SHORT COMMUNICATION

Leptin administration in physiological or pharmacological doses does not alter circulating irisin levels in humans

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Leptin is an adipokine causing browning of adipose tissue, and it thus increases energy expenditure. The same is true for irisin. We studied whether exogenously administered metreleptin affects serum irisin concentrations in humans, which would suggest a direct interplay between leptin and irisin. We performed two studies: a dose-escalating 1-day-long study and a randomized placebo-controlled study. *Study 1*: 15 healthy, normal-weight and/or obese male and female individuals participated in three 1-day-long trials of metreleptin administration in the fed state. Metreleptin was administered once at physiological and pharmacological (0.01, 0.1 and 0.3 mg per kg body weight) doses. *Study 2*: 18 apparently healthy hypoleptinemic young women with hypoleptinemia and secondary amenorrhea took part in this study. Subjects received either metreleptin in replacement doses (0.08 and/or 0.12 mg kg^{-1}) or placebo for 16 weeks. Blood samples were analyzed for leptin and irisin. We found no effect of metreleptin administration on irisin levels of subjects studied at either the fasting or the fed state either in the short or the long term. We provide evidence that leptin is not altering circulating irisin levels in humans.

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INTRODUCTION

Irisin and its precursor fibronectin type III domain containing five (FNDC5) have received attention as promising targets for the treatment of metabolic disorders. Irisin was initially proposed to be secreted from the skeletal muscle in response to exercise and/or peroxisome proliferator-activated receptor-γ coactivation 1a,[1] but adipose tissue seems to be also contributing.[2] Recently, it was shown that leptin could alter FNDC5 expression in human subcutaneous adipose tissue explants *in vitro*.[3] Irisin levels are positively associated with markers of adiposity, and obese individuals may develop resistance or tolerance to irisin, similar to leptin.[4,5] Any direct effect of leptin on circulating irisin levels in humans still needs to be elucidated. As leptin activates brown adipose tissue (BAT) to increase thermogenesis and energy expenditure,[6] it would be reasonable to speculate that the effect of leptin on BAT activation could be mediated by irisin, which has also been suggested to have similar actions.[7]

Here, we present for the first time data from studies in human volunteers who received either physiological and/or pharmacological doses of metreleptin and had their irisin serum levels measured to provide evidence on whether exogenously administered leptin affects irisin levels in humans.

MATERIALS AND METHODS

Blood samples from two different study protocols, described in detail elsewhere,[8,9] were utilized. The first study investigated increasing doses of metreleptin in normal-weight and/or obese participants over 18 h,[8] whereas the second one explored physiological metreleptin replacement in hypoleptinemic female athletes over a period of 16 weeks.[9] Both studies were approved by the Beth Israel Deaconess Medical Center Institutional Review Board, and all participants provided written informed consent.

Study 1

Fifteen individuals, five normal-weight females (age = 20.4 ± 0.7 years, body mass index (BMI) = 21.9 ± 0.7 kg m^{-2}), five normal-weight males (age = 22.2 ± 0.9 years, BMI = 22.0 ± 0.5 kg m^{-2}) and five obese males (age = 23.4 ± 1.5 years, BMI = 32.0 ± 1.0 kg m^{-2}) participated in this study. All participants were healthy, receiving no medications. They participated in three 1-day-long trials of metreleptin administration in the fed state. Metreleptin was administered subcutaneously in the abdomen at 0800 hours in the morning in ascending doses of (a) a physiological dose: 0.01 mg per kg body weight, (b) a supraphysiological dose: 0.1 mg per kg body weight and (c) a pharmacological dose: 0.3 mg per kg body weight. Blood samples were drawn at baseline (0800 hours) and at 0810, 0830, 0900, 1000, 1100, 1200, 1300, 1400, 1600, 1800, 2000 and 0200 hours thereafter. When participants were admitted to the hospital, they received an isocaloric diet. Trials were performed with 1–12 weeks' interval among them.

