METABOLISM CLINICAL AND EXPERIMENTAL 60 (2011) 1359–1362

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Metabolism

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Preliminary Report

Ghrelin levels in patients with juvenile idiopathic arthritis: relation to anti–tumor necrosis factor treatment and disease activity

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ARTICLE INFO

Article history:
Received 12 December 2010
Accepted 10 March 2011

ABSTRACT

Studies in adults with rheumatoid arthritis reported low serum ghrelin that increased following anti–tumor necrosis factor (TNF) infusion. Data on juvenile idiopathic arthritis (JIA) are lacking. The aim of this pilot study was to explore serum ghrelin levels in patients with JIA and the possible association with anti-TNF treatment, disease activity, and nutritional status. Fifty-two patients with JIA (14/52 on anti-TNF treatment) were studied. Juvenile idiopathic arthritis was inactive in 3 of 14 anti-TNF–treated patients and in 11 of 38 non–anti-TNF-treated patients. The nutritional status, energy intake/requirements, appetite, and fasting serum ghrelin levels were assessed. Ghrelin control values were obtained from 50 individuals with minor illness matched for age, sex, and body mass index. Ghrelin levels in patients with JIA were significantly lower than in controls ($P < .001$, confidence interval [CI] = –101 to –331). Analysis according to anti-TNF treatment and disease activity showed that ghrelin levels were comparable to control values only in 3 patients with anti-TNF–induced remission. Ghrelin in non–anti-TNF-treated patients in remission was low. Multiple regression analysis showed that disease activity ($P = .002$, CI = –84.16 to –20.01) and anti-TNF treatment ($P = .003$, CI = –82.51 to –18.33) were significant independent predictors of ghrelin after adjusting for other potential confounders. Ghrelin did not correlate with nutritional status, energy balance, and appetite. Serum ghrelin is low in patients with JIA and is restored to values similar to those in controls following anti-TNF–induced remission. Our study provides evidence that TNF blockade is independently associated with serum ghrelin, which possibly contributes to anti-TNF–induced remission. These preliminary results could form the basis for future research.

TKL was responsible for study funding, design, and coordination. She supervised acquisition and interpretation of data as well as statistical analysis. MT participated in study design and was responsible for patient recruitment and data collection. CA carried out literature review and data analysis, and drafted the manuscript. PPG: equal contribution to CA. AT performed ghrelin assessment. SL authored the "OKMO" software. FKT participated in study design and provided expert advice.

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doi:10.1016/j.metabol.2011.03.006

1. Introduction

Ghrelin is a growth hormone–releasing peptide mainly produced by the stomach and associated with appetite regulation and feeding behavior [1]. Furthermore, ghrelin exerts anti-inflammatory properties by inhibiting the expression of proinflammatory cytokines including tumor necrosis factor (TNF) [2-4]. The key role of TNF on rheumatoid arthritis (RA) progression led to the introduction of anti-TNF agents in the treatment of RA and juvenile idiopathic arthritis (JIA) [5]. It was found that, in patients with RA, plasma ghrelin is low [6] and it sharply increases following anti-TNF infusion [7]. So far, there is a paucity of data regarding ghrelin levels in JIA and their association with anti-TNF treatment.

The aim of this pilot study was to assess serum ghrelin in patients with JIA and its possible association with anti-TNF treatment, disease activity, and nutritional status.

2. Methods

2.1. Study population

Fifty-two patients with JIA, 8 males and 44 females aged 5 to 20 years, followed-up by the Pediatric Immunology and Rheumatology Referral Center, were enrolled in the study. The onset type of JIA was systemic in 8, polyarticular in 19, oligoarticular in 23, and enthesitis-related arthritis in 2 [8]. Fourteen patients with JIA received anti-TNF treatment (etanercept, 7; infliximab, 7). At the time of ghrelin measurement, median disease duration was 87.6 (3-180) months and median anti-TNF treatment duration was 35 (2-68) months. Juvenile idiopathic arthritis was inactive in 3 (21.4%) of 14 patients treated with anti-TNF and in 11 (28.9%) of 38 patients not treated with anti-TNF [9].

Anthropometric parameters (body mass index [BMI], triceps skin-fold thickness, midarm circumference) were expressed as centiles for age and sex. Energy requirements (indirect calorimetry, kilocalories per day) were assessed using a handheld device (Microlife, Dunedin, FL). Twenty-four-hour food intake recall was analyzed using food-analysis software (Food Processor ESHA, ESHA Research, Salem, OR). Subjective appetite-related sensations (appetite, satiety, desire for food) were rated using "OKMO," a software designed and built using free and open-source software technologies specifically for the needs of this study. It runs on a handheld device (Palm OS 4, SONY ZIRE 72S, Tokyo, Japan); and it is based on a translation to the Greek language of the EARS software, which uses a visual analogue scale (0-10) that has been previously validated in English [10]. Fasting serum total ghrelin was measured using enzyme-linked immunosorbent assay (DSL Laboratory, Oxon, UK) with 9.8% interassay variation coefficient and assay range of 6 to 600 pg/mL. Ghrelin levels assessed in 50 healthy children and adolescents who visited the general pediatric and adult clinics for minor illness, matched for age, sex, and BMI, were used as control values. The study was approved by the Institutional Research Committee, and informed consent was obtained from all patients or their parents.

