Novel Insights from Clinical Practice

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Rapid Improvement of Hepatic Steatosis after Initiation of Leptin Substitution in a Leptin-Deficient Girl

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Established Facts

- Leptin deficiency leads to severe obesity, insulin resistance, and hypertriglyceridemia.

Novel Insights

- Leptin-deficient patients can also develop hepatic steatosis.
- In humans, leptin substitution seems to influence hepatic lipid metabolism directly as has been proven for rodents.

Key Words

Leptin · Obesity · Hormone replacement therapy · Insulin resistance · Non-alcoholic fatty liver disease

Abstract

Background: Leptin deficiency is associated with severe obesity and metabolic disturbances. Increased liver fat content has been reported in only one case beforehand, even though hepatic steatosis is a typical comorbidity of common obesity. It is also frequent in patients with lipodystrophy where it resolves under leptin therapy. ***Subject and Methods:*** In 2010, we reported a leptin-deficient patient with a novel homozygous mutation in the leptin gene and severe hepatic steatosis. We have now studied serum changes and changes in liver fat content during the substitution with recombinant methionyl human leptin. ***Results:*** After 23 weeks of leptin substitution, elevated transaminases, total cholesterol and low-density lipoprotein levels normalized. After 62 weeks, homeostasis model assessment of insulin resistance

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improved from 10.7 to 6.0 and body fat mass dropped from 50.2 to 37.8%. Liver fat content was drastically reduced from 49.7 to 9.4%. The first changes in liver fat content were detectable after 3 days of therapy. ***Conclusion:*** Our patient showed a remarkable reduction of liver fat content during the treatment with recombinant methionyl human leptin. These changes occurred rapidly after initiation of the substitution, which implies that leptin has a direct effect on hepatic lipid metabolism in humans as it is seen in rodents.

Introduction

Leptin deficiency is associated with severe metabolic disturbances including insulin resistance and hypertriglyceridemia [1]. In addition to such complications, patients with low leptin levels caused by lipodystrophy frequently develop a severe hepatic steatosis [2], which is also a typical feature of common obesity.

Hepatic steatosis is also a common complication in genetically leptin-deficient *ob/ob* mice, in addition to insulin resistance, obesity, and dyslipidemia [3]. The expanded white adipose tissue mass in *ob/ob* mice promotes adipose tissue lipolysis. Thus, increased long-chain fatty acids are released into the circulation and are delivered to the liver. Furthermore, the key enzyme for fatty acid synthesis, SREBP-1c, is upregulated in the hepatocytes of *ob/ob* mice leading to excessive de novo lipogenesis [4, 5]. Adding to the problem is an insufficient increase in hepatocyte β-oxidation in *ob/ob* mice which is not capable of disposing the greater fatty acid load [6].

As increased leptin levels are independently associated with histological grading of hepatic steatosis in obese humans [7–9], leptin resistance might play a direct role in the development of hepatic steatosis in the normal obese population. However, the main link between normal obesity and hepatic steatosis is hyperinsulinemia. In obese people, insulin-sensitive and insulin-resistant individuals can be distinguished on the basis of lipid accumulation in muscles and liver, but not on the basis of subcutaneous or visceral adiposity [10]. Hyperinsulinemia leads to a hepatic insulin resistance with an impaired suppression of hepatic gluconeogenesis, but this resistance is only partial because at the same time increased insulin signaling enhances SREBP-1c expression and thus hepatic lipogenesis [11]. The association between hepatic steatosis and hyperinsulinemia is reciprocal, since excessive fat accumulation in the liver leads to a deterioration of glucose and fat metabolism via subclinical inflammation and secretion of humoral factors, so-called hepatokines [12].

Also, leptin is likely to have an effect on hepatic steatosis by influencing insulin sensitivity. There is conflicting evidence concerning the short-term influence of leptin on insulin sensitivity [13]. Long-term leptin replacement however reliably leads to an improvement of insulin sensitivity in *ob/ob* mice [14], in leptin-deficient patients [15], and in lipodystrophic patients [16, 17]. The latter also show a marked improvement of hepatic steatosis under leptin substitution [2, 16–20].

