Published in final edited form as:
Clin Endocrinol (Oxf). 2008 April ; 68(4): 547–554. doi:10.1111/j.1365-2265.2007.03095.x.

Leptin therapy for partial lipodystrophy linked to a *PPAR-γ* mutation

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Abstract

Aims/hypothesis—Partial lipodystrophy (PL) is most commonly characterized by loss of subcutaneous fat in the extremities with preservation of truncal fat and is associated with insulin resistance, diabetes and hyperlipidaemia. Recombinant human leptin (rmetHuLeptin) therapy has been shown to be effective in treating metabolic abnormalities associated with congenital or acquired generalized lipodystrophy and PL associated with lamin A/C (*LMNA*) gene mutations or highly active antiretroviral therapy (HAART). Our aim was to assess the effectiveness of leptin therapy in treating metabolic complications of PL associated with heterozygous peroxi-some proliferator activated receptor gamma (*PPARG*) mutations. This is the first report to detail the clinical response of a patient with PL due to a *PPARG* mutation treated with r-metHuLeptin.

Methods—A 36-year-old female with PL associated with a heterozygous *PPARG* mutation complicated by poorly controlled diabetes and severe, refractory hypertriglyceridaemia was enrolled in a National Institutes of Health (NIH) protocol to evaluate the role of r-metHuLeptin in lipodystrophy. The patient received escalating doses of r-metHuLeptin until a dose 0·12 mg/kg/day was reached. Metabolic parameters, including serum chemistries, fasting blood glucose, glycated haemoglobin (HbA1c), lipid profile, an oral glucose tolerance test (OGTT), an insulin tolerance test (ITT), liver volume, percentage body fat and energy expenditure were followed at regular time intervals over 18 months of therapy.

Results—Eighteen months of r-MetHuLeptin therapy was associated with a marked improvement in glucose homeostasis as evidenced by normalization of the fasting blood glucose (baseline = 8·3 mmol/l; 18 months = 4·9 mmol/l), lowering of HbA1c (baseline = 9·9%; 18 months = 7·2%) and improved tolerance to an oral glucose load. In addition, a striking amelioration in the patient's refractory, severe hypertriglyceridaemia was observed (baseline = 21·15 mmol/l; 18 months = 5·96 mmol/l).

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Conclusion—r-MetHuLeptin is effective in treating metabolic complications associated with PL due to *PPARG* mutations. In the context of previously published work, our findings suggest that the response to r-MetHuLeptin is independent of the aetiology in lipodystrophy.

Introduction

Lipodystrophy is a group of conditions characterized by generalized or partial loss of adipose tissue and associated with severe insulin resistance, hypertriglyceridaemia and diabetes. Considerable progress in defining the specific genetic alterations that underlie congenital forms of lipodystrophy has been made. Three genes responsible for the inherited partial form of lipodystrophy (PL) have been identified. In the most extensively studied form, PL-*LMNA*, also referred to as Dunnigan's partial lipodystrophy or familial partial lipodystrophy (FPLD) subtype-2 (OMIM ID 151660), the lamin A/C gene (*LMNA*)[1-3] was found to be involved. A mutated peroxisome proliferator activated receptor-γ gene (*PPARG*)[4-12] was identified as the genetic basis for PL-*PPARG*, also referred to as PPAR-γ ligand resistance syndrome or FPLD3 (OMIM ID 604367). In a recent report, a heterozygous *AKT2* mutation was found to cosegregate with insulin resistance and PL in a family.[13]

Despite these advances, treatment of metabolic disturbances associated with lipodystrophy has remained difficult. The severe abnormalities in triglyceride metabolism have proven particularly challenging to treat. In this regard, use of recombinant human leptin (r-metHuLeptin) was shown to confer unique benefits, in both the generalized[14-16] and the PL-*LMNA* forms.[17]

In the present study, we extend these observations and report for the first time on the efficacy of r-metHuLeptin treatment for PL-*PPARG*.

Patient and methods

Lipodystrophic patient NIH-29

The patient was a 36-year-old female of East Indian origin living in France (Fig. 1). Her family history was negative for parental consanguinity but positive for two first-degree relatives (mother and youngest brother) with diabetes and hypertriglyceridaemia. A short account of the patient's early clinical history has been published previously.[18] In brief, the patient underwent menarche at age 10 and a half and cycled normally until age 12. At this age, she developed signs and symptoms of hyperandrogenism that included growth of terminal hair on the face, chest and extremities, a deepening of the voice and oligomenorrhoea. Loss of subcutaneous fat and the appearance of a defined, hypertrophic musculature in the limbs and gluteal region was first noted by the patient at age 15. A diagnosis of lipodystrophy was made at this age based on these and other presenting features, which included: acanthosis nigricans, impaired fasting glucose, fasting hyperinsulinaemia, polycystic ovaries, mild hepatomegaly, elevated testosterone and triglyceride levels.

Between ages 18 and 20, she had repeated hospital admissions for episodes of acute pancreatitis secondary to severe hypertriglyceridaemia [triglyceride levels > 46 mmol/l (3000 mg/dl)]. Skin lesions consistent with eruptive xanthomas were noted on several of these admissions. Her impaired fasting glucose progressed to diabetes during this time. At age 21, the patient was referred to the Hôtel-Dieu Hospital, Nutrition Department (Paris), for management of her severe metabolic abnormalities. The patient's diabetes was initially managed by a sulfonylurea drug [Glimepiride (Amaryl®) 6 mg per day (drug name and dose at the time of protocol enrolment)], followed by an alpha-glucosidase inhibitor [Miglitol (Glyset®) 100 mg three times per day] and finally by addition of a thiazolidinedione [Rosiglitazone (Avandia®) 8 mg per day]. On this oral regimen, the patient's diabetes remained poorly controlled with an average

glycated haemoglobin (HbA1c) of 10%. Insulin therapy was recommended but the patient was reluctant to start. To treat her severe hypertriglyceridaemia, the patient was instructed to follow a low-fat, hypocaloric diet and was started on a fibrate class of medication [Ciprofibrate (Lipanor®) 100 mg per day]. Despite this treatment, she had persistent, severe hypertriglyceridaemia with average triglyceride levels above 15 mmol/l (1000 mg/dl). At age 26, the patient was diagnosed with hypertension, severe proliferative diabetic retinopathy requiring laser therapy and mild proteinuria ranging from 0·3 to 0·5 g of protein/24 h. She presented to the National Institutes of Health (NIH) on four antihypertensive drugs, which included a diuretic, an angiotensin II receptor blocker [Valsartan/hydrochlorothiazide (Cotareg®) 160/25 (mg)], a calcium channel blocker, and a beta-blocker [felodipine/ metoproplol (Logimax®) 5/47·5 mg].

