Leptin Therapy in a Congenital Leptin-Deficient Patient Leads to Acute and Long-Term Changes in Homeostatic, Reward, and Food-Related Brain Areas

Sabine Frank, Martin Heni, Anja Moss, Julia von Schnurbein, Andreas Fritsche, Hans-Ulrich Häring, Sadaf Farooqi, Hubert Preissl, and Martin Wabitsch

MEG Center (S.Fr., H.P.), University of Tübingen, 72076 Tübingen, Germany; Graduate School of Neural and Behavioural Sciences (S.Fr.), International Max Planck Research School, 72074 Tübingen, Germany; Department of Internal Medicine (M.H., A.F., H.-U.H.), Division of Endocrinology, Eberhard Karls University Tübingen, 72076 Tübingen, Germany; German Centre for Diabetes Research DZD (M.H., A.F., H.-U.H.), 72076 Tübingen, Germany; Division of Pediatric Endocrinology (A.M., J.v.S., M.W.), Department of Pediatrics and Adolescent Medicine, University of Ulm, 89081 Ulm, Germany; Metabolic Research Laboratories (S.Fa.), Institute of Metabolic Science, Addenbrooke's Hospital, University of Cambridge, Cambridge, United Kingdom; and Department of Obstetrics and Gynecology (H.P.), University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Context: Mutations that lead to congenital leptin deficiency cause severe obesity, hyperphagia, and impaired satiety due to malfunctions of peripheral and brain-related mechanisms.

Design and Patient: In a leptin-deficient adolescent girl, we investigated brain-related changes before and at two time points after leptin therapy (3 d and 6 months). Functional magnetic resonance imaging was performed during visual stimulation with food (high and low caloric) and nonfood pictures.

Results: Results show acute and long-term effects in the amygdala, the orbitofrontal cortex, and the substantia nigra/ventral tegmental area for the comparison of food and nonfood pictures. For the comparison of high and low caloric pictures, pure acute effects in the ventral striatum and the orbitofrontal cortex could be observed as well as acute and long-term effects in the hypothalamus.

Conclusion: This study gives additional insight in the influence of leptin therapy on brain functions in leptin deficiency. (***J Clin Endocrinol Metab*** **96: E1283–E1287, 2011)**

Congenital leptin deficiency is characterized by hyperphagia, impaired satiety, and severe obesity as well as immunological differences and abnormal pubertal development (1). Since 1997, 14 cases of congenital leptin deficiency have been described. After leptin replacement therapy with recombinant human leptin, all treated patients showed normalization of their symptoms (1–3).

Leptin signaling plays a crucial role in the maintenance of metabolic homeostasis and energy expenditure (4), is processed in various brain regions, especially in the hypothalamus (5), and is important for cortical food processing (6). In healthy obese subjects, it was shown that leptin application after a 10% weight loss led to similar brain activations elicited by food cues as were seen before weight loss including increased activation, *e.g.* in hypothalamus, frontal and limbic areas, and reduced activation in the brainstem. Weight loss-induced changes could be observed, for example, in reduced amygdala and hypothalamic activation. The authors concluded that subjects after weight loss are in a leptin-deficient state (7).

Two studies investigated the effect of leptin therapy in congenital leptin-deficient patients. In the first study, three subjects (substituted with leptin for 57 months) were measured before interruption of the leptin treatment, 33 d after

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-2713 Received November 17, 2010. Accepted April 27, 2011.
First Published Online May 18, 2011

Abbreviations: fMRI, Functional magnetic resonance imaging; OFC, orbitofrontal cortex; SN/VTA, substantia nigra/ventral tegmental area.

discontinuation of leptin treatment and 14 d after resubstitution with leptin. During discontinuation of leptin treatment, these patients showed increased brain activation for high *vs.* low caloric pictures in areas linked to hunger (insula, parietal, and temporal areas) and decreased activity mainly in prefrontal areas linked to inhibition (8). The second study included two subjects and examined acute functional differences before and after the start of leptin therapy. Differences were reported mainly in reward-related areas (ventral striatum), showing increased brain responses in the leptin-deficient state to food stimuli 7 d after the start of treatment (9).

To further elucidate brain-related changes due to leptin therapy and consecutive weight loss, functional magnetic resonance imaging (fMRI) measurements were performed with an adolescent patient before and after leptin therapy at three time points. Like the two aforementioned fMRI studies, we used visual presentation of food pictures and additionally differentiated acute and long-term effects. Based on the previous studies (7–9), we assume increased activity in reward- and food-related areas in the acute and chronic state. In contrast, homeostatic areas, especially the hypothalamus, should show no acute effect but appropriate food-related response after chronic leptin substitution.

