Leptin Replacement Reestablishes Brain Insulin Action in the Hypothalamus in Congenital Leptin Deficiency

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OBJECTIVE

Human obesity is associated with impaired central insulin signaling, and in very rare cases, severe obesity can be caused by congenital leptin deficiency. In such patients, leptin replacement results in substantial weight loss and improvement in peripheral metabolism.

RESEARCH DESIGN AND METHODS

In a leptin-deficient patient, we investigated the impact of leptin substitution on central insulin action, as quantified by changes in neuronal activity after intranasal insulin application. This was assessed before and during the first year of metreleptin substitution.

RESULTS

After only 1 year, treatment with metreleptin reestablishes brain insulin sensitivity, particularly in the hypothalamus and, to a lesser degree, in the prefrontal cortex. Results are depicted in comparison with a control group. In our patient, brain activation changes were accompanied by substantial weight loss, reduced visceral adipose tissue, reduced intrahepatic lipid content, and improved whole-body insulin sensitivity.

CONCLUSIONS

Leptin replacement and weight loss improved homeostatic insulin action in the patient in question.

Patients affected by monogenetic leptin deficiency, whose leptin levels are therefore practically indiscernible, suffer from severe obesity, hyperphagia, impaired satiety, and metabolic impairments such as peripheral insulin resistance (1). Leptin replacement therapy is known to improve metabolism, normalize body weight, and introduce changes in brain processes (2–5). In recent years, we and others have ascertained that brain insulin action is crucial to various aspects of behavior and metabolism, such as eating behavior, cognition, and whole-body metabolism (6,7). However, brain insulin action, which is quantified by changes in neuronal activity in the functional MRI, had never been assessed both before and after leptin replacement therapy in congenital leptin-deficient patients. On the basis of the close molecular interaction of brain leptin and insulin signaling (8), we hypothesized that the untreated leptin-deficient patient will be brain insulin resistant, which can be reverted by substitution of leptin with metreleptin, a synthetic leptin analog.

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RESEARCH DESIGN AND METHODS

We performed resting functional MRI measurements on a leptin-deficient 31-year-old Pakistani woman before therapy (leptin-deficient untreated state), as well as 7 days after and 1 year after onset of substitution with 1.8 mg metreleptin. Brain insulin sensitivity was assessed by the quantification of cerebral blood flow (CBF) pre– and 30 min post–intranasal insulin application (160 units) by a nasal spray (details in 9). Blood samples were taken before the first measurement to determine peripheral glucose and insulin levels. Fasting HOMA of insulin resistance (HOMA-IR) was calculated for each measurement day [fasting insulin (pmol/L/6.945) * fasting glucose (mg/dL)/405] to estimate peripheral insulin sensitivity.

Scanning was performed on a 3T whole-body scanner with a standard 20-channel head coil (MAGNETOM Prisma; Siemens, Erlangen, Germany). CBF was measured with a three-dimensional fast spin-echo sequence using the FAIR QII perfusion mode with three-dimensional readout (3D GRASE). In addition, a high-resolution T1-weighted anatomical image was acquired.

CBF image processing was performed using FSL (http://fsl.fmrib.ox.ac.uk) and SPM12 (http://www.fil.ion.ucl.ac.uk./spm). Images from each session were realigned and resliced using FLIRT. Perfusion images were calculated by pairwise subtraction of the motion-corrected control and label images with ASL_FILE. The OXFORD_ASL module in FSL was used to calculate resting-state perfusion maps. Perfusion analysis was restricted by including only voxels that were classified with a probability of at least 75% of being gray matter on the basis of T1-weighted, anatomical images (10). The perfusion maps of each session and day were then individually coregistered to the anatomical image and normalized to Montreal Neurological Institute space.

To investigate brain insulin sensitivity, we created masks to extract perfusion values of the hypothalamus and the prefrontal cortex (PFC) within the right middle frontal gyrus, as a previous study showed decreased hypothalamic activity but increased prefrontal activity 30 min after intranasal insulin application in an obese group in comparison with a placebo condition (9). The hypothalamic mask was defined as the upper part of the hypothalamus (11). We used the peak activation voxel (x = 39, y = 29, z = 31) as a center with a sphere of 6 mm for the PFC mask (9). Perfusion values were extracted for each voxel within the masks for all measurements. Furthermore, whole-brain mean perfusion was calculated to standardize each extracted perfusion value for global perfusion differences between measurements (relative perfusion values). Data were further analyzed in SPSS 24 (IBM Corporation, Armonk, NY) using a sign test to investigate the change in relative perfusion in each voxel within each region of interest ($P < 0.05$, Bonferroni corrected).

For the quantification of visceral adipose tissue (VAT) and intrahepatic lipids (IHL), abdominal MRI and localized proton MRS measurements were performed (12).