PPARG mutation screen

PPARG was screened for mutations after amplification of genomic DNA by polymerase chain reaction (PCR) followed by single-stranded conformation polymorphism (SSCP) analysis and direct sequencing of abnormal conformers. PCR was performed using specific oligonucleotides flanking each of the seven exons of the gene, as described previously.[19] PCR products were denatured and electrophoresed on 10% acrylamide mini-gels at 7°C, and at 20°C for 6-7 h at 16 mA. The gels were silver-stained and dried. The exon 6-PCR product showed an abnormal migration profile and was directly sequenced (GENOME Express, France). Results from the sequencing data were compared to the normal sequence using Blast (http://www.ncbi.nlm.gov/cgi-bin/BLAST).

Co-segregation of the *R425C* mutation with metabolic abnormalities was tested in the patient's family by PCR amplification of exon 6 and restriction endonuclease digestion of the PCR product with *Aci*-I. PCR was used to amplify the region of genomic DNA containing exon 6 of *PPARG* in all first-degree relatives after obtaining their informed consent. A 10 μl aliquot of the PCR product was digested with 10 units of the *Aci*-I restriction enzyme for 3 h at 37°C. The digestion products were resolved on a 3% agarose gel and visualized by use of ethidium bromide (Fig. 1). The presence of the mutation leads to loss of an *Aci*-I restriction site in the PCR fragment, resulting in a different migration pattern of the digested PCR product.

Recombinant leptin therapy protocol

r-metHuLeptin therapy was given as a self-administered, twice-daily subcutaneous injection for 12 months as described previously[14] for patients with generalized lipodystrophy. In this patient, the dose was increased to 0·08 mg/kg/day after 2 months, then to 0·12 mg/kg/day after 12 months in an attempt to simulate the normal to high physiological range. r-metHuLeptin was then changed to once daily subcutaneous injections after studies found this to be effective. The patient was evaluated at the Clinical Research Center of the NIH at baseline, every 4 months for 1 year, and every 6 months thereafter. No medication changes, other than leptin dose adjustment, were made in the first year of study. The protocol was approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases. Informed consent was obtained prior to the initiation of the study.

Biochemical analyses

Serum leptin levels were determined by immunoassays with the use of a commercial kit (Linco Research, St Charles, MO). HbA1c values were measured by ion-exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA). Serum TSH, GH, IGF-1 and C-reactive protein (CRP) levels were measured with a two-site chemiluminescent immunometric assay on DPC Immulite 2000 equipment (Diagnostic Products, Los Angeles, CA). Free thyroxine (FT4) was measured with an electrochemiluminescent competitive immunoassay on Elecsys 2010 equipment (Roche Diagnostics, Indianapolis, IN). Insulin was determined by

immunoassay (Abbott Imx Instrument, Abbott Park, IL). Serum glucose and lipid values were determined according to standard methods with the use of automated equipment (Beckman, Fullerton, CA). All values represent morning fasting levels.

Experimental procedures

Resting energy expenditure (REE; Deltatrac equipment, Sensormedics, Yorba Linda, CA) was measured between 0600 and 0800 h after an overnight fast of at least 8 h, while the patient remained at rest. After an overnight fast, an oral glucose tolerance test (OGTT) in which 1·75 g/kg up to 75 g of dextrose was administered and plasma glucose levels were followed over 180 min. A high-dose insulin tolerance test (ITT) was performed with the use of 0·2 U of regular insulin per kilogram administered intravenously to assess the patient's sensitivity to insulin. Percentage body fat was determined using dual-energy X-ray absorptiometry (DEXA; QDR 4500, Hologic, Inc., Bedford, MA). Axial T1-weighted magnetic resonance imaging (MRI) of the patient's liver was performed with the use of a 1·5-T scanner (General Electric Medical Systems, Milwaukee, WI). The liver volumes were estimated with the use of the MEDx image-analysis software package (Sensor Systems, Sterling, VA).

Results

Genetic screening of patient and family

Previous genetic screens for mutations in genes associated with severe insulin resistance syndromes had been carried out in this patient. No mutations in the genes coding for the insulin receptor (*INSR*),[20] lamin A/C (*LMNA*),[21] seipin (*BSCL2*),[22] 1-acylglycerol-3-phosphate *O*-acyltransferase 2 (*AGPAT2*)[23] or in the regulatory subunit 3A of protein phosphatase 1 (*PPP1R3A*) were identified.

A heterozygous, single base-pair substitution (C to T) at nucleotide *c*. 1273 in exon 6 of *PPARG* was found (Fig. 1a). This mutation changes codon CGC to TGC, resulting in an arginine to cysteine substitution at position 425 (*R425C*) of the PPAR-γ2 isoform (R397C of the PPAR-γ1, -γ3 and -γ4 transcript variants). This mutation had been reported previously in another case of PL.[5]

The *Aci*-I restriction enzyme was used to evaluate for segregation of the mutation with metabolic abnormalities in the patient's family (Fig. 1b). The *R425C* substitution was found in the patient's mother (A) and in one of the patient's brothers (H), who both display metabolic alterations. The mother has diabetes, hypertension and hirsutism while the brother carries a diagnosis of hypertriglyceridaemia since childhood and diabetes since the age of 20. The *R425C* substitution was not observed in the patient's father (J) or older brother (F). These two first-degree relatives have no metabolic abnormalities. Inheritance of metabolic traits follows an autosomal dominant pattern.

