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

C. Vatier, S. Fetita, P. Boudou, C. Tchankou, L. Deville, Jp. Riveline, J. Young, L. Mathivon, F. Travert, D. Morin, et al.

1   One-year metreleptin treatment improves insulin secretion

2   in patients with diabetes linked to genetic lipodystrophic syndromes

3

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

5   C. Vatier[1,2], S. Fetita[3], P. Boudou[4], C. Tchankou[3], L. Deville[5], JP. Riveline[3,6], J. Young[7], L. Mathivon[8], F.

6   Travert[9], D. Morin[10], J. Cahen[11], O. Lascols[1,2,12], F. Andreelli[2,13], Y. Reznik[14], E. Mongeois[15], I. Madelaine[5], MC.

7   Vantyghem[16], JF. Gautier[3,6,17]*, C. Vigouroux[1,2,12]*

8

9   [1] Sorbonne Universités, UPMC Univ Paris 06, and INSERM, UMR_S938, Centre de Recherche Saint-Antoine,

10   F-75012, Paris, France

11   [2] ICAN, Institute of Cardiometabolism and Nutrition, F-75013, Paris, France

12   [3] AP-HP, Groupe Hospitalier Lariboisière-Saint-Louis, Service de Diabétologie et Endocrinologie, F-75010,

13   Paris, France

14   [4] AP-HP, Hôpital Saint-Louis, Service de Biochimie, F-75010, Paris, France

15   [5] AP-HP, Hôpital Saint-Louis, Département de Pharmacie, F-75010, Paris, France

16   [6] INSERM, UMR_S1138, Centre de Recherche des Cordeliers, Paris, F-75006 France

17   [7] AP-HP, Hôpital Bicêtre, Service d'Endocrinologie et des Maladies de la Reproduction, F-94275, Le Kremlin-

18   Bicêtre, France

19   [8] Centre Hospitalier de Meaux, Service de Pédiatrie, F-77104, Meaux, France

20   [9] AP-HP, Hôpital Bichat, Service d'Endocrinologie, Diabétologie, Nutrition F-75018, Paris, France

21   [10] CHRU Montpellier, Hôpital Arnaud de Villeneuve, Service de Pédiatrie, 34295 Montpellier, France

22   [11] Centre Hospitalier, Service d'Endocrinologie et Métabolismes, 95100 Argenteuil, France

23   [12] AP-HP, Hôpital Saint-Antoine, Laboratoire Commun de Biologie et Génétique Moléculaires, F-75012, Paris,

24   France

25   [13] AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Diabétologie, F-75013 Paris, France

26   [14] Service d'Endocrinologie, Centre Hospitalier Universitaire Côte-de-Nacre, F-14033, Caen, France; Equipe

27   d'Accueil 2608, Université de Caen-Basse Normandie, F-14032, Caen, France; Unité sous Contrat 2006, Institut

28   National de la Recherche Agronomique, F-14032 Caen, France;

29   [15] Centre Hospitalier Régional d'Orléans, Service d'Endocrinologie, F-45000 Orléans, France

30   [16] Centre Hospitalier Régional Universitaire de Lille, Service d'Endocrinologie et Métabolisme, F-59037 Lille,

31   France

32   [17] Université Paris-Diderot Paris-7, Paris, France

33

34   * These authors contributed equally to this work

35

36

37   Corresponding authors:

38   Corinne Vigouroux, INSERM UMR_S938, Faculté de Médecine Pierre et Marie Curie, site Saint Antoine, 27

39   rue Chaligny, 75571, Paris Cedex 12, France.

40   Fax: +33 1 40 01 14 32, Phone: +33 1 40 01 14 84, corinne.vigouroux@inserm.fr

41

42

43 **ABSTRACT**

44 Recombinant methionyl human leptin (metreleptin) therapy was shown to improve hyperglycemia, dyslipidemia

45 and insulin sensitivity in patients with lipodystrophic syndromes, but its effects on insulin secretion remain

46 controversial.

