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Beneficial effects of leptin substitution on impaired eating behavior in lipodystrophy are sustained beyond 150 weeks of treatment

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ABSTRACT

Aim: Metreleptin treatment in lipodystrophy patients improves eating behavior with increased satiety and reduced hunger. However, no data are available whether effects are maintained beyond 52 weeks of treatment.
Methods: A prospective study with measurements at baseline and at > 150 weeks of metreleptin treatment was performed. Five female lipodystrophy patients with indication for metreleptin were included. Behavioral aspects of hunger- and satiety regulation were assessed by validated eating behavior questionnaires and visual analog scales assessing hunger and satiety feelings before and after a standardized meal.
Results: Hunger rated on visual analog scales at 120 min after the meal significantly decreased from 46 ± 10 mm at baseline to 17 ± 6 mm at long-term assessment. Furthermore, satiety at 5 and 120 min after the meal significantly increased from baseline to long-term assessment (5 min: 70 ± 7 mm to 87 ± 3 mm; 120 min: 43 ± 10 mm to 79 ± 8 mm). On the Three Factor Eating Questionnaire, the mean value of factor 3 (hunger) significantly decreased from 9.2 ± 0.2 at baseline to 2.6 ± 1.5 at long-term assessment. In the Inventory of Eating Behavior and Weight Problems Questionnaire, mean values for scale 2 (strength and triggering of desire to eat) and scale 7 (cognitive restraint of eating) significantly decreased from baseline (31.6 ± 4.8 and 11.4 ± 2.2, respectively) to long-term assessment (14.0 ± 2.1 and 10.0 ± 1.9).
Conclusion: First evidence is presented that long-term metreleptin treatment of > 150 weeks has sustained effects on eating behavior with increased satiety, as well as reduced hunger and hunger-related measures.

1. Introduction

Adipocytes secrete various proteohormones collectively called adipokines. Among those, leptin has been introduced as an adipokine affecting metabolic and neurocognitive control of the human body including insulin sensitivity, lipid metabolism, and eating behavior. Various effects of leptin in humans have been elucidated by studying patients with leptin-deficiency. Among those, lipodystrophy (LD) is a rare disease state in which reduced to absent subcutaneous adipocytes result in leptin-deficiency with reduced to non-measurable leptin blood concentrations. LD patients often suffer from severe diabetes mellitus and hypertriglyceridemia, and leptin substitution in the form of the analogue metreleptin has beneficial effects on these metabolic complications [1,2]. Furthermore, patients with LD describe a disturbed eating behavior with reduced satiety after food consumption, leading to an increase in meal frequency. Impaired eating behavior can be improved by metreleptin substitution [3].

Our group has recently characterized neurobehavioral and brain functional changes in LD patients over 52 weeks of metreleptin treatment [4]. Here, decreases in hunger in the fasted state and increases in satiety after a meal through metreleptin treatment were observed. Furthermore, hunger-associated measures as assessed with eating

Abbreviations: BMI, body mass index; FEV, *Fragebogen zum Essverhalten*, German version of the Three Factor Eating Questionnaire; HbA1c, Hemoglobin A1c; IEG, inventory of eating behavior and weight problems (German title: *Inventar zum Essverhalten und Gewichtsproblemen*); LD, lipodystrophy; TFEQ, three factor eating questionnaire; TG, triglycerides

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behavior questionnaires significantly decreased with ongoing treatment. These neurobehavioral changes were accompanied by alterations of hedonic and homeostatic central nervous networks regulating eating behavior [4]. However, neurobehavioral assessments were only performed for up to 52 weeks of the substitution program and no study in LD patients so far has assessed hunger and satiety beyond this period of time.

Therefore, in the current study the same LD patients on metreleptin substitution of [4] were re-assessed after > 150 weeks of treatment in the current study. We hypothesized that behavioral changes observed during the first 52 weeks of metreleptin treatment in LD would also persist at this long-term assessment. Since approaches to overcome leptin resistance in obesity have recently been successful in animal models [5,6], the significance of our study findings on long-term effects of metreleptin go well beyond leptin-deficient disease states including LD, congenital leptin-deficiency, hypothalamic amenorrhea, and various eating disorders. Furthermore, elucidating chronic effects of leptin on hunger regulation is an important pillar in understanding the hormonal regulation of long-term energy homeostasis in healthy humans.

