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Influence of etanercept on leptin and ghrelin secretion in children with juvenile idiopathic arthritis

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Abstract

Objective: To assess possible changes in leptin and ghrelin secretion due to etanercept in juvenile idiopathic arthritis (JIA).

Methods: 50 patients with JIA and 16 age-matched controls were enrolled into this prospective, cross-sectional study. Serum leptin, total and acyl ghrelin were measured in addition to white blood cell (WBC) and lymphocyte counts.

Results: 25 patients received etanercept and 25 conventional therapies (including methotrexate) for JIA. There was no difference between treatment and control groups in leptin or ghrelin levels and no evidence of a relationship between leptin and ghrelin in patients with JIA. In all children with JIA there was a correlation between leptin and body mass index (BMI). However, compared with children in the conventional treatment group, children in the etanercept group showed a positive correlation between total ghrelin and BMI and those with a low BMI showed a negative correlation between acyl ghrelin and BMI.

Conclusion: No differences in leptin and ghrelin concentrations were found when patients with JIA and controls were compared or when patients who received etanercept were compared with those who received conventional treatment for JIA.

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Introduction

Juvenile idiopathic arthritis (JIA) is the most frequent chronic form of arthropathy in children. [1] The disease is characterized by joint pain and inflammation that develops in a child younger than 16 years of age, lasts longer than six weeks and is of unknown cause.[2] Some patients experience aggressive, progressive and treatment resistant forms of JIA. [1] For patients whose disease has responded inadequately to, or who are intolerant of the disease modifying drug, methotrexate, current guidelines recommend the tumour necrosis factor α (TNFα) inhibitor, etanercept. [3] TNF is thought to play an important role in the pathogenesis of JIA and high levels have been found in both the serum and synovial fluid of children with JIA [2] Etanercept is a receptor protein p75 Fc that binds to TNFα thereby blocking its interaction with cell surface receptors and attenuating its pro-inflammatory effects.[2] Etanercept may also modulate biological responses controlled by additional downstream molecules (i.e., cytokines, adhesive molecules and proteinases) which are induced or regulated by TNFα. [4]

Leptin and ghrelin are hormones which have a major influence on energy balance.[5] Leptin synthesis depends on the obesity (ob) gene and one of the factors stimulating ob gene expression is TNFα. [6,7] Therefore, any pharmacological influence leading to a weakening of TNFα activity may cause a change in leptin synthesis. [8,9] In addition, leptin regulates endocrine and immune functions.[10-12] It also intensifies the inflammatory response by monocyte and macrophage activation, which, under its influence, increases production of pro-inflammatory cytokines, such as TNFα, interleukin (IL)-1 and IL-6. [12] Ghrelin is a growth hormone–releasing peptide and associated with appetite regulation. [13] Acylated ghrelin constitutes about 10-20% of total ghrelin and is a powerful stimulant of appetite.[14] Interestingly, loss of appetite is among the most common nonspecific symptoms of JIA. [15] Ghrelin also exerts anti-inflammatory properties by inhibiting the expression of pro-inflammatory cytokines including TNFα [13] this action may be of benefit in the treatment of JIA. [14]

Therefore, treatment with anti-TNF agents may influence serum levels of leptin and ghrelin. [13] The aim of this current study was to assess possible changes in leptin and ghrelin secretion following treatment with the anti-TNF drug, etanercept in children with JIA.

Methods

This was a prospective, cross-sectional study and involved children 6–18 years of age with JIA who were treated at the Paediatric Centre, Sosnowiec, Poland from 2009. The JIA was classified according to International League of Associations for Rheumatology (ILAR) criteria.[16,17] Nutritional status of each child was assessed by the calculation of body mass index (BMI).[18] Healthy, age-matched subjects with BMI $< 23\,kg/m^2$ were chosen for the control group.

In accordance with treatment guidelines,[3] children experiencing an acute course of JIA (i.e., symptoms from at least five affected joints and at least three joints affected by mobility dysfunction with accompanying pain and sensitivity) were prescribed etanercept. The drug was given subcutaneously at

0.4 mg/kg (up to 25 mg maximum dose).[3] Dosing was twice weekly, the first dose was administered on day zero and the second dose was given on days 3 or 4. Subsequent doses were given in following weeks at 3 to 4 day intervals.

