r-metHuLeptin improves highly active antiretroviral therapy-induced lipoatrophy and the metabolic syndrome, but not through altering circulating IGF and IGF-binding protein levels: observational and interventional studies in humans

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Abstract

Objective: Leptin is an adipocyte secreted hormone and an important regulator of neuroendocrine, metabolic, and immune function. Both r-metHuLeptin and IGF1 administration result in reduced central adipose tissue in subjects with highly active antiretroviral therapy-induced metabolic syndrome (HAART-MS) but whether the effects of leptin are mediated through increasing IGF levels remains unknown.

Methods: To assess whether r-metHuLeptin improves the HAART-MS by regulating circulating IGF and IGFBPs, we first conducted a cross-sectional study of 118 men and women with HIV infection and 6 months of exposure to antiretroviral medications to examine any association between circulating IGF1 and leptin levels. We also performed a randomized, double-blinded, placebo-controlled, crossover trial of recombinant human leptin (r-metHuLeptin) administration to seven HIV positive men with lipoatrophy and leptin deficiency (leptin < 3 ng/ml) related to antiretroviral medication use.

Results: In the observational study, leptin levels were inversely associated with circulating IGF1 levels after adjusting for age and gender (r = 0.27, P = 0.002), but this inverse association became non-significant after adjustment for % body fat and exercise. In the interventional leptin study, leptin levels increased significantly during r-metHuLeptin treatment (from 1.34 ± 0.20 ng/ml at baseline to 17 ± 5.05 ng/ml after 8 weeks, P = 0.046) and metabolic parameters improved including reduced fasting insulin levels and reduced homeostasis model assessment-insulin resistance (HOMA-IR). Despite the increase in circulating leptin levels, there was no change in IGF1, IGF2, free IGF1, or IGFBP levels during the 2-month treatment period.

Conclusion: The effects of r-metHuLeptin in patients with HAART-MS are not mediated through increasing IGF or IGFBP levels.

Introduction

Highly active antiretroviral therapy (HAART)-induced lipoatrophy, currently the most common form of lipoatrophy worldwide, is associated with hypoleptinemia, insulin resistance, and elevated triglycerides in the context of the HAART-induced metabolic syndrome (HAART-MS). HAART-MS is also associated with abnormalities in the GH–insulin-like growth factor (IGF) axis including reduced GH response to stimulatory testing and reduced IGF1 levels (1). Interventions which increase IGF1 have recently been demonstrated to result in improvements in body composition and lipid profile in patients with this syndrome (2). Similarly, administration of recombinant human leptin (r-metHuLeptin), an adipocyte-secreted hormone with important regulatory role in metabolic, immune, and neuroendocrine function, has been shown to blunt the decline in IGF1 levels in response to acute starvation and to increase IGF1 levels after chronic administration in hypoleptinemic subjects (3–5), and to also result in reduced trunk fat and improved lipid profile and insulin resistance in hypoleptinemic patients with HAART-MS (6). Whether the beneficial effects of r-metHuLeptin administration in subjects with the HAART-MS are mediated by or largely independent from increased IGF1
levels remains unknown and this could have potential therapeutic implications. If the therapeutic effects of these two agents are independent of each other combination treatments with both could have additive effects.

To evaluate whether leptin is an important regulator of circulating IGF1 levels in HAART-MS, we first conducted a cross-sectional study in 118 HIV-positive individuals. This study was followed by an interventional crossover study involving administration of placebo or r-metHuLeptin in replacement doses to evaluate whether normalizing leptin levels in seven patients with HAART-MS and lipoatrophy would result in changes in circulating IGFs or IGF-binding proteins (IGFBPs).

**Research design and methods**

**Observational study**

Consecutively, 118 enrolled subjects were recruited from the infectious diseases clinics at the Beth Israel Deaconess Medical Center (BIDMC) from August 2000 through January 2004, as previously described (7). Inclusion criteria were age greater than 16 years, documented HIV-1 infection, and cumulative exposure to HAART for a minimum of 6 months. Each subject provided a detailed medical history and underwent physical examination and anthropometric measurements. Fasting blood samples were obtained for measurement of total IGF1 and leptin. Dual-energy X-ray absorptiometry was used to measure body composition. Exercise was assessed by questionnaire as described previously (7).