Materials and Methods

Case history

We report the case of a 15-yr-old girl with a homozygous mutation in the *LEP* gene (10). Despite being obese (body mass index 35 kg/m^2), she gained much less weight compared with other leptin-deficient children. Since the age of 14 yr, she underwent a leptin substitution therapy with human metreleptin (0.6 mg twice a day = 0.024 mg/kg lean body mass), which caused substantial weight loss and an increase in leptin level. Human metreleptin for this patient was provided by Amylin (San Diego, CA).

Experimental design and procedure

We conducted fMRI measurements at three visits: 6 d before leptin substitution (pre), 3 d after the beginning of the therapy (3 d), and 6 months afterward (6 months).

At each measurement day, the subject was measured at the same time in the morning after an overnight fast of at least 12 h. Before scanning, hunger was examined on a rating scale.

During fMRI the measurements, the subject was stimulated visually with food (F) and nonfood (NF) pictures. Food pictures were divided in high caloric (HC) and low caloric (LC) (11).

Imaging analysis

After standard preprocessing, a fixed-effect analysis to all time points was applied using the regressors: food (F-NF) and calorie content (HC *vs.* LC). Whole-brain analysis of the main effects of the factor food and the factor calorie content was analyzed. Interactions of these factors with the factor time were analyzed with a region-of-interest approach for brain regions involved in homeostatic control (hypothalamus), reward [ventral striatum, substantia nigra/ventral tegmental area (SN/VTA), amygdala], and visual processing of food pictures [orbitofrontal cortex (OFC)].

Behavioral data

At each measurement day, the German eating behavior questionnaire, Fragebogen zum Essverhalten, and the Beck's Depression Inventory were applied. Additionally, an implicit and an explicit test were performed to evaluate implicit and explicit attitudes toward food (12) (Supplemental Material, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Results

Behavioral data

The subject showed no depressive symptoms but high cognitive control, low irritability during eating, and very low reported hunger at any time point.

Imaging data

Analysis of the main effects of the factors food (F-NF) and calorie content (HC *vs.* LC) showed well-known activation pattern and are described in the Supplemental Material. In the following text, we focus on the interactions of these factors with the factor time.

Acute and long-term effects: food × time

The contrast food *vs.* nonfood over time revealed significant acute BOLD response differences in the amygdala bilaterally. Activation differences of food and nonfood cues were significantly greater before therapy compared with both after measurements (Fig. 1A and Table 1). Activity in SN/VTA exhibited a similar pattern (Fig. 1B and Table 1). A converse pattern was observed in the OFC bilaterally (Fig. 1C and Table 1).

Acute and long-term effects: calorie content × time

The interaction analyses calorie content × time revealed significant acute effects in the ventral striatum and the OFC as well as acute and long-term effects in the hypothalamus.

Activation in reward- and food-related areas (ventral striatum and the OFC) (Fig 1, D and E, and Table 1) revealed significant interactions. In these areas the patient showed marked activation differences in the first post-measurement in which the ventral striatum showed substantial decrease at time point 3 d in low caloric pictures. This effect is alleviated after 6 months. High caloric stimuli showed a reversed pattern with marked activation 3 d after the start of treatment.