47 We used dynamic intravenous clamp procedures to measure insulin secretion, adjusted to insulin sensitivity, at

48 baseline and after one-year metreleptin therapy, in 16 consecutive patients with lipodystrophy, diabetes and

49 leptin deficiency.

50 Patients, aged 39.2 ± 4 years (mean $\pm$ SEM), presented with familial partial lipodystrophies (n=11, 10 women)

51 or congenital generalized lipodystrophy (n=5, 4 women). Their BMI (23.9 ± 0.7 kg/m^2), HbA1c ($8.5 \pm 0.4\%$)

52 and serum triglycerides (4.6 ± 0.9 mmol/l) significantly decreased within 1 month of metreleptin therapy, then

53 remained stable. Insulin sensitivity (from hyperglycaemic or euglycemic hyperinsulinemic clamps, n = 4 and 12,

54 respectively), insulin secretion during graded glucose infusion (n=12), and acute insulin response to intravenous

55 glucose adjusted to insulin sensitivity (disposition index, n=12), significantly increased after 1 year of

56 metreleptin therapy. Increase in disposition index was related to decrease in percent total and trunk body fat.

57 Metreleptin therapy improves not only insulin sensitivity, but also insulin secretion in patients with diabetes due

58 to genetic lipodystrophies.

59

INTRODUCTION

61 Leptin deficiency, linked to reduced fat amount, is thought to importantly contribute to the metabolic

62 complications associated with lipodystrophic syndromes, as shown by studies in mice [1,2] and humans [3,4]. In

63 generalized forms of lipodystrophies, metreleptin therapy dramatically decreased liver and muscle lipid content,

64 improving insulin sensitivity, hyperglycemia and dyslipidemia, partly independently of decreased caloric intake

65 [3,4]. However, in partial forms of lipodystrophies, the effect of metreleptin on hyperglycemia has not been

66 clearly established in patients with moderate baseline metabolic alterations [5-7]. In addition, the effect of

67 metreleptin on insulin secretion, which remains controversial, has been previously investigated using only oral

68 glucose tolerance tests [3,6,8-10].

69 In this study, we evaluated the effect of metreleptin on insulin sensitivity and insulin secretion using dynamic

70 intravenous clamp procedures in 16 patients with genetic lipodystrophy syndromes, included in a compassionate

71 therapeutic programme.

72

METHODS

74 (see also Supplementary appendix)
75

Patients

77 Twenty-two non HIV-infected patients with genetic or acquired, partial or generalized lipodystrophy, diabetes

78 and low serum leptin (ie, fasting leptin $\leq$ 6 ng/ml), entered a compassionate programme of metreleptin therapy

79 approved by the National French Health Agency. Among them, sixteen consecutive patients older than 16 years

80 gave their informed consent for metabolic investigations at baseline and after one year of therapy, which were

81 approved by the local ethics committee (Comité de Protection des Personnes, Paris-St-Louis).

82 Recombinant human methionyl-leptin (r-metHuLeptin/metreleptin, Amylin/Bristol-Myers-Squibb/AstraZeneca

83 Pharmaceuticals, San Diego, CA), self-administered in one daily subcutaneous injection at the initial dose of

84 0.02 mg/kg (M0) increased to 0.08 mg/kg at one month (M1), was added to the patients' regimen, stable for at

85 least 6 weeks. Every three months, metreleptin and other medications were adapted to tolerance and

86 effectiveness, and anthropometric parameters, lipid profile, HbA1c, renal and liver function tests were collected.

87 The mean metreleptin daily dose at one year (M12) was 0.10 mg/kg $\pm$ 0.02 (SEM).

88

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

90   The first four patients (1 to 4) were evaluated with hyperglycemic clamps, and the twelve subsequent patients (5

91   to 16) with euglycemic hyperinsulinemic clamps and intravenous glucose tolerance test (IVGTT) followed by a

92   graded IV-glucose infusion (glucose ramping).

93   Hyperglycemic clamps allowed the measurement of acute insulin response to an intravenous bolus of glucose

94   (AIR), and the ratio of the glucose disposal rate to insulin concentration (M/I) at the 200 mg/dl-hyperglycemic

95   plateau, as an estimate of insulin sensitivity.