**Interventional study**

Seven men with HIV-1 infection, 6 months or more of cumulative HAART exposure, serum leptin level less than 3 ng/ml and lipoatrophy that developed after HAART initiation were enrolled in a crossover, placebo controlled study designed to evaluate the effect of r-metHuLeptin on insulin resistance (6). Exclusion criteria have been described previously (6). Subjects were randomized in a double-blind fashion, to initial treatment with either r-metHuLeptin or matching placebo at a dose of 0.04 mg/kg·d divided into two equal doses, which were self-administered twice daily by s.c. injection for 2 months. They then discontinued treatment for a 1-month washout before crossing over to the other treatment for 2 months. Subjects were advised to keep their HAART regimen (reviewed at each study visit), exercise regimen, and diet (log books) stable throughout the study. Both studies were approved by the BIDMC Institutional Review Board. All subjects provided written informed consent. The clinical trial registration number is NCT00140244.

**Assessments**

At each study visit, serum was collected and stored at –80°C until assayed in duplicate. Samples for the same subject were run in the same assay (interventional study). Standard techniques were used to measure leptin (Linco Research, St Charles, MO, USA, sensitivity: 0.5 ng/ml, intraassay coefficient of variation (CV) 8.3%), IGF1 (DPC, Los Angeles, CA, USA, sensitivity: 20 ng/ml, CV: 8.4%), IGFBP3 (DPC, sensitivity 0.1 μg/ml, CV 9.9%), IGFBP1 (DSL, Webster, TX, USA, sensitivity 0.33 ng/ml, intraassay CV 5.2%), IGFBP2 (DSL, sensitivity 0.17 ng/ml, intraassay CV 2.3%), IGFBP4 (DSL, sensitivity 0.008 ng/ml, intraassay CV 6.3%), free IGF1 (DSL sensitivity 0.15 ng/ml, intraassay CV 4.8%), and IGF2 (DSL sensitivity 0.25 ng/ml, intraassay CV 4.2%) (4, 7, 8).

**Statistical analysis**

SAS (version 8.02, SAS Institute, Cary, NC, USA) and SPSS (version 11.5, SPSS Inc., Chicago, IL, USA) were used for statistical analysis, and P<0.05 (two-tailed) was considered significant. Data are presented as means±SDs. For the observational study, leptin was log transformed to better approximate normal distribution. Pearson correlation was used to explore the associations between study variables. For multivariate analysis, we used linear regression, controlling for age, gender, body fat, and exercise. For the interventional study, we used nonparametric Wilcoxon tests to compare change under r-metHuLeptin versus placebo, with Wilcoxon’s signed rank (paired tests) as primary (on treatment analysis) and intention-to-treat as secondary analysis. We also performed multivariate analysis by fitting a mixed model with a random subject component permitting correlation within patients, and controlling for treatment sequence and changes in body weight.

**Results**

**Observational study**

The 118 individuals, 108 male, had the following characteristics: mean age 43.7±8.7 years, mean body mass index 24.4±3.5, mean body fat 19.5±8.1%, mean leptin 4.8±8.37 ng/ml, mean IGF1 90.0±38.1 ng/ml. IGF1 levels were inversely correlated with leptin \( (r=0.28, P=.002) \), which persisted after adjustment for age and gender \( (r=0.27, P=0.002) \). Circulating IGF1 levels were also inversely correlated with body fat \( (gms; r=0.19, P<0.05) \) and body fat % \( (r=0.24, P<0.01) \). Multivariate analysis adjusting for age, gender, % body fat and exercise, rendered the relationship between IGF1 and leptin non-significant \( (\beta=0.18, P=0.35) \).
Interventional study

