Leptin replacement therapy for the treatment of non-HAART associated lipodystrophy syndromes: a meta-analysis into the effects of leptin on metabolic and hepatic endpoints

Terapia de reposição de leptina para o tratamento de síndromes lipodistróficas não associadas ao uso de antirretrovirais: uma metanálise dos efeitos da leptina em parâmetros metabólicos e hepáticos

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ABSTRACT

The clinical manifestations of lipodystrophy syndromes (LS) are hypoleptinemia, hyperglycemia, insulin resistance, dyslipidemia and hepatic steatosis. Leptin replacement therapy (LRT) is effective at improving these pathologies. Currently, there are no data compiling the evidence from the literature, and demonstrating the effect of LRT in LS patients. A systematic review of the MEDLINE and Cochrane Library databases was conducted to identify studies assessing the effect of LRT on metabolic and hepatic endpoints in patients with LS not associated with highly active antiretroviral therapy (HAART) use. Standardized mean differences (SMD) and 95% confidence intervals of pooled results were calculated for overall changes in glucose homeostasis, lipid profile, and hepatic physiology, using an inverse-variance random-effects model. After screening, 12 studies were included for review. Meta-analysis of results from 226 patients showed that LRT decreased fasting glucose [0.75 SMD units (range 0.36-1.13), p = 0.0001], HbA1c [0.49 (0.17-0.81), p = 0.003], triglycerides [1.00 (0.69-1.31), p < 0.00001], total cholesterol [0.62 (0.21-1.02), p = 0.003], liver volume [1.06 (0.51-1.61), p = 0.0002] and AST [0.41 (0.10-0.73) p = 0.01]. In patients with non-HAART LS, LRT improves the outcome of several metabolic and hepatic parameters. Studies were limited by small populations and therefore large prospective trials are needed to validate these findings. Arq Bras Endocrinol Metab. 2014;58(8):783-97

RESUMO

As manifestações clínicas das síndromes lipodistróficas (SL) incluem hipoleptinemia, hiperglicemia, resistência insulínica, dislipidemia e esteatose hepática. A terapia de reposição de leptina (TRL) melhora tais parâmetros, mas atualmente não há dados compilados demonstrando tal efeito. Uma revisão sistemática dos bancos de dados MEDLINE e Cochrane Library identificou estudos avaliando os efeitos da TRL sobre parâmetros metabólicos e hepáticos em pacientes com SL não associadas ao uso de antirretrovirais. Diferenças médias padronizadas (DMP) e intervalos de confiança de 95% foram calculados a partir dos resultados, para os efeitos da TRL sobre a homeostase da glicose, perfil lipídico, e morfologia/função hepática, usando um modelo de variação inversa e efeitos randômicos. Após a triagem, 12 estudos foram incluídos para revisão. A metanálise dos resultados de 226 pacientes mostrou que a TRL reduziu a glicemia de jejum [0,75 DMP (amplitude 0,36-1,13), p = 0,0001], HbA1c [0,49 (0,17-0,81), p = 0,003], triglicerídeos [1,00 (0,69-1,31), p < 0,00001], colesterol total [0,62 (0,21-1,02), p = 0,003], volume hepático [1,06 (0,51-1,61), p = 0,0002] e AST [0,41 (0,10-0,73), p = 0,001]. Em pacientes com SL não associada ao uso de antirretrovirais, a TRL melhora vários parâmetros metabólicos e hepáticos. Os estudos avaliados foram limitados pelo pequeno número de pacientes. Maiores estudos clínicos prospectivos são necessários para validar tais achados. Arq Bras Endocrinol Metab. 2014;58(8):783-97

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INTRODUCTION

Lipodystrophy syndromes (LS) are congenital and acquired disorders characterized by the generalized or partial absence of subcutaneous adipose tissue. Cases of lipodystrophy not associated with the use of highly active antiretroviral therapy (HAART) are rare conditions, and most determined by molecular defects in genes that regulate adipocyte differentiation, lipid metabolism, and lipid droplet morphology (1). As the adipose tissue is an important endocrine organ that synthesizes hormones with cytokine-like actions (known as adipokines) (2,3), its absence determines several metabolic defects, such as insulin resistance, diabetes, and hypertriglyceridemia, which can lead to the development of atherosclerosis, acute pancreatitis, and nonalcoholic fatty liver disease (NAFLD) (4).

Leptin is one of the most abundant adipokines. It is an important metabolic regulator responsible for not only controlling food intake and energy expenditure, but also for maintaining glucose, insulin and lipid homeostasis (5,6). Impaired leptin action, due to leptin resistance or leptin deficiency, results in several metabolic abnormalities such as insulin resistance, hyperinsulinemia, diabetes, and hypertriglyceridemia, which are similar to those that are observed in LS. In fact, LS and leptin deficiency have similar metabolic manifestations, with low leptin levels (7-9).

Leptin replacement therapy is the treatment of choice for LS. Numerous animal models have shown that exogenous supplementation of leptin, in order to achieve physiological levels of the hormone, is effective in restoring normal glycemic control, normal serum lipid profile and liver function (10,11). In humans, leptin replacement therapy (LRT) with recombinant methionyl human leptin (r-metHuLeptin, metreleptin) has been evaluated by several clinical trials (12), and has been recently approved by the FDA for the treatment of patients with generalized lipodystrophy (13). Administered as a subcutaneous injection once or twice daily, metreleptin reverses the metabolic abnormalities that are seen in LS, leading to significant improvements in overall health (14-16).

Currently, there is no data that comprehensively and succinctly demonstrate the effect of LRT on metabolic and hepatic parameters in LS patients. Therefore, this meta-analysis sought to survey the literature in this area, and quantitatively assess the effect of LRT in patients with all forms of LS (generalized and partial, congenital and acquired; excluding HAART-related LS) on various clinical endpoints. We sought data from clinical trials and case series on blood concentrations of glucose, insulin and glycated hemoglobin (Hb1Ac) as markers of glycemic control; triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol as markers of lipid control; and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin as markers of liver function. Also, total liver volume and liver fat were assessed as radiological findings of liver health in response to LRT.

MATERIALS AND METHODS

Literature search

We conducted a literature review of published work in accordance with the PRISMA guidelines (17). Relevant studies were retrieved on 9/26/2013 from the MEDLINE (January 1966 to September 2013) and Cochrane Library of Clinical Trials (January 1980 to September 2013) databases. In order to identify studies assessing the outcomes of LRT for the treatment of all forms of LS not related to HAART, we applied as search terms the following title/abstract phrases: ["leptin" AND ("lipodystrophy" OR "lipoatrophy")] with no language restriction. Titles and abstracts of identified searches were screened. In addition, reference lists of all articles were manually scanned and the 'related article tool' provided following a term search was used to identify other potentially relevant studies. We termed these the "grey literature". The full texts were assessed to determine eligibility for inclusion. Studies were eligible if they assessed the outcomes of systemically administered leptin as a treatment for LS. Specific exclusion criteria were: i) study population consisting of participants whose LS were a result of HAART for the treatment of HIV; ii) studies that did not report specific data on the outcome of glycemic, lipid or hepatic measures following leptin treatment; iii) studies evaluating an agent other than leptin or a derivative of leptin (e.g. r-metHu-leptin/metreleptin); iv) case-reports of a single individual patient; v) studies published in languages other than English.

