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Circulating vaspin and visfatin are not affected by acute or chronic energy deficiency or leptin administration in humans

Eun Seok Kang[1,2], **Faidon Magkos**[1], **Elizabeth Sienkiewicz**[1], and **Christos S. Mantzoros**[1,3,4]

[1] Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

[2] Division of Endocrinology and Metabolism, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea

[3] Section of Endocrinology, Boston VA Healthcare system, Harvard Medical School, Boston, Massachusetts

[4] Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts

Abstract

Objective—Animal and in vitro studies indicate that leptin alleviates starvation-induced reduction in circulating vaspin and stimulates the production of visfatin. We thus examined whether vaspin and visfatin are affected by short- and long-term energy deprivation and leptin administration in human subjects in vivo.

Design and Methods—We measured circulating levels of vaspin and visfatin 1) before and after 72-h of starvation (leading to severe hypoleptinemia) with or without leptin administration in replacement doses in 13 normal-weight subjects, 2) before and after 72-h of starvation with leptin administration in pharmacological doses in 13 lean and obese subjects, 3) during chronic energy deficiency in 8 women with hypothalamic amenorrhea on leptin replacement for 3 months, and 4) during chronic energy deficiency in 18 women with hypothalamic amenorrhea on leptin replacement or placebo for 3 months.

Results—Acute starvation decreased serum leptin (to 21% of baseline values, P=0.002) but had no significant effect on vaspin and visfatin concentrations (P>0.05). Nor did normalization of leptin levels affect the concentrations of these two adipokines (P>0.9). Leptin replacement in women with hypothalamic amenorrhea did not significantly alter vaspin and visfatin concentrations, whether relative to baseline (P>0.15) or relative to placebo administration (P>0.9). Pharmacological doses of leptin did not affect circulating vaspin and visfatin concentrations (P>0.9).

Conclusions—Circulating vaspin and visfatin are not affected by acute or chronic energy deficiency leading to hypoleptinemia and are not regulated by leptin in human subjects, indicating that these adipocyte-secreted hormonal regulators of metabolism are independently regulated in humans.

Corresponding author: Eun Seok Kang, MD, PhD, Division of Endocrinology, Diabetes, & Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Ave - SL Room 412, Boston, MA 02215, Office: 617-667-2805, Fax: 617-667-2896. ekang@bidmc.harvard.edu, Severance Hospital Diabetes Center, Division of Endocrinology and Metabolism, Department of Internal Medicine, Yonsei University College of Medicine, 134 Shinchon Seodaemun, Seoul, Korea, Office: 82-2228-1968, Fax: 82-2-393-6884. edgo@yuhs.ac.

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Keywords

Energy deprivation; hypothalamic amenorrhea; metreleptin; adipokines

INTRODUCTION

Adipose tissue has been increasingly recognized as an important endocrine organ. Approximately 30 biologically active peptides and proteins (adipokines) are produced and secreted by adipose tissue and play key roles in the regulation of food intake and energy balance, insulin action, and glucose and lipid metabolism (1, 2). Vaspin (visceral adipose tissue-derived serine protease inhibitor) and visfatin (pre-B-cell colony-enhancing factor, PBEF) are two relatively new and less well described peptides secreted from white adipose tissue. Studies in animal models indicate that vaspin is an endogenous insulin sensitizer (3) and that visfatin has insulin-mimetic effects (4). Both adipokines are elevated in overweight and obese type 2 diabetic patients (5). Dysregulation of adipose tissue secretory function, such as that occurring in obesity (excess body fat) (6), has been linked with an array of metabolic complications. The lack of normal adipose tissue, e.g. in lipodystrophy and lipoatrophy, is also associated with dysmetabolic sequelae similar to what is seen in obesity (7). Hypoleptinemia is prevalent in energy deprivation states in humans, such as food deprivation/starvation, lipoatrophy, and exercise induced hypothalamic amenorrhea, and exogenous metreleptin administration corrects many of the metabolic and neuroendocrine abnormalities characteristic of these conditions (8–12).