Baseline characteristics

The patient was aged 35 at the time of her first NIH visit. Loss of subcutaneous fat in the limbs and gluteal regions was observed on the intake examination (Fig. 2). A defined, hypertrophic musculature in the extremities was also noted. Fat depots were present in the cheeks, submandibular, neck, supraclavicular areas and trunk. The patient had skin changes consistent with acanthosis nigricans in the neck, armpit and crural areas. Coarse terminal hair was present in the areas of the sideburns, chin, chest, lower abdomen and on the extremities. Her body mass index (BMI) was calculated to be 22 and her body fat percentage measured 14·8% by DEXA. Biochemical evaluation was significant for a low serum leptin level of 0·23 nmol/l (3·7 ng/ml). The patient's diabetes was poorly controlled, as evidenced by a fasting glucose level of 8·3 mmol/l (151 mg/dl) and an HbA1c of 9·9%, on three oral agents (Fig. 3). She had severely

elevated triglyceride levels (21·15 mmol/l; 1377 mg/dl), elevated total cholesterol (8·3 mmol/l; 321 mg/dl) and low density lipoprotein (LDL) cholesterol levels (4·1 mmol/l; 159 mg/dl). Her high density lipoprotein (HDL) cholesterol level was low (0·78 mmol/l; 30 mg/dl). Her liver volume was calculated to be 2131 cm^3. These results are summarized in Table 1.

Response to r-metHuLeptin therapy

The patient was followed for 18 months while on r-metHuLeptin therapy and improvement in several metabolic parameters were noted and are summarized in Table 1 and Fig. 3. The patient had a marked amelioration in her glycaemic control evidenced by normalization of her fasting glucose to 4·9 mmol/l (89 mg/dl) and decrease in her HbA1c level to 7·2% (Table 1). These changes were paralleled by an improvement in insulin responsiveness at 12 months as assessed by use of a standard ITT (Fig. 4). In addition, a modest improvement in glucose tolerance accompanied by a robust insulin response was seen on an OGTT performed at 18 months (Fig. 5). These changes are similar to those reported previously for cases of generalized lipodystrophy and PL-*LMNA* treated with r-metHuLeptin.

Improvement in the patient's dyslipidaemia was also noted. After 18 months on r-metHuLeptin therapy, the patient's triglyceride levels decreased from a baseline value of 21·15 mmol/l to 5·96 mmol/l (1377 to 388 mg/dl) (Table 1). She has had no episodes of pancreatitis while on therapy. Total and LDL cholesterol have also declined moderately from respective baseline values of 8·30 and 4·11 mmol/l down to 18-month values of 6·23 and 3·57 mmol/l (321 and 159 mg/dl down to 241 mg/dl and 138 mg/dl, respectively). HDL cholesterol levels have not changed (Table 1).

The patient reported a mild reduction in her appetite. A slight increase in weight, BMI and body fat percentage was noted at 12 and 18 months. Lean mass, as measured by DEXA, accounted for the majority of the weight gain (data not shown). Liver volume showed a mild decrease from baseline of 2131 cm^3 down to 1874 cm^3 (Table 1). Fasting GH, IGF-1, CRP, TSH and FT4 remained essentially unchanged during r-metHuLeptin therapy. r-metHuLeptin was well tolerated and no side-effects were reported.

Discussion

We report on the first patient with a *PPARG* mutation and PL to be treated with r-metHuLeptin therapy for her metabolic abnormalities. The partial loss of adipocytes in PL-*PPARG*, as in other partial forms of lipodystrophy, is associated with metabolic derangements. In addition, a defect in the insulin sensitizing action of PPAR-γ, specific to this form of lipodystrophy, probably contributes to the insulin resistance and overall phenotype of PL-*PPARG*. The efficacy of rmetHuLeptin has been demonstrated for the treatment of metabolic[14,15] and hyperandrogenic[24] abnormalities associated with congenital generalized lipodystrophies (CGLs) linked to either *AGPAT2* (CGL type 1) or *BSCL2* (CGL type 2) mutations and the generalized acquired forms of lipodystrophies. More recently, we have reported on the effect of r-metHuLeptin in PL-*LMNA*.[17] A modest effect of leptin therapy in reducing metabolic abnormalities associated with the highly active antiretroviral therapy (HAART)-induced PL has also been shown.[25]

Success in treating metabolic abnormalities associated with inherited (PL-*LMNA*) [26,27] or acquired partial forms of lipodystrophies [28-31] with the thiazolidenedione class of drugs has been mixed. In our patient, treatment with both rosiglitazone and ciprofibrate was inadequate to control either blood glucose or dyslipidaemia. Insulin therapy in this patient had not been used. We do not know whether improved glycaemic control by early intervention with insulin could have prevented her severe diabetic retinopathy. It has been our personal experience that insulin therapy does not control the severe triglyceride abnormalities seen in these patients.

We have previously observed that an adequate endogenous insulin response is necessary for the optimal effect of r-metHuLeptin. [17] Our patient displayed a robust endogenous insulin response prior to and on the therapy. Whether higher doses of r-metHuLeptin will completely normalize her triglyceride levels is as of yet undetermined.

In most cases of congenital lipodystrophy, leptin therapy results in weight loss. By contrast, this case was marked by fluctuations in body weight and an elevated body weight at 18 months compared to baseline. Whole-body composition of the subtotal area by DEXA at 12 months showed a greater increase in lean body mass over fat mass [change from baseline: (+) 1532·2 g of lean mass; (+) 407 g of fat]. The significance and reasons for this are not immediately clear and will have to be confirmed by additional studies.

