Correlation between insulin resistance and serum ghrelin in non-diabetic ankylosing spondylitis patients undergoing anti-TNF-α therapy

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Abstract

Objective

To evaluate whether anti-TNF-α therapy (infliximab) administration alters circulating levels of ghrelin, an anti-inflammatory gastric peptide. We also assessed possible associations of circulating ghrelin concentrations with CRP and ESR levels, metabolic syndrome, demographic characteristics and other adipokines in ankylosing spondylitis (AS) patients.

Methods

We studied 30 consecutive non-diabetic AS patients, without history of cardiovascular (CV) events, on periodical treatment with infliximab. Serum ghrelin levels were determined immediately prior to and after an infliximab infusion. Correlations of ghrelin serum levels with disease activity, systemic inflammation and metabolic syndrome were assessed. Potential changes in ghrelin concentration following an infusion of infliximab were analysed.

Results

We observed a negative correlation between ghrelin concentration and insulin resistance (HOMA-IR immediately before infliximab infusion- at time 0 and at the end of infliximab infusion- at time 120') (r=-0.496; p=0.01 at time 0; r=-0.393; p=0.047 at time 120', respectively). We also found a positive correlation with insulin sensitivity (QUICKI) (r=0.415; p=0.035 at time 0; r=0.465; p=0.017 at time 120'). A correlation was found between ghrelin and resistin prior to infliximab infusion (r=0.429; p=0.046), and a negative correlation between serum ghrelin levels at time 0 and triglycerides (r=-0.416; p=0.035). No differences in ghrelin levels according to specific clinical features of the disease were seen. A single infliximab infusion led to mild but not significant increase in ghrelin serum concentration.

Conclusion

In AS patients undergoing periodical treatment with anti-TNF-α monoclonal antibody-infliximab a link between insulin resistance and serum ghrelin concentration was observed.

Key words

ankylosing spondylitis, inflammation, anti-TNF-α antibody-infliximab, ghrelin, insulin resistance

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with increased cardiovascular (CV) mortality, 1.5-2 times higher than in the normal population (1, 2). Alterations in surrogate markers of atherosclerosis, such as endothelial dysfunction and increased common carotid artery intima-media wall thickness, have been reported in AS patients (3-5). These findings indicate the presence of subclinical atherosclerosis associated to the disease (6).

Traditional CV risk factors such as obesity and its related metabolic syndrome contribute to the increased CV morbidity and mortality in AS patients. However, the pro-inflammatory state present in AS patients is an additional CV risk factor (2). Ghrelin, the endogenous ligand for the growth hormone secretagogue receptor (GHS-R), is a peptide predominantly expressed in the stomach that regulates food intake and GH expression (7). In addition, many other biological functions have been proposed for ghrelin, one of them being a potential regulator of the immune response. Dixit *et al*. have previously demonstrated that ghrelin inhibits the expression of proinflammatory cytokines *in vitro* (8). Therefore, the possible role of ghrelin in the modulation of the immune system is of growing importance in the study of chronic inflammatory diseases.

Recently, we demonstrated that non-diabetic patients with AS treated with the anti-TNF-α monoclonal antibody-infliximab, which specifically and with high affinity binds to TNF-α and neutralises this cytokine, experience a rapid and dramatic reduction in the serum insulin levels and a rapid improvement of insulin sensitivity after administration of this drug (9). In order to establish other potential beneficial effects of the anti-TNF-α therapy on the metabolic syndrome associated to AS, we also studied serum levels of several adipokines in non-diabetic AS patients undergoing infliximab treatment. We found a positive correlation between adiponectin serum levels and insulin sensitivity, which suggested that low circulating adiponectin concentrations may be involved in the pathogenesis of the CV disease in AS (10). When we assessed visfatin serum levels in the same population, we also disclosed a significant positive correlation of this adipokine with insulin resistance (11). Because of that, we also analysed apelin serum levels, a new adipokine recently involved in CV risk, but we could not find apelin association with disease activity or with metabolic syndrome (12). Treatment with anti-TNF-α agents was reported to be effective in patients with AS and other spondyloarthropathies (13-15). As discussed before, we observed a rapid beneficial improvement of insulin sensitivity mediated by infliximab in AS patients (9). Therefore, it is plausible to think that TNF-α blockade might account for biological changes that may slow the progression of atherosclerosis in patients with AS. For this reason, an important step forward in our understanding of the effect of anti-TNF-α drugs on CV disease in AS may be to establish potential changes in metabolic syndrome-related biomarkers following the administration of these biologic agents.