Seven subjects were enrolled and contributed data for analysis but five subjects completed the entire study, as described previously (6). Leptin levels increased with treatment (1.34 ± 0.20–17.26 ± 5.05 ng/ml after 2 months, \( P=0.05 \)). There was no significant change in leptin levels during treatment with placebo (1.21 ± 0.25–1.37 ± 0.35 ng/ml, \( P=0.14 \)). Body weight tended to decrease during treatment with r-metHuLeptin (71.0 ± 5.52–69.2 ± 5.90 kg, \( P=0.08 \) compared with placebo), mainly due to reduced trunk fat (5.88 ± 1.50–5.37 ± 1.44 kg, \( P=0.04 \) compared with placebo). Insulin resistance, measured by fasting insulin levels and HOMA-IR decreased with r-metHuLeptin compared with placebo (fasting insulin levels decreased from 16.6 ± 5.61 to 11.6 ± 4.46 mcIU/ml, \( P=0.04 \) compared with placebo and HOMA-IR decreased from 3.58 ± 1.14 to 2.52 ± 0.91, \( P=0.04 \) compared with placebo) and HDL also tended to increase (31.5 ± 3.31–35.0 ± 2.23 mg/dl, \( P=0.08 \) compared with placebo) with r-metHuLeptin as reported previously (6).

Table 1 shows the changes in IGFs and IGFBPs during the study. Mean IGF1 levels were within the low normal range at baseline and did not change with r-metHuLeptin administration. Similarly, circulating levels of free IGF1, IGFBP2, IGFBP3 and IGFBP4 did not change significantly with r-metHuLeptin administration. IGFBP1 levels increased with r-metHuLeptin administration; however, this difference became non-significant after adjusting for treatment sequence and body weight changes indicating that the IGFBP1 differences observed reflect the latter.

Discussion

Our study demonstrates that there is no independent association between IGF1 and leptin levels in subjects with HIV infection. Furthermore, r-metHuLeptin administration, which improves the metabolic profile, does not increase IGF1 levels in lipoatrophic and hypoleptinemic subjects with HAART-MS.

Leptin is an important regulator of the GH–IGF axis in animals (9), but the role of leptin in the regulation of this axis in humans remains to be fully elucidated. In an uncontrolled study in subjects with congenital leptin deficiency, r-metHuLeptin administration was not associated with any significant change in IGF1 levels but was associated with dramatic weight loss which may have obscured any effect (10). By contrast, in an uncontrolled study in subjects with severe leptin deficiency and insulin resistance due to congenital lipodystrophy, leptin administration for 8–12 months resulted in a small reduction in weight and increased IGF1 levels (11). Compared to the subjects in our study, these previously studied subjects had more profound leptin deficiency and insulin resistance (11). In a carefully controlled study of hypoleptinemia induced by short term fasting, r-metHuLeptin administration partially restored total IGF1 levels (4, 8) and in a more long-term study of chronic hypoleptinemia due to hypothalamic amenorrhea, leptin administration for 3 months increased IGF1 levels (5). Thus, leptin levels may need to fall below a certain threshold in order to regulate the GH–IGF axis in humans (8). We demonstrate herein that although r-metHuLeptin administration improves metabolic parameters in subjects with HAART-induced lipoatrophy, this effect of r-metHuLeptin administration is not mediated by any significant changes in IGF1 levels.

In subjects with HIV infection, central fat accumulation is associated with reduced GH secretion and IGF1 levels through mechanisms which remain largely unclear (1, 12). Our studies do not point to an important role for leptin as a modulator of circulating IGFs in HIV positive patients with lipodystrophy and indicate that the beneficial effects of r-metHuLeptin and IGF1 administration in the HAART-MS are probably independent of each other. Whether concomitant administration of both agents would result in additive or synergistic effects in

