One-year metreleptin improves insulin secretion in patients with diabetes linked to genetic lipodystrophic syndromes

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One-year metreleptin treatment improves insulin secretion in patients with diabetes linked to genetic lipodystrophic syndromes

Short running title : Metreleptin effect on insulin secretion in lipodystrophic syndromes

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ABSTRACT

Recombinant methionyl human leptin (metreleptin) therapy was shown to improve hyperglycemia, dyslipidemia and insulin sensitivity in patients with lipodystrophic syndromes, but its effects on insulin secretion remain controversial.

We used dynamic intravenous clamp procedures to measure insulin secretion, adjusted to insulin sensitivity, at baseline and after one-year metreleptin therapy, in 16 consecutive patients with lipodystrophy, diabetes and leptin deficiency.

Patients, aged 39.2 ± 4 years (mean ± SEM), presented with familial partial lipodystrophies (n=11, 10 women) or congenital generalized lipodystrophy (n=5, 4 women). Their BMI (23.9 ± 0.7 kg/m^2), HbA1c (8.5 ± 0.4%) and serum triglycerides (4.6 ± 0.9 mmol/l) significantly decreased within 1 month of metreleptin therapy, then remained stable. Insulin sensitivity (from hyperglycaemic or euglycemic hyperinsulinemic clamps, n = 4 and 12, respectively), insulin secretion during graded glucose infusion (n=12), and acute insulin response to intravenous glucose adjusted to insulin sensitivity (disposition index, n=12), significantly increased after 1 year of metreleptin therapy. Increase in disposition index was related to decrease in percent total and trunk body fat.

Metreleptin therapy improves not only insulin sensitivity, but also insulin secretion in patients with diabetes due to genetic lipodystrophies.

INTRODUCTION

Leptin deficiency, linked to reduced fat amount, is thought to importantly contribute to the metabolic complications associated with lipodystrophic syndromes, as shown by studies in mice [1,2] and humans [3,4]. In generalized forms of lipodystrophies, metreleptin therapy dramatically decreased liver and muscle lipid content, improving insulin sensitivity, hyperglycemia and dyslipidemia, partly independently of decreased caloric intake [3,4]. However, in partial forms of lipodystrophies, the effect of metreleptin on hyperglycemia has not been clearly established in patients with moderate baseline metabolic alterations [5-7]. In addition, the effect of metreleptin on insulin secretion, which remains controversial, has been previously investigated using only oral glucose tolerance tests [3,6,8-10].

In this study, we evaluated the effect of metreleptin on insulin sensitivity and insulin secretion using dynamic intravenous clamp procedures in 16 patients with genetic lipodystrophy syndromes, included in a compassionate therapeutic programme.

METHODS

(see also Supplementary appendix)

Patients

Twenty-two non HIV-infected patients with genetic or acquired, partial or generalized lipodystrophy, diabetes and low serum leptin (ie, fasting leptin $\leq$ 6 ng/ml), entered a compassionate programme of metreleptin therapy approved by the National French Health Agency. Among them, sixteen consecutive patients older than 16 years gave their informed consent for metabolic investigations at baseline and after one year of therapy, which were approved by the local ethics committee (Comité de Protection des Personnes, Paris-St-Louis).

Recombinant human methionyl-leptin (r-metHuLeptin/metreleptin, Amylin/Bristol-Myers-Squibb/AstraZeneca Pharmaceuticals, San Diego, CA), self-administered in one daily subcutaneous injection at the initial dose of 0.02 mg/kg (M0) increased to 0.08 mg/kg at one month (M1), was added to the patients' regimen, stable for at least 6 weeks. Every three months, metreleptin and other medications were adapted to tolerance and effectiveness, and anthropometric parameters, lipid profile, HbA1c, renal and liver function tests were collected. The mean metreleptin daily dose at one year (M12) was 0.10 mg/kg ± 0.02 (SEM).

Metabolic investigations using insulin and/or glucose intravenous infusions were performed at M0 and M12.

The first four patients (1 to 4) were evaluated with hyperglycemic clamps, and the twelve subsequent patients (5 to 16) with euglycemic hyperinsulinemic clamps and intravenous glucose tolerance test (IVGTT) followed by a graded IV-glucose infusion (glucose ramping).

Hyperglycemic clamps allowed the measurement of acute insulin response to an intravenous bolus of glucose (AIR), and the ratio of the glucose disposal rate to insulin concentration (M/I) at the 200 mg/dl-hyperglycemic plateau, as an estimate of insulin sensitivity.

Euglycemic hyperinsulinemic clamps estimated the whole-body insulin sensitivity to glucose, expressed as the insulin-stimulated glucose disposal rate (M-value), further adjusted to insulin concentrations (M/I). AIR was calculated from IVGTT. Insulin secretion rates (ISR) in response to four-step graded glucose infusions evaluated the beta-cell sensitivity to glucose.

The disposition index was calculated as the product of AIR by M measured during euglycemic hyperinsulinemic clamps [11].

Statistical analyses

A favorable effect of metreleptin on glucose control was defined as a 0.5-point decrease in HbA1c, or HbA1c stability with a decrease of more than 50% in total daily insulin or oral antidiabetic doses, or discontinuation of one antidiabetic class, between M0 and M12. Results are presented as mean ± SEM, unless otherwise specified. See File S1 for supplemental methods and references.