2.2. Statistical analysis

Means and standard deviations (normal distribution, Kolmogorov-Smirnov test) or counts and proportions were calculated. One-way analysis of variance with Dunnett T3 test for multiple comparisons (equal variances not assumed), the Fisher exact test, logistic regression analysis, and the Pearson correlation coefficient were used for data analysis, as appropriate. A model of multiple regression analysis (standard linear regression analysis) was constructed with ghrelin as the dependent variable and anti-TNF treatment, disease activity, JIA subtypes at measurement, BMI, and treatment with prednisolone as predictors. Statistical analysis was performed using the SPSS software for Windows (version 13.0 LEAD Technologies, Chicago, IL) and GraphPad Instat (version 3.05 GraphPad Software, San Diego, CA).

3. Results

Clinical data (age, anthropometry, energy intake/requirements, and appetite score) did not differ between anti-TNF–treated and non–anti-TNF-treated patients.

Ghrelin levels in patients with JIA were significantly lower than in controls in all disease-subtype groups ($P = .001$, $P = .005$, and $P < .001$ for the differences between controls and patients with oligoarticular, polyarticular, and systemic course, respectively). Anti-TNF recipients had ghrelin (137 ± 92.9 pg/mL) comparable to the controls (170 ± 107 pg/mL, $P = .61$, CI = –106.9 to 42.0, Fig. 1), whereas nontreated patients had ghrelin (90.2 ± 34.0 pg/mL) significantly lower than the controls ($P < .001$, Fig. 1). However, among anti-TNF–treated patients, only 3 patients (2 on etanercept and 1 on infliximab for 12, 59, and 24 months, respectively) with disease remission had increased serum ghrelin at levels comparable to the controls ($P = .12$, CI = –42.8 to 292.4). All of these 3 patients, 1 male and 2 females aged 8 to 15 years, had polyarticular JIA. Their demographic characteristics, nutritional status, and disease subtype were comparable to those of the other subgroups. Ghrelin was significantly lower in every other subgroup compared with controls (Fig. 1). It should be noted that patients who went into remission with treatment other than anti-TNF had ghrelin comparable to patients with active disease, treated or not with anti-TNF (Fig. 1), and lower than patients with anti-TNF–induced remission ($P = .05$, CI = 0.51-402.6).

No correlation was found between ghrelin and BMI, triceps skin-fold thickness, midarm circumference, appetite, and energy intake to requirement ratio.

Multiple regression analysis showed that both the anti-TNF treatment and disease activity were significant independent predictors of ghrelin levels after adjusting for other potential confounders, that is, disease subtype, BMI, and prednisolone treatment (Table 1).

4. Discussion

The low serum ghrelin found in our patients with JIA corroborates findings in RA patients [6], which however contrasts with reports in other chronic inflammatory diseases [11]. Disease remission was associated with increased ghrelin only in anti-TNF–treated patients.