Table 1  Biochemical measures at baseline and 8 weeks.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>r-metHuLeptin</th>
<th>Wilcoxon</th>
<th>Multivariate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>8 weeks</td>
<td>Change</td>
<td>Baseline</td>
</tr>
<tr>
<td>Free IGF1 (ng/ml)</td>
<td>0.72 ± 0.20</td>
<td>0.64 ± 0.17</td>
<td>-0.08 ± 0.29</td>
<td>0.73 ± 0.61</td>
</tr>
<tr>
<td>IGF1 (ng/ml)</td>
<td>132 ± 63</td>
<td>146 ± 62</td>
<td>+14 ± 16</td>
<td>139 ± 53</td>
</tr>
<tr>
<td>IGF2 (ng/ml)</td>
<td>1741 ± 625</td>
<td>1571 ± 404</td>
<td>-169 ± 232</td>
<td>1579 ± 496</td>
</tr>
<tr>
<td>IGFBP1 (ng/ml)</td>
<td>6.9 ± 5.1</td>
<td>20.8 ± 9.8</td>
<td>+13.9 ± 14.7</td>
<td>6.0 ± 6.6</td>
</tr>
<tr>
<td>IGFBP2 (ng/ml)</td>
<td>305 ± 143</td>
<td>275 ± 116</td>
<td>-29 ± 72</td>
<td>334 ± 156</td>
</tr>
<tr>
<td>IGFBP3 (ng/ml)</td>
<td>4.48 ± 1.54</td>
<td>4.52 ± 1.25</td>
<td>+0.04 ± 0.46</td>
<td>4.18 ± 1.14</td>
</tr>
<tr>
<td>IGFBP4 (ng/ml)</td>
<td>774 ± 399</td>
<td>739 ± 265</td>
<td>-35 ± 187</td>
<td>771 ± 183</td>
</tr>
<tr>
<td>Insulin (mcIU/ml)</td>
<td>13.34 ± 2.43</td>
<td>20.26 ± 7.06</td>
<td>6.92 ± 5.86</td>
<td>16.58 ± 5.6</td>
</tr>
</tbody>
</table>

Crossover study of r-metHuLeptin versus placebo. Paired tests are comparing change from baseline to 8 weeks between placebo and r-metHuLeptin treated groups for n=5 subjects using Wilcoxon signed-rank test. *P values from linear mixed models including a random subject effect to account for within-subject correlated measurements and between-subject independence and controlling for treatment order and body weight. Data are presented as mean ± S.E.M.
terms of body composition and metabolic improvements remains to be determined by future studies.

IGFBP1 is a nutritionally regulated factor responsible for less than 5% binding of IGF in the circulation and is associated with insulin resistance, especially hepatic insulin resistance (13). Increased IGFBP1 levels have been reported following r-metHuLeptin replacement inducing weight loss in subjects with congenital leptin deficiency (10). We replicate the findings of that uncontrolled study by demonstrating an increase in IGFBP1 associated with r-metHuLeptin administration in men with HAART-MS. r-metHuLeptin’s effect appears to be indirect, mediated by changes in body weight as the association became non-significant following adjustment for treatment sequence and body weight changes. Whether leptin has an effect to influence IGFBP1 through reducing hepatic insulin resistance remains to be determined in future studies.

Strengths of this study include the combination of observational and interventional data and the utilization of a blinded, placebo-controlled, crossover design. We did not perform dynamic testing of GH secretion herein but assessed circulating levels of both IGF1 and IGF2, as well as their binding proteins using state-of-the-art methodology. The power of the observational study was sufficient (more than 80%) to demonstrate associations with a correlation of \( r > 0.22 \), i.e., clinically significant associations. The power of the interventional study was limited due to the relatively small number of subjects but was improved by the crossover study design that limits variability by comparing the subjects on placebo and active treatment. In any case, the study was sufficient to demonstrate statistically significant increased leptin levels as well as improvement of metabolic parameters in contrast to no change in IGF levels. More specifically, we had 80% power to detect a change in IGF1 levels of 50 ng/ml in the intervention study but may have missed a smaller effect of leptin. Whether smaller changes in IGF1 are of clinical significance in terms of metabolic improvements remains to be determined. In summary, our study provides important new information on the HAART-MS by demonstrating that r-metHuLeptin administration improves metabolic parameters independently of changes in the GH–IGF axis. Trials of combination treatments with leptin and treatments to increase IGF1 may be warranted on the basis of findings reported herein.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References