RESULTS

Anthropometric and metabolic markers

Patients with diabetes, attributable to familial partial lipodystrophy (FPLD) linked to *LMNA* (nine women), *PPARG* (one man) or *PLIN1* mutations (one woman), or to congenital generalized lipodystrophy attributable to *AGPAT2* mutations (CGL1) (four women, one man) were included in the study (Table S1). They presented different forms of lipodystrophic syndromes with insulin resistance and dyslipidemia, attributable to already described causative mutations (for review, see [12]). Their age and BMI were 39.2 ± 4.0 years and 23.9 ± 0.7 kg/m^2, respectively. Their serum leptin was low (2.7 ± 0.5 ng/ml), related to fat mass (r^2=0.7, p=0.003). BMI, total energy intake (but not food macronutrient distribution), HbA1c, triglycerides, aspartate aminotransferase (AST) and gamma glutamyl transferase levels significantly decreased within the first month of metreleptin

therapy, then were not significantly modified until M12 (Table 1, Figure S1, and data not shown). After one-year metreleptin therapy, proportion of total body fat and lean masses were not significantly modified, but abdominal and percentage of truncal fat decreased or tended to decrease. Patients used a lower number of antidiabetic classes, and three among nine of them stopped insulin therapy (Table 1). One-year changes in BMI, HbA1c and triglycerides were not significantly related to baseline leptin levels.

Fourteen of 16 patients met the criteria for a glucose response to metreleptin. However, one of these fourteen patients cannot be formally considered as a responder since, although her glucose control was strikingly improved after one-year metreleptin whereas glitazones were stopped, her basal insulin doses were increased from 24 to 30 U/day and her metformin dose from 1g to 3g per day (patient 9, Table S2). The two non-responder patients were the only patient with a *PLIN1*-linked partial lipodystrophy, and a patient with FPLD2 with moderately elevated baseline HbA1c (patients 6 and 12, Table S2). None of them reported any difficulties regarding the compliance with the treatment. Compared to other patients with partial lipodystrophies, they had baseline values of serum leptin, percent body fat, waist circumference and duration of diabetes above the median levels. They were the only patients who did not lose, or even gained weight (+0 and +3.2 kg, respectively) over the one-year period.

Insulin sensitivity

Insulin-stimulated glucose disposal rate during euglycaemic hyperinsulinemic clamp (n=12) significantly increased during metreleptin therapy, from 2.72 ± 0.79 to 5.44 ± 1.19 mg/kg of fat free mass/min (p=0.0005) for M-value and 0.013 ± 0.005 to 0.031 ± 0.008 mg/kg of fat free mass/min/pmol/l for M/I (p=0.02), showing that the whole-body insulin sensitivity improved. Of note, in three patients (patients 6, 8 and 13, Table S1), euglycemia was maintained without any glucose infusion during the hyperinsulinemic clamp at baseline, while a significant glucose infusion rate was mandatory after one year-metreleptin therapy, pointing to a significant improvement in insulin sensitivity. In the four patients evaluated with the hyperglycemic clamp, M/I increased by a mean of 48% (Table S3).

Insulin secretion

Acute insulin response to intravenous glucose (AIR, n=16) did not significantly increased after one-year metreleptin therapy (88.9 ± 27.5 pmol/kg/min at M0 and 128.8 ± 36.4 at M12, p=0.19). However, insulin secretion rate (ISR) during glucose ramping was higher at every hyperglycemic step (ANOVA overall effect

p<0.0001), and the disposition index (AIR x M-value), which adjusts the acute beta-cell function to insulin sensitivity [11] increased after one-year metreleptin (n=12) (Figure 1) (Table S3). The AIR x M/I index, assessed in the whole group, also significantly increased (from 6.3 ± 3.8 to 9.2 ± 3.1, p=0.02).

Although one-year changes in HbA1c, triglycerides, M-value, and AIR were not significantly associated with modifications in anthropometric parameters (data not shown), disposition index variation was significantly related to changes in percent total body fat (r^2=0.71, p=0.008), and percent trunk fat mass (r^2=0.40, p=0.05).

Metreleptin response in the *LMNA*-mutated subgroup of patients

In patients with *LMNA* mutations (n=9), BMI, HbA1c, triglycerides and liver enzymes significantly decreased after one-year metreleptin therapy (data not shown), while M-value increased from 2.88 ± 1.13 to 6.85 ± 1.65 mg/kg of fat free mass/min (n=7, p=0.02). ISR increased or tended to increase at the four steps of glucose ramping, with a mean increase of 36%, and the disposition index significantly increased, from 208.8 ± 154.3 to 1068 ± 543.9 (n=7, p=0.02) (Figure S2 and Table S3). In this group, the decrease of HbA1c at M12 was positively correlated with the initial HbA1c level (r^2=0.7, p=0.03), but was not related to the initial leptinemia.

DISCUSSION

In patients with lipodystrophy, metreleptin therapy was shown to improve insulin sensitivity [8,13,14]. However the studies that investigated its effects on insulin secretion were done using oral glucose tolerance tests, and gave heterogeneous results [3,6,8-10]. Interestingly, leptin has been shown to have dual effects on pancreatic beta-cell function: while suppressing insulin gene expression and secretion, it also inhibits ectopic lipid storage in islet cells, thus preventing lipotoxicity in rodent models [15].

The present study, which used dynamic i.v. clamp techniques in 16 patients with diabetes and endogenous hypoleptinemia due to genetically-determined lipodystrophic syndromes, shows that metreleptin treatment for 1 year significantly improved insulin secretion. It also confirms that it decreased HbA1c, triglycerides and liver enzymes and enhanced insulin sensitivity, with a two-fold increase in insulin-stimulated glucose disposal rate after one year, as reported [8]. Importantly, in this context of severe insulin resistance, we adjusted insulin secretion measurements for insulin sensitivity using the disposition index, based on the hyperbolic function linking acute insulin response and insulin sensitivity [11]. Improvement of this index showed that, in patients with lipodystrophy, metreleptin therapy increased beta-cell sensitivity to glucose.

