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ORIGINAL ARTICLE

Facial soft tissue volume decreases during metreleptin treatment in patients with partial and generalized lipodystrophy

Konstanze Miehle[1] · **Michael Stumvoll**[1] · **Mathias Fasshauer**[1,2] · **Thomas Hierl**[3]

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Abstract
Purpose Lipodystrophy (LD) patients suffer from loss or maldistribution of subcutaneous adipose tissue accompanied by dysregulation of several adipocyte-secreted factors, e.g., leptin. The effect of recombinant leptin (metreleptin) therapy on facial soft tissue volume in patients with non-human immunodeficiency virus LD has not been quantified to date.
Methods Eight LD patients (six female, two male; six familial partial LD [FPLD], two generalized LD) were treated with metreleptin over 1 year. Anthropometric parameters and 3D stereophotogrammetric imaging of the patients´ faces were assessed at baseline and after 1 year of metreleptin treatment.
Results Median fat mass was significantly reduced during metreleptin treatment from 22.3 kg at baseline to 20.0 kg at 1 year ($p = 0.031$); however, body weight, body mass index, and waist-to-hip ratio were not significantly affected. Five of the six patients with FPLD lost between 4 and 114 cm^3 of facial soft tissue volume in the pre-auricular, buccal, and submandibular area during metreleptin treatment whereas a slight volume gain was seen in one FPLD patient. The two patients with generalized LD developed a volume loss of 20 and 8 cm^3 in the buccal region between baseline and 1 year of metreleptin therapy, respectively.
Conclusions Metreleptin replacement leads to loss of facial soft tissue volume in FPLD and generalized LD. However, volume changes in most patients are not visible by the naked eye.

Keywords 3D stereophotogrammetric imaging · Adipokine · Lipodystrophy · Metreleptin treatment · Obesity

Introduction

Non-human immunodeficiency virus (HIV) lipodystrophy (LD) is a rare disease of acquired or congenital origin [1]. Besides severe metabolic complications including diabetes mellitus, hypertriglyceridemia, hepatic steatosis, pancreatitis, liver cirrhosis, and cardiovascular disease, LD patients suffer from maldistribution and loss of subcutaneous adipose tissue [1]. In generalized LD, considerable reduction or complete loss of subcutaneous adipose tissue can be found from birth or early childhood onwards. Patients with familial partial LD (FPLD) type 2 and—less pronounced—type 3, develop loss of subcutaneous adipose tissue from upper and lower limbs and, to variable extent, from the trunk during puberty or later. Excess fat deposition within the face, neck, and intraabdominal region occurs in FPLD resulting in a Cushingoid appearance [1]. The physical changes in appearance are often a source of psychological distress. LD patients frequently report a reduced quality of life and perceive body shape changes as a social stigma

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Mathias Fasshauer and Thomas Hierl contributed equally to this work.

✉ Konstanze Miehle
konstanze.miehle@medizin.uni-leipzig.de

[1] Department of Internal Medicine (Endocrinology and Nephrology), University of Leipzig, Leipzig, Germany

[2] Leipzig University Medical Center, IFB Adiposity Diseases, Leipzig, Germany

[3] Department of Oral and Maxillofacial Plastic Surgery, University of Leipzig, Leipzig, Germany

[2, 3]. Current treatment options for phenotypic alterations in LD include fat grafting, filler injections, and silicone prostheses for lipoatrophic areas, as well as liposuction or lipectomy procedures for lipohypertrophic areas [4–6].

In all LD types, circulating levels of the adipocyte-secreted factor leptin are decreased due to adipose tissue loss [7] Administration of recombinant leptin improves metabolic homeostasis in both humans and animal models with LD [8, 9]. Moreover, leptin reduces weight and fat mass in various leptin-deficient conditions [9–11]. Thus also an apparent reduction in facial fat mass should be expected in patients with LD during leptin treatment. However, changes in facial volume in LD patients receiving leptin supplementation have not been quantified to date. Therefore, we studied facial soft tissue volume changes in LD patients treated with recombinant leptin (metreleptin) over 1 year. A rapid and non-invasive 3D stereophotogrammetric imaging system was used for highly accurate and reproducible quantification [12, 13].