2. Material and Methods

2.1. Patients with LD

We performed a long-term assessment of LD patients investigating changes in eating behavior after > 150 weeks of metreleptin treatment. The study was approved by the ethics committee of the University of Leipzig (approval No. 147/10-ek). Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used. Data about changes during the first 52 weeks of metreleptin substitution were already reported in [4]. Five (all female) of the initial nine (seven female) patients of [4] were eligible for long-term assessment. Baseline characteristics, laboratory data, and numbering of patients in [4] for these five patients are given in Table 1. Four of the nine patients in [4] were not eligible for the long-term assessment for the following reasons: discontinuation of (patient 2), insufficient adherence to (patient 4), or not reaching > 150 weeks of (patient 7) metreleptin treatment; and inability to attend the long-term study visit due to progressive immobilization (patient 8).

2.2. Medication and experimental design

Metreleptin was provided by Amylin (San Diego, CA)/ Bristol-Myers-Squibb (Munich, Germany)/ AstraZeneca (London, UK)/ Aegerion Pharmaceuticals (Cambridge, MA), respectively, Medication and experimental design have been described in [4]. In brief, metreleptin was administered following the manufacturer's instructions; the dose was uptitrated according to metabolic (i.e., triglyceride and HbA1c) effects to 5 (patient C) and 7.5 mg/d (patient A, B, D, E). The drug was administered once daily subcutaneously. Blood was drawn in the fasted state. On the day of the baseline and long-term visits at our study center, visual analog scales assessing hunger and satiety were completed in the fasted state, as well as 5 and 120 min after a meal consisting of 20% of the daily energy requirements. Furthermore, the Three Factor Eating Questionnaire (TFEQ [7], German version: *Fragebogen zum Essverhalten*, FEV [8]) and the Inventory of Eating Behavior and Weight Problems (German title: *Inventar zum Essverhalten und Gewichtsproblemen*, IEG [9]) were filled in at baseline and long-term assessment. For comparison of the TFEQ results of our patients with values of healthy persons, we used TFEQ sample 3 of the test manual [8] as the sample best representing the normal population. All participants of sample 3 were females recruited by newspaper advertisements (n = 1097; mean age 30.1 years; mean body mass index [BMI] 22.8 kg/m^2) [8]. The median values of sample 3 are depicted as dotted lines in Fig. 2A. For comparison of the IEG questionnaire results of our patients with values of healthy persons, normal values of the IEG were obtained from the female normal sample of the test manual representing the normal population of Germany (n = 355; mean age 38.1 years; mean BMI 22.3 kg/m^2) [9]. The median values of this normal sample are depicted as dotted lines in Fig. 2B.

3. Results

3.1. Anthropometric and metabolic parameters

In our study, no treatment-emergent adverse events including T-cell lymphoma occurred in the enrolled patients. Mean ± SEM BMI was 26.5 ± 1.5 kg/m^2 at baseline and 25.8 ± 1.7 kg/m^2 at the long-term assessment. Mean ± SEM hemoglobin A1c was 7.2 ± 0.4% at baseline and 7.4 ± 0.5% at the long-term assessment (Table 1). Mean ± SEM triglycerides were 13.6 ± 6.3 mmol/L at baseline and 8.4 ± 2.3 mmol/L at the long-term assessment (Table 1). Differences between the two time points were not statistically significant for all three parameters ($p > 0.05$).

3.2. Valence of food/non-food pictures and hunger/satiety ratings

The average rating score of food pictures was 2.7 ± 0.2 and of non-food pictures 2.6 ± 0.2 at baseline (Fig. 1A). These values were not significantly affected by metreleptin treatment at the long-term assessment (food pictures 2.7 ± 0.3; non-food pictures 2.3 ± 0.1; $p > 0.05$; Fig. 1A).

Hunger rated on visual analog scales at 120 min after the meal significantly decreased from 46 ± 10 mm at baseline to 17 ± 6 mm at long-term assessment ($p < 0.05$, Fig. 1B). Numerically, decreased hunger ratings from baseline to long-term assessment were also found in the fasted state (70 ± 9 mm to 39 ± 10 mm) and at 5 min after the meal (25 ± 11 mm to 7 ± 2 mm); however, statistical significance was not reached ($p > 0.05$; Fig. 1B).

Satiety rated on visual analog scales at 5 and 120 min after the meal significantly increased from baseline to long-term assessment (5 min: 70 ± 7 mm to 87 ± 3 mm; 120 min: 43 ± 10 mm to 79 ± 8 mm; both $p < 0.05$; Fig. 1C). The numerical increase in satiety ratings in the fasted state from 26 ± 12 mm to 48 ± 8 mm did not reach statistical significance ($p > 0.05$; Fig. 1C).

Table 1
Baseline characteristics and laboratory data of LD patients with long-term assessment (n = 5, all female). FPLD, familial partial lipodystrophy; General., Generalized; HbA1c, Hemoglobin A1c; LD, Lipodystrophy; n.d., not detected; Pat., Patient; TG, Triglycerides.

