediators of DNL	Peripheral insulin sensitivity ($mg/kg_{LBM}/min$)	r	-0.74	-0.84
		p	0.022	0.005
	Insulin dose, insulin users only[A] (U/day)	r	0.87	0.88
		p	0.023	0.022
	Hemoglobin A1c (%)	r	0.20	0.75
		p	0.604	0.019
	Branched chain amino acids (mmol/L)	r	0.60	0.62
		p	0.097	0.086
Consequences of change in DNL	Triglycerides (mg/dL)	r	0.79	0.51
		p	0.012	0.165
	Liver fat (%)	r	0.70	0.65
		p	0.035	0.061

n=9 except as noted. FFM, fat free mass

Univariate analysis was performed using Pearson and Spearman correlations for normally and non-normally distributed data, respectively.

[A]n=6.

Table 3. Correlations with lipolysis at baseline and after 6-months of metreleptin administration

Predictor	Time Point		Response Variable	
			Liver Fat (%)	Serum Triglycerides (mg/dL)
R_a Glycerol (µmol/kg$_{LBM}$/min)	Pre-leptin	r	-0.06[A]	-0.06[A]
		p	0.860[A]	0.860[A]
	Post-leptin	r	-0.30	-0.15
		p	0.407	0.682
R_a Palmitate (mmol/ kg$_{LBM}$ /min)	Pre-leptin	r	0.23	0.14
		p	0.531	0.710
	Post-leptin	r	0.45	0.49
		p	0.195	0.151

n=10 except as noted. LBM, lean body mass; R_a, rate of appearance

Univariate analysis was performed using Pearson and Spearman correlations for normally and non-normally distributed data, respectively.

[A]n=11

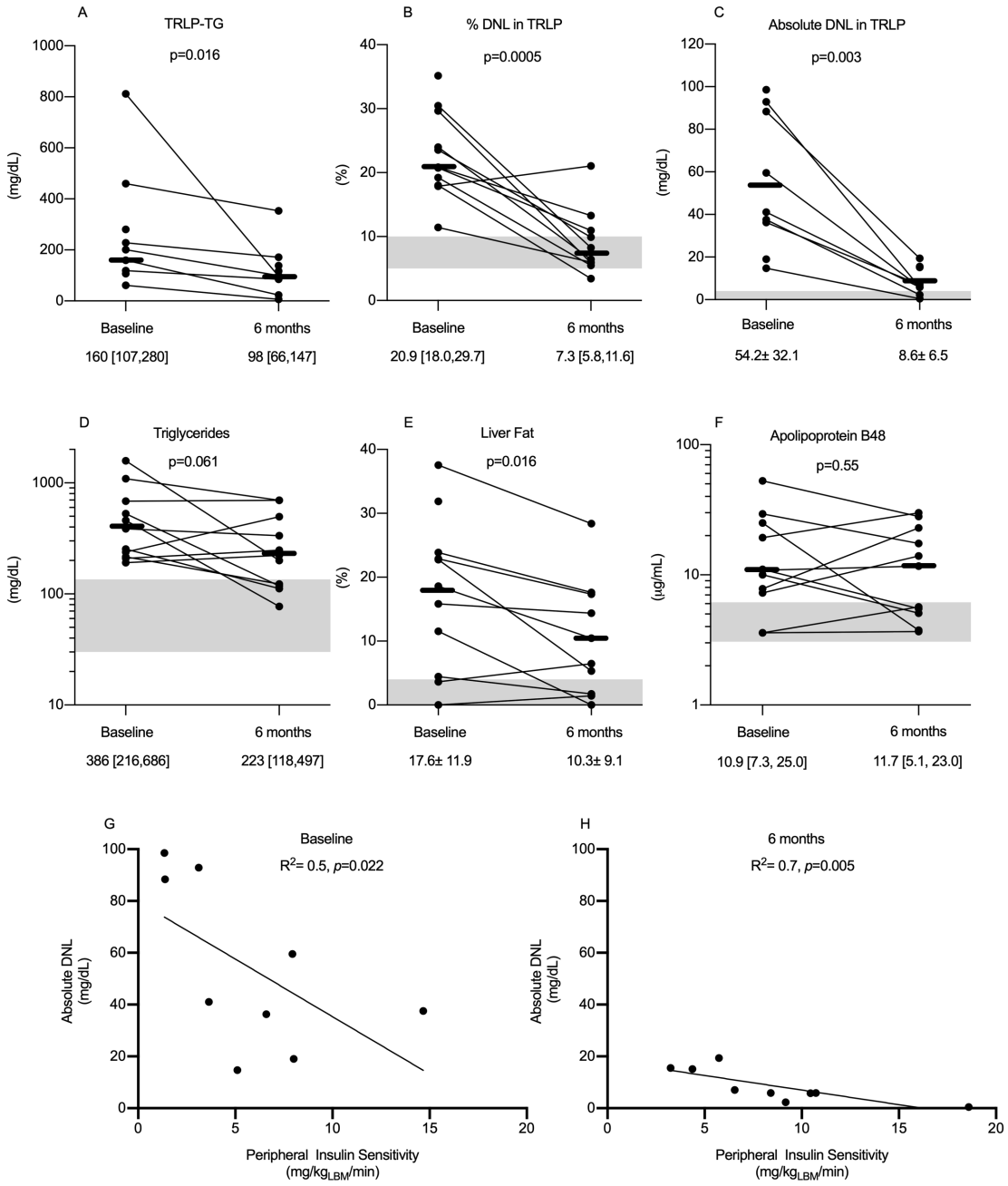

Figure 1. Effects of metreleptin in patients with lipodystrophy (A) Triglyceride (TG) in TG-rich lipoproteins (TRLP-TG) (n=7). (B) Fraction of TG in TRLP derived from DNL (%DNL) (n =10). (C) Absolute DNL as the product of TRLP-TG and %DNL (n =7). (D) Hepatic-TG (n =10). (E) Serum-TG (n =11). (F) Plasma apolipoprotein B48 (n =11). Correlation between DNL and peripheral insulin sensitivity before (G) and after (H) metreleptin (n =9). Gray shaded areas represent normal ranges for healthy individuals. Comparisons were made using paired, 2-tailed Student's t test or Wilcoxon signed-rank test for normally and non-normally distributed data, respectively. Univariate analysis was performed using Pearson and Spearman correlations for normally and non-normally distributed data, respectively.