Leptin substitution in patients with lipodystrophy: neural correlates for long-term success in the normalization of eating behavior

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Key Words
Leptin, neuroimaging, fMRI, resting-state, lipodystrophy

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Abstract

Lipodystrophy (LD) is a rare disease with a paucity of subcutaneous adipocytes and leptin-deficiency. Patients often develop severe diabetes mellitus and show disturbed eating behavior with reduced satiety that can be restored by substitution with the leptin analogue metreleptin. However, long-term effects of metreleptin on resting-state brain connectivity in treatment-naïve LD patients have not been assessed. In this study, resting-state functional magnetic resonance imaging (fMRI) scans and extensive behavioral testing assessing changes in hunger/satiety regulation were performed during the first 52 weeks of metreleptin treatment in nine LD patients. Resting-state connectivity significantly increased over the course of metreleptin treatment in three brain areas, i.e. hypothalamus, insula/superior temporal gyrus, and medial prefrontal cortex. Behavioral tests demonstrated that perceived hunger, importance of eating, eating frequencies, and liking ratings of food pictures significantly decreased during metreleptin therapy. Taken together, leptin substitution was accompanied by long-term changes of hedonic and homeostatic central nervous networks regulating eating behavior, as well as decreased hunger feelings and diminished incentive value of food. It needs to be assessed in future studies whether metreleptin treatment in LD restores physiological processes important for the development of satiety.
Abbreviations

BMI - Body mass index
CNS - Central nervous system
EC - Eigenvector centrality
EPI - Echo-planar imaging
FDR - False discovery rate
FEV - Fragebogen zum Essverhalten, German version of the Three factor eating questionnaire
FMRI - Functional magnetic resonance imaging
HbA1c - Hemoglobin A1c
IEG - Inventory of eating behavior and weight problems (German title: Inventar zum Essverhalten und Gewichtsproblemen)
LD - Lipodystrophy
LMNA - Lamin A/C
MNI - Montreal Neurological Institute
PPARγ - Peroxisome proliferator-activated receptor γ
SPM - Statistical parametric mapping
TFEQ - Three factor eating questionnaire
TG - Triglycerides
VAS - Visual analogue scale
Lipodystrophy (LD) is a rare disease with a paucity of subcutaneous adipocytes and reduced leptin blood concentrations. Several genetic mutations are known to cause partial or generalized forms of the disease, and also cases of acquired LD are reported (1). LD is frequently accompanied by type 2 diabetes mellitus and dyslipidemia. Leptin substitution in the form of the analogue metreleptin has shown beneficial metabolic effects. In the so far biggest clinical trial on metreleptin treatment in LD, hemoglobin A1c (HbA1c) on average decreased by 1.5 % and serum triglycerides (TG) fell by >50 % after one year of treatment (2). Additionally, patients with LD often develop a disturbed eating behavior with reduced satiety after food consumption, leading to an increase in meal frequency (3). Impaired eating behavior can be improved by leptin substitution (4). Humoral leptin crosses the blood brain barrier by active transport in the proximity of the mediobasal hypothalamus where the blood brain barrier is well permeable for peripheral hormones (3,5). Via receptors in the arcuate nucleus, leptin inhibits food intake through direct activation of anorexigenic cocaine and amphetamine regulated transcript and pro-opiomelanocortin neurons (6,7), as well as inhibition of orexigenic neuropeptide Y neurons (8).

In a widely accepted model of the control of eating behavior, the hypothalamus is considered to govern the homeostatic component of human eating regulation, i.e. the drive to eat to meet the bodily demands for energy (9, 10).

In addition, the leptin receptor is also expressed in neurons of the mesolimbic dopamine system, which is involved in the processing of motivation and reward. Here, leptin reduces dopamine signaling (reviewed in 11). These findings suggest an influence of leptin also on the other component contributing to the formation of human eating behavior, i.e. hedonic eating which is the drive to eat for pleasure in the absence of an energy deficit (12). Human behavioral data support the hypothesis that leptin affects both components of eating control (4,13).