Taking together these considerations, in the present study we aimed to establish whether inflammation and/or metabolic syndrome have any influence on circulating ghrelin concentrations in a series of non-diabetic AS patients undergoing anti-TNF-α-infliximab therapy. We also studied possible associations of circulating ghrelin concentrations with clinical and demographic characteristics of these patients. Moreover, we investigated whether an infliximab infusion altered circulating ghrelin concentrations in non-diabetic AS patients who required this therapy because of disease refractory to non-steroidal anti-inflammatory drugs (NSAIDs).

Patients

We assessed a series of 30 consecutive patients with AS attending hospital outpatient clinics seen over 14 months (January 2009 to March 2010), who fulfilled the modified New York diagnostic criteria for AS (16). They were treated by the same group of rheumatologists and were recruited from the Hospital Xeral-Calde, Lugo, Spain.

For ethical reasons, patients included in the present study were not randomised

to a placebo group. The same procedure has been found acceptable and followed in studies on the short term effect of infliximab therapy on the lipid profile, adipokines and biomarkers of endothelial cell activation in patients with rheumatoid arthritis (RA) (17-19).

Patients on treatment with infliximab seen during the period of recruitment with diabetes mellitus or with plasma glucose levels greater than 110 mg/dl were excluded. None of the patients included in the study had hyperthyroidism or renal insufficiency. In addition, patients seen during the recruitment period who had experienced CV events, including ischemic heart disease, heart failure, cerebrovascular accidents or peripheral arterial disease were excluded. Hypertension was diagnosed in patients with a blood pressure of ≥140/90 mmHg and in those taking antihypertensive agents. Obesity was defined if body mass index (BMI) (calculated as weight in kilograms divided by height in squared meters) was greater than 30.

In all cases the anti-TNF-α monoclonal antibody-infliximab was prescribed because of active disease. All patients included in the current study had begun treatment with NSAIDs immediately after the disease diagnosis. All of them were still being treated with these drugs at the time of the study. At the time of this study, most patients were on treatment with naproxen: 500-1000 mg/d. However, since the criterion for initiation of infliximab therapy was severe disease refractory to NSAIDs, all of them had been treated with at least three NSAIDs prior to the onset of infliximab therapy (20).

A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index- BASDAI- range of 0 to 10) (21) was evaluated in all patients at the time of the study. Clinical information on hip involvement, history of synovitis or enthesitis in other peripheral joints, history of anterior uveitis, presence of syndesmophytes and HLA-B27 status (typed by cell cytotoxicity) was assessed. Moreover, CRP- by a latex immunoturbidity method, ESR-Westergren, serum glucose, total cholesterol, HDL and LDL cholesterol and triglycerides (fasting overnight determinations) were assessed in all the patients at the time of the study.

The main demographic, clinical and laboratory data of this series of 30 AS patients at the time of the study are shown in Table I. Since at that time all patients were undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab (median duration of periodical treatment with this biologic agent: 23 months), the mean BASDAI ± standard deviation (SD) was only 2.94±2.11.

The local institutional committee approved the anti-TNF-α therapy. Patients gave informed consent to participate in this study. Neither this study nor the former studies on the short-term effect of infliximab therapy on insulin resistance in AS (9) or adipokines (10, 11) were supported by any pharmaceutical drug company.