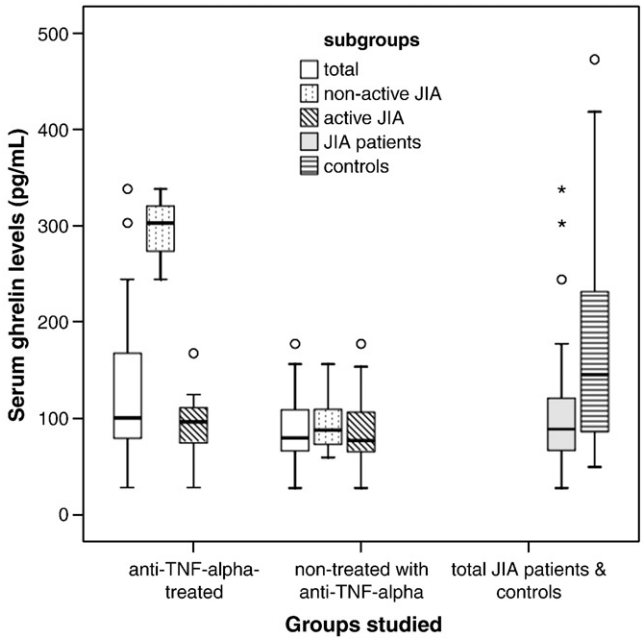

Fig. 1 – Serum ghrelin levels in relation to anti-TNF treatment and disease activity. Significant differences: (1) patients with JIA vs controls ($P < .001$, CI = –101 to –331, (2) non–anti-TNF-treated patients vs controls ($P < .001$, CI = –119 to –40.5, (3) anti-TNF–treated patients with active disease vs controls ($P = .002$, CI = –129.9 to –20.6, (4) non–anti-TNF-treated patients with active disease vs controls ($P < .001$, CI = –129.5 to –33.2, (5) non–anti-TNF-treated patients with inactive disease vs controls ($P = .001$, CI = –127.7 to –25.8, and (6) anti-TNF–treated patients with inactive disease vs anti-TNF–treated patients with active disease ($P = .043$, CI = 11.2 to 389) (analysis of variance with Dunnett T3 test for multiple comparisons).

Tumor necrosis factor and ghrelin exert opposite effects on the immune system and metabolism [12]. Existing data indicate a bidirectional influence between ghrelin and TNF [2,13]. Exogenous ghrelin inhibits the production of TNF from lipopolysaccharide-stimulated macrophages [2], whereas TNF administration in mice decreases plasma ghrelin and induces hypophagia, effects that are reversed by preadministration of ghrelin [13]. These findings indicate that inhibition of TNF could possibly increase ghrelin and improve appetite and nutrition. However, precise data on the role of ghrelin in disease activity are missing.

Table 1 – Multiple regression analysis (standard linear regression) with serum ghrelin levels as dependent variable

Predictors	Standardized coefficients (β)	P	95% CIs	
			Lower bound	Upper bound
JIA activity (active/nonactive)	–0.395	.002	–84.16	–20.01
Anti-TNF treatment (yes/no)	–0.382	.003	–82.51	–18.33
JIA subtypes (oligoarticular/ polyarticular/systemic)	0.155	.203	–7.18	32.80
BMI centiles	–0.127	.310	–0.69	0.23
Prednisolone treatment (yes/no)	–0.038	.758	–33.82	24.80

In line with studies in RA patients [7], we found that JIA recipients of anti-TNF had increased ghrelin levels. Further analysis revealed that anti-TNF treatment was associated with increased ghrelin only in patients with JIA remission. This finding could be interpreted as restoration to normal of endocrine processes following JIA inactivation [14]. However, this interpretation is not supported by our results showing that disease remission without TNF blockade was not associated with increased ghrelin. Furthermore, multiple regression analysis revealed the anti-TNF treatment as a significant independent predictor of ghrelin, suggesting a direct effect of anti-TNF on ghrelin, independent of that on disease activity. These results combined with published data demonstrating the anti-inflammatory properties of ghrelin [2,3] and the suppressive effect of TNF-α on ghrelin [7] indicate that anti-TNF treatment directly induces an increase of ghrelin that possibly contributes to disease remission. Evidence supporting an important role of ghrelin on RA pathophysiology is given by a recent study associating genetic variations of ghrelin with disease onset and rheumatoid factor positivity [15].

The stimulating effect of ghrelin on food intake and energy homeostasis [1] and the inverse correlation between circulating ghrelin and BMI [16,17] have been previously documented. However, in our study, ghrelin was not correlated with nutritional status, energy balance, or appetite. These findings might suggest that, in patients with JIA, the effect of nutritional status on ghrelin is overridden by the impact of inflammatory process [6,7] or by the suggested defect in endocrine regulation [14].

Previous studies demonstrated an improvement of growth velocity in patients with JIA treated with anti–TNF-α [18]. In our study, no correlation was found between anti-TNF treatment and nutritional status or food intake. These findings could be due to the fact that growth failure/cachexia was uncommon in our patients with JIA, and their energy intake exceeded the measured requirements (mean energy intake/requirement = 1.24, range = 0.31-2.9), possibly reflecting the high incidence of childhood obesity in Greece.

The main strength of this study is the assessment of a considerable number of parameters concerning the association between ghrelin and nutritional status, energy balance, and appetite. Therefore, our conclusion that ghrelin is not primarily influenced by nutritional factors is strengthened by the lack of correlation with any of these parameters.

A limitation of this pilot study is the small number of patients with anti-TNF–induced remission. Larger multicenter studies are needed to confirm or dispute our findings. In addition, simultaneous measurement of TNF levels could add a valuable dimension to a future study.

Our study provides evidence that TNF blockade is independently associated with serum ghrelin, which possibly contributes to anti-TNF–induced disease inactivation. From a clinical point of view, should our preliminary results be

confirmed in a larger study, this may suggest that ghrelin could be used as a novel therapeutic approach in JIA, in which case it would be especially useful for patients who do not tolerate anti-TNF treatment.

Acknowledgment

Funding statement: The project is cofunded by the European Social Fund and National Resources- EPEAEK II ARCHIMIDIS.

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