The *PPARG* mutation in this patient is similar to one that has been described previously.[5] The reported case, like the index case, had trunk-sparing lipodystrophy (Fig. 2). In contrast to the original case, however, no marked facial atrophy was noted and gluteal fat was decreased. These findings most probably represent a variant phenotype of PL-*PPARG R425C*. The reasons behind this observation have not been determined and are likely to involve complex interplay between numerous factors, one being differences in genes other than *PPARG* among individuals. Phenotype heterogeneity across cases of PL associated with other *PPARG* mutations[6,10-12] has been documented. A molecular mechanism to explain the *R425C*-associated PPAR-γ dysfunction has been proposed recently. Formation of a salt bridge between arginine 425 and glutamic acid 352, located in the ligand-binding domain region of the PPAR-γ, is predicted from modelling studies to be an important determinant of tertiary protein structure and function. A recent report[32] has indeed shown that, *in vitro*, the *R425C* mutation affects receptor heterodimerization, ligand and coactivator binding, resulting in decreased transcriptional activity of the receptor and abnormal adipocyte differentiation. Two mechanisms have been suggested to explain the occurrence of lipodystrophy and metabolic abnormalities in the presence of only one mutant allele. The first involves a dominant negative effect of the mutated receptor[4] and the second invokes haploinsufficiency.[11,32] The *R425C* mutation was not found to affect wild-type receptor function *in vitro*, suggesting haploinsufficiency.[32] *In vivo*, mechanistic interpretation has been complicated by the fact that a mouse model of a human (*P467L*)-related *PPARG* mutation (e.g. heterozygous *P465L/wt*) failed to replicate the human phenotype.[33] To explain this discrepancy, interspecies differences in the function of metabolically relevant tissues and the presence of compensatory pathways in the mouse model were invoked. Superimposing leptin deficiency (i.e. extreme positive energy challenge) on this mouse model by creating a double heterozygous *P465L/ob* mouse, however, resulted in a phenotype similar to human lipodystrophy.[34]

The response to r-metHuLeptin appears to be independent of the aetiology (i.e. genetic forms or acquired forms) as the drug seems to be effective in treating all forms of lipodystrophy. Both the optimal therapeutic dose and the upper endogenous serum leptin concentration that still allow for a response to r-metHuLeptin in cases of lipodystrophy have yet to be determined.

Acknowledgements

We are grateful to the patient and her family, and to Prof. Jean-Jacques Robert for initially referring the patient, Marc Delépine and Mark Lathrop for sequencing *PPP1R3A* (Centre National de Génotypage, CNG), and the nurses and endocrine fellows of the National Institutes of Health Clinical Center for their support of patient care. This work was supported by the Intramural Research Program of the National Institute of Diabetes, Digestive and Kidney Diseases and grants from Aide aux Jeunes Diabétiques (AJD), Association de Langue Française de l'Etude du Diabète et des Maladies Métaboliques (ALFEDIAM), Assistance Public-Hôpitaux de Paris (AP-HP), and Ministère de la Recherche et de la Technologie.

References

1. Peters JM, Barnes R, Bennett L, Gitomer WM, Bowcock AM, Garg A. Localization of the gene for familial partial lipodystrophy (Dunnigan variety) to chromosome 1q21-22. Nature Genetics 1998;18:292–295. [PubMed: 9500556]
2. Shackleton S, Lloyd DJ, Jackson SN, Evans R, Niermeijer MF, Singh BM, Schmidt H, Brabant G, Kumar S, Durrington PN, Gregory S, O'Rahilly S, Trembath RC. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nature Genetics 2000;24:153–156. [PubMed: 10655060]
3. Cao H, Hegele RA. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. Human Molecular Genetics 2000;9:109–112. [PubMed: 10587585]
4. Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, Maslen GL, Williams TD, Lewis H, Schafer AJ, Chatterjee VK, O'Rahilly S. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. Nature 1999;402:880–883. [PubMed: 10622252]
5. Agarwal AK, Garg A. A novel heterozygous mutation in peroxisome proliferator-activated receptor-gamma gene in a patient with familial partial lipodystrophy. Journal of Clinical Endocrinology and Metabolism 2002;87:408–411. [PubMed: 11788685]
6. Hegele RA, Cao H, Frankowski C, Mathews ST, Leff T. PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy. Diabetes 2002;51:3586–3590. [PubMed: 12453919]
7. Savage DB, Tan GD, Acerini CL, Jebb SA, Agostini M, Gurnell M, Williams RL, Umpleby AM, Thomas EL, Bell JD, Dixon AK, Dunne F, Boiani R, Cinti S, Vidal-Puig A, Karpe F, Chatterjee VKK, O'Rahilly S. Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-{gamma}. Diabetes 2003;52:910–917. [PubMed: 12663460]
8. Al-Shali K, Cao H, Knoers N, Hermus AR, Tack CJ, Hegele RA. A single-base mutation in the peroxisome proliferator-activated receptor gamma4 promoter associated with altered in vitro expression and partial lipodystrophy. Journal of Clinical Endocrinology and Metabolism 2004;89:5655–5660. [PubMed: 15531525]
9. Francis GA, Li G, Casey R, Wang J, Cao H, Leff T, Hegele RA. Peroxisomal proliferator activated receptor-gamma deficiency in a Canadian kindred with familial partial lipodystrophy type 3 (FPLD3). BMC Medical Genetics 2006;7:3. [PubMed: 16412238]
10. Hegele RA, Ur E, Ransom TP, Cao H. A frameshift mutation in peroxisome-proliferator-activated receptor-gamma in familial partial lipodystrophy subtype 3 (FPLD3; MIM 604367). Clinical Genetics 2006;70:360–362. [PubMed: 16965332]
11. Monajemi H, Zhang L, Li G, Jeninga EH, Cao H, Maas M, Brouwer CB, Kalkhoven E, Stroes E, Hegele RA, Leff T. Familial partial lipodystrophy phenotype resulting from a single-base mutation in deoxyribonucleic acid-binding domain of peroxisome proliferator-activated receptor-{gamma}. Journal of Clinical Endocrinology and Metabolism 2007;92:1606–1612. [PubMed: 17299075]
12. Ludtke A, Buettner J, Wu W, Muchir A, Schroeter A, Zinn-Justin S, Spuler S, Schmidt HHJ, Worman HJ. Peroxisome proliferator-activated receptor-{gamma} C190S mutation causes partial lipodystrophy. Journal of Clinical Endocrinology and Metabolism 2007;92:2248–2255. [PubMed: 17356052]
13. George S, Rochford JJ, Wolfrum C, Gray SL, Schinner S, Wilson JC, Soos MA, Murgatroyd PR, Williams RM, Acerini CL, Dunger DB, Barford D, Umpleby AM, Wareham NJ, Davies HA, Schafer AJ, Stoffel M, O'Rahilly S, Barroso I. A family with severe insulin resistance and diabetes due to a mutation in AKT2. Science 2004;304:1325–1328. [PubMed: 15166380]
14. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A. Leptin-replacement therapy for lipodystrophy. New England Journal of Medicine 2002;346:570–578. [PubMed: 11856796]
15. Javor ED, Cochran EK, Musso C, Young JR, Depaoli AM, Gorden P. Long-term efficacy of leptin replacement in patients with generalized lipodystrophy. Diabetes 2005;54:1994–2002. [PubMed: 15983199]
16. Ebihara K, Kusakabe T, Hirata M, Masuzaki H, Miyanaga F, Kobayashi N, Tanaka T, Chusho H, Miyazawa T, Hayashi T, Hosoda K, Ogawa Y, DePaoli AM, Fukushima M, Nakao K. Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with

generalized lipodystrophy. Journal of Clinical Endocrinology and Metabolism 2007;92:532–541. [PubMed: 17118991]