96   Euglycemic hyperinsulinemic clamps estimated the whole-body insulin sensitivity to glucose, expressed as the

97   insulin-stimulated glucose disposal rate (M-value), further adjusted to insulin concentrations (M/I). AIR was

98   calculated from IVGTT. Insulin secretion rates (ISR) in response to four-step graded glucose infusions evaluated

99   the beta-cell sensitivity to glucose.

100   The disposition index was calculated as the product of AIR by M measured during euglycemic hyperinsulinemic

101   clamps [11].

102

103   **Statistical analyses**

104   A favorable effect of metreleptin on glucose control was defined as a 0.5-point decrease in HbA1c, or HbA1c

105   stability with a decrease of more than 50% in total daily insulin or oral antidiabetic doses, or discontinuation of

106   one antidiabetic class, between M0 and M12. Results are presented as mean ± SEM, unless otherwise specified.

107   See File S1 for supplemental methods and references.

108

109

110   **RESULTS**

111   **Anthropometric and metabolic markers**

112   Patients with diabetes, attributable to familial partial lipodystrophy (FPLD) linked to *LMNA* (nine women),

113   *PPARG* (one man) or *PLIN1* mutations (one woman), or to congenital generalized lipodystrophy attributable to

114   *AGPAT2* mutations (CGL1) (four women, one man) were included in the study (Table S1). They presented

115   different forms of lipodystrophic syndromes with insulin resistance and dyslipidemia, attributable to already

116   described causative mutations (for review, see [12]). Their age and BMI were 39.2 ± 4.0 years and 23.9 ± 0.7

117   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,

118   total energy intake (but not food macronutrient distribution), HbA1c, triglycerides, aspartate aminotransferase

119   (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

148   p<0.0001), and the disposition index (AIR x M-value), which adjusts the acute beta-cell function to insulin

149   sensitivity [11] increased after one-year metreleptin (n=12) (Figure 1) (Table S3). The AIR x M/I index, assessed

150   in the whole group, also significantly increased (from 6.3 ± 3.8 to 9.2 ± 3.1, p=0.02).

151   Although one-year changes in HbA1c, triglycerides, M-value, and AIR were not significantly associated with

152   modifications in anthropometric parameters (data not shown), disposition index variation was significantly

153   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).

154

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

156   In patients with *LMNA* mutations (n=9), BMI, HbA1c, triglycerides and liver enzymes significantly decreased

157   after one-year metreleptin therapy (data not shown), while M-value increased from 2.88 ± 1.13 to 6.85 ± 1.65

158   mg/kg of fat free mass/min (n=7, p=0.02). ISR increased or tended to increase at the four steps of glucose

159   ramping, with a mean increase of 36%, and the disposition index significantly increased, from 208.8 ± 154.3 to

160   1068 ± 543.9 (n=7, p=0.02) (Figure S2 and Table S3). In this group, the decrease of HbA1c at M12 was

161   positively correlated with the initial HbA1c level (r^2=0.7, p=0.03), but was not related to the initial leptinemia.

162

163   **DISCUSSION**

164   In patients with lipodystrophy, metreleptin therapy was shown to improve insulin sensitivity [8,13,14]. However

165   the studies that investigated its effects on insulin secretion were done using oral glucose tolerance tests, and gave

166   heterogeneous results [3,6,8-10]. Interestingly, leptin has been shown to have dual effects on pancreatic beta-cell

167   function: while suppressing insulin gene expression and secretion, it also inhibits ectopic lipid storage in islet

168   cells, thus preventing lipotoxicity in rodent models [15].