Pat.	Pat. No. in [4]	LD Phenotype	Age [years]	Treatment [weeks]	HbA1c [% (mmol/mol)]		TG [mmol/L]	
			Baseline	Long-term Assessment	Baseline	Long-term Assessment	Baseline	Long-term Assessment
A	1	Partial	23	210	6.4 (46)	5.8 (40)	16.3	4.0
B	3	Partial	48	208	6.6 (49)	6.8 (51)	2.5	1.8
C	5	Partial	41	160	8.0 (64)	9.1 (76)	7.9	13.7
D	6	General.	33	151	8.1 (65)	7.5 (58)	37.1	12.1
E	9	Partial	53	156	7.0 (53)	7.7 (61)	4.3	10.5

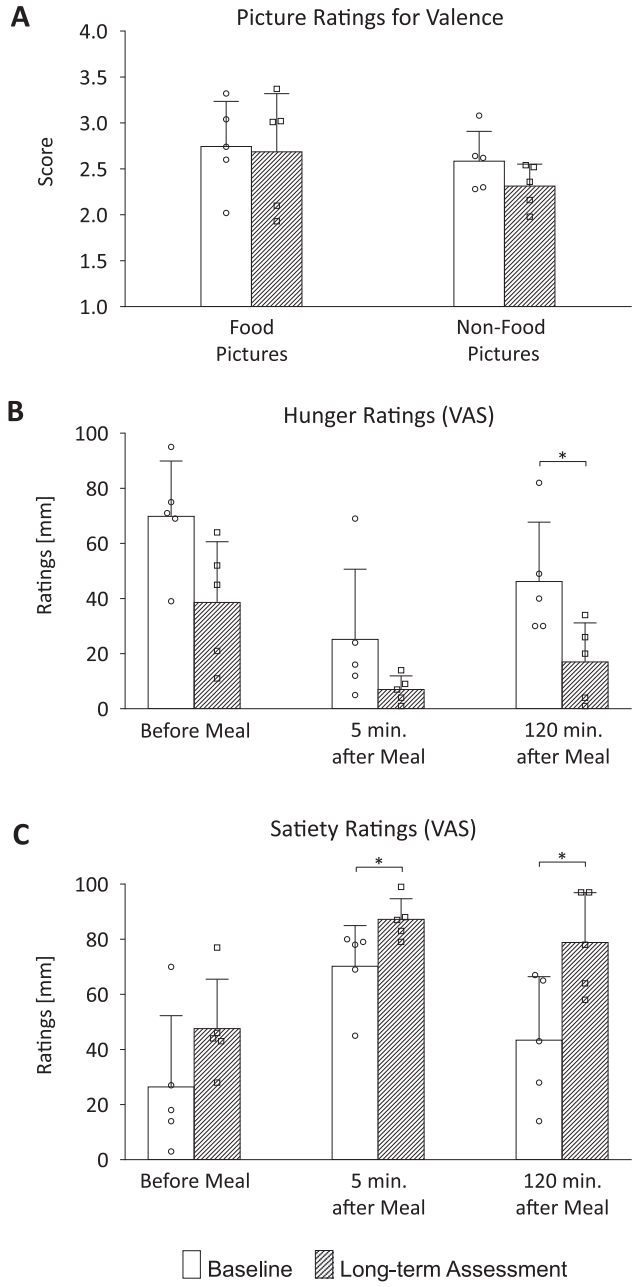

Fig. 1. Scatter plots with bars of mean values and plotted values of individual patients of (A) valence ratings of food and non-food pictures and (B, C) visual analog scales for (B) hunger and (C) satiety before, as well as 5 and 120 min after a standardized meal. Error bars indicate standard deviation. Assessments were performed at baseline (before start of metreleptin treatment; ○; white bars) and long-term (after > 150 weeks; □; hatched bars). *p < 0.05 as compared to baseline as assessed by two-tailed paired Student's *t* test. VAS, Visual analog scale.