It remains unknown whether these adipocyte-secreted hormones are acting independently from each other or whether secretion or administration of one influences the other. In rats, fasting decreases serum vaspin levels which are partly normalized by exogenous leptin administration (13). Leptin in physiological doses has also been reported to stimulate the production and secretion of visfatin from murine and human omental adipose tissue ex vivo and in mouse 3T3-L1 adipocytes in vitro (14) but animal and in vitro data cannot be directly translated into human biology. There is currently no information on whether leptin, which decreases in energy deprivation states, mediates changes in circulating vaspin and visfatin in humans in vivo. Furthermore, nothing is known regarding the in vivo regulation of vaspin and visfatin levels in humans by energy deficit per se, whether acute or chronic. Thus, in this study we first assessed circulating vaspin and visfatin in healthy men and women in response to acute energy deficit with and without leptin administration in physiological replacement and pharmacological doses which would alleviate the significant hypoleptinemia induced by acute energy deficit. We then assessed circulating vaspin and visfatin in women with exercise-induced chronic energy deficit and resultant hypoleptinemia, with or without exogenous leptin given at replacement doses.

MATERIALS AND METHODS

The study protocols were approved by the institutional review board of the Beth Israel Deaconess Medical Center (BIDMC) and recombinant methionyl human leptin (metreleptin; formerly r-metHuLeptin) was administered under an FDA-approved, investigator-initiated IND. The same clinical quality leptin was supplied initially by Amgen, Inc. (Thousand Oaks, CA) and later on by Amylin Pharmaceuticals, Inc. (San Diego, CA). Administration regimes were chosen on the basis of leptin pharmacokinetics (15–17) and modified as per protocol demands. All subjects were informed about the risks associated with the study procedures and gave their written informed consent.

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Study 1: Short-term energy deprivation (clinical trial registration: n/a)

Six men (age 23.5±1.5 years) and seven women (age 23.7±1.5 years) with a body mass index (BMI) < 25 kg/m^2 were studied during three separate admissions in the BIDMC General Clinical Research Center (GCRC). These studies were carried out between 2002 and 2006 and were designed to evaluate the role of leptin in the neuroendocrine and immune response to fasting (18, 19). Each subject completed three 3-day trials in random order: a 72-h control trial (fed state), a 72-h total fasting with administration of placebo, and a 72-h total fasting with administration of replacement doses of metreleptin. Briefly, for each trial, subjects were admitted to the GCRC the night before starting the study. Blood samples were obtained at 0800 h on day 1 and at 0800 h on day 3 for measurement of leptin, vaspin, and visfatin concentrations. Each admission was separated by at least 7 weeks to permit recovery of leptin levels and weight to baseline. Metreleptin was administered at a dose of 0.04 (men) or 0.08 (women) mg/kg/day on the first day and 0.1 (men) or 0.2 (women) mg/kg/day on the second and third days, to account for the progressive decrease in leptin concentration with additional days of fasting (on the basis of our pharmacokinetic studies) (15–17). The total daily metreleptin dose for each 24-h period was divided into four equal doses given every 6 hours by subcutaneous injection (administered by nursing staff) to mimic the normal diurnal variation in leptin levels (17). Placebo was administered according to the same schedule as metreleptin.

Study 2: High-dose leptin treatment (clinical trial registration: clinicaltrials.gov, NCT00140205)

The effect of high-dose leptin treatment on vaspin and visfatin were evaluated as part of a pharmacokinetic study of metreleptin carried our between 2006 and 2008 (15). Five healthy men (age: 22.2±0.9 years) and five healthy women (age: 20.4±0.7 years) with a BMI <25 kg/m^2, and five obese but otherwise healthy men (age: 23.4±1.5 years) with a BMI >30 kg/m^2, were admitted to the GCRC at the BIDMC for a 3-day fasting study with subcutaneous administration of metreleptin at a pharmacological dose of 0.3 mg/kg, once daily at 0800 h for 3 consecutive days. Blood samples were obtained at 0800 h on days 1 and 3 for measurement of leptin, vaspin, and visfatin concentrations. Details of this protocol have been described previously (15).

Study 3: Chronic energy deprivation - open label (clinical trial registration: n/a)

Eight women (age 24.8±1.9 years) with relative hypoleptinemia (<5 ng/ml) and hypothalamic amenorrhea for at least six months due to strenuous exercise or low weight were enrolled, and were studied before and after treatment with metreleptin for 3 months. The study was carried out between 2002 and 2004; the clinical characteristics and methods have been previously described in detail (9). Subjects self-administered metreleptin (0.08 mg/kg/day) subcutaneously for 3 months, with 40% of the daily dose given at 0800 h and 60% at 2000 h to mimic the normal diurnal variation in leptin concentrations (17). They were instructed not to change their diet and exercise habits for the duration of the study. Blood samples were obtained monthly for the measurement of leptin, vaspin and visfatin concentrations.