169   The present study, which used dynamic i.v. clamp techniques in 16 patients with diabetes and endogenous

170   hypoleptinemia due to genetically-determined lipodystrophic syndromes, shows that metreleptin treatment for 1

171   year significantly improved insulin secretion. It also confirms that it decreased HbA1c, triglycerides and liver

172   enzymes and enhanced insulin sensitivity, with a two-fold increase in insulin-stimulated glucose disposal rate

173   after one year, as reported [8]. Importantly, in this context of severe insulin resistance, we adjusted insulin

174   secretion measurements for insulin sensitivity using the disposition index, based on the hyperbolic function

175   linking acute insulin response and insulin sensitivity [11]. Improvement of this index showed that, in patients

176   with lipodystrophy, metreleptin therapy increased beta-cell sensitivity to glucose.

177   Metreleptin-induced changes in disposition index and body fat mass and distribution were correlated, suggesting

178   that improvement in beta-cell secretory function could result from decreased lipotoxicity. In accordance, the two

179   non-responder patients did not lose, or even gained weight under metreleptin. Leptin could also inhibit glucagon

180   oversecretion, which was reported in insulin-deficient mice [16] and remains to be investigated in patients with

181   lipodystrophy under metreleptin therapy.

182   In patients with partial forms of lipodystrophies as a result of mutations in the *LMNA* gene, defects of insulin

183   secretion have been suspected to prevent the beneficial effect of metreleptin on glucose homeostasis [5]. Our

184   present results show that metreleptin therapy also increases insulin secretion in this subgroup of patients,

185   affected by typical Dunnigan syndrome (FPLD2) [17] or by previously described mixed laminopathic

186   phenotypes associating lipodystrophic syndrome and progeroid or cardiomyopathic signs [18,19]. In addition, in

187   accordance with recent findings showing that, in patients with partial lipodystrophy, metreleptin was mainly

188   useful if metabolic derangements were severe [7], the present study shows that effect of metreleptin on glucose

189   control in these patients was related to the baseline level of HbA1c.

190   In conclusion, 1 year of metreleptin therapy improves beta-cell function in patients with lipodystrophy, leptin

191   deficiency and diabetes. Improved insulin secretion, related to fat mass and distribution changes, probably

192   contributes to the metabolic benefits of metreleptin. Further studies are required to investigate whether these

193   effects are maintained over time and to study mechanisms by which metreleptin affects the islets. Leptin has

194   been proposed to protect the islets by acting on several pathophysiological steps involved in beta-cell

195   lipotoxicity and in glucagon production during diabetes [20]. These leptin-regulated pathways, which control,

196   among others, *de novo* ceramide synthesis, could be further studied in response to metreleptin therapy.

197

198
199   **ACKNOWLEDGEMENTS**

206

207   **CONFLICT OF INTEREST**

218

219 **SUPPORTING INFORMATION**

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

221 File S1. Supplemental methods and references.

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

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

224 Table S1. Baseline characteristics of the 16 studied patients.

225 Table S2. Use of antidiabetic medications during metreleptin therapy.

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

227

228

229 **REFERENCES**

230 1.   Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and
231 diabetes mellitus in mice with congenital lipodystrophy. Nature 1999; 401: 73-76.
232
233 2.   Colombo C, Cutson JJ, Yamauchi T *et al.* Transplantation of adipose tissue lacking leptin is unable to
234 reverse the metabolic abnormalities associated with lipoatrophy. Diabetes 2002; 51: 2727-2733.
235
236 3.   Javor ED, Cochran EK, Musso C, Young JR, Depaoli AM, Gorden P. Long-term efficacy of leptin
237 replacement in patients with generalized lipodystrophy. Diabetes 2005; 54: 1994-2002.
238
239 4.   Chong AY, Lupsa BC, Cochran EK, Gorden P. Efficacy of leptin therapy in the different forms of
240 human lipodystrophy. Diabetologia 2010; 53: 27-35.
241
242 5.   Simha V, Subramanyam L, Szczepaniak L *et al.* Comparison of efficacy and safety of leptin
243 replacement therapy in moderately and severely hypoleptinemic patients with familial partial lipodystrophy of
244 the Dunnigan variety. J Clin Endocrinol Metab 2012; 97: 785-792.
245