3.3. Food questionnaires

On the TFEQ, the mean value of factor 3 (hunger) significantly decreased from 9.2 ± 0.2 at baseline to 2.6 ± 1.5 at long-term assessment (p < 0.05; Fig. 2A). Furthermore, there was a trend (p = 0.07) towards a decrease of factor 2 (disinhibition) from 7.6 ± 1.4 at baseline to 4.0 ± 0.3 long-term (Fig. 2A). In contrast, there were no significant differences in the values in factor 1 (cognitive restraint of eating) between baseline and long-term assessment (9.8 ± 0.8 and 7.2 ± 1.5; p > 0.05; Fig. 2A). For the IEG questionnaire, all scales which showed significant changes during the first

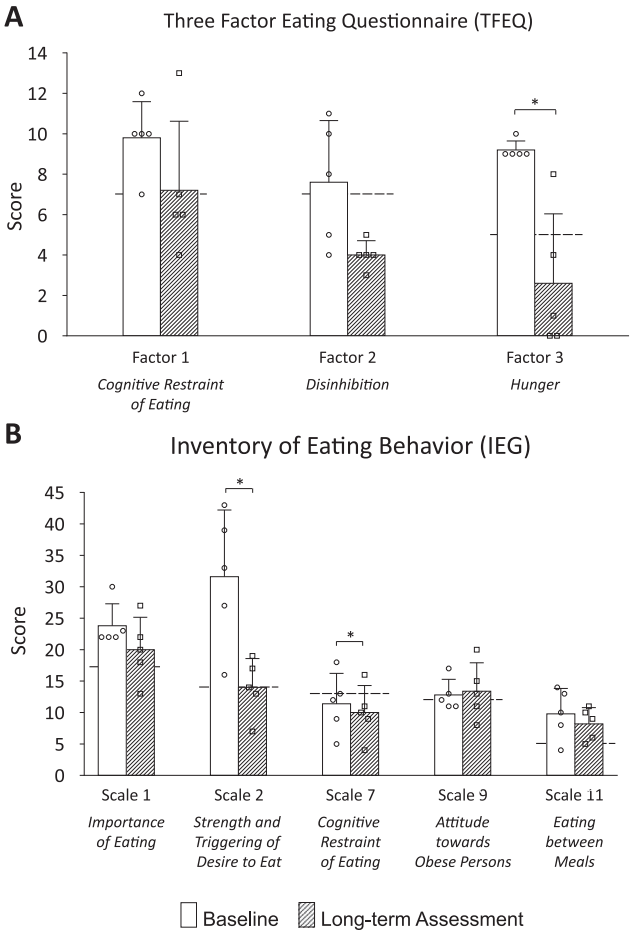

Fig. 2. Scatter plots with bars of mean values and plotted values of individual patients of (A) all scales of the Three-Factor Eating Questionnaire (TFEQ); and (B) the five (out of 14) scales of the Inventory of Eating Behavior and Weight Problems questionnaire (German title: *Inventar zum Essverhalten und Gewichtsproblemen,* IEG) which have shown significant changes after initiation of metreleptin treatment in [4]. Error bars indicate standard deviation. Assessments were performed at baseline (before start of metreleptin treatment; ○; white bars) and long-term (after > 150 weeks; □; hatched bars). *p < 0.05 as compared to baseline as assessed by two-tailed paired Student's *t* test. Dotted lines represent medians of normal tables of the validation cohorts of the questionnaires as described in the Materials and Methods section.

52 weeks of metreleptin treatment [4] are shown. Mean values for scale 2 (strength and triggering of desire to eat) and scale 7 (cognitive restraint of eating) significantly decreased from baseline (31.6 ± 4.8 and 11.4 ± 2.2, respectively) to long-term assessment (14.0 ± 2.1 and 10.0 ± 1.9, respectively; for both scales p < 0.05, Fig. 2B). Furthermore, there was a trend (p = 0.06) towards decreased average scores for scale 1 (importance of eating) from baseline (23.8 ± 1.6) to long-term assessment (20.0 ± 2.3; Fig. 2B). There were no significant differences between baseline versus long-term assessment for scale 9 (attitude towards obese persons; 12.8 ± 1.1 versus 13.4 ± 2.0) and scale 11 (eating between meals; 9.8 ± 1.8 versus 8.2 ± 1.2; p > 0.05; Fig. 2B).

4. Discussion

In the current study, we demonstrate for the first time that metreleptin in initially treatment-naïve LD patients increases satiety and decreases hunger, as well as hunger-associated measures, in the long-term, i.e., after > 150 weeks of treatment. On visual analog scales, hunger ratings are significantly decreased 120 min after a meal and

satiety ratings are significantly increased 5 and 120 min after a meal. Furthermore, strong and disturbing hunger feelings are reported in leptin treatment-naïve LD patients (TFEQ, factor 3); the mean score of 9.2 points of LD patients at baseline is well beyond the median of the normal population (5 points) and is significantly reduced to 2.6 points upon > 150 weeks of metreleptin therapy. These results suggest that hunger feelings are pathologically increased in LD patients at baseline and normalized by metreleptin treatment also in the long-term.