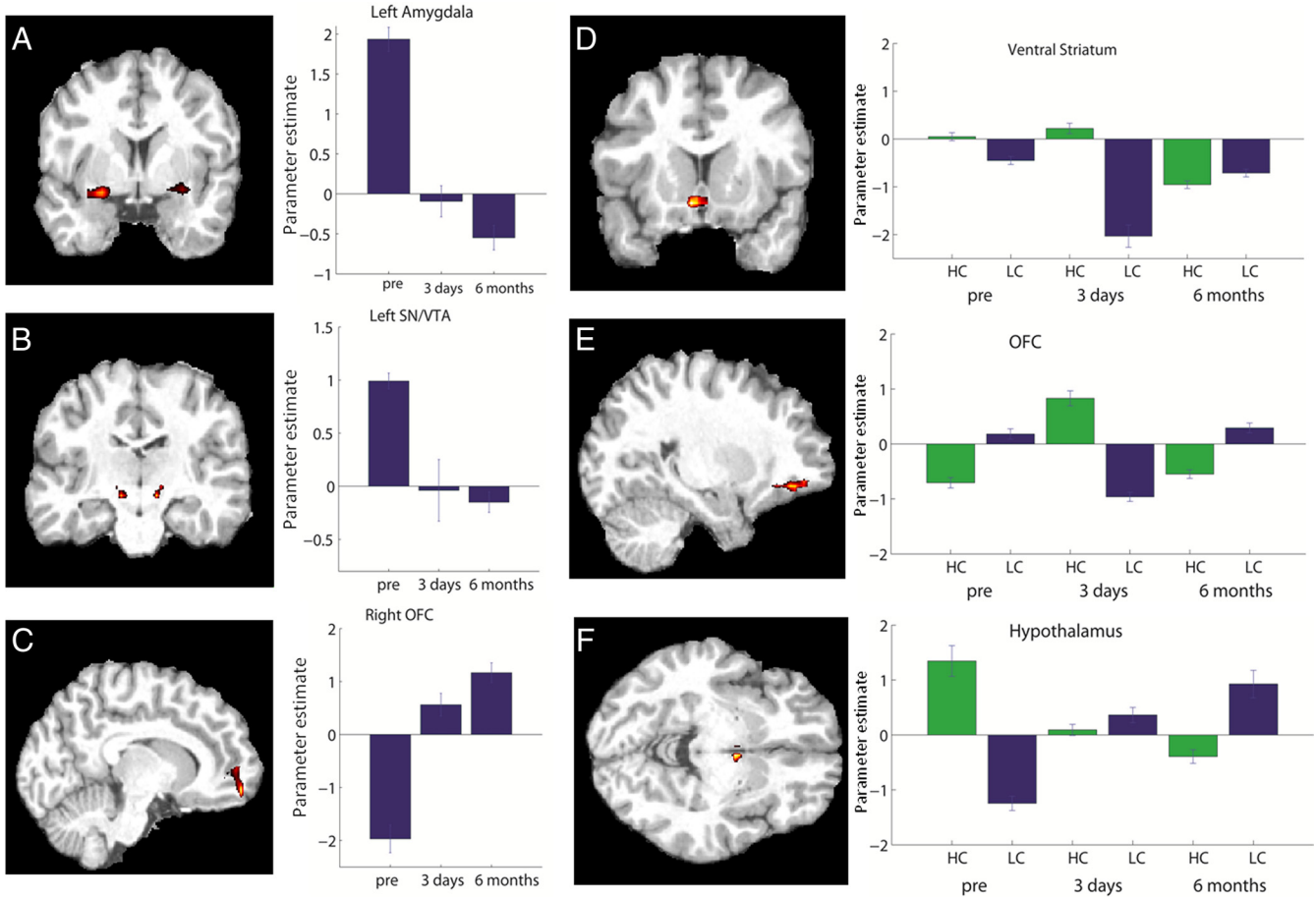

FIG. 1. A, *left panel*, Coronal view of amygdala activation for interaction F-NF over time. B, *left panel*, Coronal view SN/VTA activation for interaction F-NF over time. C, *left panel*, Sagittal view of OFC activation for interaction F-NF over time. A–C, *right panel*, Activation difference of F and NF pictures at three time points (only the hemisphere showing the stronger effect is represented). Contralateral hemisphere shows a similar pattern. D, *left panel*, Coronal view of the ventral striatum activation for the interaction HC *vs.* LC over time. E, *left panel*, Sagittal view of the OFC activation for the interaction HC *vs.* LC over time. F, *left panel*, Transversal view of hypothalamus activation for the interaction HC *vs.* LC over time. D–F, *right panel*, Activation difference of HC and LC displayed separately for both calorie contents. The *bar plots* represent parameter estimates ± SEM of the different sessions.

In the hypothalamus the activation to high caloric pictures decreased over time, whereas low caloric stimuli led to increased activation (Fig. 1F and Table 1).

A comparison with a normal-weight female control group is shown descriptively in Supplemental Material (Supplemental Fig. 2). The control group showed greater OFC activation for food *vs.* nonfood pictures. This was also observed in the patient after 6 months of treatment. High caloric pictures elicited a marked activation in the hypothalamus before treatment. This response was reduced by leptin therapy and resembled the observed activation in the control group.