In contrast to the *ob/ob* mouse, among the 35 leptin-deficient patients reported so far in the literature [1, 21–26], only our patient and a 3-year-old boy from Egypt [24] have been shown to suffer from hepatic steatosis.

We hereby present the first report on the effect of leptin substitution on hepatic steatosis in a patient with congenital leptin deficiency.

Subject and Methods

Case Report

The patient is the first child of 2 healthy, non-obese Austrians without known consanguinity. She was born at term with a normal weight (3,440 g), but gained weight rapidly thereafter. She presented at our clinic at the age of 13 years and 9 months with a body mass index (BMI) of 31.5 kg/m^2 (2.46 standard deviation score (SDS)) and a body fat content of 44.1%. In addition to metabolic changes, both a hypogonadotropic hypogonadism and an insufficient growth hormone secretion was found in the patient. No distinct immunological changes could be found. Serum levels of leptin were at the limit of detection and direct sequencing of the leptin gene revealed a hitherto unknown homozygous transition (TTA to TCA) in exon 3, resulting in a L72S replacement in the leptin protein [27]. Treatment with recombinant methionyl human leptin was initiated at the age of 14 years and 9 months at a weight of 103.4 kg (BMI 35.9 kg/m^2, BMI-SDS 3.04) with a dose of 0.6 mg twice daily (0.012 mg/kg/day, 0.024 mg/kg BW/day; Amylin Pharmaceuticals, Inc., San Diego, Calif., USA) in accordance to the treatment doses of the other leptin-deficient patients [1, 15]. In addition to the metabolic changes documented below, leptin substitution also led to the induction of menstrual cycles and normalization of growth hormone secretion [28]. For treatment and all investigations, informed parental consent and informed assent from the patient was obtained. Ethical permission was granted by the ethical committee of the University of Ulm.

Blood Analysis

Blood samples were obtained by venous puncture and processed shortly after withdrawal. Leptin was analyzed via RIA (Millipore, Billerica, Mass., USA) with a detection limit of 0.5 ng/ml. Serum was assayed for insulin by electrochemiluminescence immunoassay (Modular E170; Roche Diagnostics, Mannheim, Germany). Routine chemical analyses were performed by standard methods. Age-related reference values were obtained from

the instructions of the commercial analysis. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using the Dimension RxL system (Dade Behring, Eschborn, Germany).

Oral Glucose Tolerance Test

All tests were performed after a 12-hour overnight fast. An intravenous catheter was inserted for blood sampling. A baseline sample for the measurement of glucose and insulin was drawn. The patients then received 75 g of flavored glucose (Accu Check Dextro OG-T solution; Roche). Blood samples were drawn every 30 min for the measurement of glucose and insulin.

As a model for insulin resistance, we calculated homeostasis model assessment of insulin resistance (HOMA-IR; product of fasting plasma insulin level (mU/l) and fasting plasma glucose levels (mg/dl) divided by 405) [29].

Ultrasound

Ultrasound examinations of the liver were performed by a single examiner using a Siemens Versa Plus (Siemens, Munich, Germany) equipped with a 3.5-MHz convex array probe and a 9-MHz linear array probe. Scans were performed after a fasting period of at least 12 h.

Body Fat Percentage

Body fat percentage was assessed using dual X-ray energy absorptiometry (Lunar Prodigy Primo™, Software en Core™; GE Healthcare, Munich, Germany).

Intra-Abdominal Fat and Liver Fat Content

Magnetic resonance examinations for quantification of visceral adipose tissue (VAT) and hepatic lipids were performed in the early morning after overnight fasting on a 3-T whole-body imager (Magnetom Tim Trio; Siemens Healthcare, Erlangen, Germany). Magnetic resonance imaging (MRI) was performed as previously described [30] with a slightly longer echo time (TE) of 38 ms. The abdominal region was scanned from thigh to shoulders, resulting in a dataset of 30 images for this patient. VAT in liters was quantified between thighs and diaphragm.