17. Park JY, Javor ED, Cochran EK, DePaoli AM, Gorden P. Long-term efficacy of leptin replacement in patients with Dunnigan-type familial partial lipodystrophy. Metabolism 2007;56:508–516. [PubMed: 17379009]
18. Robert JJ, Rakotoambinina B, Cochet I, Foussier V, Magre J, Darmaun D, Chevenne D, Capeau J. The development of hyperglycaemia in patients with insulin-resistant generalized lipoatrophic syndromes. Diabetologia 1993;36:1288–1292. [PubMed: 8307257]
19. Vigouroux C, Fajas L, Khallouf E, Meier M, Gyapay G, Lascols O, Auwerx J, Weissenbach J, Capeau J, Magre J. Human peroxisome proliferator-activated receptor-gamma2: genetic mapping, identification of a variant in the coding sequence, and exclusion as the gene responsible for lipoatrophic diabetes. Diabetes 1998;47:490–492. [PubMed: 9519760]
20. Desbois-Mouthon C, Magre J, Amselem S, Reynet C, Blivet MJ, Goossens M, Capeau J, Besmond C. Lipoatrophic diabetes: genetic exclusion of the insulin receptor gene. Journal of Clinical Endocrinology and Metabolism 1995;80:314–319. [PubMed: 7829633]
21. Vigouroux C, Magre J, Vantyghem MC, Bourut C, Lascols O, Shackleton S, Lloyd DJ, Guerci B, Padova G, Valensi P, Grimaldi A, Piquemal R, Touraine P, Trembath RC, Capeau J. Lamin A/C gene: sex-determined expression of mutations in Dunnigan-type familial partial lipodystrophy and absence of coding mutations in congenital and acquired generalized lipoatrophy. Diabetes 2000;49:1958–1962. [PubMed: 11078466]
22. Magre J, Delepine M, Khallouf E, Gedde-Dahl T Jr, Van Maldergem L, Sobel E, Papp J, Meier M, Megarbane A, Bachy A, Verloes A, d'Abronzo FH, Seemanova E, Assan R, Baudic N, Bourut C, Czernichow P, Huet F, Grigorescu F, de Kerdanet M, Lacombe D, Labrune P, Lanza M, Loret H, Matsuda F, Navarro J, Nivelon-Chevalier A, Polak M, Robert JJ, Tric P, Tubiana-Rufi N, Vigouroux C, Weissenbach J, Savasta S, Maassen JA, Trygstad O, Bogalho P, Freitas P, Medina JL, Bonnicci F, Joffe BI, Loyson G, Panz VR, Raal FJ, O'Rahilly S, Stephenson T, Kahn CR, Lathrop M, Capeau J. Identification of the gene altered in Berardinelli-Seip congenital lipodystrophy on chromosome 11q13. Nature Genetics 2001;28:365–370. [PubMed: 11479539]
23. Magre J, Delepine M, Van Maldergem L, Robert JJ, Maassen JA, Meier M, Panz VR, Kim CA, Tubiana-Rufi N, Czernichow P, Seemanova E, Buchanan CR, Lacombe D, Vigouroux C, Lascols O, Kahn CR, Capeau J, Lathrop M. Prevalence of mutations in AGPAT2 among human lipodystrophies. Diabetes 2003;52:1573–1578. [PubMed: 12765973]
24. Musso C, Cochran E, Javor E, Young J, Depaoli AM, Gorden P. The long-term effect of recombinant methionyl human leptin therapy on hyperandrogenism and menstrual function in female and pituitary function in male and female hypoleptinemic lipodystrophic patients. Metabolism 2005;54:255–263. [PubMed: 15690321]
25. Lee JH, Chan JL, Sourlas E, Raptopoulos V, Mantzoros CS. Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipoatrophy and metabolic syndrome induced by the highly active antiretroviral therapy. Journal of Clinical Endocrinology and Metabolism 2006;91:2605–2611. [PubMed: 16636130]
26. Owen KR, Donohoe M, Ellard S, Hattersley AT. Response to treatment with rosiglitazone in familial partial lipodystrophy due to a mutation in the LMNA gene. Diabetic Medicine 2003;20:823–827. [PubMed: 14510863]
27. Ludtke A, Heck K, Genschel J, Mehnert H, Spuler S, Worman HJ, Schmidt HH. Long-term treatment experience in a subject with Dunnigan-type familial partial lipodystrophy: efficacy of rosiglitazone. Diabetic Medicine 2005;22:1611–1613. [PubMed: 16241930]
28. Hadigan C, Yawetz S, Thomas A, Havers F, Sax PE, Grinspoon S. Metabolic effects of rosiglitazone in HIV lipodystrophy: a randomized, controlled trial. Annals of Internal Medicine 2004;140:786–794. [PubMed: 15148065]
29. Sutinen J, Hakkinen AM, Westerbacka J, Seppala-Lindroos A, Vehkavaara S, Halavaara J, Jarvinen A, Ristola M, Yki-Jarvinen H. Rosiglitazone in the treatment of HAART-associated lipodystrophy: a randomized double-blind placebo-controlled study. Antiviral Therapy 2003;8:199–207. [PubMed: 12924536]

30. Carr A, Workman C, Carey D, Rogers G, Martin A, Baker D, Wand H, Law M, Samaras K, Emery S, Cooper DA. No effect of rosiglitazone for treatment of HIV-1 lipoatrophy: randomised, double-blind, placebo-controlled trial. Lancet 2004;363:429–438. [PubMed: 14962523]