Apart from the clinical need for metreleptin treatment, patients with decreased leptin blood concentrations provide a unique model to study the central nervous effects of leptin. However, in
vivo investigations of metreleptin effects on brain activation assessed with functional magnetic resonance imaging (fMRI) have not been performed in treatment-naïve LD patients so far. Furthermore, the impact of leptin on resting-state brain function which is a useful tool for the assessment of connectivity of brain regions and of long-term state changes (14) has not been investigated yet. To address these open points, long-term effects of leptin substitution on resting-state brain connectivity in treatment-naïve LD patients were assessed with task-free resting-state fMRI before and at five time points over 52 weeks after initiation of metreleptin treatment. Since anchoring of fMRI findings with behavioral results is crucial for a correct interpretation (15), all patients underwent an extensive neuropsychological assessment accompanied by metabolic tests at each study visit.

We hypothesized that with resting-state fMRI long-term increases in connectivity in both homeostatic and hedonic brain areas can be observed, and that these changes are accompanied by behavioral changes including perceived hunger/satiety and liking ratings of food pictures. For the first time, we show combined long-term metreleptin treatment-related effects on behavior and resting-state brain connectivity in initially leptin-deficient and treatment-naïve patients. These findings are particularly important in view of recent successful approaches of overcoming leptin resistance (16) and hopes of using metreleptin as a therapeutic agent in obesity in the future.
Research Design and Methods

LD patients
Nine patients with LD (7 female) eligible for metreleptin treatment at the University Hospital Leipzig participated in the MRI study. Baseline characteristics and laboratory data of included patients are summarized in Table 1. Inclusion criteria for leptin replacement were established LD, age ≥5 years at baseline, insufficiently controlled diabetes mellitus and/or hypertriglyceridemia despite adequate antihyperglycemic and lipid-lowering medication, respectively. Exclusion criteria included pregnancy or lactation, severe renal insufficiency, active malignant disease, primary hematologic abnormalities, infectious liver disease, HIV infection, and hypersensitivity to E. coli-derived proteins. All patients were metreleptin treatment-naïve and consented to participating in the MRI study.

Medication
The leptin analogue metreleptin was used for treatment. Metreleptin was provided by Amylin (San Diego, CA)/ Bristol-Myers-Squibb (Munich, Germany)/ AstraZeneca (London, UK)/ Aegerion Pharmaceuticals (Cambridge, MA), respectively, and applied subcutaneously. Dosing was recommended by the respective manufacturer in order to achieve physiological replacement. Patients 1 to 3 (all female) administered metreleptin BID at 0.04 mg/kg body weight per day for the first week and, thereafter, at 0.08 mg/kg body weight per day resulting in doses between 2.9 and 7.8 mg per day. For patients 4 to 9, dosing changed due to modified instructions by the manufacturer. Metreleptin was administered once daily at 2.5 mg per day for men and 5 mg per day for women independent of body weight.

Experimental design
Experiments were performed between 2010 and 2014. Behavioral tests and MRI scanning were performed at six different time points, i.e. 1) one day before start of metreleptin supplementation, and after 2) one, 3) four, 4) 12, 5) 26, and 6) 52 weeks of metreleptin treatment. On each
measurement day, the same set of questionnaires and behavioral tests was performed as further indicated below. All patients were asked to have a small lunch at 12 pm (noon) and thereafter stay fasted until 5 pm. Then, a standard meal consisting of 20% of the daily energy requirements calculated for each patient was consumed. Since leptin physiologically mediates satiety, differences between leptin deficiency and imitated physiological leptin levels due to metreleptin treatment were expected to be most pronounced in the sated state and, therefore, the mentioned calorie amount was chosen to create a state of moderate satiety. We did not choose a higher percentage of daily energy requirements in order to avoid both a ceiling effect in extreme fullness and postprandial tiredness during the following fMRI scan. Before and after the meal, patients filled in visual analogue scales (VAS). At 6 pm, the MRI scan was performed. The next morning at 8 am, a fasting blood sample was taken for assessment of metabolic parameters including fasting TG, HbA1c, and leptin serum concentrations.