During the study, venous blood samples were taken from the children and white blood cell (WBC) and lymphocyte counts were assessed using the Mindray BC 2300 Haematology Analyser (Diamond Diagnostics, MA, USA). Levels of serum leptin, total ghrelin, acyl ghrelin were assessed and the presence of antinuclear antibody (ANA)-HEp-2 determined Leptin was measured using an enzyme-linked immunosorbent assay (ELISA) test (Human Leptin Elisa Kit, Millipore, Missouri, USA; Human Leptin Receptor Elisa, BioVendor Laboratory Medicine, Inc, Czech Republic) according to the manufacturer's advised methodology. Total ghrelin and acyl ghrelin concentrations were assessed using ELISA tests (Linco Research Inc., Missouri, USA). Serum levels of ANA–Hep2 antibodies were detected under a fluorescent microscope using the Colorzyme HEp-2 ANA test system (Immuno Concepts N.A. Ltd., California, USA).

All patients and parents/guardians of children under 16 years provided written informed consent and the research was approved by the Bioethical Commission of Medical University of Silesia, Katowice no KNW/0022/KB1/6/I/99.

Statistical analyses

To examine relationships between variables both parametric methods and non-parametric tests were used depending on the data. Data analyses were performed using STATISTICA (data analysis software system; version 12 (2014) StatSoft, Inc. USA). A P-value <0.05 was considered to indicate statistical significance.

Results

Study population

Fifty children with JIA (34 girls and 16 boys) were enrolled into the study. Eighteen children had BMI $\geq 23\,kg/m^2$ and 32 had BMI $<23\,kg/m^2$. Two children had systemic JIA, 17 had oligoarticular JIA and 31 had polyarticular JIA. Overall, 35 (70%) children with JIA received oral glucocorticosteroids with approximately the same number in each treatment group. Clinical characteristics of the study groups are shown in Table 1. Twenty-five children received etanercept and 25 conventional therapies for JIA which included methotrexate. The control group consisted of 16 healthy, age-matched children (11 girls and 5 boys) all with BMI $< 23\,kg/m.^2$ The ANA Hep-2 antibodies were detected in 17 (68%) children in the etanercept group, in 16 (64 %) children in the conventional treatment group and none of the controls.

For all children with JIA and BMI $< 23\,kg/m^2$, the WBC count was statistically significantly higher compared with healthy controls ($P < 0.05$). The children in the etanercept had statistically significant lower BMI measurements and higher WBC counts compared with children in the control group ($P < 0.05$).

Leptin results

In the control group, a moderately positive correlation was observed between leptin and acyl ghrelin (Kendall's τ; $P <= 0.03$). This correlation was not detected in the two treatment groups nor in the subgroup of JIA patients with BMI $<23\,kg/m^2$.

In the etanercept group, a moderately positive correlation was detected between leptin and BMI (Spearman's rank correlation; $P <= 0.001$). Similarly, in the conventional treatment group, a strong positive correlation was observed between leptin and

Table 1. Clinical characteristics the study population of children with juvenile idiopathic arthritis treated with etanercept or conventional treatments (including methotrexate) and healthy, age-matched controls.

	Etanercept treatment	Conventional treatments (including methotrexate)	Healthy controls
Patients	25	25	16
Sex (girls/boys)	15/10	9/16	8/8
Age, years	13.5 ± 3.75	13.8 ± 3.2	13.2 ± 2.4
Systemic JIA	1 (4)	1 (4)	–
Oligoarticular JIA	8 (32)	9 (36)	–
Polyarticular JIA	16 (64)	15 (60)	–
Glucocorticosteroids	18 (72)	17 (68)	–
BMI, kg/m^2	17.1 ± 4.7*	18.4 ± 5.1	18.3 ± 1.0
BMI < 23 kg/m^2	18 (72)	14 (56)	16 (100)
Leptin, ng/ml	2 (0.6, 52.1)	4.4 (1.1, 35.9)	2.9 (2.4, 3.7)
Ghrelin total, pg/ml	1127 (436, 3016)	1471 (689, 4017)	1542 (1197, 1957)
Acyl ghrelin, pg/ml	559 (126, 1605)	471 (111, 1432)	627 (298, 910)
WBC, x 10^9 /l	7.8 (5.5, 12.9)*	7.3 (4.7, 11.0)	7.4 (5.4, 7.5)
Lymphocytes, x 10^9 /l	2.73 ± 0.88	2.75 ± 0.76	2.5 ± 0.38
ANA-Hep-2	17 (68)	16 (64)	0