Further on, volume-selective proton magnetic resonance spectroscopy (^{1}H-MRS) of the liver was performed for quantification of hepatic lipid content applying a single voxel STEAM technique with a TE of 20 ms [31, 32]. Signal integrals of water at 4.7 ppm and methylene/methyl (lipids) at 1.3 and 0.9 ppm were determined and the ratio of lipids/(water + lipids) was calculated to determine the lipid ratio in percent. The coefficient of variation for this technique lies between 8.5% [33] and 10% of the measured liver fat content [31]. The 95th percentile of hepatic triglyceride content in a population of healthy adults without identifiable risk factors for hepatic disease lies at 5.56% [33].

Anthropometric Measurements (Patient and Parents)

Weight was assessed to the nearest 0.1 kg with a calibrated balanced scale while the subjects were wearing only undergarments. Height was measured to the nearest 0.1 cm using the Ulm Stadiometer (Busse Design, Ulm, Germany). On the basis of these data, BMI was calculated as weight over the square of height. The degree of overweight was quantified using Cole's least mean square method, which normalizes the BMI-skewed distribution in childhood and expresses BMI as a SDS (BMI-SDS).

Energy Intake

A cafeteria-style breakfast was offered to the patient after an overnight fast including items such as rolls, jam, cereal, milk, cheese, and fruit. For each test meal, the items offered remained the same. The patient was allowed to eat ad libitum. Food items were covertly weighed before and after the meal to assess the total calories consumed.

During the hospital admission, the patient consumed a regular hospital diet. On three occasions (before and during therapy) food items were covertly weighed before and after the meal to assess the total calories consumed over the whole day. Energy intake was calculated using the software FCMS/Diät 2000 (Soft & Hard D. Beyer, Rimbach, Germany).

Results

Baseline Characteristics

In former medical reports on the patient, ultrasound demonstrated increased echogenicitiy compatible with hepatic steatosis starting from the age of 8 years, and elevated transaminases from 13 years of age. At the first presentation in our clinic, liver enzymes were mildly elevated. We did not find any evidence for immunological (ANA, AMA, LKM, LC, SLA antibodies normal) or infectious origin of the elevated transaminases (antibodies against hepatitis A and C negative, hepatitis B surface antibodies positive after vaccination, CMV and EBV immunoglobulin G positive and M negative, compatible with old infection). In addition, no clinical sign or symptom suggestive of a progressive hepatic disease was found. Values for γ-glutamyltransferase, alkaline phosphatase, creatine kinase, LDH, and total bilirubin were unremarkable. Hepatic lipids determined by ^{1}H-MRS were distinctly increased with 49.7% (table 1; fig. 1a). Ultrasound examination revealed an increased echogenicity of the liver, normal vessel structure, and increased midclavicular line diameter of 209 mm. Overall, the findings were compatible with a steatosis grade II and hepatomegaly.

The patient also showed a dyslipidemia with elevated cholesterol (5.4 mmol/l) and triglycerides (2.8 mmol/l) as well as decreased high-density lipoprotein (HDL) cholesterol (1.0 mmol/l). In addition, while her fasting blood glucose was normal, she showed a hyperinsulinemia with an elevated HOMA-IR of 10.7 (table 1).

Metabolic Status of the Parents

Both parents are heterozygous for the mutation in the leptin gene (leptin level of the father 0.7 ng/ml, leptin level of the mother 5.1 ng/ml) [27]. They had a normal weight with a BMI of 25.0 kg/m^2 (father) and 22.9 kg/m^2 (mother). The father showed unremarkable liver enzymes, HOMA-