246   6.   Park JY, Javor ED, Cochran EK, DePaoli AM, Gorden P. Long-term efficacy of leptin replacement in
247   patients with Dunnigan-type familial partial lipodystrophy. Metabolism 2007; 56: 508-516.
248
249   7.   Diker-Cohen T, Cochran E, Gorden P, Brown RJ. Partial and generalized lipodystrophy: comparison of
250   baseline characteristics and response to metreleptin. J Clin Endocrinol Metab 2015; 100: 1802-1810.
251
252   8.   Ebihara K, Kusakabe T, Hirata M *et al.* Efficacy and safety of leptin-replacement therapy and possible
253   mechanisms of leptin actions in patients with generalized lipodystrophy. J Clin Endocrinol Metab 2007; 92: 532-
254   541.
255
256   9.   Guettier JM, Park JY, Cochran EK *et al.* Leptin therapy for partial lipodystrophy linked to a PPAR-
257   gamma mutation. Clin Endocrinol (Oxf) 2008; 68: 547-554.
258
259   10.   Muniyappa R, Brown RJ, Mari A *et al.* Effects of Leptin Replacement Therapy on Pancreatic beta-Cell
260   Function in Patients With Lipodystrophy. Diabetes Care 2014; 37: 1101-1107.
261
262   11.   Kahn SE, Prigeon RL, McCulloch DK *et al.* Quantification of the relationship between insulin
263   sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 1993; 42:
264   1663-1672.
265
266   12.   Vatier C, Bidault G, Briand N *et al.* What the genetics of lipodystrophy can teach us about insulin
267   resistance and diabetes. Curr Diab Rep 2013; 13: 757-767.
268
269   13.   Oral EA, Simha V, Ruiz E *et al.* Leptin-replacement therapy for lipodystrophy. N Engl J Med 2002;
270   346: 570-578.
271
272   14.   Beltrand J, Beregszaszi M, Chevenne D *et al.* Metabolic correction induced by leptin replacement
273   treatment in young children with Berardinelli-Seip congenital lipoatrophy. Pediatrics 2007; 120: e291-e296.
274
275   15.   Lee YH, Magkos F, Mantzoros CS, Kang ES. Effects of leptin and adiponectin on pancreatic beta-cell
276   function. Metabolism 2011; 60: 1664-1672.
277
278   16.   Yu X, Park BH, Wang MY, Wang ZV, Unger RH. Making insulin-deficient type 1 diabetic rodents
279   thrive without insulin. Proc Natl Acad Sci U S A 2008; 105: 14070-14075.
280
281   17.   Vigouroux C, Magré J, Vantyghem MC *et al.* Lamin A/C gene: sex-determined expression of mutations
282   in Dunnigan-type familial partial lipodystrophy and absence of coding mutations in congenital and acquired
283   generalized lipoatrophy. Diabetes 2000; 49 : 1958-1962.
284
285   18.   Caron M, Auclair M, Donadille B *et al.* Human lipodystrophies linked to mutations in A-type lamins
286   and to HIV protease inhibitor therapy are both associated with prelamin A accumulation, oxidative stress and
287   premature cellular senescence. Cell Death Differ 2007; 14: 1759-1767.
288
289   19.   Garg A, Speckman RA, Bowcock AM. Multisystem dystrophy syndrome due to novel missense
290   mutations in the amino-terminal head and alpha-helical rod domains of the lamin A/C gene. Am J Med 2002;
291   112: 549-55.
292
293   20.   Unger RH, Roth MG. A new biology of diabetes revealed by leptin. Cell Metab 2015; 21: 15-20.
294

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 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