Study protocol

In all cases, the drug was given to patients as an intravenous infusion in a saline solution over 120 minutes. All measurements were made in the fasting state. Blood samples were taken at 0800 hours for determination of the ESR (Westergren), CRP (latex immuno-turbidimetry), lipids (enzymatic colorimetry), plasma glucose and serum insulin (DPC, Dipesa, Los Angeles, CA, USA). As previously described, insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula= (insulin (μU/ml) x glucose (mmol/l)÷22.5[7] (9). A commercial ELISA kit was used to measure serum ghrelin levels (Millipore, EZGRT-89K; assay sensitivity=100 pg/ml; intra- and interassay coefficients of variation were 1.32% and 6.62%, respectively) (Linco Research, St. Charles, MO, USA) according to the manufacturer's instructions. Serum levels of ghrelin were measured in samples obtained immediately prior to an infliximab infusion and 120 minutes later. Commercial ELISA kits (Linco Research, St. Charles, MO, USA) were used to measure total plasma adiponectin (Millipore, EZHADP-61K; assay sensitivity=0.5 ng/ml; intra-and interassay coefficients of variation were 3.3% and 5.5%, respectively), serum resistin (Millipore, EZHR-95K; assay sensitivity=0.16 ng/ml; intra- and interassay coefficients of variation were <5% and <7%, respectively), and serum leptin levels (Millipore, EZHL-80SK; assay sensitivity=0.135 ng/ml ± 2 SD; intra-and interassay coefficients of variation were 3.7% and 4%, respectively), according to the manufacturer's instructions, immediately prior to an infliximab infusion and at the end of the infliximab infusion (at time 120 minutes) (10, 11). Commercially available ELISA/EIA kits (Phoenix pharmaceuticals, Burlingame, CA, USA) were also used to determine serum visfatin (Phoenix pharmaceuticals, EK-003-80; assay sensitivity = 2.68 ng/ml; intra- and interassay coefficients of variation were <10% and <15%, respectively) (11) and serum apelin levels (Phoenix pharmaceuticals, EK-057-23; assay sensitivity=0.06 ng/ml; intra-and interassay coefficients of variation were <10% and <15%, respectively) (12), according to the manufacturer's instructions, immediately prior to and after an infliximab infusion. Angpt-2 serum levels were also determined prior to and after an infliximab infusion by commercially available ELISA (Abcam, AB99971; assay sensitivity=10 pg/ml; intra-and interassay coefficients of variation were <10% and <12%, respectively) (Human Immunoassay Quantikine, R&D Systems, Cambridge, UK) according to the manufacturer's instructions. In addition, a commercial ADMA ELISA kit was used to measure serum ADMA levels immediately before and after infliximab infusion (Immundiagnostik, K7860; assay sensitivity=0.04 μmol/l; intra-and interassay coefficients of variation were 6.9% and 9.2%, respectively) (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions.

Statistical analyses

Variables were expressed as mean ± SD, median (interquartile [IQ] range) or percentages. Correlation between basal ghrelin at time 0 and with selected continuous variables in this series of non diabetic AS patients was performed adjusting by age at the time of the study, sex, and classic cardiovascular risk fac-

tors (dyslipemia, smoking, obesity, hypertension) via estimation of the Pearson partial correlation coefficient (r).
The associations between baseline characteristics and serum ghrelin concentrations were assessed by the Student's paired *t*-test for categorical variables. Differences in ghrelin levels between men and women and patients with hypertension or not were assessed by Mann-Whitney U-test.
Ghrelin serum levels before (at time 0) and postinfusion (at time 120') were compared using the Student's *t*-test.
Two-sided *p*-values ≤0.05 were considered to indicate statistical significance. Analyses were performed using Stata 12/SE (StataCorp, College Station, TX).

Table I. Demographic, clinical and laboratory data of 30 patients with ankylosing spondylitis.