Questionnaires and behavioral tests

Prior to all other measurements, the German versions of the Three factor eating questionnaire (TFEQ [17], German version: Fragebogen zum Essverhalten, FEV [18]) and the Inventory of eating behavior and weight problems (German title: Inventar zum Essverhalten und Gewichtsproblemen, IEG [19]) were filled in. Since FEV and IEG refer more to long-term than acute attitudes and feelings, both tests were not performed 1 week after initiation of metreleptin treatment. A complete list of the 14 scales of the IEG with German titles and English translations is shown as Suppl. Table 1. VAS were bars of 100 mm length for assessment of hunger and satiety feelings. The very left at 0 mm indicated no hunger or satiety whereas the very right at 100 mm indicated extreme hunger or satiety. The picture rating task was performed in a designated room with as little distraction as possible. On a computer screen, 200 food and 50 non-food pictures were presented in randomized order and rated via a keypad (four keys) for valence (Food pictures: “How tasty do you find the depicted food item?”, 1 = not at all tasty to 4
= extremely tasty; Non-food pictures: “How much do you like the depicted object?”, 1 = not at all to 4 = very much).

**FMRI paradigm, technical parameters, and data processing**

The fMRI scan was performed in resting-state. Patients inside the scanner were in a supine position and looking at a black screen with a white fixation cross. A whole-body 3T TIM Trio scanner (Siemens, Erlangen, Germany) with a 32-channel head coil was used. In each scanning session, resting-state fMRI data were acquired using a gradient-echo echo-planar imaging (EPI) sequence. The following parameters were used: 300 whole brain volumes, acquisition matrix = 64 x 64, slice thickness = 4 mm (1 mm gap), resulting in a nominal voxel size of 3 x 3 x 5 mm³. Further imaging parameters were: 30 axial slices, TR = 2300 ms, TE = 30 ms, flip angle = 90° and bandwidth = 1817 Hz/pixel.

Preprocessing of the fMRI data was performed using statistical parametric mapping (SPM) 8 including estimation and correction for motion and EPI deformation. The normalization was performed by registering the individual 3D high-resolution T1-weighted structural image onto the functional images. This individual anatomical image was further processed by the unified segmentation algorithm (20), and the resulting deformation field was applied onto the functional images. After normalization, the resulting voxel size of the functional images was interpolated to an isotropic voxel size of 3 x 3 x 3 mm³. In the final step of the preprocessing, the functional images were smoothed using a Gaussian smoothing kernel of 8 mm full width at half maximum.

To identify treatment-related connectivity changes, Eigenvector centrality (EC) mapping was performed using the LIPSIA software package (21). EC provides a measure for detecting central hubs within a brain network using an algorithm similar to Google’s PageRank algorithm (22). For all voxels, a similarity matrix was generated including Pearson's correlation coefficients between all resting-state fMRI time courses. In order to use a similarity matrix with only positive numbers, all negative entries were set to zero before computing the EC. In a second approach,
we also used the absolute value taking all values of the similarity matrix into account when computing the EC measure. Note that according to the theorem of Peron and Frobenius (23) the similarity matrix has a unique real largest Eigenvalue, and the corresponding Eigenvector has strictly positive components. Then, the EC map was generated using the components of this Eigenvector to determine the EC of all voxels.

Within SPM, the statistical analysis was performed on the group level using all six EC maps for all subjects using the general linear model with a flexible factorial design with factors subject and time. A weighted sum of the parameter estimates was statistically assessed using a contrast vector generated by the a priori hypothesis of an increased EC over time. The resulting statistical parametric map was processed using a voxel-wise threshold of p<0.005. To take the multiple comparison problem into account, clusters were detected with a minimum size of 80 voxels in order to obtain clusters with a false discovery rate (FDR)-corrected p<0.05.
Results

**Anthropometric and metabolic parameters in LD patients**

Baseline characteristics of all patients are summarized in Table 1. Mean ± standard error of the mean (SEM) age was 38 ± 4 years and body mass index (BMI) was 27.0 ± 1.7 kg/m². Baseline leptin was 5.3 ± 1.2 µg/l, HbA1c was 7.4 ± 0.3 %, and TG were 13.2 ± 3.8 mmol/l. Changes in BMI, HbA1c, and TG during metreleptin treatment are summarized in Suppl. Table 2.