Metreleptin-induced changes in disposition index and body fat mass and distribution were correlated, suggesting that improvement in beta-cell secretory function could result from decreased lipotoxicity. In accordance, the two non-responder patients did not lose, or even gained weight under metreleptin. Leptin could also inhibit glucagon oversecretion, which was reported in insulin-deficient mice [16] and remains to be investigated in patients with lipodystrophy under metreleptin therapy.

In patients with partial forms of lipodystrophies as a result of mutations in the *LMNA* gene, defects of insulin secretion have been suspected to prevent the beneficial effect of metreleptin on glucose homeostasis [5]. Our present results show that metreleptin therapy also increases insulin secretion in this subgroup of patients, affected by typical Dunnigan syndrome (FPLD2) [17] or by previously described mixed laminopathic phenotypes associating lipodystrophic syndrome and progeroid or cardiomyopathic signs [18,19]. In addition, in accordance with recent findings showing that, in patients with partial lipodystrophy, metreleptin was mainly useful if metabolic derangements were severe [7], the present study shows that effect of metreleptin on glucose control in these patients was related to the baseline level of HbA1c.

In conclusion, 1 year of metreleptin therapy improves beta-cell function in patients with lipodystrophy, leptin deficiency and diabetes. Improved insulin secretion, related to fat mass and distribution changes, probably contributes to the metabolic benefits of metreleptin. Further studies are required to investigate whether these effects are maintained over time and to study mechanisms by which metreleptin affects the islets. Leptin has been proposed to protect the islets by acting on several pathophysiological steps involved in beta-cell lipotoxicity and in glucagon production during diabetes [20]. These leptin-regulated pathways, which control, among others, *de novo* ceramide synthesis, could be further studied in response to metreleptin therapy.

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CONFLICT OF INTEREST

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C. Vatier, JF. Gautier and C. Vigouroux designed the study, managed the metreleptin therapy, performed the metabolic clamps, collected and analysed the data and wrote the manuscript. S. Fetita, C. Tchankou and JP. Riveline performed the metabolic clamps. P. Boudou performed the biochemical tests. L. Deville and I. Madelaine managed the metreleptin delivery. J. Young, L. Mathivon, F. Travert, D. Morin, J. Cahen, F. Andreelli, Y. Reznik, E. Mongeois, MC. Vantyghem and C. Vigouroux referred the patients and collected data. O. Lascols performed the genetic analyses. All authors approved the final version of the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article :

File S1. Supplemental methods and references.

Figure S1. Longitudinal effects of metreleptin therapy in the 16 patients.

Figure S2. Effects of metreleptin on insulin secretion in patients with *LMNA* mutations.

Table S1. Baseline characteristics of the 16 studied patients.

Table S2. Use of antidiabetic medications during metreleptin therapy.

Table S3. Insulin secretion and insulin sensitivity indexes before and one year after metreleptin therapy.

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FIGURE 1 LEGEND

Effect of metreleptin therapy on insulin secretion

A. Insulin secretion rates (ISR) during graded glucose infusion were calculated for each of the four glucose infusion steps (of 4, 8, 10 and 12 mg/ kg of body weight of glucose per min) and plotted against the corresponding mean glucose concentration, in patients 5 to 16. Mean values obtained at baseline are represented as empty circles, and those obtained at one year-metreleptin therapy as filled triangles. Whiskers represent SEM.

* $p<0.05$ *versus* M0

B. Disposition index, indicating the insulin secretion capacity relative to insulin sensitivity, was calculated in the same patients as the product of M value measured during the euglycemic hyperinsulinemic clamp by AIR. Rectangles represent 25^{th} and 75^{th} percentile values, with the median values depicted in-between. Whiskers represent the lowest datum still within 1.5 IQR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile (Tukey boxplot).