31. van Wijk JP, de Koning EJ, Cabezas MC, op't Roodt J, Joven J, Rabelink TJ, Hoepelman AI. Comparison of rosiglitazone and metformin for treating HIV lipodystrophy: a randomized trial. Annals of Internal Medicine 2005;143:337–346. [PubMed: 16144892]

32. Jeninga EH, van Beekum O, van Dijk AD, Hamers N, Hendriks-Stegeman BI, Bonvin AM, Berger R, Kalkhoven E. Impaired peroxisome proliferator-activated receptor gamma function through mutation of a conserved salt bridge (R425C) in familial partial lipodystrophy. Molecular Endocrinology 2007;21:1049–1065. [PubMed: 17312272]

33. Tsai YS, Kim HJ, Takahashi N, Kim HS, Hagaman JR, Kim JK, Maeda N. Hypertension and abnormal fat distribution but not insulin resistance in mice with P465L PPARgamma. Journal of Clinical Investigation 2004;114:240–249. [PubMed: 15254591]

34. Gray SL, Nora ED, Grosse J, Manieri M, Stoeger T, Medina-Gomez G, Burling K, Wattler S, Russ A, Yeo GS, Chatterjee VK, O'Rahilly S, Voshol PJ, Cinti S, Vidal-Puig A. Leptin deficiency unmasks the deleterious effects of impaired peroxisome proliferator-activated receptor gamma function (P465L PPARgamma) in mice. Diabetes 2006;55:2669–2677. [PubMed: 17003330]

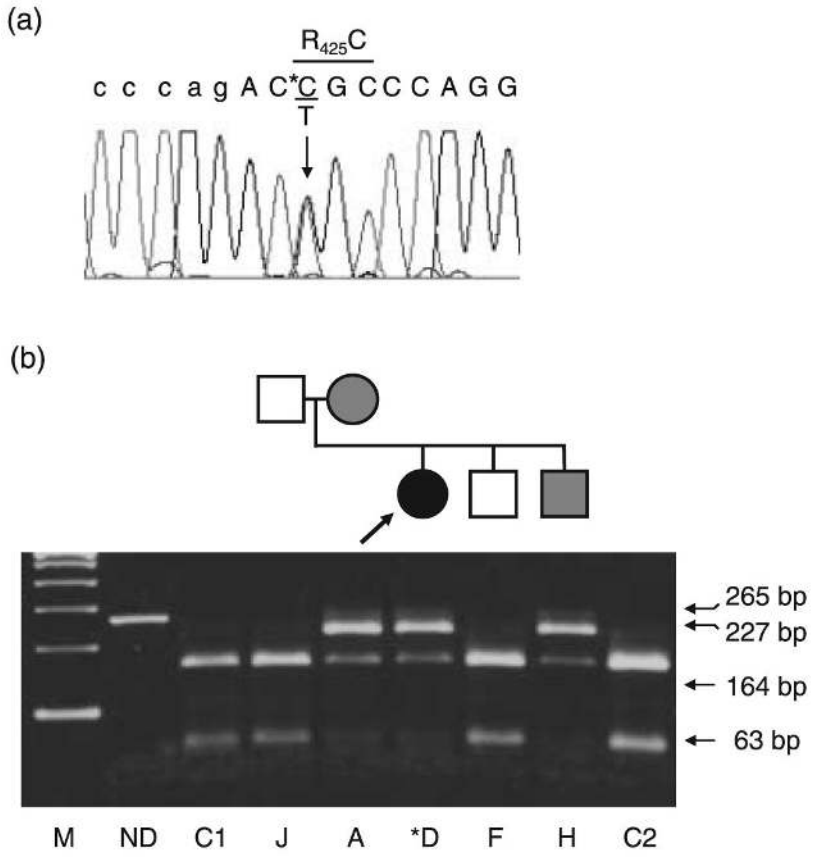

Fig. 1.

Detection of a heterozygous *PPARG* mutation that cosegregates with insulin resistance and dyslipidaemia (a) Direct sequencing of genomic DNA from the proband. The asterisk indicates the single base pair (C to T) substitution that results in an arginine to cysteine exchange at codon 425 of PPAR-γ2. (b) Family pedigree and electrophoretic pattern of digested PCR fragment containing the heterozygous *R425C* mutation demonstrating cosegregation of metabolic abnormalities with the mutation. Carriers of the *PPARG* mutation have diabetes and dyslipidaemia as indicated by grey fill. The proband is indicated by black fill and an asterisk. M, molecular weight marker; ND, nondigested PCR product; C1 and C2, digested PCR products from controls 1 and 2; J, A, D, F and H, digested PCR products from the proband and first-degree relatives.