Subjects and methods

Study population and clinical assessment

Eight patients with non-HIV LD (six female, two male) were included in the current study. Six patients (five female, one male; patient no. 1, 2, and 5–8) had FPLD. Four of the FPLD patients suffered from FPLD2 (with established genetic diagnosis of LMNA mutation in two patients). Two patients had FPLD3 (mutation in PPARγ). Two patients (one male, one female; patient no. 3 and 4) suffered from generalized LD of autoimmune origin. Patients were recruited from the Outpatient Care Unit of the Endocrine Department, University of Leipzig. Diagnosis of LD was made according to the American Association of Clinical Endocrinologists consensus statement criteria 2013 [14]. All patients met the inclusion criteria for metreleptin treatment i.e., insufficiently controlled hypertriglyceridemia and/or diabetes mellitus despite adequate lipid-lowering and/or antihyperglycemic treatment as well as low serum leptin levels (baseline leptin serum concentration was 0.24 µg/l in the two patients with generalized LD and ranged between 3.6 and 11.2 µg/l in the six FPLD patients). Further exclusion and inclusion criteria for metreleptin therapy have been described previously in detail [15–17]. Patients 1 and 2 (both female) administered metreleptin subcutaneously at 0.04 mg/kg body weight and day for the first week and thereafter at 0.08 mg/kg body weight and day. Subsequently, dosing instructions by the manufacturer changed. Patients 3–8 received 2.5 mg metreleptin per day (men) and 5 mg metreleptin per day (women) from day 1 onwards. Metreleptin doses after the first week of treatment ranged from 2.5 to 7.8 mg per day. Patients underwent clinical examination and imaging with the 3D scan camera before and after 1 year of metreleptin supplementation.

Blood was taken after an overnight fast. Metabolic routine laboratory parameters were determined by standard laboratory methods in a certified laboratory. For determination of serum leptin and adiponectin levels, commercially available enzyme-linked immunosorbent assays (Mediagnost, Reutlingen, Germany) were used according to the manufacturer´s instructions. Calculation of homeostasis model assessment of insulin resistance (HOMA-IR) and estimated glomerular filtration rate was performed as described previously [18, 19].

Age of the patients varied from 16 to 55 years. Body mass index calculated by body weight in kilogram divided by squared height in meters ranged between 17.4 and 33.5 kg/m^2 at baseline. Waist-to-hip ratio was determined after measurement of waist and hip circumferences. Fat mass and fat free mass were measured by bioelectrical impedance analysis (MEDICAL Health Care, Karlsruhe, Germany) according to the instructions of the manufacturer. The study was approved by the Ethics Committee of the University of Leipzig. All patients gave written informed consent before taking part in the study.

3D stereophotogrammetric imaging

The 3D stereophotogrammetric imaging system Vectra-3M (Canfield Scientific, Parsippany, NJ) was used according to the manufacturer´s instructions. In brief, patients were seated on a chair facing the imaging system and the face was centered on a computer screen preview image. Pictures were captured with closed mouth and with a neutral facial expression. Capture time for each image was 3.5 ms. Before analysis, the 3D image was trimmed, i.e., erasing ears, hair, and décolleté.

Rigid registration of the 3D stereophotogrammetric scans was conducted by means of iterative closest point algorithm (100 steps, 0.001 mm). Manually operated adjustment was used to refine 3D images via shortcut and zoom-control in steps of 0.5 mm for reaching maximal superposition in the stable regions of the face (forehead, bridge of the nose) in a 4-window panel (coronal, axial, sagittal, and 3D-views) for best control. The 3D stereophotogrammetric imaging data were analyzed by Facial Analysis Tool (FAT) software (https://facial-analysis.jimdo.com) which has been developed by the Department of Oral and Maxillofacial Plastic Surgery, University of Leipzig, Germany. The analyzed soft tissue facial area included cheek (pre auricular—nasolabial folds—lateral canthus), submandibular area (lower bound: skin fold approx. 4 cm below the submandibular rim), and submental area (bound in line with the lower submandibular rim) (Supplementary Fig. 1). The neck caudal of the lower

submandibular rim, as well as the lower lids, were excluded from analysis. For visualizing volume differences and the degree of contour changes, a color-coded distance map was generated in FAT. Colored images of the patients´ faces and color histograms were produced for visualizing changes. In these images and histograms, blue areas show "negative" changes, i.e., loss of volume whereas red areas show "positive" changes, i.e., gain of volume. Areas without change in facial volume are colored in green. Volume differences were calculated as follows: After registration the follow-up mesh was marked in the anatomically defined region with a brush tool (Supplementary Fig. 1). The outline was then projected on the pre-treatment surface mesh. Next the gaps between pre-treatment and post-treatment outlines were closed and a volume was created (formed by pre-treatment and post-treatment surface and closing) and measured in FAT software.