For interpretation of the results of the patient from the perspective of "healthy" women, unpublished data of a female control group from a previous study (9) are included for results presentation (N = 21; mean $\pm$ SD BMI 26.5 $\pm$ 5.5 kg/m^2 and age 26.1 $\pm$ 3.6 years) (methods described in 9).

RESULTS

Analyses showed increased relative perfusion after intranasal insulin application in the hypothalamus in the leptin-deficient state and 7 days after initiation of metreleptin treatment. After 1 year, intranasal insulin had effected a marked reduction of relative perfusion in the hypothalamus (Fig. 1*A*). No significant effect of insulin application was observed for the PFC in either the leptin-deficient state or 7 days after the onset of treatment. After 1 year, however, insulin had induced an increase in relative PFC perfusion (Fig. 1*B*).

As can be observed in Fig. 1*C*, hypothalamic activity increase was indeed high compared with a healthy female control group and showed reduced activity after 1 year. Activity in the PFC was less prominent, since the subject was within the range of the control sample.

After initiation of leptin replacement, the patient's BMI was markedly reduced. She was, however, still severely obese after 1 year. Peripheral insulin sensitivity, assessed as HOMA-IR, also improved in the course of leptin treatment (Fig. 1*E*). Furthermore, VAT and IHL were already reduced 7 days after leptin substitution (Fig. 1*F*).

CONCLUSIONS

Congenital leptin deficiency is characterized by various metabolic impairments, in particular those associated with energy homeostasis and peripheral insulin action. Earlier studies have shown not only weight loss but also a marked improvement in whole-body metabolic functions, e.g., insulin sensitivity, reduction of visceral and liver fat, and behavioral changes once metreleptin substitution was initiated (12,13).

In the current study, we show that brain insulin action was impaired in a leptin-deficient patient before, as well as 7 days after, metreleptin substitution. Interestingly, 1 year after metreleptin treatment, brain insulin action showed a marked reduction in hypothalamic CBF but higher PFC activity after insulin application.

Before and especially after 7 days of metreleptin substitution, our patient showed an increase in hypothalamic CBF that exceeded even the highest increase of subjects of a healthy control group. By contrast, after 1 year of treatment, our patient showed the anticipated decrease in perfusion after intranasal insulin application. This result corresponds to the control subjects with the highest brain insulin sensitivity (and thus with reduced hypothalamic activity after insulin application) (Fig. 1*C*). It is well established that the two anorectic hormones insulin and leptin closely interact with each other in the hypothalamus and share molecular signaling pathways in neurons and nonneuronal cells (8). Furthermore, animal (8) and first human (6,7,14) data indicate a modulation of peripheral insulin sensitivity by the brain. Thus, the improvement in peripheral insulin sensitivity after 1 year of metreleptin treatment observed in our patient might be due to restored brain insulin action. However, improved insulin action in the brain was detected only after 1 year—not after 7 days of metreleptin treatment. We reported previously that saturated nonesterified fatty acids (NEFAs) are associated with reduced action of insulin in the brain (15). Soon after initiation of metreleptin substitution, systemic lipolysis is stimulated (12), as a consequence of which circulating NEFA concentrations increase. Brain effects of these NEFAs might therefore mask enhanced insulin effects in the brain immediately after initiation of treatment (12).

Both visceral and intrahepatic fat were reduced after only 7 days of treatment.