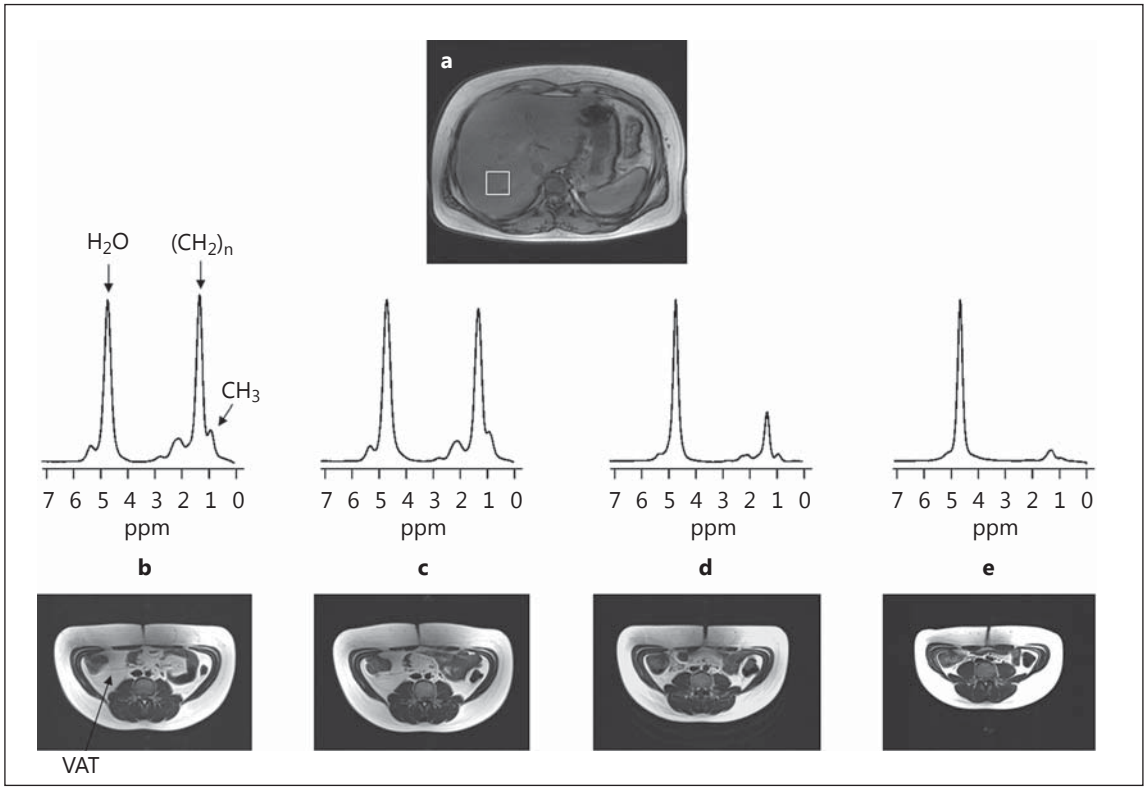

Fig. 1. Magnetic resonance images and spectra of the patient during leptin substitution. **a** An axial T1-weighted MR image of the liver with indicated volume of interest for spectroscopic examination. **b–e** Different spectra of the liver during the course of therapy (before therapy, after 3 days, 11 weeks and 15 months of substitution, respectively). Below, the concomitant changes in VAT in axial T1-weighted images at the umbilical level are shown. A marked decrease of the lipid signals (CH_2 + CH_3) in relation to the water signals (H_2O) can be seen as well as a decrease of VAT.

Table 1. Metabolic changes under substitution with metreleptin

	Time from start of leptin substitution						
	±0 days	+3 days	+7 days	+11 weeks	+23 weeks	+62 weeks	ref. values
Weight, kg	103.4	102.5	102.2	90.5	86.7	80.5	
BMI, kg/m^2	35.4	35.1	35.0	31.1	29.6	26.9	
Test meal, kcal	1,899		1,794	1,630	1,517	1,495	
Fat content of test meal, kcal	616		705	658	609	551	
Energy intake in hospital, kcal/day	2,268		2,203		2,202		
Hepatic lipid content, %	49.7	46.5		24.0	9.6	9.4	<5.6
Visceral adipose tissue, l	3.84	3.44		3.01	2.61	1.60	
Fat mass, %	50.1			44.9	40.2	37.8	
Leptin, ng/ml	0.4	0.7		4.3	10.1	21.3	2.0–5.6
Cholesterol, mmol/l	5.3	4.1		4.1	3.9	3.5	<5.2
Triglycerides, mmol/l	2.6	3.3		3.1	2.3	3.0	<2.3
HDL cholesterol, mmol/l	0.9	0.7		0.9	0.9	0.8	>1.2
LDL cholesterol, mmol/l	3.2	1.8		1.8	2.0	1.3	<4.1
AST, U/l	58.0	62.0		25.0	24.0	13.0	<50
ALT, U/l	95.0	120.0		39.0	21.0	14.0	<35
Fasting insulin, mU/l	36.8	57.7		58.2	29.8	27.6	2.6–24.9
HOMA-IR	10.7			13.7	7.1	6.0	<3