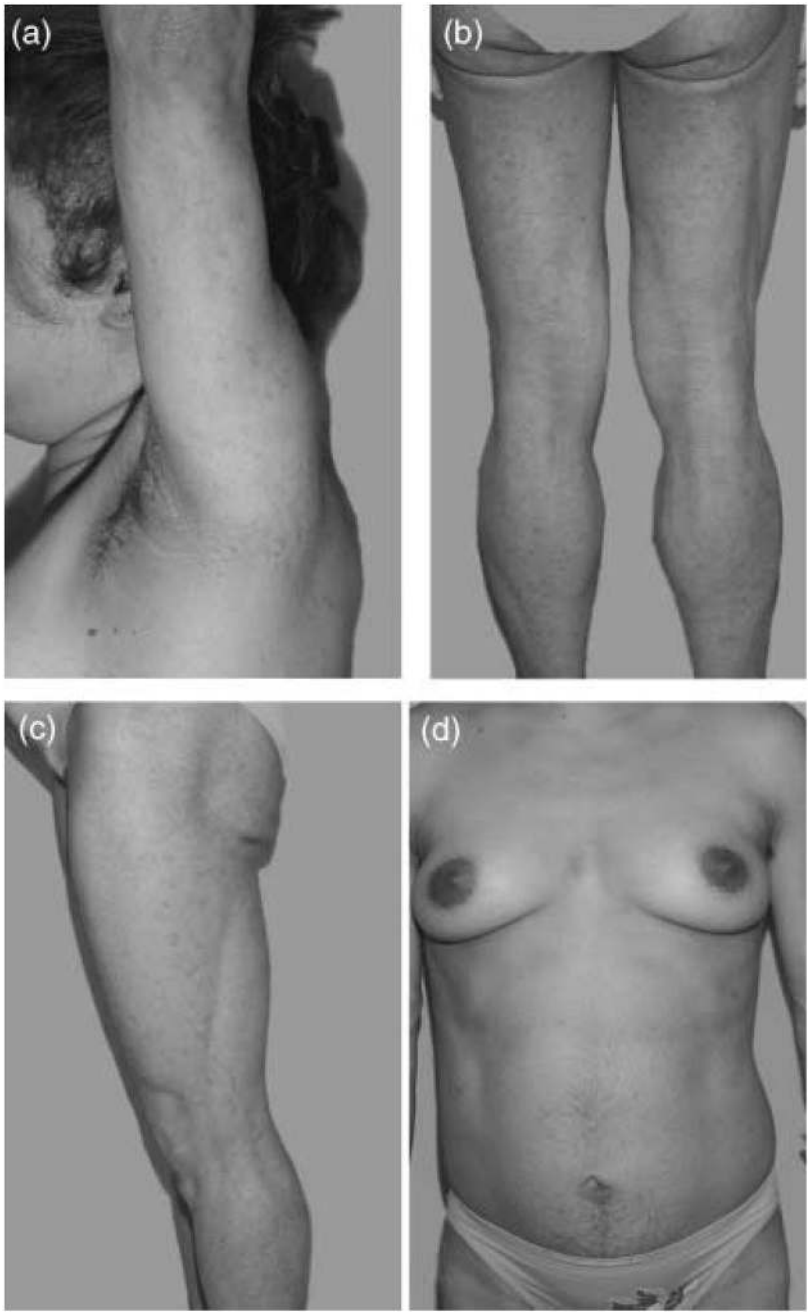

Fig. 2.

Clinical features of the proband Photographs show partial loss of fat affecting the upper (a) and lower extremities as well as the gluteal regions (b and c). Facial (a), truncal and mammary fat (d) is present. Hyperandrogenism is evidenced by muscular hypertrophy in the lower limbs (b and c) and abdomen (d) as well as by coarse terminal hair on the chest and lower abdomen (d). Features of insulin resistance include the presence of acanthosis nigricans in the neck and axilla (a). Skin changes consistent with a previous history of eruptive xanthomas are also apparent (a and c).

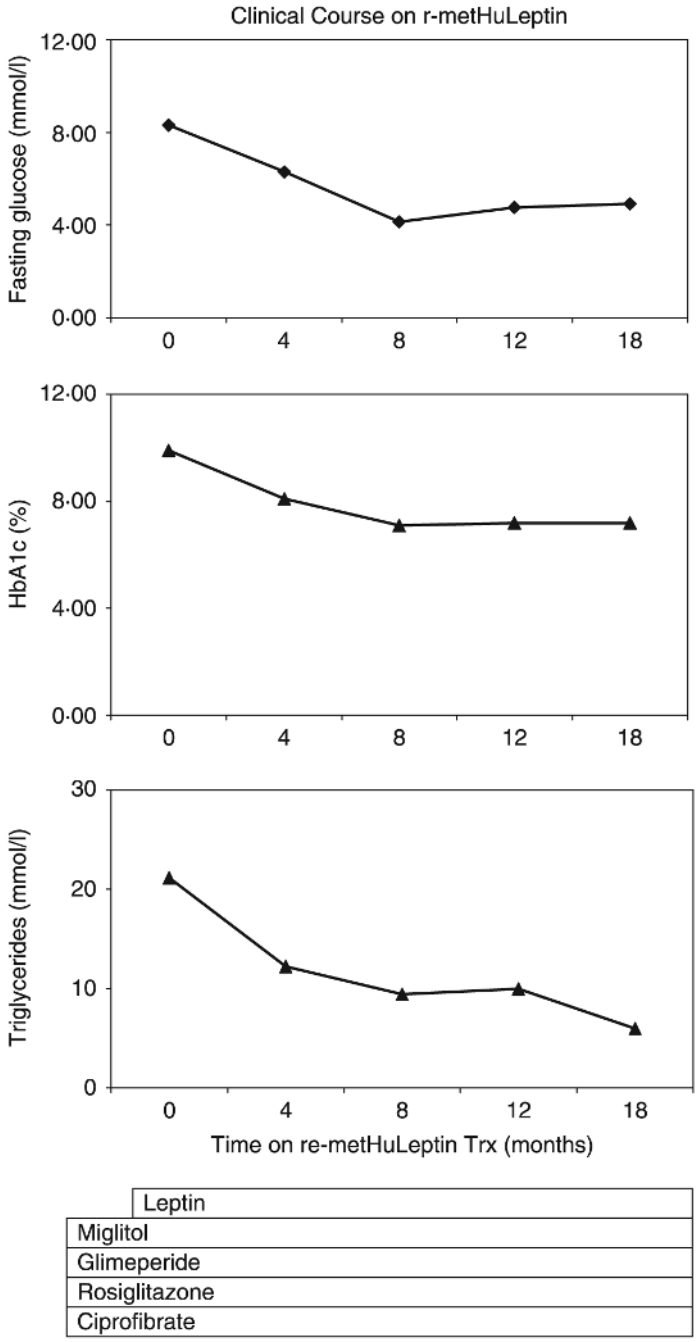

Fig. 3.

Clinical course on r-metHuLeptin Fasting glucose, HbA1c and triglycerides levels over 18 months of leptin therapy. Medications used to treat both diabetes and dyslipidaemia prior to the start of leptin therapy are indicated (doses: miglitol 100 mg/day, glimepiride 6 mg/day, rosiglitazone 8 mg/day, ciprofibrate 100 mg/day). No medication changes besides leptin dose adjustments were made during this time period.