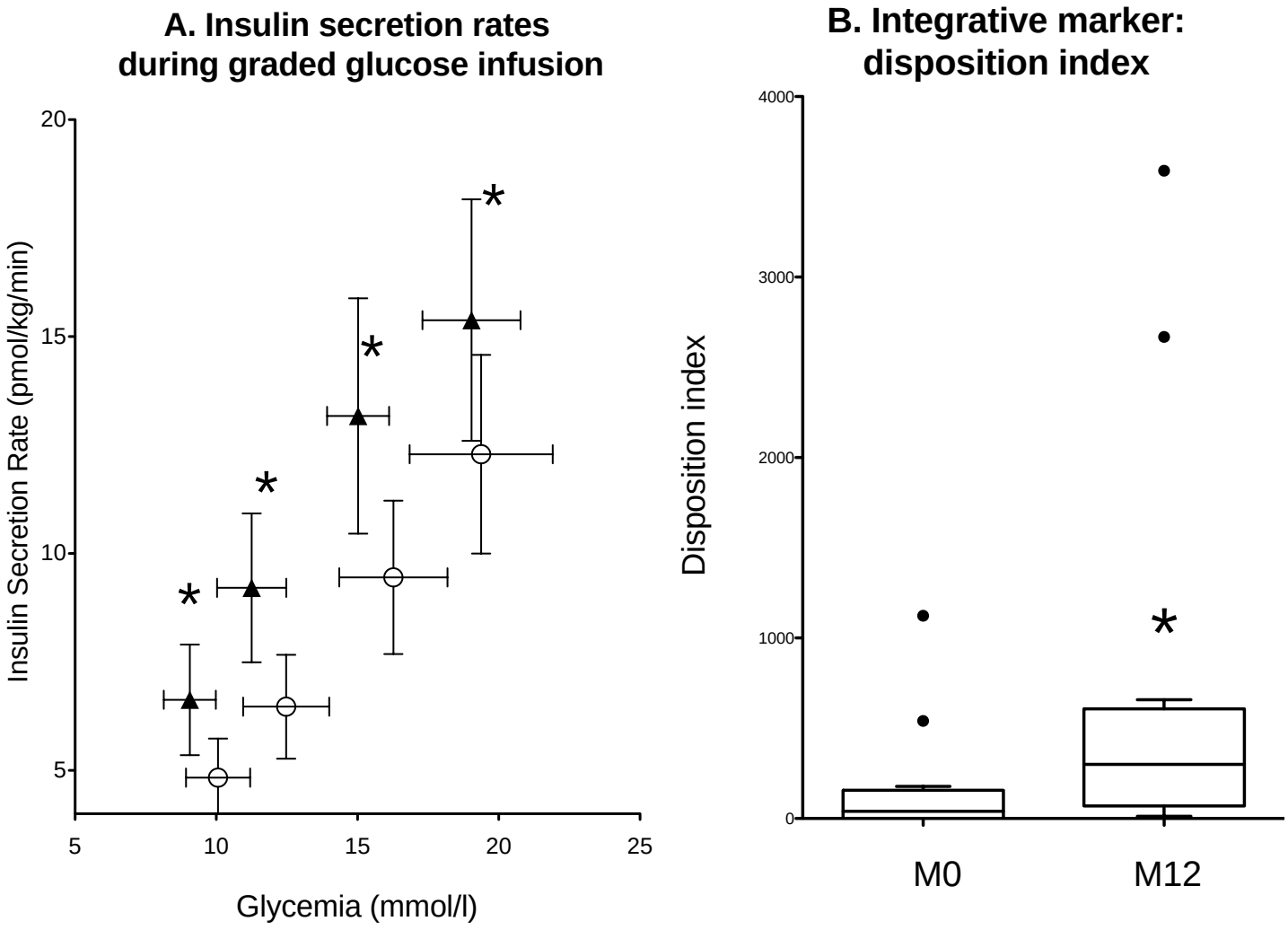
M0: baseline, M12: after 12 months of metreleptin therapy; * $p<0.05$ *versus* M0

Table 1: Metabolic markers at baseline and after one year of metreleptin treatment in the sixteen studied patients

Variable	Baseline	12 month-metreleptin	p
Body weight and body composition			
BMI (kg/m^2)	23.9 (0.7)	22.6 (0.8)	**0.003**
Waist circumference (cm)	81.8 (7.0)	77.5 (8.5)	**0.004**
Abdominal total adipose tissue area (cm^2, CT-scan)	195.9 (29.3)	144.0 (24.4)	**0.03**
Abdominal visceral adipose tissue area (cm^2, CT-scan)	122.2 (17.7)	94.6 (15.7)	**0.0005**
Abdominal subcutaneous adipose tissue area (cm^2, CT-scan)	73.7 (13.6)	49.4 (11.8)	**0.006**
Fat mass (% of total body mass, DEXA)	15.4 (1.6)	14.7 (1.5)	0.6
Trunk fat mass (% of total body mass, DEXA)	18.7 (2.4)	17.8 (2.4)	0.08
Lean mass (% of total body mass, DEXA)	82.1 (1.7)	85.5 (2.5)	0.8
Energy intake			
Total food intake (Kcal/day)	1970 (108.1)	1717 (112.8)	**0.03**
Metabolic, renal and liver parameters			
Systolic blood pressure (mmHg)	130.1 (3.4)	125.1 (3.0)	0.23
HbA1c (%)	8.5 (0.4)	7.5 (0.3)	**0.005**
Fasting Glucose (mmol/l)	7.5 (0.5)	7.0 (0.8)	0.5
Fasting Insulin (pmol/l)	259.2 (81.2)	380.9 (125.8)	0.7
LDL-cholesterol (mmol/l)	2.5 (0.3)	2.0 (0.02)	0.14
HDL-cholesterol (mmol/l)	0.7 (0.04)	0.8 (0.04)	0.24
Triglycerides (mmol/l)	4.6 (0.9)	3.4 (0.9)	**0.03**
Aspartate aminotransferase (AST) (IU/l)	47.8 (8.5)	32.4 (2.9)	**0.03**
Alanine aminotransferase (ALT) (IU/l)	70.9 (17.2)	49.4 (6.9)	0.3
Gamma glutamyl transferase (GGT) (IU/l)	84.9 (21.7)	57.6 (19.9)	**0.05**
Creatinine (µmol/l)	62.4 (4.8)	66.7 (4.9)	**0.03**
Albumin excretion rate (mg/l)	297.5 (166.5)	83.4 (55.9)	0.12
Therapy			
Antidiabetic medication classes per patient	2.2 (0.28)	1.6 (0.26)	**0.008**
Insulin users (n)	9/16	6/16	
Lipid-lowering medications per patient	Fibrate 9/16 Statin 6/16	Fibrate 9/16 Statin 4/16	

Values are expressed as mean (SEM). p values are depicted in bold when considered significant ($p < 0.05$).
Total, visceral and subcutaneous abdominal adipose tissue areas were evaluated form 1cm-reconstructed CT-scan slices at the L4 level. DEXA: dual energy x-ray absorptiometry

Figure 1: Effect of metreleptin therapy on insulin secretion



▲ After one-year metreleptin therapy (M12)

○ Baseline (M0)

Additional Supporting Information

File S1. Supplemental methods and references

Methods

Biochemical analyses

HbA1c was measured using high performance liquid chromatography and plasma insulin using a immuno-radiometric assay (BI-INSULIN IRMA, Cis Bio-International, Gif-Sur-Yvette, France).