Variable	n (%)
Mean age (years) ±SD	
At the time of study	50.47 ± 14.85
At the time of onset of symptoms	28.23 ± 10.40
Delay to the diagnosis (years) ±SD	11.48 ± 9.01
Men/Women	21(70) / 9(30)
Mean disease duration (years) ±SD*	21.97 ± 13.16
History of classic cardiovascular risk factors	
Hypertension (n=30)	12 (40)
Dyslipidemia (n=30)	11 (36.67)
Obesity (BMI >30 kg/m^2) (n=30)	3 (10.00)
Current smokers (n=30)	13 (43.33)
Mean blood pressure (mm Hg) ±SD*	
Systolic	123.17 ± 18.17
Diastolic	75.67 ± 12.51
Mean body mass index (kg/m^2) ±SD	26.70 ± 3.26
Mean BASDAI ±SD*	2.94 ± 2.11
Mean VAS ±SD*	31.13 ± 24.23
Hip involvement, n (%)(n=30)	6 (20)
Synovitis and/or enthesitis in other peripheral joints, n (%)(n=27)	11 (36.67)
Anterior uveitis, n (%)(n=30)	6 (20.00)
Syndesmophytes, n (%)(n=30)	10 (33.33)
Mean CRP (mg/l) ±SD**	
At the time of disease diagnosis	24.01 ± 33.43
At the time of study	6.24 ± 8.65
Mean ESR (mm/1st hour) ±SD***	
At the time of disease diagnosis	30.10 ± 28.23
At the time of study	19.00 ± 15.18
Mean cholesterol or triglycerides (mg/dl) ±SD*	
Total cholesterol	199.10 ± 30.61
HDL cholesterol	53.17 ± 12.81
LDL cholesterol	126.77 ± 26.54
Triglycerides	93.97 ± 56.70
Mean fasting serum glucose (mg/dl) ±SD*	92.77 ± 8.63
HLA-B27 positive (n=27)	20 (74.07)

*At the time of the study. **Normal value <5 mg/l. ***Normal value < 20 mm/1st hour.
BASDAI: Bath ankylosing spondylitis disease activity index; BMI: Body mass index; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HLA: human leukocyte antigen; LDL: low-density lipoprotein; SD: standard deviation; VAS: visual analogue scale

Results

Relationships of demographic features, inflammation, adiposity and adipokines with circulating ghrelin concentration

Ghrelin concentration (either at time 0 or at time 120') did not show significant correlation with age at the onset of symptoms, BMI, CRP and ESR at the time of the study or at the time of disease diagnosis (Table II). A significant positive correlation was found between ghrelin and resistin at time 0 (r=0.429; *p*=0.046). However, no association was found with adiponectin, leptin, visfatin, Angpt-2, apelin and ADMA (Table II). In addition, no difference was observed when we compared ghrelin levels between men and women (Table III).

Relationships of ghrelin concentration with metabolic syndrome features other than adiposity

No significant correlation between ghrelin concentration (either at time 0 or at time 120') with systolic or diastolic blood pressure, total cholesterol, HDL and LDL-cholesterol and serum glucose levels was observed (Table II). A negative correlation was found between serum ghrelin levels at time 0 and triglycerides (r=-0.416; *p*=0.035) (Table II). No significant differences in ghrelin concentration were seen when patients were stratified according to the presence or absence of arterial hypertension, dyslipidemia or obesity (Table III). Interestingly, a significant negative correlation between ghrelin concentration and insulin resistance (HOMA-IR at time 0 and 120') was observed (r=-0.496; *p*=0.01 at time 0; r=-0.393; *p*=0.047 at time 120', respectively) (Table II). We also found a positive correlation with insulin sensitivity (QUICKI) (r=0.415; *p*=0.035 at time 0; r=0.465; *p*=0.017 at time 120') (Table II).

Relationships of ghrelin concentration with other recorded baseline characteristics

Circulating ghrelin concentration (either at time 0 or at time 120') did not correlate with disease duration, BASDAI and VAS spinal pain at the time of the study (Table II). Likewise, no difference in ghrelin concentration was observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis and/or enthesitis in other peripheral joints was compared with the remaining patients who did not exhibit these features (Table III). It was also the case when patients were compared according to HLA-B27 status (Table III).