Study 4: Chronic energy deprivation - double blinded (clinical trial registration: clinicaltrials.gov, NCT00130117)

Eighteen women (age 25.0±2.2 years) with hypothalamic amenorrhea for at least six months and relative hypoleptinemia (<5 ng/ml) due to strenuous exercise or low weight were evaluated as part of a larger study on the effects of metreleptin on neuroendocrine function, carried out between 2008 and 2010 (20). Patient characteristics and detailed methods have been described elsewhere (20). The participants were randomized in a 1:1 ratio to receive

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either metreleptin or placebo. Metreleptin (0.08 mg/kg/day) or matching placebo doses were self-administered by subcutaneous injection daily between 1900 h and 2300 h for 3 months. According to our previous studies (15–17), the dose of metreleptin was calculated based on each subject's weight to achieve physiologic leptin concentrations in blood, but given as a single injection to facilitate compliance. Blood samples for the measurement of leptin, vaspin, and visfatin concentrations were obtained at baseline and after 3 months of treatment.

Hormone measurements

All samples used were stored at −80°C until assayed. Leptin concentration was measured by radioimmunoassay (Millipore, Billerica, MA) with a sensitivity of 0.5 ng/ml. Vaspin concentration was measured by using a commercially available ELISA kit (ALPCO Diagnostics, Salem, NH) with a sensitivity of 12 pg/ml. Visfatin concentration was measured by using a commercially available EIA kit (ALPCO Diagnostics, Salem, NH) with a sensitivity of 30 pg/ml. All samples were run in duplicate with quality controls, and inter- and intra-assay coefficients of variation for all analytes were <15%. In routine evaluation in our laboratory, as well as previously published data (21–24), suggest that long-term storage and freezing/thawing does not affect levels of the molecules of interest.

Statistical analysis

The results are presented as mean ± SEM. Statistical analyses were performed by using SPSS 11.0 (SPSS, Chicago, IL), and a two-tailed $P < 0.05$ was considered statistically significant. For study 1 (short-term energy deficit), paired *t* tests were used to assess changes in hormone levels within each condition. To determine whether these changes varied between conditions, we used a mixed-effects model repeated-measure ANOVA. For study 2 (high-dose leptin treatment), paired *t* tests were used to assess changes in hormone levels. For study 3 and study 4 (the two chronic energy deficit studies), changes in hormone levels were analyzed by using a mixed-effects model repeated-measures ANOVA and mixed model analysis. For comparing changes between the metreleptin- and placebo-treated groups, we used repeated measures analysis as primary analysis to evaluate changes in hormone levels by overall *P*-values for the main effect of treatment and treatment by time interaction. One-way ANOVA followed by the protected least significant-differences technique was used to compare differences between the two treatment groups for baseline characteristics and for follow-up measurements. Statistical analyses were conducted using on-treatment analysis.

RESULTS

Study 1: Short-term energy deprivation

Leptin concentration was significantly reduced after 3 days of fasting (from 8.5 ± 2.3 ng/ml to 1.8 ± 0.4 ng/ml, $P = 0.002$) and metreleptin administration reversed this change (both $P = 0.002$); vaspin and visfatin concentrations were not affected by short-term fasting nor leptin replacement (Table 1).

Study 2: High-dose leptin treatment

Pharmacological doses of leptin during 3 days of fasting had no significant effects on serum vaspin (0.68 ± 0.05 vs. 0.68 ± 0.05 ng/ml on days 1 and 3, respectively; $P = 0.981$) and visfatin (4.36 ± 0.29 vs. 4.31 ± 0.35 ng/ml on days 1 and 3, respectively; $P = 0.907$) concentrations.

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Study 3: Chronic energy deprivation - open label leptin treatment

Open-label metreleptin treatment for 3 months in women with hypothalamic amenorrhea significantly decreased body weight (54.7±4.5 kg to 52.2±3.5 kg; P <0.001) and increased serum leptin concentrations to physiologic levels during the first 2 months and to mildly supraphysiologic levels during the third month at the higher dose (Table 2). There were no significant changes in circulating vaspin or visfatin (Table 2).

Study 4: Chronic energy deprivation - double blind, placebo controlled, randomized leptin treatment

In the double blind, placebo controlled, randomized study, metreleptin treatment decreased body weight only slightly but significantly (55.6±2.0 kg to 55.5±1.7 kg; P = 0.046) whereas no change was observed with placebo administration (55.4±2.3 kg to 56.9±1.8; P =0.727). Despite the significant increase in serum leptin concentration, there were no significant changes in circulating vaspin or visfatin (Table 3).