Study 2

Eighteen apparently healthy hypoleptinemic young women with secondary amenorrhea induced by strenuous exercise and/or low body weight for at least 6 months before entering the study, fasting morning leptin levels less than 5 ng ml^{-1}, stable body weight for at least 6 months prior study initiation and baseline weight within 15% of ideal body weight completed this randomized double-blinded, placebo-controlled study. Participants were randomly assigned in a 1:1 ratio to two different groups: (a) the metreleptin group (*n* = 10, age = 26.6 ± 1.4 years, BMI = 20.9 ± 0.6 kg m^{-2}) and (b) the placebo group (*n* = 8, age = 25.4 ± 1.2 year, BMI = 19.8 ± 0.7 kg m^{-2}). Treatment was self-administered, performed subcutaneously on a daily basis between 1900–2300 hours for 16 weeks. The initial metreleptin/ placebo dose was a physiological one of 0.08 mg kg^{-1}. Participants stayed on this dose for the whole 16-week period if menstruation occurred during the 12-week assessment, but if not they were

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switched to a supraphysiological dose of 0.12 mg kg^{-1} from the 12 weeks thereafter. Supplements of calcium (600 mg twice daily) and Vitamin D (400 IU daily) were provided to the study subjects. Fasting blood samples were drawn at every visit, that is, 0, 4, 8 and 16 weeks.

Blood sample analysis

Leptin concentrations were measured as previously described.[8,9] Irisin concentrations were measured using a previously validated[10,11] ELISA kit (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA; EK- 067-52) with a sensitivity of 0.03 ng ml^{-1} per ng ml^{-1} and intra-assay and inter-assay coefficient of variations (CVs) of 4.9–7.5% and 5.2–7.3%, respectively. Samples were assayed in duplicate and samples for the same subject were run within the same assay.

Statistical analysis

Normality was tested with histograms and pp plots, and non-normally distributed data were log-transformed and/or ranked. Total and incremental areas under the curve (AUC and IAUC, respectively) were calculated and compared using two-sided univariate analysis of variance using Bonferroni's *post hoc* test for comparing one intervention to another. Levene's test was nonsignificant for all analyses of variance performed. Total AUC was calculated with the trapezoid rule, that is, sum of the areas under and over the baseline, whereas IAUC was calculated as: AUC − (baseline × 1080 min)/2 for Study 1 and as AUC − (baseline × 16 weeks/2) for Study 2. SSPS, v.17 was used, and the significance level was set for 0.05. Data are presented as mean ± s.d. or as median (1st to 3rd quartiles).

RESULTS

Study 1 (short-term leptin administration)