Data abstraction

The primary outcome of this review was to quantitatively assess and compare the impact of LRT on the

blood concentrations of glucose, insulin, HbA1c, TG, TC, HDL, LDL, ALT, AST and albumin, and on liver fat and volume. Data capture was performed independently by two examiners (AJR, AGG). Data relating to the study design (including population, patient sampling, follow-up time and leptin replacement dosage), and baseline characteristics of study participants (including age, body mass index, LS etiology and other risk factors) were extracted. Further, we evaluated laboratory methods used to determine levels of systemic markers of leptin replacement outcomes. Additionally the quality of each study was assessed using a quality assessment questionnaire, which was modified from the previously validated QUADAS 2 tool as there is currently no accepted quality assessment tool for studies examining leptin to treat LS (18). Our questionnaire contained eight points relating to study design, outcome measurement and analysis. Each point was marked as 'yes', 'no' or 'unclear', and the quality percentage score was based on the percentage of 'yes' responses that was obtained by each study. Sample copies of the data extraction form and quality assessment questionnaire are available upon request. Any questions or inconsistencies regarding these data were resolved through iteration and consensus. We included patients with acquired, congenital, partial and generalized forms of LS, and evaluated the effects of LRT collectively and separately by LS subtype.
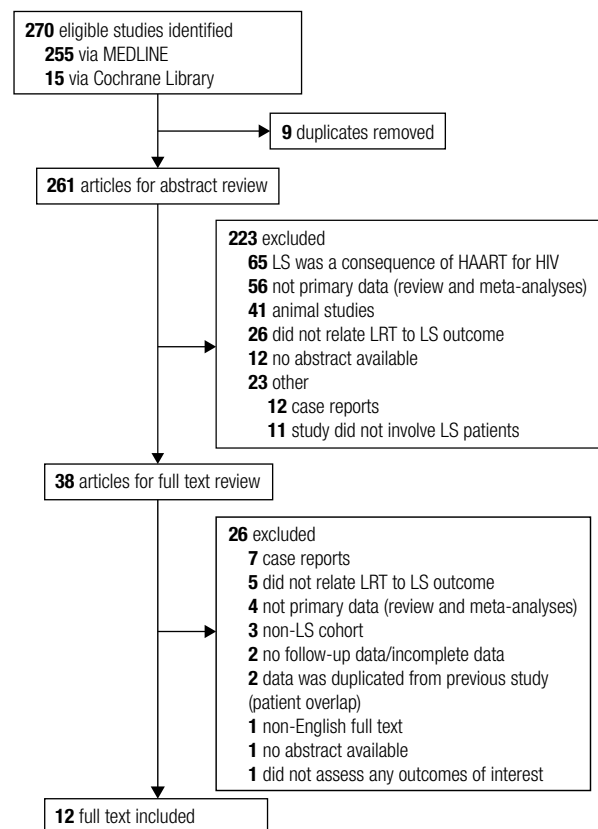
Statistical methods

To mitigate against differences in the way marker concentrations were measured and reported, the absolute levels of the aforementioned markers were converted into a common unit by calculating a standardized mean difference (SMD). Standardized mean differences were derived from the reported p-values, by calculating the t-statistic and dividing by the square root of the population size. Standard errors were estimated as the inverse of the square root of the population size. We did not include data where significance tests were not performed (i.e. where p-values were not reported). Heterogeneity was determined by the I^2 index, which provides an indication into the inconsistencies between the studies (19). Results were presented as mean ± standard deviation (SD) or median and interquartile range (IQR). Meta-analysis was performed using an inverse-variance random effects model with RevMan v5.1 software (The Nordic Cochrane Centre, The Cochrane Collaboration, 2012).

This software allows for the automated output of forest plots, I^2 index, test for overall effect, effect significance and a funnel plot as an indication into publication bias.

RESULTS

Included literature

The initial database search yielded 270 abstracts, of which 261 unique abstracts were reviewed for eligibility. Following abstract screening, 223 were excluded because they did not fit the inclusion criteria; mainly because they evaluated only HAART-associated LS (n = 65), or did not include primary original data (n = 56). Thirty-eight articles were eligible for full-text review. Of those, 26 were excluded mainly due to not being population-based data (case reports) or review articles and two of these studies were excluded as they represented data sets based on duplicated results from previous studies (20,21). Overall, 12 studies were included for data analysis (14,16,22-31). Figure 1 illustrates the flow diagram outlining literature search strategy and study selection.

270 eligible studies identified
255 via MEDLINE
15 via Cochrane Library

9 duplicates removed

261 articles for abstract review

223 excluded
65 LS was a consequence of HAART for HIV
56 not primary data (review and meta-analyses)
41 animal studies
26 did not relate LRT to LS outcome
12 no abstract available
23 other
12 case reports
11 study did not involve LS patients

38 articles for full text review

26 excluded
7 case reports
5 did not relate LRT to LS outcome
4 not primary data (review and meta-analyses)
3 non-LS cohort
2 no follow-up data/incomplete data
2 data was duplicated from previous study (patient overlap)
1 non-English full text
1 no abstract available
1 did not assess any outcomes of interest

12 full text included

LRT: leptin replacement therapy; LS: lipodystrophy syndromes.

Figure 1. Flow diagram outlining literature search strategy and study selection.

Quality assessment

Quality percentage scores based on a modified QUADAS 2 tool ranged from 25% (30) to 75% (16,23), and the overall mean score was 56.3 ± 14.6% (Table 1).

Study participants

Pooled together, the evaluated studies represented a composite population of 226 participants (45 males, 181 females). Studies had samples sizes ranging from 4 to 55, where the mean number of participants was 19.8 ± 16.8. Follow-up times ranged from 3 to 100 months, where the mean time was 26.9 ± 27.2 months.

Table 1 summarizes the characteristics of the selected studies. The average age of the participants was 26.5 ± 10.0 years. As shown in table 2, the average percentage of male participants was 22.4 ± 25.7%, and four studies did not include males in the study population (27-30). Where reported, the mean baseline BMI (body mass index) of study participants was 20.9 ± 1.9 kg/m², mean baseline body fat percentage was 11.6 ± 6.14%, and mean baseline fasting leptin concentration was 1.87 ± 0.95 ng/mL. The number of diabetic patients was reported in only two studies (28,30) (n = 8/9 and 1/9 participants, respectively) (Table 2).

Table 1. Studies characteristics

Author (reference)	Study details			LS etiology				Outcome assessment	Quality score (%)
	n	Design	Follow-up (months)	AGL (n)	CGL (n)	Partial (n)	Other (n)		
Beltrand and cols. (22)	7	Prospective	4	n/a	7	n/a	n/a	MWU test	50
Beltrand and cols. (23)	7	Prospective	28	n/a	7	n/a	n/a	MWU test	75
Chan and cols. (14)	55	Prospective	36	13	23	19	n/a	t-test	50
Chong and cols. (24)	48	Prospective	100	12	31	n/a	4	t-test	62.5
Ebihara and cols. (25)	7	Prospective	36	2	5	n/a	n/a	ANOVA	37.5
Javor and cols. (26)	15	Prospective	12	4	11	n/a	n/a	t-test, ANOVA	50
Moran and cols. (27)	14	Prospective	12	5	7	n/a	2	t-test, ANOVA	62.5
Oral and cols. (28)	9	Prospective	12	3	5	1	n/a	t-test, ANOVA	62.5
Park and cols. (29)	4	Prospective	48	n/a	n/a	4	n/a	t-test	62.5
Petersen and cols. (30)*	9	Case-control	3	1	2	n/a	n/a	t-test	25
Safar Zadeh and cols. (31)	27	Prospective	26	5	10	12	n/a	t-test	62.5
Simha and cols. (16)	24 (SH)	Prospective	6	n/a	n/a	24	n/a	ANOVA	75
	24 (MH)	Prospective	6	n/a	n/a	24	n/a	ANOVA	75

AGL: acquired generalized lipodystophy; CGL: congenital generalized lipodystrophy; MWU: Mann-Whitney U test; ANOVA: analysis of variance; n/a: not applicable.
Simha and cols. (16) divided analysis into patient with severe (SH) and mild (MH) hypoleptinemia.
* The etiology of all cases was not completely reported.