DISCUSSION

We found that neither short-term energy deprivation (72 hours of total fasting) nor chronic energy deficit (hypothalamic amenorrhea) affect serum concentrations of vaspin and visfatin. Since energy deprivation states are typically associated with hypoleptinemia, we also studied whether metreleptin administration in either replacement doses to normalize circulating leptin levels or pharmacological doses that would increase serum leptin levels at or beyond the physiological range would alter the concentrations of these two adipokines. We observed that metreleptin has no significant effect on circulating vaspin and visfatin levels. We thus conclude that: 1) vaspin and visfatin are not likely involved in the development of metabolic and neuroendocrine changes which are typical in states of energy deficiency in humans, and 2) leptin does not modulate the levels of these two adipokines.

Leptin is a pleiotropic hormone; in addition to regulating energy homeostasis, it has important roles in neuroendocrine, reproductive and immune functions (8, 25, 26). Leptin conveys information to the central nervous system (CNS) regarding energy availability in the body, a system that is particularly sensitive to energy deprivation. In both humans and animals, states of acute and chronic energy deprivation are characterized by hypoleptinemia and metabolic and neuroendocrine dysfunction, which are normalized with the administration of leptin (9, 27, 28). Leptin exerts its actions by binding to its receptors in both the CNS and the periphery and activating signal transduction pathways that overlap with but are distinct from insulin signaling pathways (29). As vaspin and visfatin are novel adipokines exhibiting insulin sensitizing and/or mimetic effects (3, 4), we examined whether leptin regulates the circulating levels of these adipokines by conducting interventional studies in humans involving acute and chronic energy deficiency and leptin administration.

Vaspin is a recently discovered adipocytokine (3). The administration of recombinant vaspin in mice was shown to improve glucose tolerance and insulin sensitivity, identifying this novel adipokine as an insulin sensitizer (3). However, recent cross-sectional studies in humans do not confirm the association between circulating vaspin and glucose tolerance status or insulin sensitivity measured with the hyperinsulinemic clamp technique (30). Still, serum vaspin concentrations are elevated in obesity and type 2 diabetes, and this adipokine has thus been suggested to serve as a biomarker of obesity-related impairment in insulin sensitivity (5, 31, 32). Recent studies in humans have shown significant and direct associations between leptin and vaspin concentrations (23, 33). Also, a study in rats observed that fasting for 24 h or 48 h decreases circulating vaspin and this effect is partly abolished by exogenous leptin administration (13). Contrary to these findings, a recent

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observational study in a small number of human subjects reported a preprandial rise and postprandial fall in vaspin levels (34) indicating that either feeding or feeding-related factors such as insulin, which also regulates leptin levels (35) and/or leptin may be reciprocally related to these daily changes in vaspin levels. To test and expand the hypothesis raised by the above observational study in a systematic and methodical manner, we performed interventional studies involving short- and long-term energy deficit with or without leptin replacement in physiologic and pharmacologic doses. We found that neither total fasting for 72 h nor leptin administration in replacement or pharmacologic doses significantly affected vaspin concentrations in healthy lean and obese men and women. Likewise, we observed no effect of chronic energy deficiency per se or leptin replacement in a condition of chronic energy deficit (hypothalamic amenorrhea). The inconsistency of the results between animals and humans could be related to differences in the relative contribution of visceral fat (secreting vaspin) to total body fat. Alternatively, it is also possible that the cellular regulation of vaspin production and secretion in rodents is not similar to that in humans. The fact we did not detect any changes in vaspin levels over a few days of fasting and/or in response to leptin replacement may indicate that only short term signals of food intake such as insulin may have an effect in altering vaspin levels in the short term, as previously suggested (34).

Visfatin was originally identified as an adipokine exhibiting insulin-mimetic effects via binding to and activating the insulin receptor (4). The results of observational studies on the association between circulating visfatin concentrations and obesity and insulin resistance are conflicting (4, 5, 36–40). In an observational study of obese human subjects, plasma visfatin levels increased following intestinal bypass surgery, and post-surgery visfatin levels were significantly correlated with post-surgery leptin levels and change in leptin levels following surgery (41). Additionally, in vitro and ex vivo studies have shown that leptin increases visfatin production in 3T3-L1 adipocytes and adipose tissue extracts from humans and mice (14). In our short-term interventional studies, however, we found no effect of energy deficit on circulating visfatin levels with or without exogenous metreleptin in replacement or pharmacologic doses. In our model of chronic energy deprivation (exercise induced energy deficiency and hypothalamic amenorrhea), visfatin levels also remained unchanged, even with the normalization of leptin levels through administration of metreleptin for up to 3 months. These results are consistent with the absence of a significant change in circulating visfatin following a long-term, calorie-restricted diet in women (42), as well as the absence of a significant difference in serum visfatin levels between untreated patients with anorexia nervosa and bulimia nervosa and healthy control subjects (43).