**Valence of food/non-food pictures and hunger/satiety ratings**

Average rating score of food pictures was 2.76 ± 0.12 and for non-food pictures was 2.55 ± 0.08 at baseline (Figure 1A). Valence of food pictures decreased after initiation of metreleptin treatment with significant decreases observed at 1 week, 4 weeks, and 52 weeks (p<0.05), as well as trends at 12 weeks (p=0.06) and 26 weeks (p=0.07). In contrast, rating scores of non-food pictures did not change significantly throughout the 52 weeks of metreleptin treatment (Figure 1A).

Fasting hunger rated on VAS was 54 ± 9 mm at baseline (Figure 1B). Hunger ratings continuously decreased over the first 26 weeks of metreleptin treatment with lowest scores detectable at 26 weeks (18 ± 6 mm; p=0.002). Fasting hunger at 52 weeks was significantly higher as compared to 26 weeks (44 ± 9; p=0.04). Satiety rated 5 min after the meal was 72 ± 8 mm at baseline. Satiety ratings 5 min after the meal continuously increased over the first 26 weeks of metreleptin treatment with highest scores detectable at 26 weeks (93 ± 2 mm; p=0.03). Satiety rated 5 min after the meal at 52 weeks (72 ± 8 mm) was significantly lower as compared to 26 weeks (p=0.04) and equal to baseline levels. Satiety rated 120 min after the meal was 56 ± 9 mm at baseline and increased to 78 ± 5 mm after 1 week (p=0.03) and 77 ± 8 mm after 4 weeks (p=0.051). Satiety ratings 120 min after the meal at 12, 26, and 52 weeks were not significantly different as compared to baseline with similar ratings seen at 26 weeks (72 ± 8 mm) and 52 weeks (70 ± 9 mm), respectively (Figure 1B). Ratings for tastiness of the test meal were between
63 and 70 mm on the VAS and did not change significantly throughout the 52 weeks of metreleptin treatment (data not shown).

*Food questionnaires*

In the TFEQ, average baseline score for scale 2 ("Disinhibition") was 7.0 ± 1.0, which significantly decreased to 4.2 ± 1.1 as early as 4 weeks after initiation of metreleptin treatment (p<0.05; Figure 2A). The average score remained significantly decreased as compared to baseline up to 26 weeks (p<0.05) and a strong trend was also seen after 52 weeks (4.3 ± 0.8; p=0.052). Average baseline score for scale 3 ("Hunger") was 8.9 ± 0.9 which also decreased to 4.4 ± 0.9 already after 4 weeks of metreleptin treatment (p<0.01). The average score further decreased with lowest values detectable at 26 weeks (2.7 ± 0.8; p<0.001) and significantly lower score also detectable after 52 weeks (3.1 ± 1.1; p<0.001). In contrast, average scores for scale 1 ("Cognitive restraint of eating") did not significantly change throughout the 52 weeks of metreleptin treatment (Figure 2A).

In the IEG questionnaire, average scores for scale 1 ("Importance of eating"), scale 2 ("Strength and triggering of desire to eat"), scale 9 ("Attitude towards obese persons"), and scale 11 ("Eating between meals") all significantly decreased at least at one time point after initiation of metreleptin treatment as compared to baseline levels (Figure 2B). Furthermore, the value for scale 7 ("Cognitive restraint of eating") was significantly higher at 52 weeks of metreleptin treatment (11.8 ± 2.1) as compared to baseline (9.0 ± 1.9; p=0.02; Figure 2B). In contrast, values in all other scales were not significantly affected by metreleptin treatment (data not shown; for German original wording and English translation of scale titles, please refer to Suppl. Table 1).

*FMRI data*

Using resting-state fMRI and EC mapping, a significant increase of brain connectivity was detected over the course of metreleptin treatment. A significant EC increase over all six measurements was found in three brain regions: hypothalamus (Montreal Neurological Institute
[MNI] coordinates in mm; x, y, z; maxima at 15, 23, -8, T=4.22; 3, 5, -11, T=4.20; -6, 11, -11, T=3.24, p on cluster level=0.040, FDR-corrected), insula/superior temporal gyrus (STG; MNI coordinates local maximum 51, -13, 7; T=4.48, p on cluster level=0.004, FDR-corrected), and medial prefrontal cortex (mPFC; MNI coordinates local maximum -6, 56, 16, T=4.82, p on cluster level<0.001, FDR-corrected) (Figure 3). To further ascertain that the significant EC increases found in the midbrain cluster map the anatomical region hypothalamus, a conjunction between whole brain ECM analysis and a hypothalamus mask created using the WFU pickatlas (24) was performed. Here, voxels within the mask of hypothalamus showed an increase of EC over time (Suppl. Fig. 1). The maximum of the cluster was located in [6 2 -17] and both peak and cluster were significant (p<0.05, family wise error [FWE]-corr.; Suppl. Fig. 1).