Leptin levels displayed a dose–response effect in the overall sample and in the three sample subgroups (that is, normal-weight females, normal-weight males and obese males) after metreleptin administration in the fed state. In the overall sample, leptin AUCs (0.01 mg kg^{-1}: 13 788.2 (4521.7–20 061.8) ng ml^{-1} versus 0.1 mg kg^{-1}: 65 647.3 (33 652.0–79 997.3) ng ml^{-1} versus 0.3 mg kg^{-1}: 16 2764.8 (78 891.3–196 274.5) ng ml^{-1}, $P \leqslant 0.001$) and IAUCs (0.01 mg kg^{-1}: 8814.8 (3205.5–11 909.5) ng ml^{-1} versus 0.1 mg kg^{-1}: 50 268.0 (32 474.8–72 779.4) ng ml^{-1} versus 0.3 mg kg^{-1}: 1 54 961.8 (77 471.1–1 89 742.5) ng ml^{-1}, $P \leqslant 0.001$; Figure 1a) differed significantly among metreleptin doses, and the same was observed for all study subgroups ($P \leqslant 0.001$ in all cases). *Post hoc* comparisons revealed significant differences between curves after all three metreleptin doses ($P \leqslant 0.001$ in all cases). However, irisin levels were not significantly different between the three trials either in the overall sample (AUC: 0.01 mg kg^{-1}: 1 99 229.7 (1 72 291.1–2 19 218.6) ng ml^{-1} versus 0.1 mg kg^{-1}: 2 05 267.1 (1 67 905.4–2 18 813.2) ng ml^{-1} versus 0.3 mg kg^{-1}: 1 86 143.9 (1 66 571.7–2 25 884.8) ng ml^{-1}, $P = 0.889$); IAUC: 0.01 mg kg^{-1}: 100 865.5 (88 838.6–1 22 435.3) ng ml^{-1} versus 0.1 mg kg^{-1}: 1 05 654.2 (92 311.8–1 11 543.3) ng^{-1}ml^{-1} versus 0.3 mg kg^{-1}: 98 024.8 (83 569.8–1 36 349.5) ng ml^{-1}, $P = 0.804$; Figure 1b) or the separate subgroups. When we analyzed separately obese and normal-weight participants, results remained the same, that is, leptin levels were significantly
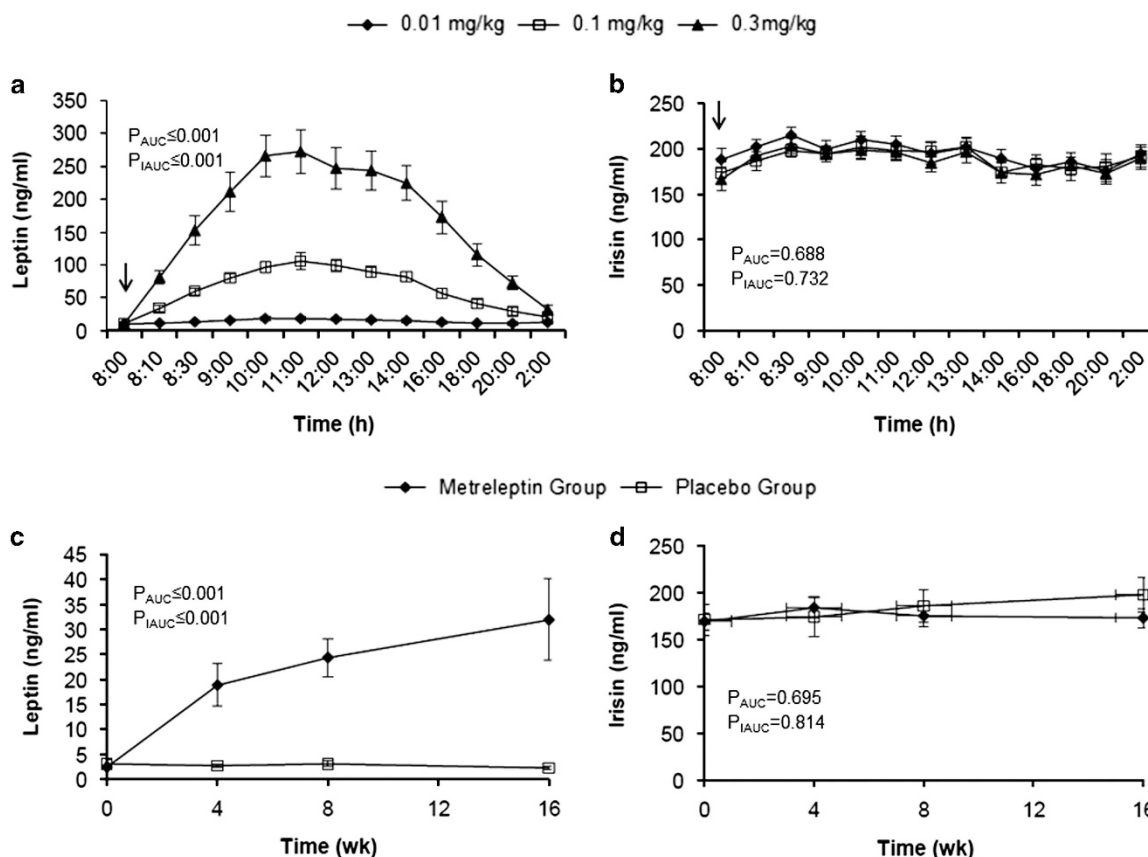

Figure 1. Serum irisin levels in response to metreleptin treatment. (**a**) Circulating leptin and (**b**) irisin concentrations of the overall group, after one dose of metreleptin (either 0.01, 0.1 or 0.3 mg per kg body weight) at 0800 hours. (**c**) Circulating leptin and (**d**) irisin concentrations after metreleptin (0.08 and/or 0.12 mg kg^{-1}) or control administration on a daily basis between 1900 and 2300 hours for 16 weeks in hypoleptinemic women. The arrow indicates the time when metreleptin was administered. AUC and IAUC were compared using two-sided univariate analysis of variance using Bonferroni's *post hoc* test for comparing one intervention to another.

different between trials in both groups ($P \leqslant 0.001$ in both cases), whereas irisin levels did not change ($P > 0.05$).