Although our studies were performed in relatively small sample sizes and thus the possibility of a type II error cannot be fully excluded, a recently published study that demonstrated effects of short term caloric intake and/or food deprivation on vaspin levels was even smaller (34). Moreover, the various approaches taken, the convergence of similar results from several independent studies and especially the randomized, double-blinded, placebo-controlled design of both the short term and the chronic energy deprivation studies add to the robustness of our results. In addition, we assessed circulating vaspin and visfatin in models of both acute and chronic energy deficit and hypoleptinemia, with administration of physiologic and pharmacologic doses of metreleptin. The range of leptin concentrations achieved and the breadth of our analysis of these hormones strengthens the validity of our results. Leptin, visfatin and vaspin were measured using established, state of the art methodology with low laboratory error by technicians that were blinded to the study hypothesis. However, in this study, we only assessed a potential effect of leptin but have not studied any potential effects of insulin, as previously suggested (34). This remains to be tested in the future.

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In summary, we have shown that 1) short- and long-term energy deficit in humans does not affect circulating vaspin and visfatin, indicating that these adipokines are likely not involved in the metabolic and neuroendocrine abnormalities characteristic of energy deprivation states, and 2) leptin administration to alleviate hypoleptinemia does not significantly alter vaspin or visfatin levels. Thus, our results argue against the existence of a cross-talk between these adipokines and leptin which is not only of physiological significance but also may have implications on whether these hormones could be administered and/or co-administered, if and when they find their way in our therapeutic armamentarium.

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Table 1

Effects of total fasting for 72 hours with or without leptin replacement on circulating vaspin and visfatin

	Fed		Fasting		Fasting + Leptin	
	Day 1	**Day 3**	**Day 1**	**Day 3**	**Day 1**	**Day 3**
Leptin (ng/ml)	7.2 ± 1.6	10.1 ± 2.3	8.5 ± 2.3	1.8 ± 0.4*	7.0 ± 1.7	15.9 ± 3.8*
Vaspin (ng/ml)	0.68 ± 0.21	0.69 ± 0.18	0.61 ± 0.14	0.40 ± 0.16	0.47 ± 0.15	0.46 ± 0.15
Visfatin (ng/ml)	3.32 ± 0.82	3.66 ± 1.41	4.00 ± 1.27	2.56 ± 0.94	2.68 ± 0.67	2.78 ± 0.79

Values are means ± SEM.

* Value is significantly different from corresponding value on Day 1, $P < 0.05$.

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Table 2

Effects of open-label leptin replacement for 3 months on circulating vaspin and visfatin in women with hypothalamic amenorrhea

	Baseline	1 month	2 months	3 months	*P*-value
Leptin (ng/ml)	3.9 ± 0.8	10.0 ± 1.8*	22.1 ± 7.3*	39.1 ± 13.1*	<0.001
Vaspin (ng/ml)	1.07 ± 0.42	1.23 ± 0.39	1.34 ± 0.42	1.06 ± 0.33	0.520
Visfatin (ng/ml)	3.01 ± 1.16	2.28 ± 1.02	2.12 ± 0.60	1.54 ± 0.61	0.265

Values are means ± SEM. The *P*-value (effect of time) from the repeated measures ANOVA is shown.

* Value is significantly different from corresponding value at baseline, $P < 0.05$.

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Table 3

Effects of double-blinded placebo or leptin replacement for 3 months on circulating vaspin and visfatin in women with hypothalamic amenorrhea

	Placebo		Leptin		*P*-values		
	Baseline	**3 months**	**Baseline**	**3 months**	**Treatment**	**Time**	**Interaction**
Leptin (ng/ml)	2.77 ± 0.60	3.24 ± 0.30	2.67 ± 0.56	23.88 ± 5.06*	0.003	0.001	0.002
Vaspin (ng/ml)	0.94 ± 0.08	1.01 ± 0.18	0.88 ± 0.31	1.02 ± 0.28	0.952	0.278	0.715
Visfatin (ng/ml)	5.80 ± 0.92	6.35 ± 0.82	4.22 ± 0.69	6.22 ± 0.43	0.921	0.824	0.361

Values are means ± SEM. *P*-values from the repeated measures ANOVA are shown.

* Value is significantly different from corresponding value at baseline, $P < 0.05$.