Values are shown as *n* (%), median (range) or mean ± standard deviation.
Abbreviations: JIA, juvenile idiopathic arthritis; BMI, body mass index; WBC, white blood cells; ANA-HEp-2, antinuclear antibody Hep-2
*$P = 0.002$ compared with the control group (Wald-Wolfowitz test)

BMI (Spearman's rank correlation, $P < 0.001$). No such correlation was observed in the control group.

Children in the etanercept group with a low BMI ($n = 18$) had statistically significantly lower levels of leptin compared with age matched controls ($P < 0.05$)

Ghrelin results

In all children with JIA, a strong positive correlation was found between BMI and total ghrelin (Pearson's linear correlation; $P <= 0.03$) and multiple regression analysis showed a relationship between age and BMI with total ghrelin as the dependent variable ($P = 0.004$). No such correlation was observed in the control group.

Children in the etanercept group with a low BMI ($n = 18$) showed a moderately negative correlation between BMI and acyl ghrelin (Spearman's rank correlation; $P = 0.04$).

In the etanercept group, a strong positive correlation between number of lymphocyte and total ghrelin was detected (Spearman's rank correlation; $P = 0.0002$) as well as a strong positive correlation between number of lymphocyte and acyl ghrelin (Spearman's rank correlation; $P = 0.0003$). These correlations were not found in the conventional treatment group and controls.

Differences in types of JIA

The two children with systemic onset JIA had high WBC counts and high CRP and leptin concentrations (Table 2). Children with polyarthritic JIA had statistically significantly higher leptin concentrations (Mann–Whitney U test; $P = 0.005$), lower total ghrelin (Mann–Whitney U test;

Table 2. Serum levels of leptin, ghrelin, white blood cells, lymphocytes and C-reactive protein in patients according to type of juvenile idiopathic arthritis.

	Systemic JIA	Oligoarthritic JIA	Polyarthritic JIA
Patients	2	17	31
Leptin, ng/mL	26.1 (17.9, 34.2)	2.55 (0.62, 20.8)	2.74 (0.78, 52.1)
Ghrelin total, pg/ml	1747 (478, 3016)	1292 (645, 4017)	1122 (436, 2712)
Acyl ghrelin, pg/ml	654 (109, 1199)	635 (193, 1605)	460 (126, 1497)
WBC, x 10^9 /l	19.2 (5.6, 32.8)	7.4 (4.9, 10.3)	7.8 (4.1, 14.8)
Lymphocytes, x 10^9 /l	1.85 (1.51, 2.18)	2.71(1.61, 4.38)	2.5 (0.66, 8.16)
CRP, mg/l	36.1 (10.9, 61.2)	2.36 (0.2, 59.5)	3.17 (0.24, 34)

Values are shown as *n* or median (range).
Abbreviations: JIA, juvenile idiopathic arthritis; WBC, white blood cells; CRP, C-reactive protein

Table 3. Serum levels of leptin, ghrelin, white blood cells, lymphocytes and C-reactive protein in patients according to treatment with glucocorticoids.