Table 2. Patients' characteristics

Reference	Age (y)	Male (%)	BMI (kg/m²)	Fat (%)	Leptin (ng/mL)	DM (n)
Beltrand and cols. (22)	10 ± 4	86	NR	2.0 ± 0.8	0.7 ± 0.4	0
Beltrand and cols. (23)	12 ± 4	71	NR	4.5 ± 0.4	1.85 ± 1.0	0
Chan and cols. (14)	25 (7-68)	20	22.2 ± 3.4	NR	2.8 ± 2.8	0
Chong and cols. (24)	18 (8-68)	19	NR	11.7 ± 1.0	2.53 ± 0.4	0
Ebihara and cols. (25)	21 ± 3	28	17.6 ± 1.1	NR	1.09 ± 0.08	0
Javor and cols. (26)	23 ± 3	13	21.5 ± 0.9	7.9 ± 0.5	1.6 ± 0.2	0
Moran and cols. (27)	30 ± 3	21	21.7 ± 0.8	9.5 ± 1.6	1.7 ± 0.3	0
Oral and cols. (28)	26 ± 10	0	NR	15.2 ± 6.0	1.21 ± 1	8
Park and cols. (29)	46 ± 4	0	23.3 ± 0.8	21.6 ± 1.9	3.8 ± 0.3	0
Petersen and cols. (30)	27 ± 9	0	19.2 ± 2.0	7.1 ± 2.1	0.6 ± 0.1	1
Safar Zadeh and cols. (31)	29 ± 3	22	NR	NR	2.72 ± 0.5	0
Simha and cols. (16) (SH)	41 ± 13	6	NR	17.2 ± 3.4	NR	0
Simha and cols. (16) (MH)	36 ± 17	6	NR	18.8 ± 2.9	NR	0
Average ± SD	26.5 ± 10	22.4 ± 25.7	20.9 ± 1.9	11.6 ± 6.1	1.86 ± 0.95	n/a

BMI: body mass index; DM: *diabetes mellitus*; NR: not reported; SH: severe hypoleptinemia; MH: mild hypoleptinemia; n/a: not applicable.

LRT details

All twelve studies included for review used r-metHu-Leptin as the form of leptin supplementation. Administration method was uniform, as all patients received r-metHuLeptin via subcutaneous injections. Most studies (n = 10) performed r-metHuLeptin administration twice daily (Table 3). Dosage of r-metHuLeptin ranged from 0.02 to 0.24 mg/kg/d for males and 0.04 to 0.24 mg/kg/d for females. For studies that did not stratify leptin dose according to gender, dosages ranged from 0.03 to 0.24 mg/kg/d (16,22,23,28,31). Instead of reporting daily leptin dosages per body weight, Chan and cols. reported the mean total dose received by patients, which was 2.75 ± 0.43 mg for males and 5.55 ± 0.33 mg for females (14). One study did not provide sufficient data on leptin administration dosages (30). These data are summarized in table 3.

Comparative data analysis

Glycemic control

Seven studies reported significant reductions in blood glucose (24-26,28-31). The largest reduction was seen in the study by Petersen and cols. (30), where patients with LS had an initial mean baseline fasting glucose concentration of 234 ± 14 mg/dL. Following the three-month intervention, this declined to 122 ± 21 mg/dL.

Three studies reported a reduction in fasting insulin following LRT but did not report whether this was significant (16,22,30). Four studies reported a significant reduction in HbA1c values at follow-up (24,26,28,31), with the highest reduction being equal to 1.90% (28). One study reported an increase of 1.77% from baseline mean HbA1c, but did not report whether this was significant (23). Supplementary table 1 illustrates the changes in fasting plasma glucose, insulin and HbA1c.

Hepatic outcomes

Three studies reported significant reductions in mean liver volume following leptin therapy (22,26,27). Liver fat was only reported in a single study and after stratifying results according to the participants' degree of hypoleptinemia, either severely or mildly hypoleptinemic, both groups of patients exhibited significant decreases in mean liver fat percentage (16) (Supplementary Table 2).

Serum albumin concentrations were evaluated by a single study, which reported no significant changes after LRT (27). Two studies reported significant decreases in ALT and AST (22,31). In the study by Simha and cols., neither cohort of participants showed significant reductions in ALT, but severely hypoleptinemic patients showed a significant reduction in AST levels (16). Supplementary table 3 shows the changes in albumin and liver enzymes levels.

Table 3. Leptin administration and liver assessment details

Reference	Leptin dosage (mg/kg/d)	Frequency	Liver assessment
Beltrand and cols. (22)	0.03	Once daily	CT
Beltrand and cols. (23)	0.06-0.12	Once daily	CT
Chan and cols. (14)	2.75 ± 0.43* (m); 5.55 ± 0.33* (f)	1-2x daily	n/a
Chong and cols. (24)	(1) 0.06; (2) 0.08-0.12; (3) 0.24	Twice daily	n/a
Ebihara and cols. (25)	0.02 (m); 0.03 (y); 0.04 (f)	Twice daily	CT/MRI
Javor and cols. (26)	0.06-0.08 (f); 0.04 (m)	Twice daily	MRI
Moran and cols. (27)	0.03 (y); 0.04 (f); 0.02 (m)	Twice daily	MRI
Oral and cols. (28)	0.03 (y); 0.04 (f)	Twice daily	n/a
Park and cols. (29)	0.08	Twice daily	MRI
Petersen and cols. (30)	Unclear	Twice daily	n/a
Safar Zadeh and cols. (31)	0.06-0.24	1-2x daily	Biopsy
Simha and cols. (16) (SH)	0.08	Twice daily	MRI
Simha and cols. (16) (MH)	0.08	Twice daily	MRI

m: male data; f: female data; y: youth data; SH: severe hypoleptinemia; MH: mild hypoleptinemia; n/a: not available. "1", "2" and "3" refer to subgroups of the study. CT: computed tomography, MRI: magnetic resonance imaging.
* Dose shown as mg/day.

Supplementary Table 1. Comparison of fasting plasma glucose, insulin and HbA1c at baseline and follow-up

Reference	Fasting plasma glucose (mg/dL)			Fasting insulin (pmol/L)			HbA1c (%)		
	B	F	P	B	F	P	B	F	P
Beltrand and cols. (22)	80 ± 7	80 ± 5	NS	135 ± 84	91 ± 78	NS	NR	NR	NR
Beltrand and cols. (23)	NR	NR	NR	212 ± 92	601 ± 1055	NR	5.4 ± 1.0	7.2 ± 4.0	NR
Chan and cols. (14)	184 ± 91	124 ± 13	NR	NR	NR	NR	8.5 ± 2.1	6.3 ± 0.3	NR
Chong and cols. (24)	164 ± 13	129 ± 12	0.014	NR	NR	NR	8.4 ± 2.0	6.9 ± 1.7	< 0.001
Ebihara and cols. (25)	172 ± 2	113 ± 9	< 0.01	NR	NR	NR	9.3 ± 0.4	NR	NR
Javor and cols. (26)	205 ± 19	126 ± 11	< 0.001	NR	NR	NR	9.0 ± 0.4	7.1 ± 0.5	< 0.001
Moran and cols. (27)	NR	NR	NR	NR	NR	NR	9.1	7.1	NR
Oral and cols. (28)	230 ± 74	132 ± 47	< 0.001	NR	NR	NR	9.1 ± 0.5	7.2 ± 0.5	0.001
Park and cols. (29)	190 ± 26	151 ± 15	0.006	NR	NR	NR	8.4 ± 0.6	8.0 ± 0.4	NS
Petersen and cols. (30)	234 ± 14	122 ± 21	< 0.05	181 ± 28	160 ± 28	NS	8.5 ± 1.0	8.2 ± 1.3	NS
Safar Zadeh and cols. (31)	178 ± 15	117 ± 9	0.004	NR	NR	NR	7.9 ± 0.4	6.3 ± 0.2	0.0009
Simha and cols. (16) (SH)	111 ± 39	106 ± 32	NS	276 ± 223	209 ± 166	NS	6.7 ± 1.7	6.3 ± 1.5	NS
Simha and cols. (16) (MH)	111 ± 4	97 ± 24	NS	432 ± 315	298 ± 366	NS	6.4 ± 1.8	6.7 ± 2.6	NS

B: baseline; F: follow-up; P: P-value; NS: not significant; NR: not reported; SH: severe hypoleptinemia; MH: mild hypoleptinemia.