Interestingly, a significant EC increase in all three brain regions was detected with both approaches of dealing with negative values in the correlation matrix, i.e. 1) setting all negative values to zero and 2) using the absolute value. When the two male subjects were excluded from the analysis, results remained similar (Suppl. Fig. 2). Furthermore, the same brain regions were also seen in all subjects (n=9) when contrasting baseline (V1) against 52 weeks of metreleptin treatment (V6) (data not shown). In our contrast with linear increase over time [-2.5 -1.5 -0.5 0.5 1.5 2.5], metreleptin treatment over 1 year also increased connectivity with other EC measures as calculated with the fast ECM SPM-toolbox (25), i.e. in the hypothalamus as assessed by norm ECM and degree ECM, as well as in the insula/STG and mPFC as assessed by norm ECM and rank ECM (Suppl. Fig. 3).

In connectivity analysis with region-wise pairs using the AAL atlas (26), connectivity for the insula/STG was also increased over the course of metreleptin treatment (Suppl. Fig. 4). The hypothalamus and the mPFC are not defined by the AAL atlas.

Using the inverse contrast of decreased EC over time, we obtained a region in the vicinity of the precuneus, a part of the so-called default mode network of the human brain (Suppl. Fig. 5).
Discussion

In the current study, we demonstrate for the first time that metreleptin treatment in LD patients over 52 weeks is associated with significantly increased resting-state connectivity in the hypothalamus, insula/STG, and mPFC, i.e. in both homeostatic and hedonic brain areas. These observed effects are accompanied by significant decreases in self-reported pre- and post-prandial hunger feelings as rated with VAS – a measure for homeostatic hunger – and food liking ratings in the fed state – a measure for the hedonic perception of food.

Firstly, we observed a connectivity increase in the homeostatic center of the brain, the hypothalamus, over the 52 weeks of metreleptin treatment. Behavioral data showed reduced hunger ratings in the fasted state and increased satiety ratings after the standard meal during the first 26 weeks of metreleptin treatment. These findings are in accordance with independent studies on metreleptin treatment in LD patients (4,13). Furthermore, in the TFEQ we found decreased scores for scale 3 (“Hunger”) after initiation of metreleptin. Lower scores indicate that hunger feelings (which are often perceived as disturbing) are decreased (18). It is interesting to note in this context that another physiological anorexigenic peptide hormone, i.e. the glucagon-like peptide-1 agonist exenatide, had similar effects on the hypothalamus in fMRI connectivity analysis, as well as on VAS-assessed hunger and satiety, in obese persons in another study from our group (27). These current and published results are in accordance with the hypothesis that leptin substitution improves homeostatic satiety signaling via the hypothalamus in our patients. However, causality cannot be established with our current design and further investigations are required.

Secondly, connectivity increased in the insula/STG during the 52 weeks of metreleptin treatment. The insula is the gustatory cortex of the brain and involved in interoception, i.e. internal sensing of food qualities like odor, taste, and nutrient composition (28). Furthermore, it is supposed to map the ongoing physiological state of the body via thalamocortical pathways (29). Moreover,
the insula is discussed as a region important in reward processes, believed to play a role in the valuation of reward, and alterations are found in diseases with disrupted reward and valuation-like addictive behavior (29). In the leptin-deficient state, our patients anecdotally described an addiction-like affinity to food with large parts of their free daytime revolving around food preparation and consumption. After a meal, satiety only persisted for approximately one hour. After metreleptin treatment, patients initially experienced longer periods of satiety after a meal, reduced meal frequencies, and lost interest in thoughts about food. These descriptions are underlined by significant decreases of IEG scores for scale 1 (“Importance of eating”), scale 2 (“Strength and triggering of desire to eat”), and scale 11 (“Eating between meals”). Furthermore, our behavioral data indicate a stronger self-control of eating behavior after initiation of leptin substitution, i.e. IEG scores for scale 7 (“Cognitive restraint on eating”) significantly increased during metreleptin treatment. The score of the identically named scale 1 of the TFEQ also increased but did not reach the level of statistical significance. Unfortunately, we did not assess cognitive abilities which have been linked to the STG (30) since the behavioral focus of our study was primarily on eating regulation. Overall, our findings are in agreement with the hypothesis that metreleptin might improve physiological interoception of food via an increase of connectivity of the insula/STG as compared to a leptin-deficient state. Future studies are needed to further investigate this hypothesis.