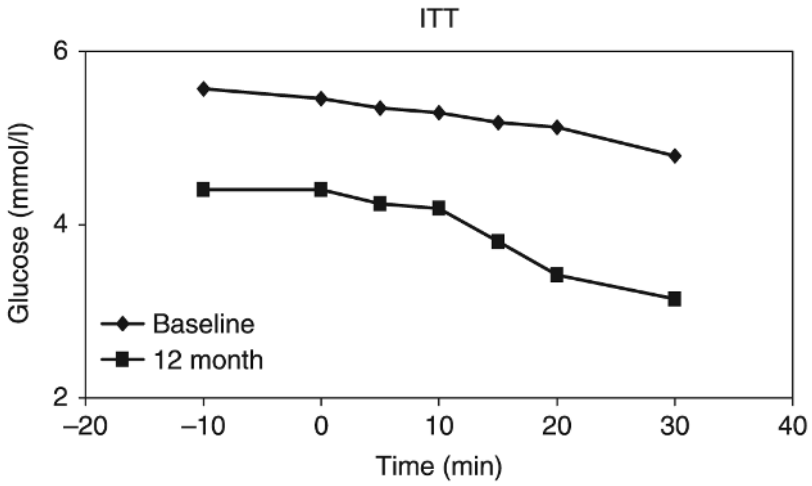

Fig. 4.

Insulin tolerance test (ITT). Fasting glucose response to intravenous insulin (0·2 U/kg) at baseline (filled diamonds) and 12 months (filled squares) of r-metHuLeptin treatment. Slope of the response and absolute decrease in serum glucose at 12 months suggest improvement in insulin responsiveness. Calculated rates of glucose disposal (K_{ITT}) at baseline and 12 months were 1·6%/min and 4·8%/min, respectively. Normal fasting blood glucose precluded testing at 18 months.

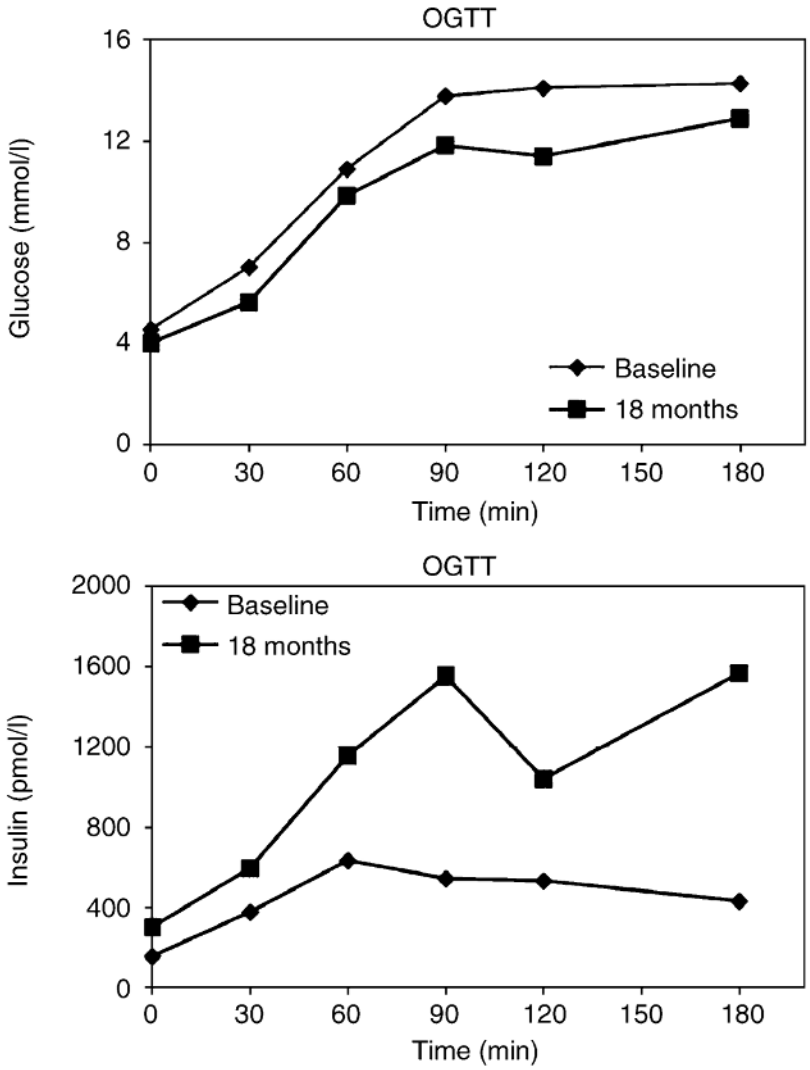

Fig. 5.

Oral glucose tolerance test (OGTT). Glucose and insulin response to 1·75 g/kg oral dextrose challenge. Improved glucose tolerance and a robust insulin response are seen after 18 months of r-metHuLeptin treatment (filled diamonds).

Table 1

Summary of clinical course over 18 months of r-metHuLeptin therapy

	Baseline	4 months	8 months	12 months	18 months
Leptin (nmol/l)	0.23	-	2.13	2.33	-
Fasting glucose (mmol/l)	8.32	6.28	4.13	4.74	4.90
HbA1c (%)	9.90	8.10	7.10	7.20	7.20
Triglycerides (mmol/l)	21.15	12.26	9.46	10.00	5.96
HDL (mmol/l)	0.78	0.75	0.80	0.78	0.65
LDL (mmol/l)	4.11	3.18	2.84	3.13	3.57
Total cholesterol (mmol/l)	8.30	6.00	5.15	6.21	6.23
Fasting GH (μg/l)	0.10	1.00	0.20	4.40	1.00
IGF-1 (μg/l)	-	208.00	180.00	185.00	216.00
C-reactive protein	< 0.40	< 0.40	< 0.40	< 0.40	< 0.40
TSH (mU/l)	1.31	1.10	1.07	1.45	1.10
Free thyroxine (pmol/l))	20.59	20.59	23.17	21.88	24.45
Liver volume (cm^3)	2131	2042	1872	1929	1874
Weight (kg)	54.10	55.70	56.70	54.20	57.50
BMI (kg/m^2)	21.90	23.30	23.90	22.40	23.60
Body fat (%)	14.8	14.8	-	15.1	-
Energy expenditure (kcal/24 h)	1310	1160	1190	1220	1230

Fasting glucose values were obtained on admission after an overnight fast and reflect usual diet. Elevated HbA1c levels confirm inadequate home glycaemic control. Discrepancies between the admission and the OGTT or ITT fasting glucose values for the same admission reflect longer fast times and in-hospital dietary changes [1800 calorie diet (carbohydrate 40%; fat 40%; protein 20%)].