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

A. Insulin secretion rates during graded glucose infusion

B. Integrative marker: disposition index
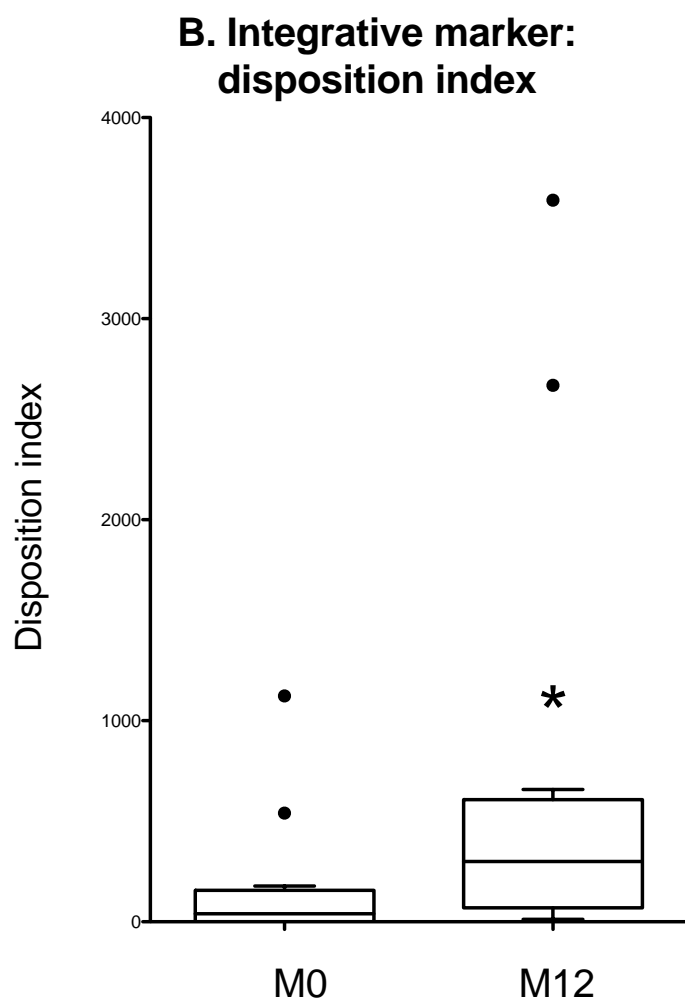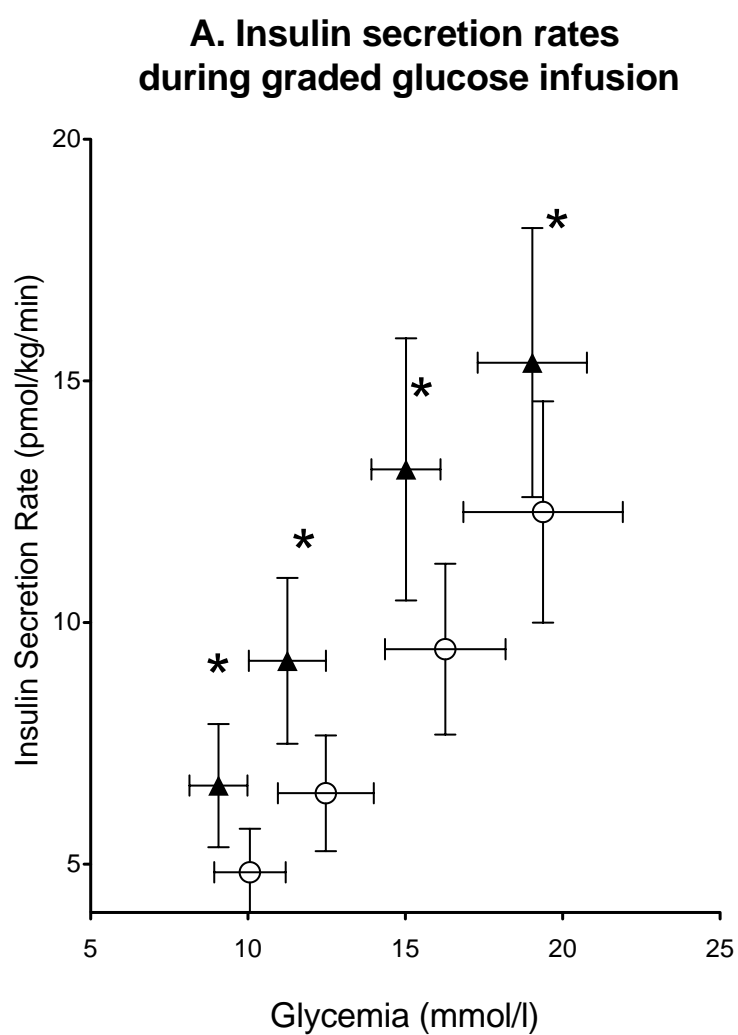
▲ After one-year metreleptin therapy (M12)

○ 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 160th, 170th and 180th 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) x 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.