The values of LDL-cholesterol were determined from total cholesterol, triglycerides and HDL-cholesterol levels using the Friedewald formula (LDL-cholesterol = total cholesterol minus HDL-cholesterol minus triglycerides (mg/dl) /5), when triglycerides levels were below 400 mg/dl (n=9 patients).

Evaluation of body composition

Total fat and lean masses, and body fat segmental distribution, were measured by dual energy x-ray absorptiometry (DEXA), and abdominal subcutaneous and visceral adipose tissue surfaces (SAT and VAT) were calculated from 1 cm-reconstructed CT-scan slices at the L4 level as previously described [1].

Caloric and macronutrient intakes were evaluated by three-day food records performed at M0 and M12.

Metabolic investigations

Metabolic investigations were performed after a 12h-overnight fast at M0 and M12. When fasting glycemia was above 7 mmol/l, a 2h-insulin infusion was performed before the investigations. Intravenous glucose tolerance tests (IVGTT, n=16), hyperglycemic and euglycemic hyperinsulinemic clamps (n= 4 and 12, respectively), and graded glucose infusion tests (glucose ramping, n=12) were performed as previously described [2-6].

Acute insulin response to an intravenous bolus of glucose (AIR) (n=16 patients)

In all patients, a solution of 20% glucose of [weight (kg) x (200 – fasting glycemia (mg/dl)) x 1.5] / 200 ml was given within 30 seconds intravenously and measurements of plasma glucose and insulin were performed at -5, 0, 2, 4, 6, 8 and 10 min. AIR was defined as the incremental area under the curve of plasma insulin concentration above baseline between 2 and 10 min after intravenous glucose administration according to the trapezoid method. Baseline insulin (InsB) was the mean insulin level between -5 and 0min [(Ins_{-5min}+Ins_{0min})/2]. AIR=

[(IT0min+ IT2min)/2 – IT0min] +[(IT2min + IT4min)/2 – IT0min]+ [(IT4min + IT6min)/2 – IT0min]+ [(IT6min +

IT8min)/2 – IT0min]+ [(IT8min + IT10min)/2 – IT0min] and expressed as pmol/kg/min.

Hyperglycemic clamp (patients 1 to 4)

Following the intravenous bolus of glucose for the AIR determination, we maintained plasma glucose at 200 mg/dl for 180 min by infusing 20% glucose at varying rates according to blood glucose measurements reformed at 5min-intervals. Blood samples were collected at 160^{th}, 170^{th} and 180^{th} min for the measurement of plasma insulin and C peptide concentrations. We calculated the glucose disposal rate from the glucose infusion rate during the last 20 min of the hyperglycemic plateau after accounting for inter-individual differences in glucose space [3] (in mg/kg body fat-free mass/min). Glucose space correction was calculated as $(G_2-G_1) \times 0.095$ with G_2 and G_1 being the glucose concentrations in mg/dl at the end and at the beginning of each 5-min period during the last 20 min of the clamp. The ratio of the glucose disposal rate to insulin concentration at the 200 mg/dl-hyperglycemic plateau (M/I) was used as an estimate of insulin sensitivity.

Euglycemic hyperinsulinemic clamp (patients 5 to 16)

The insulin-stimulated glucose disposal rate (M-value) was measured during a 100 min-step of 80 $mU/m^2/min$ insulin infusion, while blood glucose was clamped at 100 mg/dl using variable infusion of 20% glucose. Blood samples were collected before the clamp and every 10 min during the last 20 min, for the measurement of plasma glucose and insulin. The M-value was calculated according to DeFronzo et al. [3], after accounting for inter-individual differences in glucose space, and was expressed in mg/kg of fat-free mass/min, using the formula described above. We also calculated the M/I ratio, which adjusted the M-value to the mean insulin concentration during the last 20 min of the test.

Glucose ramping (graded glucose infusion test) (patients 5 to 16)

This test consisted of four consecutive 40-min intravenous infusion of 4, 8, 10 and 12 mg/kg/min of glucose as previously described [2,4]. Blood samples were collected every 10 minutes during the whole procedure (200 min). The insulin secretion rates (ISR), which evaluate the beta-cell sensitivity to glucose, were assessed from the changes in C-peptide concentrations and the pre-hepatic insulin secretion rate for each of the four glucose infusion steps. ISR was derived by deconvolution, assuming a two-compartmental model of C-peptide clearance kinetic, using the ISEC software version 3.4a designed by Hovorka R et al (see [5] for more details). Mean ISR for each glucose infusion step was adjusted to fat-free mass and plotted against the

corresponding mean glucose concentration, thereby establishing a dose-response relationship between plasma glucose and insulin secretion rate for each patient.

The ***disposition index (patients 5 to 16)*** was calculated as the product of AIR by M-value measured during euglycemic hyperinsulinemic clamps [6].

Statistical analyses

Statistical analyses were performed using GraphPad PRISM (GraphPad Software, Inc, CA, USA) and Statview (SAS Institute Inc., CA, Austria) statistical softwares. We used the Fisher exact test to compare categorical variables and the non-parametric Mann Whitney U test. Analysis of variance (ANOVA) and Wilcoxon rank-sum test for quantitative variables were performed for comparisons over time. The relationship between ISR and glucose levels during glucose ramp was analyzed using mixed model analysis of covariance. Correlations of different measures of glucose metabolism with body composition or age were evaluated using Spearman's rank correlation test or linear regression analysis. *P* values <0.05 were considered significant.