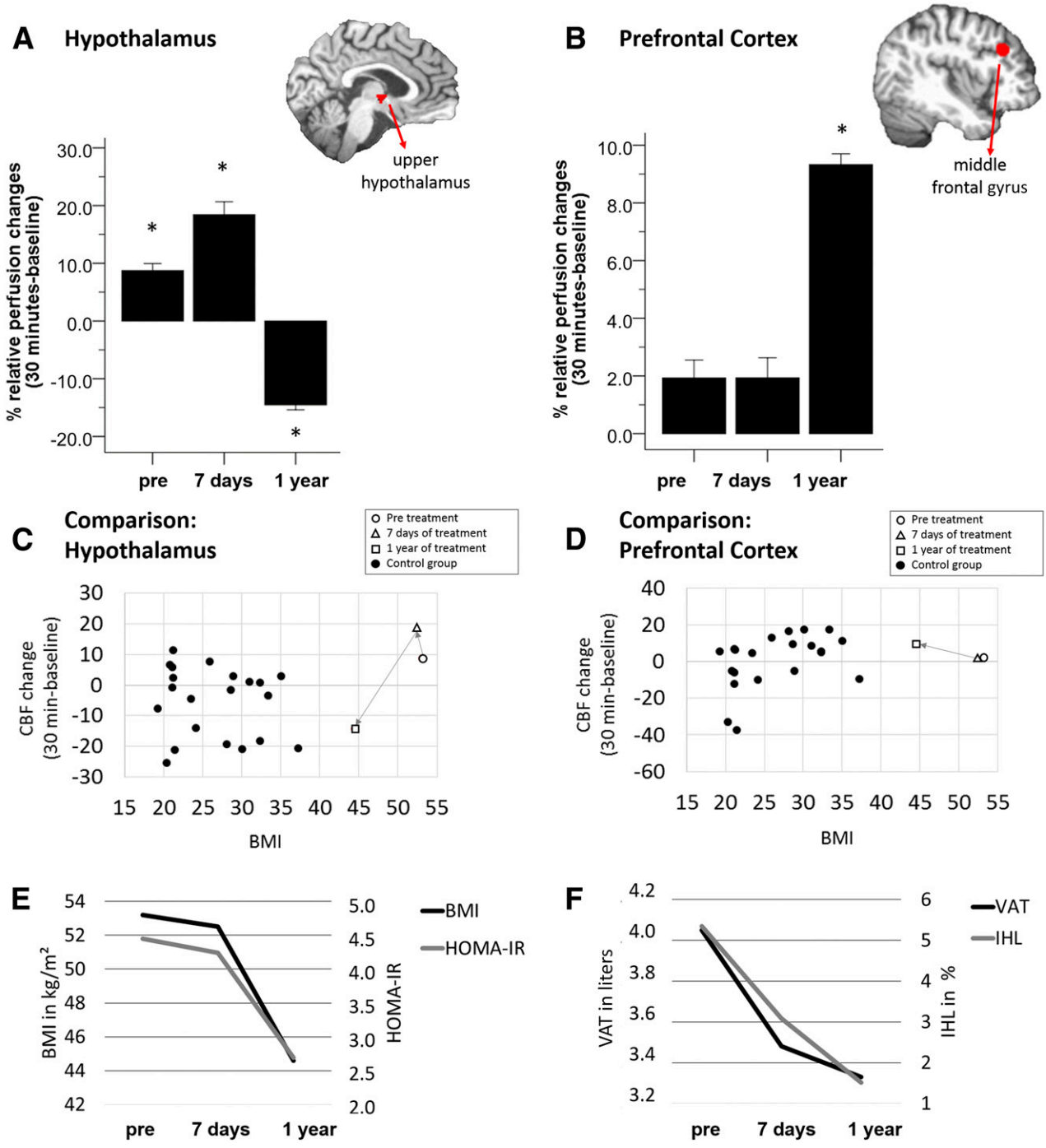

Figure 1—*A* and *B*: Brain insulin sensitivity in the hypothalamus and prefrontal cortex before and after leptin substitution. Bar graphs show relative perfusion changes (30 min − baseline) for each measurement day. For illustration purposes, % mean relative perfusion (standardized on overall perfusion) was calculated over the number of voxels in the regions of interest, and the error bar represents SEM. An asterisk (*) indicates significantly different relative perfusion changes after intranasal insulin application. *C* and *D*: Results of the patient are depicted with data from a control group who also received intranasal insulin (on one measurement day only), and baseline-corrected values are shown for the hypothalamus and prefrontal cortex. For the leptin-deficient patient, all three time points are included in the graph. Empty circle = pretreatment initial measurement, triangle = 7 day measurement, and square = 1 year measurement. *E* and *F*: Change in BMI and HOMA-IR (*E*) as well as in VAT and IHL content (*F*) over the time course of 1 year, commencing in the leptin-deficient state. pre, pretreatment.

We had already observed such a rapid decrease in another leptin-deficient female patient after leptin substitution (12). The rapid decrease of visceral fat and liver fat might again be explained by the leptin-induced stimulation of lipolysis (12). Thus, the observed reestablishment of brain insulin sensitivity in the hypothalamus may be triggered by the restored leptin signaling directly in this brain area or may be secondary to reduction of body fat.

In the PFC, intranasal insulin induced a minimal increase in relative perfusion in the PFC, which did not change during the

7 days after onset of treatment. However, 1 year after initiation of leptin replacement, the increase in relative perfusion after insulin administration was found to be further strengthened—similar to the effect found in an obese control group from our previous study (9). The direct comparison with the control group, however, showed only a marginal effect in comparison with healthy subjects (Fig. 1*D*). Thus, impaired brain insulin sensitivity might be particularly applicable to homeostatic rather than to prefrontal regions in this patient.

In conclusion, these data suggest that leptin contributes to the modulation of insulin sensitivity in the human brain. Leptin replacement and subsequent changes in weight in this leptin-deficient patient improve insulin sensitivity in the periphery and central homeostatic areas.

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