	Glucocorticosteroid treatment	Without glucocorticosteroids
Patients	35	15
Leptin, ng/ml	3.23 (0.78, 52.1)	2.88 (0.62, 40.1)
Ghrelin total, pg/ml	1097 (478, 4017)	1439 (436, 2761)
Acyl ghrelin, pg/ml	530 (109, 1605)	542 (207, 1432)
WBC, x 10^9 /l	8 (4.1, 32.8)	7.4 (4.1, 11.2)
Lymphocytes, x 10^9 /l	2.28 (0.66, 7)	3.35 (1.45, 8.2)
CRP, mg/l	3.39 (0.2, 61.2)	2.49 (0.24, 7.95)

Values are shown as *n* or median (range).
Abbreviations: JIA, juvenile idiopathic arthritis; WBC, white blood cells; CRP, C-reactive protein

$P = 0.003$) and lower acyl ghrelin (Mann–Whitney U test; $P = 0.006$) compared with children with oligoarthritic JIA (Table 2).

Influence of glucocorticosteroids

Children treated with oral glucocorticoids had statistically significantly higher WBC counts (Mann–Whitney U test; $P = 0.0023$) and CRP concentrations (Mann–Whitney U test; $P = 0.0002$) compared with those children who did not receive glucocorticosteroids (Table 3).

A moderately positive correlation between CRP concentrations and number of lymphocytes was found in children treated with oral glucocorticoids, (Spearman's rank correlation; $P = 0.05$).

Discussion

To our knowledge, few studies have examined levels of leptin and ghrelin in children with JIA treated with etanercept.[13] Previous studies have confirmed that long-term treatment with etanercept with or without

methotrexate is effective and well tolerated in children with JIA [19-22] In this current study, the population of children with JIA was typical, in that it was characterized by high levels of ANA Hep-2 and WBC counts. [15] In addition, the children with JIA and low BMI had high WBC counts compared with controls. These low leukocyte counts may have been caused by the JIA or treatment with glucocorticoids. [23] In addition, leptin levels were significantly higher in the children with JIA who received oral glucocorticoids compared with those who did not.

Overall, we found no difference in leptin or ghrelin levels between treatment groups and control subjects and no evidence of a relationship between leptin and ghrelin in the patients with JIA. Both treatment groups exhibited positive correlations between leptin and BMI. However, compared with children in the conventional treatment group, children in the etanercept group showed a positive correlation between total ghrelin and BMI and those with a low BMI showed a negative correlation between acyl ghrelin and BMI. In addition, children in the etanercept group also showed a positive correlation between WBC count and total and acyl ghrelin which was not observed in the conventional treatment group or in the control group.

Although earlier studies examining leptin levels in relation to BMI in patients with JIA have been negative, leptin levels may well be a marker of nutritional status in these patients. [24,25] For example, studies in patients with rheumatoid arthritis (RA), have shown that serum leptin levels were directly related to percent body fat but not to disease activity.[26,27] In our previous study, we showed that children with JIA and BMI $< 23\,kg/m^2$ had lower leptin concentrations compared with healthy subjects and children with a short-term disease (2–18 months) had a higher diversification of leptin concentration per BMI unit compared with healthy controls.[12] Serum ghrelin levels and the possible association with anti-TNF treatment have been explored in a previous pilot study involving 52 patients with JIA.[13] The study showed that serum ghrelin was low in patients with JIA and following anti-TNF therapy values were comparable with those in the control group. However, a relationship between BMI and ghrelin was not observed. Interestingly, in this present study we found a positive and significant correlation between BMI and total ghrelin in all patients with JIA. In addition, we also showed a negative correlation between BMI and acyl ghrelin in patients receiving etanercept with BMI $< 23\,kg/m^2$

While our sample size was small, in this current study, positive correlations were shown in the etanercept group between the number of lymphocytes and both total and acyl ghrelin. These correlations occurred irrespective of the differences in lymphocyte counts and ghrelin concentrations between study and control groups. Although the role of lymphocytes in the pathogenesis of JIA has been confirmed, [15] the finding of a relationship between ghrelin concentrations and lymphocytes during anti-TNF treatment encourages further studies in this area, particularly because ghrelin receptors have been demonstrated in murine spleen T lymphocytes.[28]

In conclusion, no differences in leptin and ghrelin concentrations were found between patients with JIA and control subjects or between those treated with etanercept and conventional treatment. Nevertheless, further studies with a large sample size are required to confirm these results.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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