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Table S1: Baseline characteristics of the sixteen studied patients

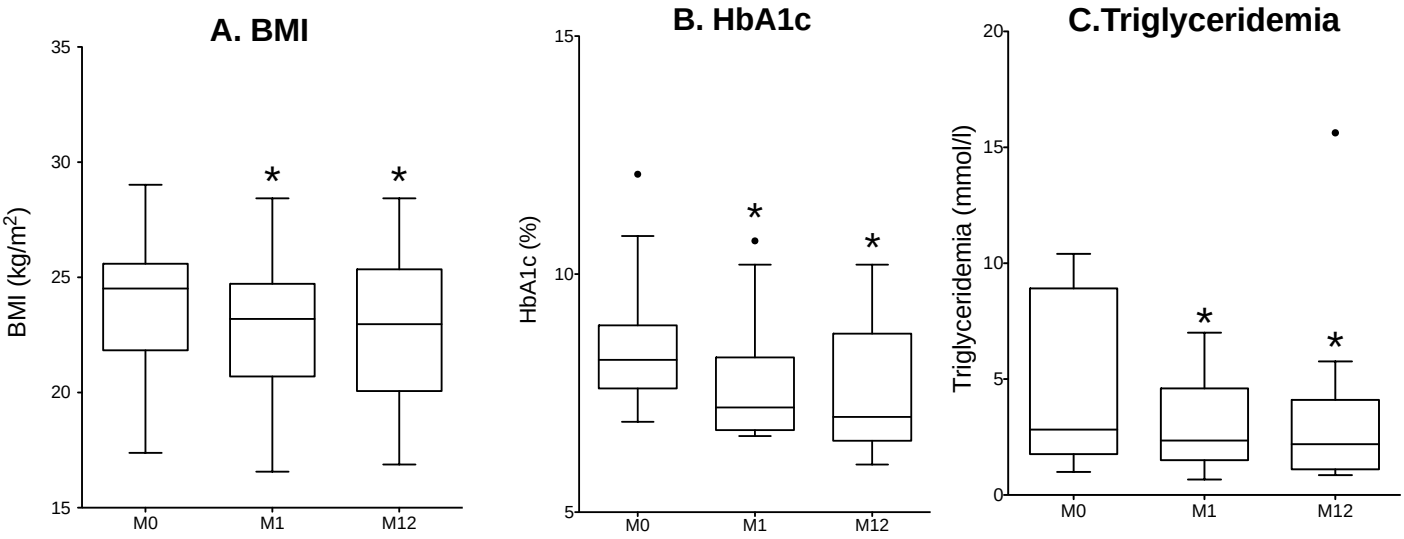
Patients	Age (years)	Sex (M/F)	Disease (gene mutation)	BMI (kg/m^2)	Total body fat mass (%, DEXA)	Serum leptin (ng/ml)	Known duration of diabetes (years)
1	60	F	FPLD2 (HTZ *LMNA* p.R482W)	22.8	21.8	0.6	23
2	37	F	FPLD2 (HTZ *LMNA* p.R482W)	25.7	18.1	1.4	16
3	24	F	CGL1 (HMZ *AGPAT2* p.L165-Q196del)	26.8	8.8	0.1	6
4	17	F	CGL1 (HMZ *AGPAT2* p.Q196fsX228)	20.3	11.2	1.7	3
5	52	F	CGL1 (HMZ *AGPAT2* p.K216X)	21.5	8.4	0.1	38
6	51	F	FPLD4 (HTZ *PLIN1* p.V398GfsX166)	26.0	22.2	5.0	30
7	50	F	FPLD2 (HTZ *LMNA* p.R482W)	24.5	21.3	4.1	6
8	41	F	FPLD2 (HTZ *LMNA* p.R482W)	23.0	13.4	3.4	22
9	19	F	FPLD2 (HTZ *LMNA* p.R482W)	25.0	22.6	5.6	3
10	50	F	FPLD2 (HTZ *LMNA* p.R482W)	25.2	13.8	3.6	1
11	16	F	Progeroid laminopathy (HTZ *LMNA* p.D47Y)	17.4	7.2	1.1	2
12	36	F	FPLD2 (HTZ *LMNA* p.R482W)	24.5	24.2	3.9	22
13	45	M	FPLD3 (HTZ *PPARG* p.L339X)	29.0	22.6	4.0	22
14	29	F	CGL1 (HMZ *AGPAT2* p.Q196fsX228)	24.1	10.1	2.2	29
15	70	M	CGL1 (HMZ *AGPAT2* p.E172K)	21.4	4.2	0.34	42
16	31	F	Mixed laminopathy (HTZ *LMNA* p.R28W)	24.8	16.9	6	20
Mean ± SEM	39.2 ± 4.0			23.9 ± 0.7	15.4 ± 1.6	2.7 ± 0.5	17.8 ± 3.3

FPLD: Familial Partial Lipodystrophy; CGL: Congenital Generalized Lipoatrophy; AGL: Acquired Generalized Lipoatrophy; HTZ: heterozygous; HMZ: homozygous; DEXA: dual energy x-ray absorptiometry