TABLE 1. Brain imaging results

Contrast	Brain region	Hemisphere	Coordinates			Cluster size (voxels)	F value (before *vs.* 3 d)	F value (before *vs.* 6 months)
			x	*y*	*z*			
F-NF	Amygdala	L	−24	−3	−15	38	14.99	16.10
		R	30	−3	−12	20	10.34	9.27
F-NF	SN/VTA	L	−15	−21	−9	7	9.09	9.26
		R	15	−21	−6	4	8.20	7.91
F-NF	OFC	L	−21	51	−3	28	12.43	9.19
		R	21	54	−9	18	13.76	17.06
HC-LC	Ventral striatum	L	−3	12	−9	4	7.55	n.s.
HC-LC	OFC	L	−21	39	−12	42	8.94	n.s.
HC-LC	Hypothalamus	R	3	−6	−12	6	11.07	12.13

Contrast, brain region, Montreal Neurological Institute coordinates, hemisphere, cluster size, and F value. All data are familywise error corrected significant at $P < 0.05$. L, Left; R, right; n.s., not significant.

Discussion

Behavioral data

The patient showed extremely high scores on the eating behavior questionnaire (Fragebogen zum Essverhalten) scale for cognitive control as well as changes in the unconscious attitude toward low caloric food over time from highly positive to negative in the implicit test (Supplemental Material, Supplemental Fig. 1). This is in line with her comparably very low initial weight, which is unique for a leptin-deficient patient. Since early infancy the patient was on a low caloric diet, which probably caused the high cognitive control and consequently a positive attitude to low caloric food, even unconsciously before therapy. Interestingly, explicit ratings of palatability showed an increase for high caloric food after 6 months. This might be related to a less restrictive diet, allowing the consumption of high caloric food.

Imaging data

Acute effects

Purely acute effects were observed in the OFC (food processing) and ventral striatum (reward) in the HC *vs.* LC contrast (Fig. 1, D and E). This effect indicates a pure leptin effect independent of weight loss. Farooqi *et al.* (9) reported increased activity in the ventral striatum in the leptin-deficient state elicited by food pictures. We could not replicate this finding for the food *vs.* nonfood contrast. However, in our patient this effect was restricted to low caloric pictures. This may be due to our patient's strong focus on low caloric food based on her diet. Leptin as a regulator of the dopamine pathway also affects the ventral striatum (13). Therefore, it is a possible explanation that high caloric food revealed higher and low caloric food lower reward shortly after treatment. The decline of this effect later on might be due to assimilation of endocrine changes to leptin and alterations in synaptic plasticity.

Acute and long-term effects

The hypothalamus as a key regulator of energy homeostasis showed a clear interaction of high and low caloric food over time (Fig. 1F). Based on the segmentation in subdivisions of the hypothalamus (14), peak activation of this interaction was localized in the lateral hypothalamus, which integrates sensory information about energy homeostasis. Additionally, the lateral hypothalamus is also integrated in the information about the reward value of food (15). Changes to leptin and weight loss in the hypothalamus are in line with previous findings (7).

For the comparison of food and nonfood pictures, acute and long-term reduction could be observed in the amygdala and SN/VTA. Because the amygdala is an important area for evaluation of emotional salience, this indicates decreasing emotional appraisal of food stimuli. A coherent explanation is the change in reward value of food stimuli based on the release of her low caloric diet. As a nonhomeostatic area, the amygdala is not directly involved in food processing. However, the amygdala has interconnections to homeostatic areas like the hypothalamus (16). Therefore, both leptin and weight loss are likely to influence this region. This is in line with previous findings of reduced amygdala activation due to weight loss (7). Additionally, the amygdala has connections to other nonhomeostatic regions like the SN/VTA (16). The SN/VTA showed similar pattern in the activation (Fig. 1B) and is known to be influenced by leptin effects on neuronal dopamine function (17).

Like Baicy *et al.* (8), we found greater activation in the OFC with leptin supplement in the HC greater than LC contrast. Additionally, OFC activity showed an inverse correlation with body mass index, suggesting greater difficulties of response inhibition in adolescent girls (18). This is in line with our findings of higher OFC activity with decreasing body mass index (Fig. 1C).

Baicy *et al.* (8) also reported reduced activity in hunger-related brain regions due to leptin. We did not find similar results. This may be due to the constant low hunger rating of our subject, which is in contrast to the changes in the hunger rating in the mentioned study. Furthermore, it is questionable whether the leptin-deficient state of already supplemented patients is equivalent to the leptin-deficient state of our subject.

However, these studies might be heavily conditioned by the exact therapeutic protocol and the previous dietary patterns of individual patients. These factors must be taken into account in attempting to interpret the aggregate group of studies on leptin effects on brain fMRI.