IR, glucose tolerance, and fasting lipid levels (total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides); however, he had an impaired fasting glucose with 103 mg/dl. The mother had a normal fasting glucose, glucose tolerance and HOMA-IR and also normal transaminases, but an increased total cholesterol (6.1 mmol/l) and LDL cholesterol (3.1 mmol/l) with normal triglycerides and HDL levels. None of them showed signs of hepatic steatosis on ultrasound examination.

Changes under Leptin Therapy

Anthropometry

Over 62 weeks, the patient showed a dramatic reduction in weight and BMI (from 35.4 to 26.9 kg/m^2) and in body fat content (from 50.1 to 37.8%; table 1).

Energy Intake

The patient's energy intake in a standardized test meal reduced from 1,899 to 1,495 kcal in 62 weeks. The fat content of the test meal (before treatment 616 kcal, 32%) rose in the beginning of the therapy to fall to 551 kcal (37%) after 62 weeks (table 1). However, the patient's average food intake during a day assessed by weighed food protocols did not show any significant changes.

Metabolic Changes

During therapy, total cholesterol normalized and LDL cholesterol decreased within the normal range. HDL cholesterol, however, remained persistently decreased around 0.9 mmol/l and triglycerides remained persistently elevated (3.0 mmol/l after 62 weeks of treatment). The patient's HOMA-IR decreased from 10.7 to 6.0 and transaminases normalized (table 1).

Hepatic Lipids and Visceral Adipose Tissue

Hepatic lipids determined by ^{1}H-MRS were markedly increased (49.7%) prior to leptin therapy. Within 3 days after the start of leptin therapy, there was a slight but obvious decrease in hepatic lipids to 46.5%, which continued to 24.0% after 3 months and to 9.4% after 15 months (table 1; fig. 1). In addition, VAT mass dropped under leptin substitution from 3.84 to 3.44 liters after 3 days, 3.04 liters after 3 months, and 1.6 liters after 15 months (table 1; fig. 1).

Discussion

Here we report a severe form of liver steatosis and rapid changes of hepatic fat content under leptin substitution in a patient with congenital leptin deficiency. With the exception of one Egyptian boy [24], transaminases levels are not reported for the other published leptin-deficient patients [1, 21–26, 34] or for the published patients with leptin receptor mutation [35, 36]. Given the fact that *ob/ob* mice typically develop hepatic steatosis [3], it is surprising that this has not been reported for the other leptin-deficient patients. Possibly like *ob/ob* mice [37], human patients actually display a hepatic steatosis without signs of inflammation and elevated transaminases. Therefore, increased liver fat content might have escaped notice. However, these are merely speculations; at least for some patients we know that neither transaminases levels nor hepatic fat content were examined prior to leptin substitution [1, 35].

Concerning further metabolic disturbances, the other leptin-deficient patients showed only a mild dyslipidemia with mostly normal total cholesterol levels and only mildly decreased HDL levels [1, 15, 21–23] despite their severe obesity. One adult patient was diagnosed with type 2 diabetes prior to leptin therapy [21]. Apart from this single case, fasting glucose levels among the other leptin-deficient patients were reported to be normal, though nearly all patients showed signs of insulin resistance with a HOMA-IR between 4.1 and 8.8 [1, 15, 21–23].

Compared to the other patients, our patient showed a severe form of insulin resistance with a HOMA-IR of 10.7 while her triglyceride levels were within the upper range reported for leptin-deficient patients. This finding is remarkable since our patient had an unusual low BMI-SDS for a leptin-deficient patient [1, 21, 22, 34, 38].