Study 2 (long-term leptin administration)
In this study, leptin levels were gradually increased from week 0 to 16 when metreleptin was administered, whereas they remained stable with placebo (Figure 1c). Consequently, both leptin AUC (metreleptin group: 365.2 (240.2–515.1) ng ml^{-1} versus placebo group: 40.7 (23.3–58.0) ng ml^{-1}, $P \leqslant 0.001$) and IAUC (metreleptin group: 344.1 (225.9–494.4) ng ml^{-1} versus placebo group: 16.5 (1.7–26.5) ng ml^{-1}, $P \leqslant 0.001$) differed significantly between the two groups. On the other hand, no differences in irisin AUC (metreleptin group: 2814.4 ± 441.4 ng ml^{-1} versus placebo group: 2941.2 ± 788.6 ng ml^{-1}, $P = 0.666$) and IAUC (metreleptin group: 1462.5 ± 348.3 ng ml^{-1} versus placebo group: 1573.6 ± 641.1 ng ml^{-1}, $P = 0.640$) were noticed between groups (Figure 1d). When we analyzed separately those who stayed on the 0.08-mg kg^{-1} metreleptin dose and those who increased the dose to 0.12 mg kg^{-1}, we found no significant differences for irisin levels in either group ($P > 0.05$).

DISCUSSION

We present for the first time data on the effect of increasing doses of metreleptin administration on irisin levels in humans, and we report no significant effect. Leptin is known to control BAT activity primarily through increasing catecholamine levels;[12] however, other pathways cannot be excluded. Although the effects of irisin on adipose tissue browning in humans have not been unambiguously documented, irisin is still considered to drive BAT formation and activation.[7,13–15] A potential effect of leptin on irisin is reasonable to speculate and could provide a possible unifying pathway of leptin action on BAT. Leptin has been shown to affect the expression of FNDC5 in both in vitro and/or in vivo experiments in animals. Specifically, leptin significantly downregulated FNDC5 mRNA expression in a dose-dependent manner in explants of the subcutaneous adipose tissue of non-obese individuals after a 24-h incubation period.[3] Furthermore, leptin administration affected FNDC5/irisin regulation in mice, in both skeletal muscle and subcutaneous adipose tissue but in an opposite manner.[16] In humans, leptin was highly correlated and, among other variables, best explained the FNDC5 mRNA expression in adipose tissue of morbidly obese individuals.[3] Furthermore, leptin was strongly and positively correlated with irisin levels in children,[17] although this was not confirmed by another study.[18] In the present study we went one step further and utilized samples from studies in which we had administered metreleptin to humans in order to investigate whether leptin affects irisin levels in vivo and whether irisin could be a mediator of leptin's effect on adipose tissue browning in humans. Contrary to the previous studies, we failed to demonstrate any effect of metreleptin, in either physiological or non-physiological doses, in the short and the long term, as well as at the fasting and the fed state on serum irisin in humans. However, tissue irisin expression was not studied herein.

The sample size was sufficient to demonstrate significant changes of leptin levels, and the study had 80% power to demonstrate an effect size of 0.218 for the first study and an effect size of 0.286 for the second one at the conventional $a = 0.05$ level. Although the lack of a control group in the dose-escalation study could be considered a limitation, the administration of the low metreleptin dose, which is considered a physiological one, could serve as the control situation. Furthermore, an obese group of women is lacking from that study; however, there is no reason to believe that physiology in men is different from that in women in this respect.

In conclusion, we showed that metreleptin administration does not affect circulating irisin levels, and thus a hypothesized mechanism that leptin may affect adipose tissue browning through altering circulating irisin concentrations could not be supported.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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