Thirdly, metreleptin treatment was also associated with increased connectivity in the mPFC which is a brain region involved in the assignment of incentive motivational value to food stimuli together with the orbitofrontal cortex and, thus, drives feeding behavior (31,32). In accordance with these imaging findings, behaviorally we found a decrease of liking ratings of food pictures with metreleptin therapy. Furthermore, values for scale 2 (“Disinhibition”) of the TFEQ which indicates how vulnerable a person’s eating behavior is through external (e.g. smell and sight of food) and internal (e.g. emotions) distractors, decreased. Our data are supported by
an independent study with an event-related fMRI paradigm investigating acute brain activity changes during presentation of food pictures in two adolescents with congenital leptin deficiency. The authors demonstrate that positive correlations of brain activations in nucleus accumbens and caudate nucleus (important regions of the dopaminergic reward system) observed in leptin deficiency were not anymore seen in the metreleptin-treated state. This suggests that leptin is necessary to suppress the incentive motivational value to food stimuli in the fed state (33).

We did not find a significant correlation between changes of the metabolic parameters HbA1c, TG, as well as total, HDL, and LDL cholesterol, and brain imaging results (data not shown). These findings do not support the hypothesis that the metabolic changes seen with metreleptin significantly impact the central nervous findings. However, we cannot exclude the possibility that our methods are not sensitive enough or sample size is too small.

Strengths of our study include being the first fMRI study in treatment-naïve LD patients, studying the effects of metreleptin on brain connectivity, as well as performing the analysis at five time points after initiation of treatment ranging from 1 to 52 weeks. Furthermore, with a wide range of neuropsychological assessments we were able to differentially picture the effects of metreleptin treatment on the different facets of hunger regulation and eating behavior. However, some limitations also have to be discussed. These include the lack of a placebo or healthy control group. Therefore, the experimental design does not allow the distinction between metreleptin treatment effects from non-specific order effects. Results of measurements may well change across repeated assessments, for a variety of reasons, e.g. habituation. In addition, phase of the menstrual cycle also impacts eating behaviors and might influence our findings. However, only in two out of our nine patients intra-individual variation in sex hormone levels can be suspected between some of the six measurements. Furthermore, metabolic improvements in LD patients under metreleptin therapy have already been demonstrated in the past in larger samples.
Moreover, the sample size of n=9 is rather small for an fMRI study, especially when taking into account that resting-state fMRI connectivity involves numerous statistical operations on the 3D volume space. However, no study with n>1 has been published so far in metreleptin treatment-naïve leptin-deficient subjects. Furthermore, our study is the first describing metreleptin treatment-associated alterations in resting-state connectivity as compared to published fMRI studies with event-related designs ranging from n=1 to 3 (33-36) and one report with n=10 but not studying treatment-naïve patients (13). Clearly, a replication of obtained fMRI results in larger samples in the future would be valuable.

Taken together, we have elucidated for the first time long-term effects of metreleptin on brain connectivity in leptin-deficient patients in the current study. Leptin substitution causes long-term changes of hedonic and homeostatic central nervous networks regulating eating behavior which are accompanied by decreased hunger feelings and diminished incentive value of food. It needs to be assessed in future studies whether metreleptin treatment in LD restores physiological processes important for the development of satiety via these mechanisms.
Acknowledgements

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References


(18) Pudel V, Westhöfer J. *Fragebogen zum Essverhalten (FEV)*. Göttingen, Toronto, Zürich, Verlag für Psychologie Dr. C. J. Hogrefe, 1989