Statistical analysis

Statistical analyzes of anthropometric and laboratory data were performed with SPSS Statistics Version 24.0 (IBM, Armonk, NY). For assessment of differences in anthropometric and metabolic parameters during metreleptin therapy, Wilcoxon signed rank test was used. A p-value of <0.05 was considered as statistically significant.

Results

Anthropometric and metabolic changes during metreleptin treatment

At baseline, the LD patients showed signs of impaired glucose homeostasis and lipid metabolism with increased levels for glycosylated hemoglobin A1c (HbA1c), fasting glucose (FG), fasting insulin (FI), HOMA-IR, and triglycerides, as well as decreased high-density lipoprotein cholesterol concentrations (Supplementary Table 1). Median serum adiponectin (1.8 mg/l) and leptin (5.4 µg/l) levels were low.

During 1 year of metreleptin treatment, median total fat mass decreased from 22.3 to 20.0 kg ($p = 0.031$; Supplementary Table 1 and Supplementary Fig. 2). In contrast, no significant differences in body weight, body mass index, waist-to-hip ratio, and fat free mass were found at 1 year of treatment as compared to baseline (Supplementary Table 1). Parameters of glucose and lipid metabolism improved, but did not reach statistical significance in the entire study cohort (Supplementary Table 1).

Facial volume changes during metreleptin treatment in LD patients

Patients with FPLD

Patients 1 (Fig. 1a), 2, 5, 6, and 7 showed a decrease in facial volume in the pre-auricular, buccal, and submandibular area at both sides of the face during the course of 1 year metreleptin treatment (Table 1). Total volume loss ranged from 4 cm^3 (patient 7, FPLD2) to 114 cm^3 (patient 1, FPLD2) (Table 1). Patient 8 (FPLD3) showed a total volume gain of 3.5 cm^3 after 1 year of metreleptin treatment as compared to baseline (Table 1).

Patients with generalized LD

Patient 3 developed a volume loss of 20 cm^3 in the buccal region between baseline and 1 year of metreleptin therapy (Fig. 1b; Table 1). Other regions of the face showed no

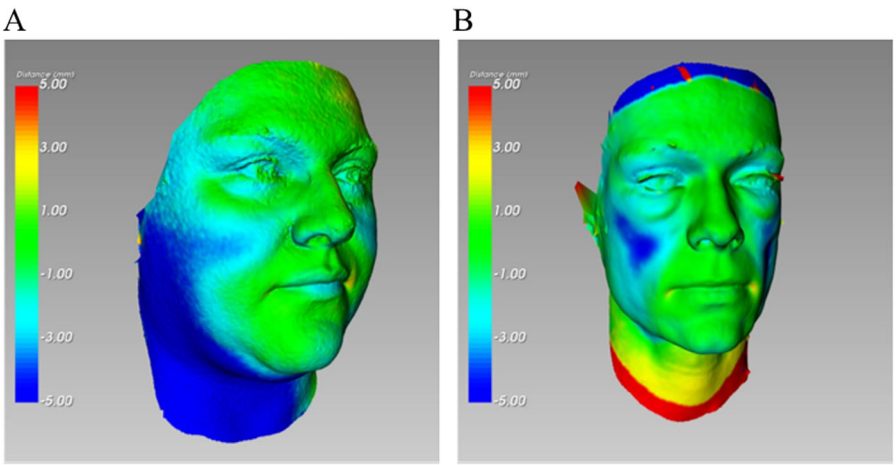

Fig. 1 Visualization of facial adipose tissue volume changes in LD patients during 1 year of metreleptin treatment using 3D stereophotogrammetric face imaging. Blue areas show "negative" changes, i.e. loss of volume, whereas red areas show "positive" changes, i.e., gain of volume. Areas without change in facial volume are colored in green. **a** Patient 1 (female, 34 years old, FPLD2) shows a decrease in facial soft tissue volume of 114 cm^3 in the pre-auricular, buccal, and submandibular area of the face. **b** Patient 3 (female, 33 years old, generalized LD) shows a volume loss of 20 cm^3 in the buccal fat pad region