Supplementary Table 2. Comparison of liver volume and fat liver percentage at baseline and follow-up

Reference	Liver volume (L)			Liver fat (%)		
	B	F	P	B	F	P
Beltrand and cols. (22)*	4.5 ± 1.4	3.2 ± 0.9	0.002	NR	NR	NR
Ebihara and cols. (25)	1.9 ± 0.1	1.5 ± 0.1	NR	NR	NR	NR
Javor and cols. (26)	3.7 ± 0.3	2.2 ± 0.1	< 0.001	NR	NR	NR
Moran and cols. (27)	3.0 ± 0.3	2.2 ± 0.2	< 0.001	NR	NR	NR
Park and cols. (29)	2.1 ± 0.2	2.0 ± 0.3	NS	NR	NR	NR
Simha and cols. (16) (SH)	NR	NR	NR	8.8 (5.2-11.9)	4.9 (1.6-11.6)	< 0.001
Simha and cols. (16) (MH)	NR	NR	NR	23.7 (10.2-34.2)	9.2 (7.3-35.5)	< 0.01

B: baseline; F: follow-up; P: P-value; NS: not significant; NR: not reported; SH: severe hypoleptinemia; MH: mild hypoleptinemia.
* Result provided as z-score.

Supplementary Table 3. Comparison of liver enzymes and albumin at baseline and follow-up

Reference	ALT (U/L)			AST (U/L)			Albumin (g/L)		
	B	F	P	B	F	P	B	F	P
Beltrand and cols. (22)	105 ± 99	35 ± 17	0.02	47 ± 41	25 ± 7	0.04	NR	NR	NR
Beltrand and cols. (23)	67 ± 43	51 ± 23	NR	35.1 ± 15	40.4 ± 17	NR	NR	NR	NR
Chan and cols. (14)	100 ± 120	29 ± 4	NR	71 ± 77	24 ± 1	NR	NR	NR	NR
Ebihara and cols. (25)	81 ± 24	32 ± 5	NR	42 ± 11	22 ± 4	NR	18 ± 11	NR	NR
Moran and cols. (27)	NR	NR	NR	NR	NR	NR	40 ± 1	37 ± 2	NS
Safar Zadeh and cols. (31)	99 ± 19	53 ± 12	0.002	66 ± 11	35 ± 5	0.002	NR	NR	NR
Simha and cols. (16) (SH)	25 ± 11	21 ± 8	NS	24 ± 9	20 ± 8	< 0.05	NR	NR	NR
Simha and cols. (16) (MH)	34 ± 17	35 ± 29	NS	26 ± 8	245 ± 14	NS	NR	NR	NR

AST: aspartate aminotransferase; ALT: alanine aminotransferase; B: baseline; F: follow-up; P: P-value; NS: not significant; NR: not reported; SH: severe hypoleptinemia; MH: mild hypoleptinemia.

Supplementary Table 4. Comparison of blood lipids at baseline and follow-up

Reference	Triglycerides (mg/dL)			Total cholesterol (mg/dL)			HDLc (mg/dL)			LDLc (mg/dL)		
	B	F	P	B	F	P	B	F	P	B	F	P
Beltrand and cols. (22)	6.8 ± 3.2*	2.5 ± 1.0*	0.017	0.5 ± 2.1*	0.9 ± 0.2*	0.009	NR	NR	NR	NR	NR	NR
Beltrand and cols. (23)	NR	NR	NR	151 ± 41	188 ± 73	NR	32 ± 5	33 ± 8	NR	NR	NR	NR
Chan and cols. (14)	479 ± 80	164 ± 26	NR	244 ± 148	142 ± 9	NR	32 ± 9	34 ± 2	NR	118 ± 48	75 ± 10	NR
Chong and cols. (24)	NR	NR	NR	253 ± 20	164 ± 8	< 0.001	32 ± 2	31 ± 2	NS	110 ± 12	267 ± 66	NS
Ebihara and cols. (25)	700± 272	178 ± 58	< 0.01	233 ± 18	NR	NR	NR	NR	NR	NR	NR	NR
Javor and cols. (26)	1380 ± 500	516 ± 236	< 0.001	NR	NR	NR	31 ± 3	29 ± 2	NS	139 ± 16	85 ± 7	0.01
Oral and cols. (28)	1405 ± 2926	563 ± 338	< 0.001	NR	NR	NR	NR	NR	NR	NR	NR	NR
Park and cols. (29)	749 ± 331	510 ± 269	0.026	280 ± 49	231 ± 41	0.012	40 ± 3	36 ± 5	NS	135 ± 4	118 ± 8	NS
Petersen and cols. (30)	5851 ± 5079	1134 ± 523	< 0.003	360 ± 130	161 ± 12	NS	44 ± 24	20 ± 2	NS	NR	65 ± 13	NR
Safar Zadeh and cols. (31)	952 ± 291	303 ± 65	0.0002	NR	NR	NR	NR	NR	NR	NR	NR	NR
Simha and cols. (16) (SH)	228 (180-506)	183 (116-220)	< 0.05	185 ± 46	169 ± 28	NS	34 ± 8	35 ± 9	NS	NR	NR	NR
Simha and cols. (16) (MH)	423 (295-813)	339 (275-359)	< 0.05	237 ± 66	242 ± 134	NS	33 ± 7	33 ± 5	NS	NR	NR	NR

NS: not significant; NR: not reported; * Reported as z score.

Lipid profile

Seven studies reported significant reductions in serum TG (22,25,26,28-31). The largest reduction was shown by Petersen and cols., where patients had a mean baseline TG level of 5,851 ± 5,079 mg/dL, decreasing to 1,134 ± 523 mg/dL following three months of LRT (30). When stratified by hypoleptinemic status, significant reductions in TG concentration were observed in participants with mild and severe hypoleptinemia (16). Two studies showed significant reductions in TC concentrations (24,29), and one study reported a significant increase in TC levels following LRT (22). Six studies recorded changes in HDL following LRT (7,22,24,26,28,30), but none of them reported significant changes. One study reported a significant decrease in LDL from a mean of 139 ± 16 mg/dL at baseline and 85 ± 7 mg/dL following 12 months of leptin replacement (26). Supplementary table 4 illustrates all the changes in blood lipids following LRT.