(23) Frobenius G. Über Matrizen aus nicht negativen Elementen. *Berl Ber* 1912;456–477


### Table 1: Baseline characteristics and laboratory data of the study population

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<tr>
<th>Patient</th>
<th>Phenotype of LD</th>
<th>Mutation</th>
<th>Sex</th>
<th>Age [years]</th>
<th>BMI [kg/m²]</th>
<th>Leptin [µg/l]</th>
<th>HbA1c [% (mmol/mol)]</th>
<th>TG [mmol/l]</th>
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BMI, Body mass index; F, Female; HbA1c, Glycated hemoglobin A1c; LD, Lipodystrophy; LMNA, Lamin A/C; M, Male; ND, Not detected; PPARγ, Peroxisome proliferator-activated receptor γ; TG, Triglycerides.
Figure legends:

**Fig. 1**  
**A)** Liking-ratings of food and non-food pictures and **B)** Visual analogue scales (VAS) for hunger and satiety before, 5 min after and 120 min after the standardized meal. *indicates p<0.05 and **p<0.01 as compared to baseline, †indicates p<0.05 as compared to 26 weeks as assessed by 2-tailed paired Student’s t test.

**Fig. 2**  
**A)** All scales of the three factor eating questionnaire and **B)** the five (out of 14) scales of the IEG showing significant changes after initiation of metreleptin treatment. Values were obtained as described in Research Design and Methods and Suppl. Table 1. *indicates p<0.05, **p<0.01, and ***p<0.001 as compared to baseline as assessed by 2-tailed paired Student’s t test.

**Fig. 3**  
Parametric contrast with increase of Eigenvector centrality (EC) over time with six measurements [-2.5 -1.5 -0.5 0.5 1.5 2.5] over 52 weeks of metreleptin treatment (color-coded in yellow/orange). EC values were obtained using repeated sessions of resting-state fMRI before metreleptin therapy, as well as after 1, 4, 12, 26, and 52 weeks of treatment. Local maxima are given in mm MNI coordinates for x, y, and z axis. In addition, mean ± SEM fitted EC values at local maxima are given for every single measurement. FDR-corr, False discovery rate-corrected.
**Picture Ratings for Valence**

- Food pictures
- Non-food pictures

**Hunger and Satiety Ratings (VAS)**

- Hunger before meal
- Satiety 5 min after meal
- Satiety 120 min after meal

Figure 1
**Figure 2**

A. Three Factor Eating Questionnaire (TFEQ)

- Factor 1: Cognitive restraint of eating
- Factor 2: Disinhibition
- Factor 3: Hunger

B. Inventory of Eating Behavior (IEG)

- Scale 1: Importance of eating
- Scale 2: Strength and triggering of desire to eat
- Scale 7: Cognitive restraint of eating
- Scale 9: Attitude towards obese persons
- Scale 11: Eating between meals
Voxel-wise, p < 0.05 (FDR-corr)

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<th></th>
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<th>$k_E$</th>
<th>t</th>
<th>Z</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Fitted EC value V1-6</th>
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Online appendix

Table S1: Complete list of the 14 scales of the IEG with German titles and English translations.

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<tr>
<th>Scale</th>
<th>German original wording</th>
<th>English translation</th>
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<tr>
<td>1</td>
<td>Stellenwert des Essens</td>
<td>Importance of eating</td>
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<tr>
<td>2</td>
<td>Stärke und Auslösbarkeit des Essbedürfnisses</td>
<td>Strength and triggering of desire to eat</td>
</tr>
<tr>
<td>3</td>
<td>Sozial-situative Auslöser für Mehressen</td>
<td>Socio-situative triggers for overeating</td>
</tr>
<tr>
<td>4</td>
<td>Wirkung des Essens</td>
<td>Effects of eating</td>
</tr>
<tr>
<td>5</td>
<td>Essen als Mittel gegen (soziale) Belastung</td>
<td>Eating as an instrument against (social) stress</td>
</tr>
<tr>
<td>6</td>
<td>Essen und Gewicht als Problem</td>
<td>Eating and weight as problem</td>
</tr>
<tr>
<td>7</td>
<td>Zügelung des Essens</td>
<td>Cognitive restraint of eating</td>
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<tr>
<td>8</td>
<td>Einstellung zur gesunden Ernährung</td>
<td>Attitude towards healthy eating</td>
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<tr>
<td>9</td>
<td>Einstellung zu Übergewichtigen</td>
<td>Attitude towards obese persons</td>
</tr>
<tr>
<td>10</td>
<td>Essgeschwindigkeit</td>
<td>Speed of eating</td>
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<td>11</td>
<td>Essen zwischen den Mahlzeiten</td>
<td>Eating between meals</td>
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<td>Nächtliches Essen</td>
<td>Night eating</td>
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<td>13</td>
<td>Esszwänge in der Kindheit</td>
<td>Compulsions of eating in childhood</td>
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<td>14</td>
<td>Belastung durch Übergewicht</td>
<td>Stress through overweight</td>
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IEG, Inventory of eating behavior and weight problems; German title: Inventar zum Essverhalten und Gewichtsproblemen
Table S2: BMI, HbA1c, and fasting TG over 52 weeks of metreleptin treatment