In summary, leptin therapy resulted in weight loss-independent activity changes in homeostatic and reward-related brain areas. Given the rarity of this syndrome and the opportunity to perform imaging before and after therapy, this study contributes strongly to the aim of understanding the influence of leptin on reward processing and homeostatic control.

Acknowledgments

We thank Amylin (San Diego, CA) for the kind gift of human metreleptin provided for this patient. We also thank Maike Borutta for excellent technical assistance.

Address all correspondence and requests for reprints to: Sabine Frank, MEG Center, Otfried Müller Strasse 47, 72076 Tübingen, Germany. E-mail: sabine.frank@med.uni-tuebingen.de.

This work was supported by the Kompetenznetz Adipositas (Competence Network for Adiposity) funded by the German Federal Ministry of Education and Research (FKZ: 01GI0837, 01GI0849, and 01GI0851).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Farooqi S, O'Rahilly S** 2006 Genetics of obesity in humans. Endocr Rev 27:710–718
2. **Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, DePaoli AM, McCann SM, Wong ML** 2004 Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci USA 101:4531–4536
3. **Galgani JE, Greenway FL, Caglayan S, Wong ML, Licinio J, Ravussin E** 2010 Leptin replacement prevents weight loss-induced metabolic adaptation in congenital leptin-deficient patients. J Clin Endocrinol Metab 95:851–855
4. **Williams KW, Scott MM, Elmquist JK** 2009 From observation to experimentation: leptin action in the mediobasal hypothalamus. Am J Clin Nutr 89:985S–990S
5. **Leshan RL, Opland DM, Louis GW, Leinninger GM, Patterson CM, Rhodes CJ, Münzberg H, Myers Jr MG** 2010 Ventral tegmental area leptin receptor neurons specifically project to and regulate cocaine- and amphetamine-regulated transcript neurons of the extended central amygdala. J Neurosci 30:5713–5723
6. **Morrison CD** 2009 Leptin signaling in brain: a link between nutrition and cognition? Biochim Biophys Acta 1792:401–408
7. **Rosenbaum M, Sy M, Pavlovich K, Leibel RL, Hirsch J** 2008 Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. J Clin Invest 118:2583–2591
8. **Baicy K, London ED, Monterosso J, Wong ML, Delibasi T, Sharma A, Licinio J** 2007 Leptin replacement alters brain response to food cues in genetically leptin-deficient adults. Proc Natl Acad Sci USA 104:18276–18279
9. **Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC** 2007 Leptin regulates striatal regions and human eating behavior. Science 317:1355
10. **Fischer-Posovszky P, von Schnurbein J, Moepps B, Lahr G, Strauss G, Barth TF, Kassubek J, Mühleder H, Möller P, Debatin KM, Gierschik P, Wabitsch M** 2010 A new missense mutation in the leptin gene causes mild obesity and hypogonadism without affecting T cell responsiveness. J Clin Endocrinol Metab 95:2836–2840
11. **Frank S, Laharnar N, Kullmann S, Veit R, Canova C, Hegner YL, Fritsche A, Preissl H** 2010 Processing of food pictures: influence of hunger, gender and calorie content. Brain Res 1350:159–166
12. **Czyzewska M, Graham R** 2008 Implicit and explicit attitudes to high- and low-calorie food in females with different BMI status. Eat Behav 9:303–312
13. **Narayanan NS, Guarnieri DJ, DiLeone RJ** 2010 Metabolic hormones, dopamine circuits, and feeding. Front Neuroendocrinol 31: 104–112
14. **Smeets PA, de Graaf C, Stafleu A, van Osch MJ, van der Grond J** 2005 Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories. Am J Clin Nutr 82: 1011–1016
15. **Kampe J, Tschöp MH, Hollis JH, Oldfield BJ** 2009 An anatomic basis for the communication of hypothalamic, cortical and mesolimbic circuitry in the regulation of energy balance. Eur J Neurosci 30:415–430
16. **Grill HJ, Skibicka KP, Hayes MR** 2007 Imaging obesity: fMRI, food reward, and feeding. Cell Metab 6:423–425
17. **DiLeone RJ** 2009 The influence of leptin on the dopamine system and implications for ingestive behavior. Int J Obes (Lond) 33(Suppl 2):S25–S29
18. **Batterink L, Yokum S, Stice E** 2010 Body mass correlates inversely with inhibitory control in response to food among adolescent girls: an fMRI study. Neuroimage 52:1696–1703