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Circulating follistatin displays a day–night rhythm and is associated with muscle mass and circulating leptin levels in healthy, young humans

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ABSTRACT

Purpose. Follistatin may affect lean and fat mass and be implicated in metabolic diseases. We aimed to elucidate physiological predictors of circulating follistatin variation in healthy young humans.

Procedures. This was an observational, cross-sectional study with two additional prospective observational arms (circadian, seasonal sub-studies) and one prospective interventional arm (mixed meal sub-study). Healthy, young individuals of both sexes (n = 122) were subjected to anthropometric and body composition measurements and their eating and exercise behavior profiles were assessed by validated questionnaires. Sub-groups were subjected to standardized meal ingestion (n = 36), day–night rhythm (n = 20) and seasonal variation (n = 20) studies. Main outcome of the study were circulating follistatin levels.

Results. At baseline follistatin levels were correlated with creatinine ($r = 0.24$; $p = 0.01$), creatine phosphokinase ($r_s = 0.22$; $p = 0.02$), and with lean body mass ($r_s = 0.19$; $p = 0.04$) and were higher in males than females ($p = 0.004$) after adjustment for leptin, which was its major predictor. Follistatin levels showed a circadian ($p < 0.001$), but not a seasonal, variation, and were also affected by the phase of menstrual cycle in females ($p = 0.034$). Follistatin levels were not affected by dietary or exercise habits but levels increased after a standardized meal ingestion (250 kcal) ($p = 0.002$).

Conclusions. In healthy young individuals circulating follistatin levels are correlated with muscle mass. Follistatin levels are associated with circulating leptin levels and display a day–night rhythm and a menstrual cycle, but not a seasonal, variation.

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body mass index; CK, creatine phosphokinase; DXA, dual-energy X-ray absorptiometry; GGT, gamma-glutamyltransferase; HOMA-IR, HOmeostatic Model of Assessment — Insulin Resistance; IGF-BP3, insulin-like growth factor-binding protein 3; LBM, lean body mass; NAFLD, nonalcoholic fatty liver disease; PCOs, polycystic ovary syndrome.

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1. Introduction

Follistatin, a glycoprotein expressed in almost all human tissues, is a known inhibitor of several members of the TGF-beta superfamily, including myostatin and activin [1–3]. Follistatin was initially linked to the reproductive system, as it was discovered in the follicular fluid and found to inhibit follicle-stimulating hormone through activin binding in the pituitary, and was thought to exert mostly autocrine/paracrine actions [4]. Thus, circulating follistatin was considered to originate mainly from such paracrine actions. However, follistatin has been proposed to be an adipokine [5] while recent data suggest an endocrine production from the liver [6] in response to stimuli such as acute exercise [7] or feeding status [8] and the physiological role of follistatin and follistatin-like molecules has expanded to the regulation of fat and lean mass and muscle growth, through activin and myostatin inhibition [2,9]. Furthermore, follistatin has been implicated in the pathogenesis of muscle atrophy [3] and of metabolic diseases, as it has been reported to be elevated in patients with type 2 diabetes [10], polycystic ovary syndrome (PCOs) [11] and nonalcoholic fatty liver disease (NAFLD) [12]. Thus, follistatin may represent an appealing therapeutic target for muscle disorders [13] and metabolic diseases. Nevertheless, the physiological determinants of follistatin and its pattern of secretion remain largely unknown.

The main aim of this study was to evaluate physiologic parameters that could affect circulating follistatin including sex, body composition, eating and activity habits, and meal ingestion. Importantly, we assessed circadian and seasonal variation of follistatin levels and investigated potential correlations with adipokines (leptin, adiponectin) and metabolic parameters.

2. Patients and Methods

2.1. Subjects

This is a post-hoc analysis of a previous clinical study (NCT01986530) [14] with an additional prospective, observational sub-study (Seasonal sub-study). At the initial study, apparently healthy, young Caucasian medical students of both sexes (50% males) were recruited. Exclusion criteria were as previously reported [14]. A sub-group of twenty randomly selected individuals was additionally subjected to seasonal evaluation. The study was approved by the Ethics Committee of 424 General Military Hospital and was in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice. Written informed consent was obtained from all the participants.

2.2. Methods

At baseline, participants had been subjected to anthropometric and body composition measurements and their eating and exercise behavior profiles had been assessed by validated questionnaires, as previously described [14]. Sub-groups of the subjects had participated in the standardized meal ingestion (Fresubin Original Drink Fresenius Kabi, Greece — energy density 1.0 kcal/mL, carbohydrates 55%, protein 15%, fat 30%) and day–night rhythm sub-studies. Morning (8–9 am), fasting venous blood samples had been obtained and metabolic parameters had been measured within 1 h after blood drawing using standard methods [14]. Additional samples had been stored at −30 °C. For the purposes of the present study, we conducted additional measurements in stored serum samples. To avoid the interference in the studied parameters of the previous freeze–thaw cycle of the samples, we defrosted a second, unused set of serum samples obtained from the participants for the new measurements. Subjects participating in the seasonal sub-study where subjected to additional, blood sampling in the middle month of each season, e.g. July, October, January and April. The samples were sent to the laboratory of the Division of Endocrinology at Beth Israel Deaconess Medical Center, Boston, at the end of the study for the measurement of follistatin (ELISA, R&D Systems, MN; sensitivity 0.03 ng/mL, intra-assay CV 4.9–7.5%, inter-assay CV 5.2–7.3%), leptin (RIA, Millipore, MA; sensitivity 0.437 ng/mL, intra-assay CV 3.4–8.3%, inter-assay CV 3.6–6.2%), adiponectin (RIA, Millipore, MA; sensitivity 1 ng/mL, intra-assay CV 1.8–6.2%, inter-assay CV 6.9–9.3%) and insulin (Immulite 1000, Siemens Healthcare Diagnostics, MA; sensitivity 1 ng/mL, intra-assay CV 5.2–6.4%, inter-assay CV 5.9–8.0%). All study samples were assayed in duplicate in one batch. For the needs of the day–night rhythm sub-study, follistatin levels were measured in duplicate twice (with two different kits) to confirm the variation and the average of the two measurements run in duplicate is presented herein. The HOmeostatic Model of Assessment — Insulin Resistance [HOMA-IR = insulin (mU/L) × glucose (mmol/L)/22.5] was used to evaluate insulin resistance.

2.3. Statistical Analysis

Data for continuous variables are presented as mean ± standard error of the mean (SEM). Data for categorical variables are presented as numbers and/or percentages. Kolmogorov–Smirnov test was used to check the normality of distribution of the continuous variables. Chi-square test or Fischer's exact test was used to compare categorical variables. Independent samples T-test or Mann–Whitney test was used for between group comparisons, in cases of two groups of continuous variables. One-way analysis of variance (ANOVA) or Kruskal–Wallis test was used in cases of more than two groups of continuous variables. Analysis of covariance (ANCOVA) was used to adjust between group differences for potential confounders. Paired-samples T-test or Wilcoxon Signed Ranks test was used for paired comparisons. Repeated measures ANOVA or Friedman's test was used for more than two paired comparisons. Repeated measures ANCOVA was used to adjust paired measurements for potential confounders. Bonferroni post-hoc correction was used to adjust for multiple pairwise comparisons. Pearson's or Spearman's coefficient was used for unadjusted binary correlations. Partial coefficient was used for binary correlations adjusted for potential confounders. Multiple linear regression analysis («enter» method) was used to identify variables independently associated with serum follistatin levels. Variables that were not normally distributed were logarithmically transformed for the need of this