Significant hunger-reducing and satiety-inducing effects of metreleptin have been shown in other studies with leptin-deficient patients; however, follow-up assessments have been much shorter. Farooqi et al. have been the first to show a reduction in energy intake in a patient with congenital leptin-deficiency undergoing metreleptin substitution for 12 months [10]. Oral et al. have demonstrated in a sample of nine female patients with LD that metreleptin treatment leads to a significant reduction in caloric intake over the course of four months [11]. McDuffie et al. have studied the effects of metreleptin in patients with LD for the first time with standardized hunger and satiety ratings. In eight female patients, four months of metreleptin therapy has bisected the time and the amount of calories patients needed to get sated while eating and has doubled the time satiety persisted after a meal [3]. In three patients with congenital leptin-deficiency, hunger and intentions to eat before a meal have been reduced after 15 weeks of metreleptin therapy [12]. In the same three patients, hunger ratings when watching food-images have increased after cessation of metreleptin substitution for about five weeks [13]. Taking these and our current findings into consideration, metreleptin increases satiety and decreases hunger in leptin-deficient patients not only in the short- to medium term but also has long-term effects of > 150 weeks.

Strengths of the current study include being the first metreleptin study in treatment-naïve LD patients to examine the effects of metreleptin on eating behavior beyond 52 weeks. However, several limitations have to be discussed. First, the study does not have a placebo or healthy control group; thus, we cannot distinguish between metreleptin treatment effects and nonspecific order effects (i.e., effects through repetition of behavioral tests or increasing age of patients throughout the course of the study). Addition of medians of validation cohorts of the eating behavior questionnaires to the figures cannot fully compensate for a healthy control group. However, given the risk of life-threatening complications in untreated LD patients, it would not be justifiable to assign LD patients to a placebo group. Second, sample size is relatively small due to the rarity of the disease to perform robust statistical analyses. Furthermore, sample size planning is difficult since the impact of long-term metreleptin treatment on eating behavior has not been assessed before. Therefore, we have included as many LD patients as possible from our center in the present long-term study. However, four out of the original nine patients [4] could not be assessed for reasons given in the Material and Methods section. Clearly, large multi-center studies similar to NIH study https://doi.org//10010769/NCT00025883 [14] are needed to more robustly define long-term effects of metreleptin substitution on eating behavior and brain function. At the time of initiation of our study in 2010, no multi-center trial was available in LD patients which assessed behavioral data combined with functional MRI scanning over the course of metreleptin treatment. Our group is part of the European Consortium of Lipodystrophies (ECLIP) and larger multi-center LD studies assessing behavioral and central nervous changes upon metreleptin treatment are planned together with partners. Third, previous studies have reported significant reductions in triglycerides and HbA1c levels after long-term metreleptin treatment (> 3 years) [14–16] which is not confirmed in our sample. However, patients with partial LD are overrepresented in our cohort as compared to other studies and metabolic effects of metreleptin are much weaker in partial as compared to generalized LD. In our cohort, four of the five patients (80%) have partial LD. In previous studies, the proportion of patients with partial LD is much smaller, i.e., 19% (9 out of 47) [15] and 35% (19 out of 55) [17]), respectively. Furthermore, the limited sample size makes it more unlikely that effects reach the level of statistical significance. Anti-metreleptin antibodies [18] with *in vitro* neutralizing activity were not assessed in the current study due to the absence of commercial assays.

Taken together, first evidence is presented in the current study that long-term metreleptin treatment of > 150 weeks has sustained effects on eating behavior with increased satiety, reduced hunger, as well as hunger-related measures as assessed with eating-behavior questionnaires. Further studies in larger samples and with non-LD and untreated control groups are necessary to more extensively assess the role of leptin in long-term regulation of eating behavior.

5. Declaration of interest

KMI received honoraria from Aegerion Pharmaceuticals GmbH, the producer of Myalept (metreleptin). JP, KMU, AV, MS, MF, and HS indicate that they have no conflicts of interest to disclose. Aegerion Pharmaceuticals did not have any role in design or conduct of the study, and was not involved in analysis and interpretation of the data, as well as in the preparation of the manuscript. Before submission, the final manuscript was sent to Aegerion Pharmaceuticals to provide the possibility for comments. No changes to the manuscript were made after receiving the comments by Aegerion Pharmaceuticals.

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7. Author contribution statement

HS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; JP, MF, and HS conceived and designed the experiments; JP and HS performed the experiments; JP and HS analyzed the data; JP, MF, and HS wrote the paper and all authors edited the manuscript.

Trial registration

The trial is registered as trial No. 147/10-ek at the ethics committee of the University of Leipzig and at the State Directorate of Saxony (Landesdirektion Sachsen).

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