References

1.   Boufassa F, Goujard C, Viard JP *et al.* Immune deficiency could be an early risk factor for altered insulin sensitivity in antiretroviral-naive HIV-1-infected patients: the ANRS COPANA cohort. Antivir Ther 2012; 17: 91-100.

2.   Gautier JF, Wilson C, Weyer C *et al.* Low acute insulin secretory responses in adult offspring of people with early onset type 2 diabetes. Diabetes 2001; 50: 1828-1833.

3.   DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979; 237: E214-E223.

4.   Sobngwi E, Boudou P, Mauvais-Jarvis F *et al.* Effect of a diabetic environment in utero on predisposition to type 2 diabetes. Lancet 2003; 361: 1861-1865.

5.   Hovorka R, Soons PA, Young MA. ISEC: a program to calculate insulin secretion. Comput Methods Programs Biomed 1996; 50: 253-264.

6.   Kahn SE, Prigeon RL, McCulloch DK *et al.* Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 1993; 42: 1663-1672.

**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

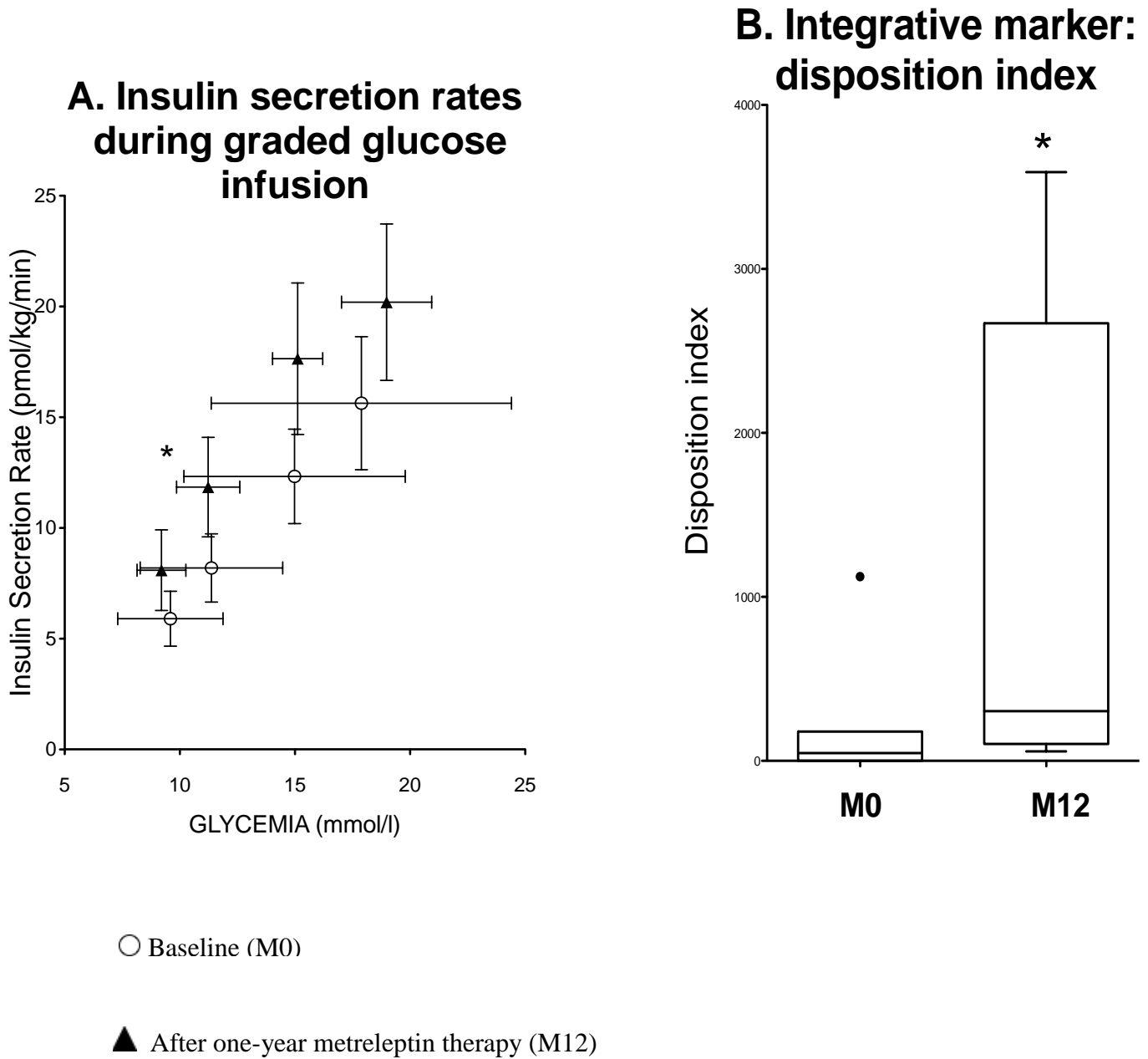
A. BMI

B. HbA1c

C.Triglyceridemia
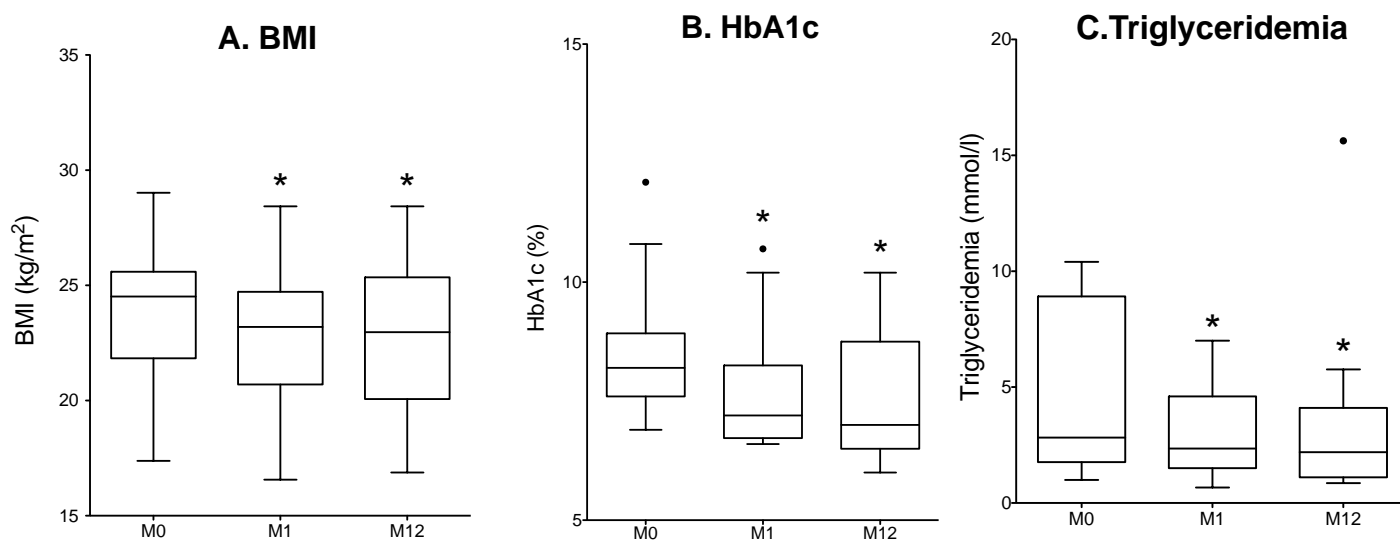
D.

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

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

A. Insulin secretion rates during graded glucose infusion

B. Integrative marker: disposition index

○ 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