Table S2: Use of antidiabetic medications during metreleptin therapy

Patients	Type of lipodystrophy	Antidiabetic treatment (except insulin)		Insulin doses (U/d)		HbA1c	
		M0	M12	M0	M12	M0	M12
1	FPLD2	Metformin 3 g/d Pioglitazone 45 mg/d Glimepiride 4 mg/d	Metformin 3 g/d Glimepiride 4 mg/d	0	0	7.7	6.9
2	FPLD2	Metformin 1.7 g/d Pioglitazone 30 mg/d Glibenclamide 2 mg/d	Metformin 1.7 g/d Glibenclamide 1 mg/d	0	0	7.7	6.9
3	CGL1	Metformin 3g/d	Metformin 3g/d	0	0	8.7	6
4	CGL1	None	None	61	0	7.6	6.4
5	CGL1	None	None	60	0	8.1	7.1
6	FPLD4	Metformin 3 g/d	Metformin 3 g/d	254	564	9	10.2
7	FPLD2	Metformin 2 g/d	Metformin 2 g/d	0	0	8.5	7.4
8	FPLD2	Metformin 3 g/d	Metformin 3g/d	140	40	6.9	6.9
9	FPLD2	Metformin 1 g/d Pioglitazone 45 mg/d Gliclazide 120 mg/d Liraglutide 1.8 mg/d	Metformin 3 g/d Gliclazide 120 mg/d Liraglutide 1.8 mg/d	24	30	12.1	9.2
10	FPLD2	Metformin 1.7 g/d	Metformin 1.7 g/d	0	0	7.1	6.2
11	Progeroid laminopathy	Metformin 1.4 g/d Pioglitazone 30 mg/d	Metformin 1.4 g/d	202	0	10.4	9
12	FPLD2	Metformin 0.7 g/d Vildagliptin 100 mg/d	Metformin 0.7 g/d Vildagliptin 100 mg/d	0	0	7.1	7.2
13	FPLD3	Metformin 3 g/d Glibenclamide 4 mg/d Liraglutide 1.8 mg/d	Metformin 3 g/d Glibenclamide 4 mg/d Liraglutide 1.8 mg/d	0	0	10.8	9.9
14	CGL1	Metformin 2.55 g/d	None	80	68	8.7	8
15	CGL1	Metformin 3 g/d	None	180	180	8.3	6.8
16	Mixed laminopathy	Metformin 3 g/d Glibenclamide 4 mg/d	Metformin 2.1 g/d	272	220	7.6	6

Figure S1 : Longitudinal effects of metreleptin therapy in the 16 patients

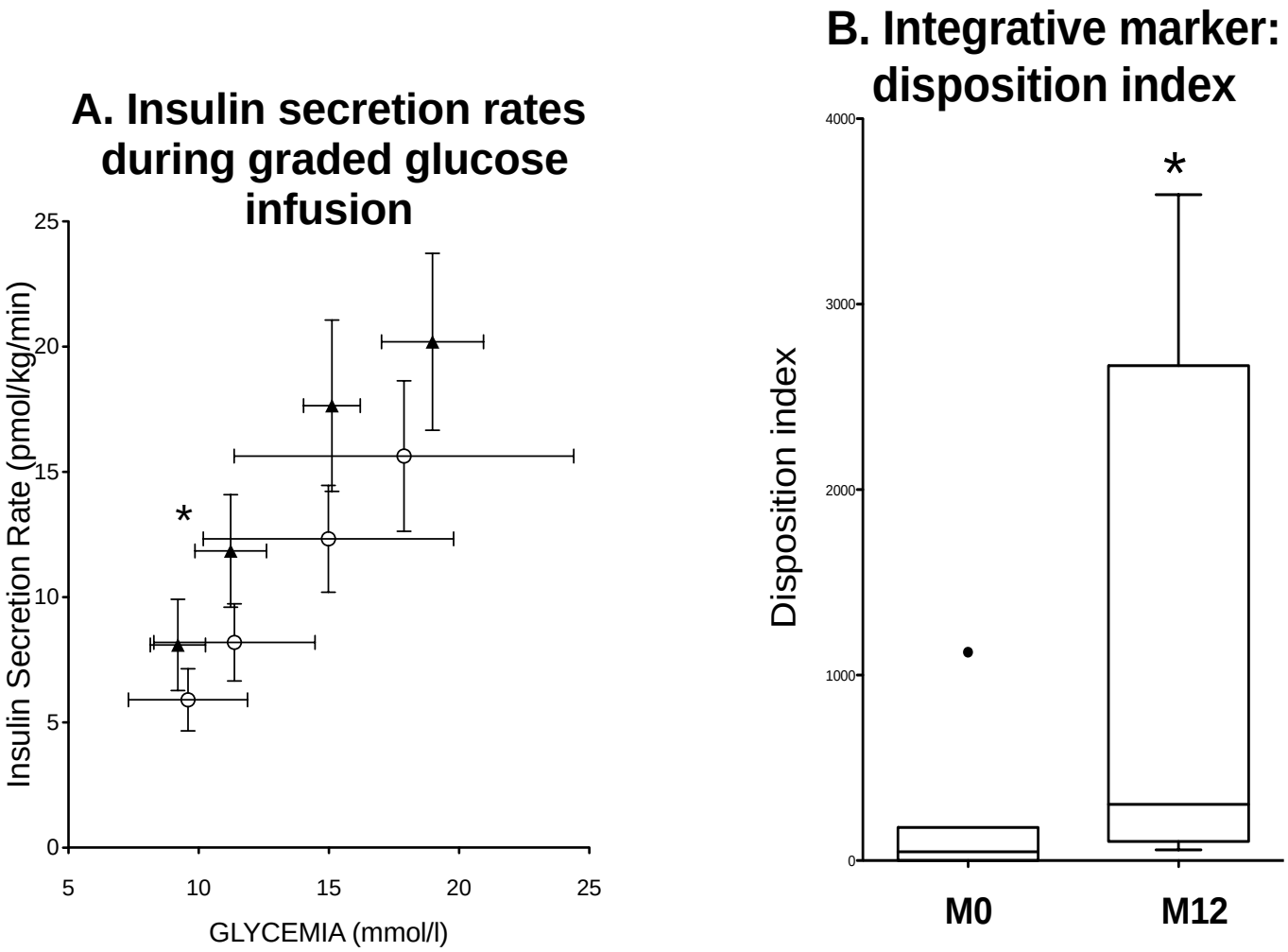


D.