Sub-analysis of LRT on LS subtype

In order to assess whether the effects of LRT varied according to the type of LS, we compiled all available individual patient data from the included studies for each of the endpoints assessed. However, besides not being large enough, results from this data set were very heterogeneous within groups, not allowing meaningful comparative analyses. For example, liver fat data were available for 8 patients with generalized LS and for zero patients with partial LS. Briefly, in endpoints with available data, median changes in glycemic parameters were larger in patients with generalized LS (n = 30), compared to patients with partial LS (n = 7): fasting glucose decreased by a median 47.50 mg/dL in patients with generalized LS, and by 28.00 mg/dL in patients with partial LS; HbA1c was reduced by 1.85% in patients with generalized LS, compared to a reduction of 0.90% in patients with partial LS. However median reductions in TG were greater in patients with partial LS (332.5 mg/dL) compared to patients with generalized LS (306.3 mg/dL). When stratifying by acquired or congenital forms, where data is available, patients with acquired LS (n = 5) showed greater median decreases in glucose (100.0 mg/dL *vs.* 57.0 mg/dL), HbA1c (1.30% *vs.* 0.90%), and TG (822.0 mg/dL *vs.* 238.0 mg/dL) relative to patients with congenital LS (n = 24).

Meta-analysis

Leptin replacement therapy produced a reduction in fasting plasma glucose in patients with LS. The standardized mean difference was 0.75, with a 95% confidence interval ranging from 0.36 to 1.13, representing an overall significant decrease (p = 0.0001). Heterogeneity for this test was high (I^2 = 84%) (Supplementary Figure 1A). No study reported a significant alteration in fasting insulin following LRT, and this was reflected in a change in SMD of 0.00 (-0.41, 0.41; p = 1.00) (Supplementary Figure 1B). As this analysis showed a heterogeneity of 0%, meta-analysis was performed using a fixed-effects model. Patients on LRT experienced an overall

decrease in HbA1c, with a SMD of 0.49 (0.17-0.81). This effect was significant (p = 0.003) and heterogeneity was high (I² = 75%) (Supplementary Figure 1C).

Liver volume of LS patients significantly decreased following LRT. Three studies had significant re-

ductions, and the overall SMD was 1.06 (0.51, 1.61), where p = 0.0002. Heterogeneity was 64% (Supplementary Figure 2A). Liver transaminases were significantly decreased when comparing mean data in cases and controls; however only AST showed a significant

A. Glucose

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Simha (MH) 2012 (n = 24)	0.0	0.2	11.3	0.00 [-0.39, 0.39]
Simha (SH) 2012 (n = 24)	0.0	0.2	11.3	0.00 [-0.39, 0.39]
Beltrand 2007 (n = 7)	0.0	0.38	8.7	0.00 [-0.74, 0.74]
Chong 2010 (n = 48)	0.37	0.14	12.1	0.37 [0.10, 0.64]
Safar Zadeh 2013 (n = 27)	0.61	0.19	11.5	0.61 [0.24, 0.98]
Peterson 2002 (n = 9)	0.75	0.33	9.4	0.75 [0.10, 1.40]
Javor 2005 (n = 15)	1.05	0.26	10.5	1.05 [0.54, 1.56]
Ebihara 2007 (n = 7)	1.32	0.38	8.7	1.32 [0.58, 2.06]
Oral 2002 (n = 9)	1.59	0.33	9.4	1.59 [0.94, 2.24]
Park 2007 (n = 4)	2.66	0.5	7.0	2.66 [1.68, 3.64]
Total (95% CI)			100.0	0.75 [0.36, 1.13]

Heterogeneity: Tau² = 0.30; Chi² = 54.67, df = 9 (P < 0.00001); I² = 84%
Test for overall effect: Z = 3.79 (P = 0.0001).

B. Insulin

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Beltrand 2010 (n = 7)	0.0	0.38	30.1	0.00 [-0.74, 0.74]
Peterson 2002 (n = 9)	0.0	0.33	39.9	0.00 [-0.65, 0.65]
Beltrand 2007 (n = 7)	0.0	0.38	30.1	0.00 [-0.74, 0.74]
Total (95% CI)			100.0	0.00 [-0.41, 0.41]

Heterogeneity: Chi² = 0.00, df = 2 (P = 1.00); I² = 0%
Test for overall effect: Z = 0.00 (P = 1.00).

C. HbA1c

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Park 2007 (n = 4)	0.0	0.5	6.5	0.00 [-0.98, 0.98]
Peterson 2002 (n = 9)	0.0	0.33	9.8	0.00 [-0.65, 0.65]
Simha (SH) 2012 (n = 24)	0.0	0.2	13.0	0.00 [-0.39, 0.39]
Simha (MH) 2012 (n = 24)	0.0	0.2	13.0	0.00 [-0.39, 0.39]
Beltrand 2010 (n = 7)	0.5	0.38	8.7	0.50 [-0.24, 1.24]
Chong 2010 (n = 48)	0.51	0.14	14.4	0.51 [0.24, 0.78]
Safar Zadeh 2013 (n = 27)	0.72	0.19	13.3	0.72 [0.35, 1.09]
Javor 2005 (n = 15)	1.05	0.26	11.5	1.05 [0.54, 1.56]
Oral 2002 (n = 9)	1.59	0.33	9.8	1.59 [0.94, 2.24]
Total (95% CI)			100.0	0.49 [0.17, 0.81]

Heterogeneity: Tau² = 0.17; Chi² = 32.26, df = 8 (P < 0.0001); I² = 75%
Test for overall effect: Z = 2.96 (P = 0.003).
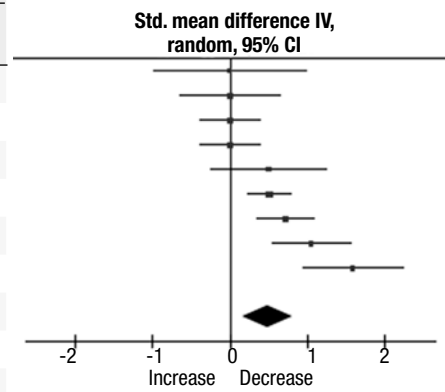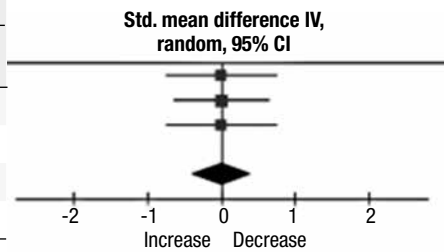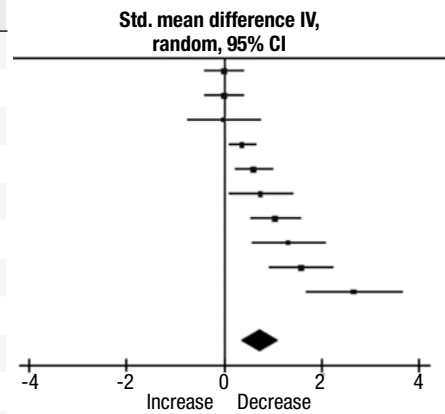
IV: inverse-variance model; CI: confidence interval.

Supplementary Figure 1. Forest plot analyzing the effects of leptin replacement therapy on fasting plasma glucose (**A**), insulin (**B**) and HbA1c (**C**).

reduction in meta-analysis 0.41 (0.10, 0.73) where p = 0.01 and heterogeneity was 52%. For ALT, the SMD was a non-significant 0.36 (-0.03, 0.75), p = 0.07 and heterogeneity was 69% (Supplementary Figure 2E). Meta-analysis was not performed from results of liver fat or albumin as these data sets were based on data from single studies only.