The reason for these marked metabolic changes might be the severe hepatic steatosis found in our patient, for which we could not identify any further risk factors. The most important finding of our study was a dramatic and instantaneous reduction of this hepatic steatosis under leptin substitution from 49.7 to 46.5% within the first 3 days and to 24.0% within 11 weeks.

Several studies in lipodystrophic patients also showed a pronounced improvement of hepatic steatosis under leptin therapy: within 6 months of therapy, a reduction of liver volume between 17 and 40% has been reported [2, 16–20, 39], as well as a reduction in liver triglyceride content between 48 and 86% [2, 19, 39]. After 8 months of therapy a normalization of liver fat content was shown [39]. Further on, paired liver biopsy specimens obtained at baseline and after approximately 6 months of treatment showed a significant improvement in steatosis and ballooning injury with a reduction of mean non-alcoholic steatohepatitis activity by 60% [2]. However, the earliest changes demonstrated in patients with lipodys-

trophy occurred after a therapy duration of at least 1 month [19].

The precise mechanisms of leptin action in relation to the changes in liver metabolism are still poorly understood. Some evidence exists that leptin might stimulate lipolysis, thus directly reducing fat content in hepatic steatosis. In rodent models, leptin activates enzymes of fatty acid oxidation, including carnitine palmitoyltransferase-1 and acyl-CoA oxidase. It inhibits enzymes of lipogenesis, including acyl-CoA carboxylase and stearoyl-CoA desaturase 1 [40–43], and also SREBP-1c, thereby suppressing insulin-induced lipogenesis [5, 44]. Whether this also applies to humans with leptin deficiency is yet unclear.

Studies of the lipid metabolism in 3 adults with congenital leptin deficiency showed no consistent difference in serum non-esterified fatty acid levels after fasting nor in a hyperinsulinemic clamp between on or off therapy [13]. The same applies to the short-term effect of a leptin treatment pause on insulin sensitivity since insulin sensitivity improved in 2 but decreased in 1 of the 3 patients whilst the patients were gaining weight rapidly [13]. This might be due to the antagonistic effect of leptin on lipid metabolism with increased lipolysis, while in the long run, decreased body fat content leads to an improvement of insulin sensitivity. In accordance, our patient initially showed a decrease in insulin sensitivity as estimated by HOMA-IR under leptin substitution. Improvement of insulin sensitivity can therefore not be the cause for the rapid improvement of liver fat content under leptin substitution in our patient. To our knowledge, there exists no other study demonstrating such a rapid effect of leptin therapy on hepatic steatosis.

The almost instantaneous reduction in liver fat content seen in our patient is unique and it strengthens the hypothesis that also in humans leptin reduces liver fat content not only via decreased energy intake or improvement of insulin sensitivity, but also by directly stimulating hepatic fat metabolism. Naturally, the immediate changes were not very large. Since the coefficient of variation for this examination lies between 8.5 and 10% of the measured liver fat content (that is 4.2–4.9% in this case), a change of 3.2% could still be due to reproducibility errors. However, since we see concomitant changes in VAT and in addition both VAT and hepatic fat content continued to decrease dramatically, it is rather likely that these early changes represent a real effect.

Due to its nature, our report has some serious restrictions. We merely examined a single patient, therefore the results cannot be generalized. Furthermore, the fact that we could not examine the liver cells ex vivo constitutes another severe limitation. Since we could not demonstrate an upregulation of enzymes of fat metabolism, we cannot exclude that changes in energy uptake at least contributed to the reduction in liver fat content. In coherence with this assumption, the energy intake in a test meal decreased within 7 days by 9.1%. However, fat uptake even increased during this time, which is consistent with observances by Farooqi and O'Rahilly [45], who report an increased choosey attitude towards food under leptin therapy. In addition, the patient's reported energy intake during the day did not change, probably due to the strict diet she has been adhering to since early childhood. Taken together, this indicates that the main effect on liver fat content can probably be attributed to a direct influence of leptin on hepatic fat metabolism.

Conclusion

Our patient showed a remarkable reduction of hepatic fat content under treatment with recombinant methionyl human leptin. These changes occurred rapidly after initiation of the substitution, which implies that leptin has a direct effect on hepatic lipid metabolism in humans as is seen in rodents.

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