Variable	Baseline	1 month-metreleptin therapy (M1)	p M1 versus baseline	12 month-metreleptin therapy (M12)	p M12 versus baseline	p M1 versus M12
BMI (kg/m^2)	23.9 (0.7)	22.9 (0.7)	**0.0009**	22.6 (0.8)	**0.003**	0.06
HbA1c (%)	8.5 (0.4)	7.6 (0.3)	**0.0005**	7.5 (0.3)	**0.005**	0.57
Triglycerides (mmol/l)	4.6 (0.9)	3.1 (0.5)	**0.004**	3.4 (0.9)	**0.03**	0.93
Total food intake (Kcal/day)	1970 (108.1)	1880 (159.4)	**0.03**	1717 (112.8)	**0.03**	0.69
Aspartate aminotransferase	47.8 (8.5)	27.3 (1.5)	**0.003**	32.4 (2.9)	**0.03**	0.06
Gamma glutamyl transferase	84.9 (21.7)	45.5 (9.2)	**0.03**	57.6 (19.9)	**0.05**	0.45

Figure S2

Effect of metreleptin on insulin secretion in patients with *LMNA* mutations



○ Baseline (M0)

▲ After one-year metreleptin therapy (M12)

Table S3

Insulin secretion and insulin sensitivity indexes before and one year after metreleptin therapy

3 A. intravenous glucose tolerance test (IVGTT) followed by hyperglycemic clamps

Patients	Type of lipodystrophy	Acute Insulin Response (pmol/kg/min)		M-value/Insulinemia (mg/kg of fat free mass/min/pmol/l)		AIR x M/I	
		M0	M12	M0	M12	M0	M12
1	FPLD2	182.7	208	0.033	0.058	6.03	12.06
2	FPLD2	31.3	50	0.184	0.125	5.76	6.25
3	CGL1	80.1	532	0.035	0.061	2.80	32.45
4	CGL1	99	81.5	0.037	0.065	3.66	6.43

3 B. Euglycemic hyperinsulinemic clamps and intravenous glucose tolerance test (IVGTT) followed by a glucose ramping

Patients	Type of lipodystrophy	Acute Insulin Response (pmol/kg/min)		M-value (mg/kg of fat free mass/min)		Disposition index (AIR x M-value)	
		M0	M12	M0	M12	M0	M12
5	CGL1	5	79.6	6.43	8.28	32.2	658.5
6	FPLD4	10	56.2	0	0.33	0	18.5
7	FPLD2	25.2	29.6	6.96	10.05	175.4	297.5
8	FPLD2	124.2	37.5	0	2.74	0	102.7
9	FPLD2	25.9	46.3	1.82	9.60	47.1	444.1
10	FPLD2	43	385.6	2.12	9.30	91.1	3586.0
11	Progeroid laminopathy	426.4	27.8	0.05	2.05	21.8	57.0
12	FPLD2	155.8	222	7.21	12.03	1123.4	2670.7
13	FPLD3	9.7	15.7	0	0.77	0	12.1
14	CGL1	179.6	97	3.01	3.96	540.6	384.1
15	CGL1	24.2	44.1	3.02	3.96	73.1	174.6
16	Mixed laminopathy	1.0	148	1.99	2.15	1.99	318.2

Supplementary Figure legends

Figure S1

Longitudinal effects of metreleptin therapy in the 16 patients

Values of BMI (A), HbA1c (B) and serum triglycerides (C) are depicted as rectangles which represent 25th and 75th percentile values, with the median values depicted in-between. Whiskers represent the lowest datum still within 1.5 IQR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile (Tukey boxplot). M: months after the onset of metreleptin therapy. *: $p<0.05$ *versus* M0

Data expressed as mean (SEM) with statistical analyses are presented in (D).

Figure S2

Effect of metreleptin on insulin secretion in patients with *LMNA* mutations

A. Insulin secretion rates (ISR) during graded glucose infusion were derived by deconvolution as described, for each of the four glucose infusion steps (of 4, 8, 10 and 12 mg per kg of body weight of glucose per min), in 7 patients (patients 7 to 12 and patient 16). ISR expressed in pmol/kg of fat-free mass/min, were plotted against the corresponding mean glucose concentration. Mean values obtained at baseline are represented as empty circles, and those obtained at one year-metreleptin therapy as filled triangles. Whiskers represent SEM.

B. Disposition index, indicating the insulin secretion capacity relative to insulin sensitivity, was calculated in the same patients as the product of M value measured during the euglycemic hyperinsulinemic clamp by AIR. Rectangles represent 25th and 75th percentile values, with the median values depicted in-between. Whiskers represent the lowest datum still within 1.5 IQR of the lower quartile, and the highest datum still within 1.5 IQR of the upper quartile (Tukey boxplot).

M0: baseline, M12: after 12 months of metreleptin therapy; * $p<0.05$ versus M0