Table 1 Characteristics of the study population and changes of facial adipose tissue volume during 1 year of metreleptin treatment

Patient number	Type of LD	Gender	Patient age at initiation of metreleptin substitution	Change of facial adipose tissue (cm^3)	Localization of facial adipose tissue change
1	FPLD2	F	34	−114.0	PA, B, SM
2	FPLD2	F	48	−66.0	PA, B, SM
3	AGL	F	33	−20.0	B
4	AGL	M	16	−8.0	B
5	FPLD2	F	41	−34.0	PA, B, SM
6	FPLD3	M	55	−7.0	PA, B, SM
7	FPLD2	F	39	−4.0	PA, B, SM
8	FPLD3	F	53	+3.5	B, IO, SM, SMT

AGL acquired generalized lipodystrophy, *B* Buccal, *F* Female, *FPLD* familial partial lipodystrophy, *IO* infraorbital, *M* male, *PA* pre-auricular, *SM* submandibular, *SMT* submental

change in volume over the course of metreleptin therapy (Fig. 1b; Table 1). Patient 4 lost 8 cm^3 of buccal soft tissue volume unilaterally at the left side (Table 1). No changes of soft tissue volume were apparent in the right buccal region or in other facial regions (Table 1).

No significant correlation between pre-treatment leptin serum concentration and changes in anthropometric parameters and facial volume measurements could be detected in the total cohort (data not shown)

Discussion

In the current study, changes in facial soft tissue volume of LD patients are quantified for the first time during metreleptin treatment via 3D stereophotogrammetric imaging. FPLD patients have excess adipose tissue deposits within the face and neck area [1]. In the current report, five of the six FPLD patients lose facial soft tissue volume during 1 year of metreleptin treatment. However, the extent of facial soft tissue loss in individual patients shows a wide variability. In most patients, changes in facial volume can hardly be detected by the naked eye. Ajluni et al. describe clinical (i.e., visible) improvement in facial fat deposition in one patient with FPLD [20]. In contrast to our study, the authors have not quantified the extent of facial volume loss in this patient. Generalized LD is characterized by loss of subcutaneous adipose tissue in large areas of the body including the face [1]. Metreleptin treatment over 1 year further decreases volume of facial soft tissue in the buccal region in two patients suffering from acquired generalized LD of autoimmune origin. Adipocyte destruction in these conditions is not well understood but is presumed to be immune-mediated [21].

Since metreleptin decreases body weight predominantly by reducing fat mass [10], the reduction in facial soft tissue volume in FPLD and generalized LD over the course of 1 year metreleptin therapy might be due to adipose tissue loss. It has to be emphasized that 3D stereophotogrammetric imaging detects volume changes but does not discriminate between different tissues. However, the facial volume changes in our LD patients occur in areas were facial adipose tissue compartments are located [22, 23]. In contrast to the volume loss in FPLD which develops in the pre-auricular, buccal, and submandibular area of the face, the 2 patients with generalized LD lose volume in the region of the buccal fat pad which is an area of deposit fat [24]. Moreover, our patients show a significant reduction in fat mass but not in fat free mass during 1 year of metreleptin treatment. Our results are in line with the study by Moran et al. showing a significant decrease in fat mass in 14 patients with LD after 4 and 12 months of metreleptin treatment as compared to baseline [25]. Taking these findings into consideration, facial volume loss in LD patients during metreleptin treatment is most likely due to loss of adipose tissue.

However, some limitations of the study have to be pointed out. Due to the rarity of the disease, the number of patients studied is very small. Moreover, there is some heterogeneity in the patient cohort since patients with different types of LD have been included in the study. Therefore, changes in metabolic parameters are not statistically significant after 1 year of metreleptin treatment in the total cohort despite considerable improvements of glucose and lipid parameters for individual patients. Furthermore, body composition indices derived by dual-energy X-ray absorptiometry were not available for our study cohort.

Clearly, studies with larger and more homogenous patient cohorts are necessary to address the question which extent of facial volume loss could be expected during metreleptin treatment.

Conclusions

A loss in facial soft tissue volume is shown in patients with FPLD and acquired generalized LD during 1 year of metreleptin treatment. Results need to be confirmed in larger patient cohorts.

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Compliance with ethical standards

Conflict of interest K.M. consults for Aegerion Pharmaceuticals. The remaining authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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