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<th>Baseline</th>
<th>1 Week</th>
<th>4 Weeks</th>
<th>12 Weeks</th>
<th>26 Weeks</th>
<th>52 Weeks</th>
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<td>27.0 ± 1.7</td>
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<td>26.7 ± 1.7</td>
<td>26.8 ± 1.7</td>
<td>26.6 ± 1.7</td>
<td>26.8 ± 1.6</td>
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<td>HbA1c [%]</td>
<td>7.4 ± 0.3</td>
<td>7.4 ± 0.3</td>
<td>6.9 ± 0.2*</td>
<td>6.7 ± 0.2*</td>
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<td>7.0 ± 0.4</td>
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<td>TG [mmol/l]</td>
<td>13.2 ± 3.8</td>
<td>7.8 ± 2.0*</td>
<td>6.0 ± 1.2*</td>
<td>9.0 ± 2.3</td>
<td>5.3 ± 1.1</td>
<td>7.6 ± 2.8</td>
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BMI, Body mass index; HbA1c, Glycated hemoglobin A1c; TG, Triglycerides. Mean ± Standard error of the mean are given; *indicates p<0.05 as compared to baseline as assessed by 2-tailed paired Student’s t test.
**Figure S1:** Conjunction between whole brain ECM analysis and a mask created using the WFU pickatlas (Maldjian et al., Neuroimage 2003;19:1233–1239), applying a 3D-dilatation of 1 voxel of the hypothalamus mask. Local maxima are given in MNI coordinates in mm for x-, y-, and z-axis. FWE-corr, Family wise error-corrected; \( k_E \), cluster size in voxels.
**Figure S2:** Main analysis as in Figure 3 with female participants only (n=7). Local maxima are given in MNI coordinates in mm for x-, y-, and z-axis. FDR-corr, False discovery rate-corrected; \( k_E \), cluster size in voxels; STG, Superior temporal gyrus.

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Figure S3: Main analysis (increase V1 to V6) for Eigenvector centrality as reported in the results of the manuscript (row 1; compare Fig. 3), and three analyses performed with the SPM-toolbox fast ECM (Wink et al., Brain Connect 2012;2:265–274): norm ECM (row 2), rank ECM (row 3), and degree CM (row 4).
**Figure S4:** Analysis with connectivity matrix using region-wise pairs over the 6 time points V1-V6 [-2.5 -1.5 -0.5 0.5 1.5 2.5]. Regions were defined using the AAL SPM-toolbox (Tzourio-Mazoyer et al., Neuroimage 2002;15:273–289). STG, Superior temporal gyrus; unc., Uncorrected.

<table>
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<th>$p_{(unc.)}$</th>
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<td>&lt;0.00001</td>
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Figure S5: Parametric contrast with decrease of Eigenvector centrality (EC) over time with six measurements over 52 weeks of metreleptin treatment (color-coded in yellow/orange). EC values were obtained using repeated sessions of resting-state fMRI before metreleptin therapy, as well as after 1, 4, 12, 26 and 52 weeks of treatment. Local maxima are given in MNI coordinates in mm for x-, y-, and z-axis. FDR-corr, False discovery rate-corrected; k_E, cluster size in voxels.