Triglycerides and total cholesterol were significantly reduced. The SMD for TG was 1.00 (0.69, 1.31), where the heterogeneity was high ($I^2 = 64\%$) and p <

0.00001 (Supplementary Figure 3A). The effect on TC was 0.62 (0.21, 1.02), where p = 0.003 and $I^2 = 80\%$ (Supplementary Figure 3B). There was no significant overall effect on HDL and LDL levels. The overall SMD for HDL was a non-significant 0.00 (-0.16, 0.17) (Supplementary Figure 3C). Similarly, a single study reported a standardized mean reduction in LDL (25). However, the overall effect of leptin on LDL levels was not significant [0.21 units (-0.20, 0.62)] (Supplementary Figure 3D).

| A. Liver volume					
Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects	Std. mean difference IV, random, 95% CI
Park 2007 (n = 4)	0	0.5	17.7	0.00 [-0.98, 0.98]	
Javor 2005 (n = 15)	1.05	0.26	29.9	1.05 [0.54, 1.56]	
Moran 2004 (n = 14)	1.11	0.27	29.3	1.11 [0.58, 1.64]	
Beltrand 2007 (n = 7)	1.81	0.38	23.1	1.81 [1.07, 2.55]	
Total (95% CI)			100.0	1.06 [0.51, 1.61]	

Heterogeneity: Tau² = 0.20; Chi² = 8.37, df = 3 (P = 0.04); I² = 64%.
Test for overall effect: Z = 3.77 (P = 0.0002).

B. ALT					
Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects	Std. mean difference IV, random, 95% CI
Simha (SH) 2012 (n = 24)	0.0	0.2	23.5	0.00 [-0.39, 0.39]	
Simha (MH) 2012 (n = 24)	0.0	0.2	23.5	0.00 [-0.39, 0.39]	
Beltrand 2010 (n = 7)	0.28	0.38	14.5	0.28 [-0.46, 1.02]	
Safar Zadeh 2013 (n = 27)	0.66	0.19	24.0	0.66 [0.29, 1.03]	
Beltrand 2007 (n = 7)	1.13	0.38	14.5	1.13 [0.39, 1.87]	
Total (95% CI)			100.0	0.36 [-0.03, 0.75]	

Heterogeneity: Tau² = 0.13; Chi² = 12.86, df = 4 (P = 0.01); I² = 69%.
Test for overall effect: Z = 1.82 (P = 0.07).

C. AST					
Study	SMD	SE	Weight (%)	SMD, 95% CI, Random effects	Std. mean difference IV, random, 95% CI
Simha (SH) 2012 (n = 24)	0.0	0.2	24.8	0.00 [-0.39, 0.39]	
Beltrand 2010 (n = 7)	0.17	0.38	12.3	0.17 [-0.57, 0.91]	
Simha (MH) 2012 (n = 24)	0.42	0.2	24.8	0.42 [0.03, 0.81]	
Safar Zadeh 2013 (n = 27)	0.66	0.19	25.8	0.66 [0.29, 1.03]	
Beltrand 2007 (n = 7)	0.95	0.38	12.3	0.95 [0.21, 1.69]	
Total (95% CI)			100.0	0.41 [0.10, 0.73]	

IV: inverse-variance model; CI: confidence interval.

Heterogeneity: Tau² = 0.06; Chi² = 8.34, df = 4 (P = 0.08); I² = 52%.
Test for overall effect: Z = 2.57 (P = 0.01).
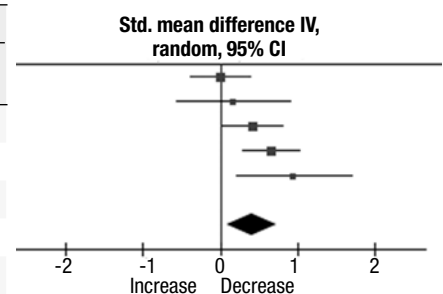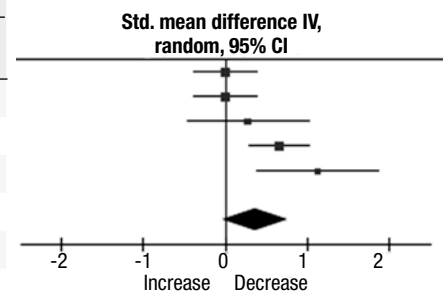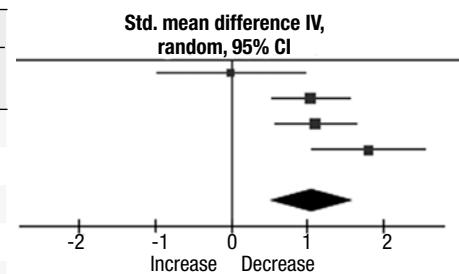
Supplementary Figure 2. Forest plot analyzing the effect of leptin replacement therapy on liver volume (**A**), ALT (**B**) and AST (**C**).

A. Triglycerides

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Simha (SH) 2012 (n = 24)	0.42	0.2	14.3	0.42 [0.03, 0.81]
Simha (MH) 2012 (n = 24)	0.42	0.2	14.3	0.42 [0.03, 0.81]
Safar Zadeh 2013 (n = 27)	0.83	0.19	14.6	0.83 [0.46, 1.20]
Javor 2005 (n = 15)	1.05	0.26	12.3	1.05 [0.54, 1.56]
Beltrand 2007 (n = 7)	1.18	0.38	8.9	1.18 [0.44, 1.92]
Ebihara 2007 (n = 7)	1.32	0.38	8.9	1.32 [0.58, 2.06]
Peterson 2002 (n = 9)	1.34	0.33	10.2	1.34 [0.69, 1.99]
Oral 2002 (n = 9)	1.59	0.33	10.2	1.59 [0.94, 2.24]
Park 2007 (n = 4)	1.73	0.5	6.4	1.73 [0.75, 2.71]
Total (95% CI)			100.0	1.00 [0.69, 1.31]

Heterogeneity: Tau² = 0.13; Chi² = 22.45, df = 8 (P = 0.004); I² = 64%.

Test for overall effect: Z = 6.39 (P < 0.00001).
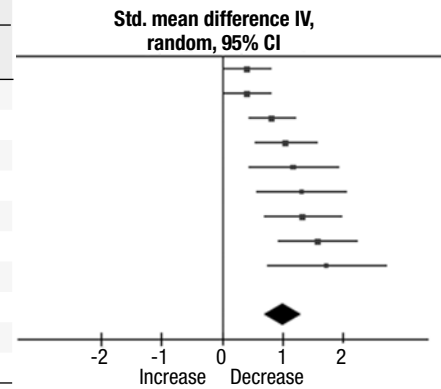
B. Total cholesterol

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Peterson 2002 (n = 9)	0.0	0.33	11.8	0.00 [-0.65, 0.65]
Simha (SH) 2012 (n = 24)	0.0	0.2	14.6	0.00 [-0.39, 0.39]
Simha (MH) 2012 (n = 24)	0.0	0.2	14.6	0.00 [-0.39, 0.39]
Chong 2010 (n = 48)	0.51	0.14	15.7	0.51 [0.24, 0.78]
Beltrand 2010 (n = 7)	0.64	0.38	10.7	0.64 [-0.10, 1.38]
Javor 2005 (n = 15)	1.05	0.26	13.3	1.05 [0.54, 1.56]
Beltrand 2007 (n = 7)	1.35	0.38	10.7	1.35 [0.61, 2.09]
Park 2007 (n = 4)	2.18	0.5	8.5	2.18 [1.20, 3.16]
Total (95% CI)			100.0	0.62 [0.21, 1.02]

Heterogeneity: Tau² = 0.25; Chi² = 35.32, df = 7 (P < 0.00001); I² = 80%.

Test for overall effect: Z = 3.00 (P = 0.003).
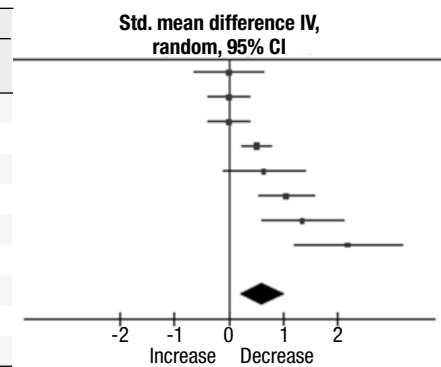
C. HDL cholesterol

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Simha (MH) 2012 (n = 24)	0.0	0.2	18.4	0.00 [-0.39, 0.39]
Simha (SH) 2012 (n = 24)	0.0	0.2	18.4	0.00 [-0.39, 0.39]
Park 2007 (n = 4)	0.0	0.5	2.9	0.00 [-0.98, 0.98]
Javor 2005 (n = 15)	0.0	0.26	10.9	0.00 [-0.51, 0.51]
Chong 2010 (n = 48)	0.0	0.14	37.5	0.00 [-0.27, 0.27]
Peterson 2002 (n = 9)	0.0	0.33	6.8	0.00 [-0.65, 0.65]
Beltrand 2010 (n = 7)	0.08	0.38	5.1	0.08 [-0.66, 0.82]
Total (95% CI)			100.0	0.00 [-0.16, 0.17]

Heterogeneity: Chi² = 0.04, df = 6 (P = 1.00); I² = 0%.
Test for overall effect: Z = 0.05 (P = 0.96).
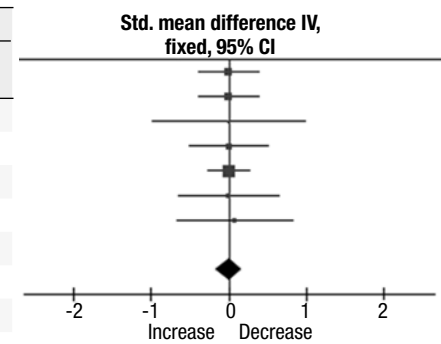
D. LDL cholesterol

Study	SMD	SE	Weight (%)	SMD, 95% CI, I-V Random effects
Chong 2010 (n = 48)	0.0	0.14	38.6	0.00 [-0.27, 0.27]
Peterson 2002 (n = 9)	0.0	0.33	21.6	0.00 [-0.65, 0.65]
Park 2007 (n = 4)	0.0	0.5	12.7	0.00 [-0.98, 0.98]
Javor 2005 (n = 15)	0.76	0.26	27.1	0.76 [0.25, 1.27]
Total (95% CI)			100.0	0.21 [-0.20, 0.62]

Heterogeneity: Tau² = 0.09; Chi² = 6.94, df = 3 (P = 0.07); I² = 57%.
Test for overall effect: Z = 0.98 (P = 0.32).
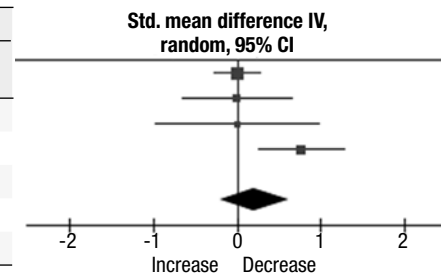
IV: inverse-variance model; CI: confidence interval.

Supplementary Figure 3. Forest plot analyzing the effect of leptin replacement therapy on triglycerides (**A**), total cholesterol (**B**), HDL cholesterol (**C**), and LDL cholesterol (**D**).

DISCUSSION

Lipodystrophy syndromes that are not associated with the use of HAART are rare conditions, and present several metabolic and hepatic abnormalities, caused by changes in circulating adipokines levels. The decrease of plasma leptin levels plays a key role in the pathophysiology of those abnormalities. Leptin replacement therapy is currently the therapy of choice for those conditions, and it was recently approved by the FDA for the treatment of patients with generalized lipodystrophy (13). Although several studies have investigated the safety and efficacy of LRT in patients with LS, no systematic meta-analysis of those results has been performed so far. This meta-analysis reports that LRT in non-HAART LS patients improves fasting plasma glucose and HbA1c values, reduces serum TG and TC levels, and reduces liver volume, and transaminases. Taken together, the results of this meta-analysis suggest that leptin is effective at ameliorating clinical and biochemical endpoints of undistinguished non-HAART LS patients, and at improving overall health in these patients.

Leptin is an adipokine with key roles in regulating food intake, energy balance and body weight. Leptin also plays important roles on lipid and glucose metabolism, the gonadal, adrenal, somatotropic and thyroid axes, sympathetic tone, biomarkers of cardiovascular disease, immunity, inflammation, and brain structure and function (5,6,32-34). In humans, leptin deficiency is observed in cases of hypothalamic amenorrhea, anorexia nervosa, genetic deficiency due to mutations in the leptin gene, and LS. The metabolic abnormalities are similar in all cases of leptin deficiency: increased insulin resistance, hyperinsulinemia, altered fasting glucose or diabetes, hypertriglyceridemia, and hepatic steatosis (35). Leptin replacement therapy has been evaluated in those conditions, showing significant metabolic improvements. In humans with genetic leptin deficiency due to mutations in the leptin gene, our group has shown that LRT improves the glucose-insulin homeostasis, decreases serum triglycerides, reduces lipogenesis, and increases lipolysis (34). In another patient, it has also been shown that LRT reduces circulating levels of transaminases, total cholesterol, LDL, insulin resistance, and liver fat content (36). Similarly, our previous research has shown that white adipose tissue transplantation from wild-type mice into genetically obese and leptin deficient ob/ob mice leads to the improvement

of several metabolic parameters, and to the reversal of nonalcoholic fatty liver disease, due to the correction of the leptin-deficient state (37).

The combined results of the studies included in this meta-analysis suggest that leptin administration is responsible for similar improvements. Analogous to the results in our mouse and human models of genetic leptin deficiency, the data presented here indicate that leptin influences the outcome of glucose and lipid metabolism in humans with LS. Fasting glucose and glycated hemoglobin, two important markers of glycemic control were decreased following intervention (p = 0.0001 and p = 0.003 respectively) (Supplementary Figure 1). Leptin therapy resulted in significant reductions in blood triglycerides (p < 0.00001) and total cholesterol (p = 0.003) (Supplementary Figure 3), which are predictive markers of cardiovascular disease. Moreover, liver volume and AST were significantly decreased (p = 0.0002 and p = 0.01) (Supplementary Figure 2), suggesting that LRT is effective in the improvement of hepatic pathology associated with LS. Liver fat was reduced in response to leptin therapy but meta-analysis was not possible as this data was based on results from a single study that reported the outcome of liver fat. However, recent studies do suggest that leptin therapy is effective at reducing hepatic steatosis associated with lipodystrophy (38).

Recently, the US Food and Drug Administration approved the use of metreleptin (the synthetic human leptin analogue used as the leptin supplement in all the trials presented here) to treat patients with only the generalized form of lipodystrophy, where the adipose tissue loss is systemic and usually subcutaneous. The FDA Endocrinologic and Metabolic Drugs Advisory Committee reviewed data from two NIH studies with a combined cohort of 72 participants: NIH Study 991265 (completed) and NIH Study 20010769 (ongoing), and from another study with a cohort of 28 participants, FHA101 Study (ongoing) (39). Similar to the parameters assessed here, these studies evaluated the effects of metreleptin on HbA1c, fasting plasma glucose, TG and liver function. Combining data from these studies, after a 12 month trial period, the following parameters were reduced: HbA1c [-1.4 ± 0.2% (-0.9, -1.8)], glucose [-42 ± 12 mg/dL (-18, -65)], TG [-673 ± 223 mg/dL or -32 ± 8% (-17, -47)], liver volume (-1001 mL); ALT (-31.4 U/L) and AST (-23.7 U/L). These reductions were more pronounced

in patients with elevated baseline levels of these end-points. For example, in patients with the generalized form with a baseline HbA1c ≥ 6%, a mean reduction of 2.3% was achieved following the 12-month trial period. However, in patients with a baseline HbA1c ≥ 8%, this reduction was even greater, at 2.7%. Similarly, in patients with partial lipodystrophy with baseline HbA1c ≥ 7%, the mean reduction in HbA1c was 0.8%, whereas mean HbA1c reduction of 1.4% was observed in patients with partial LS and baseline HbA1c ≥ 8%. Similar patterns were observed in all assessed parameters, which lead the investigators to suggest that in recovering a healthy phenotype, the effect of metreleptin is more pronounced in those patients with more severe underlying metabolic abnormalities. Furthermore, the investigators noted that the clinical efficacy of metreleptin was more related to the severity of the underlying metabolic abnormalities, rather than to the form of lipodystrophy (generalized or partial). To assess this claim, a further sub-analysis was performed by stratifying data for the two main outcomes (HbA1c and TG), according to lipodystrophy subtype (39). This sub-analysis showed that more pronounced effects were seen in patients with the generalized form, with mean reductions in serum HbA1c and TG equal to 1.4% (SE = 0.2) and 121 mg/dL (SE = not reported) respectively. Interestingly, similar results were seen across patients with congenital or acquired variants of each lipodystrophy subtype (39). Our analysis showed a similar pattern of results where patients with generalized LS showed greater improvements in the endpoints of glucose and HbA1c relative to patients with partial LS. Similarly relative to patients with congenital LS, patients with acquired LS had greater reductions in blood glucose, HbA1c and TG. However, the small size and high heterogeneity of our data set makes statistical comparisons between subtypes of LS underpowered and not significant.

Our analysis suggests that, overall, metreleptin is more effective in ameliorating the condition of patients with the generalized forms of lipodystrophy, even though it has been suggested that the drug might be useful in partial lipodystrophy, who present a more heterogeneous disease profile. Overall, improvements with metreleptin have been related to the severity of the baseline metabolic abnormalities of the patient, and not necessarily dependent on severity of leptin deficiency or the type of LS. Although, the use of metreleptin to treat partial lipodystrophy was not entirely discouraged, but

a thorough physical exam was advised by the authors of the NIH sponsored trials in determining further treatment (39).

The results of this meta-analysis were influenced by several factors. Importantly, the sample sizes of each study varied significantly, from n = 4 to n = 55 (Table 1). Smaller studies represent important exploratory trials, providing data on clinical efficacy and safety, which are useful to direct regulatory bodies in developing guidelines for metreleptin use. However, from a statistical perspective, small trials may be prone to large variances in results, as individual differences have a significant impact in final data. To overcome this, we employed the standard error (SE) of the standardized mean difference (SMD) to be inversely proportional to the study population size. In this way, the smallest trials had the largest SE, which reflected into a lower weighting of those small trials in the final analysis. In the meta-analysis, heterogeneity was generally high, ranging from I^2 = 84% for the outcome of glucose to 0% for HDL cholesterol and insulin (employed a fixed effects model in these instances). This means that a low percentage of all variability in effect size estimates was caused by sampling errors within the studies. In other words, most of the variability was caused by true heterogeneity between studies (19). In considering a consistent pattern of results seen in several parameters, large heterogeneity may be seen as strength, in that leptin improves these endpoints in several settings.

The dosage of r-metHu-leptin was uniform amongst the included trials. However, as only mean leptin dose was reported, it was not possible to determine if increases in leptin dosage may elicit greater changes. Interestingly Beltrand and cols. reported that some patients began not to respond to leptin treatment and developed resistance. This may represent some of the problems that clinicians will encounter in the future (23).

Variation in effects may also be attributed to the differing follow-up times employed by the trials included in this meta-analysis. Some studies (16,22,30) involved follow-up times less than 12 months. However, longer trials, such as the one conducted by Chong and cols., arrived at similar conclusions, suggesting that leptin has similar immediate and long-term effects (24). Baseline mean levels of leptin varied significantly amongst the studies (range 0.7-3.8 ng/mL). This may create an impact when comparing the effects of different r-metHu-leptin dosages in reaching target and physiological levels of lep-

tin. However, a similar pattern of results is seen across all the studies, which would suggest that r-metHu-leptin is effective in patients with differing states of leptin-deficiency, paralleling the findings of the NIH-sponsored trials. Therefore, it would be reasonable to assume that leptin may be applicable for clinical use in other non-lipodystrophy leptin-deficient conditions. Indeed, leptin has previously been investigated in hypothalamic amenorrhea with positive results (40,41). Finally, age- and sex-specific effects cannot be discounted as possible sources of variation and error, and future clinical trials should investigate these aspects, as some studies assessed here included only children, and others only women (Table 2).

This meta-analysis has some limitations. Most importantly, this analysis was restricted to clinical data from studies using leptin to treat forms of lipodystrophy that did not arise out of complications from HAART for HIV infection. This form of LS was excluded because it has a unique pathophysiological basis, and therefore may introduce an important confounding factor to the meta-analysis. As such, we have excluded a significant amount of data relating to the effect of leptin replacement in the treatment of lipodystrophy. Indeed, 65 articles were excluded, from the 261 abstracts eligible for inclusion from initial database searching (Figure 1). Secondly, in order to perform the meta-analysis, we pooled together data from prospective cohort studies and clinical case series that were conducted in a similar fashion. The exclusion of case reports [*e.g.* (21,22,24,27-30)] may not reflect adequately the reality of the management of lipodystrophy, but this approach was necessary in order to increase the statistical quality and power of the analyzed data, as case reports are less powerful forms of clinical experimentation and observation, and are subject to increased bias compared to larger prospective studies. Thirdly, this review obtained data from publicly available literature, and as such, we did not have access to primary data. This hindered the completion of some observations and proper comparison of baseline and follow-up results. The outcome of the meta-analysis could be strengthened by including those missing data, including the significance levels of baseline and follow-up data. For example, Beltrand and cols. (21) reported an increase in HbA1c, but did not provide a significance test, and therefore we could not include this as part of our data analysis. Those missing data also extend to other qualitative data such as reporting the use of other medications by the patients. The use of medications to treat the clinical manifestation of lipodystrophy, in particular glucose and lipid lowering drugs can significantly impact on interpreting the true effects of leptin on these endpoints. Finally, our quality assessment suggests that the studies included for meta-analysis can be improved in several aspects (range of quality scores 25-75%), and that future studies would need to address the limitations outlined here and by the authors of the studies themselves.

To our knowledge, this paper represents the first comprehensive meta-analysis into the effects of leptin in patients with non-HAART associated LS, focusing on several metabolic parameters as well as classic liver parameters. In patients with undistinguished forms of LS (congenital/acquired, partial/generalized), leptin replacement therapy was effective in improving parameters of glucose-insulin and liver homeostasis, and blood lipids. Although data suggest that metreleptin may be clinically effective for patients with partial forms of lipodystrophy, a meta-analysis into this type of LS was not possible due to the small sample size and its heterogeneity. Our findings may guide future clinical research for expansion of the use of metreleptin for partial forms of LS, and for the development of leptin-based therapies for conditions such as metabolic syndrome, nonalcoholic fatty liver disease and other conditions where patients display similar metabolic abnormalities.

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