Changes in ghrelin concentration upon infliximab therapy

Following infliximab infusion, there was a mild but not significant increase in ghrelin serum concentration. In this regard, the median (IQ range) values of ghrelin were 502.20 (373.13-704.92) μmol/l immediately prior to infliximab

Table II. Partial correlation of serum Ghrelin prior to infliximab infusion (at time 0) and immediately after infliximab infusion (at time 120') with selected continuous variables adjusting by age at the time of the study, sex, and classic cardiovascular risk factors (dyslipemia, smoking, obesity, hypertension) in 30 patients with ankylosing spondylitis.

	Ghrelin (Time 0)			Ghrelin (Time 120')	
Variable	*r*	*p*		*r*	*p*
Age at the onset of symptoms	-0.212	0.299		-0.163	0.427
Disease duration*	0.236	0.246		0.127	0.535
BMI*	0.079	0.700		0.007	0.974
Systolic blood pressure*	0.224	0.272		0.140	0.497
Diastolic blood pressure*	0.250	0.217		0.134	0.514
BASDAI*	0.047	0.820		0.094	0.646
VAS *	-0.033	0.873		-0.056	0.785
ESR* (natural-log-transformed)	0.001	0.997		-0.190	0.353
CRP* (natural-log-transformed)	0.082	0.690		-0.156	0.446
ESR** (natural-log-transformed)	-0.117	0.568		-0.117	0.568
CRP** (natural-log-transformed)	-0.238	0.241		-0.143	0.485
Total cholesterol* (natural-log-transformed)	0.042	0.837		0.101	0.622
HDL cholesterol* (natural-log-transformed)	-0.048	0.818		0.087	0.672
LDL cholesterol* (natural-log-transformed)	0.224	0.271		0.228	0.263
Triglycerides* (natural-log-transformed)	-0.416	0.035		-0.370	0.063
Serum glucose* (natural-log-transformed)	0.028	0.892		-0.104	0.613
HOMA-IR at time 0*	-0.496	0.010	HOMA-IR at time 120'	-0.393	0.047
QUICKI at time 0*	0.415	0.035	QUICKI at time 120'	0.465	0.017
Resistin at time 0	0.429	0.046	Resistin at time 120'	0.068	0.763
Adiponectin at time 0	-0.170	0.408	Adiponectin at time 120'	-0.106	0.605
Leptin at time 0	-0.164	0.423	Leptin at time 120'	0.010	0.961
Visfatin at time 0	-0.309	0.125	Visfatin at time 120'	-0.297	0.141
Angpt-2 at time 0	0.123	0.549	Angpt-2 at time 120'	0.085	0.682
Apelin at time 0	-0.278	0.169	Apelin at time 120'	-0.308	0.126
ADMA al time 0	0.086	0.684	ADMA al time 120'	0.011	0.959

*At the time of the study. **At the time of disease diagnosis.
ADMA: Asymmetric dimethylarginine; BASDAI: Bath ankylosing spondylitis disease activity index; BMI: Body mass index; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein; QUICKI: quantitative insulin sensitivity check index; VAS: visual analogue scale.

infusion (at time 0) and 511.82 (380.77-620.74) μmol/l at the end of the infusion (at time 120 minutes) (p=0.398).

Discussion

Ghrelin is a molecule which is not only involved in appetite regulation and growth hormone expression, but that also participates in hormone secretion, gastrointestinal motility, cell proliferation, cardiovascular function and many other biological functions (7, 8). As previously mentioned, ghrelin inhibits the expression of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α through binding to its receptor, GHS-R (8).

Low circulating ghrelin levels have been reported in obese people (22), a condition directly associated with hyperinsulinemia and insulin resistance (23). As reported in individuals without rheumatic diseases (24, 25), we also disclosed a significant correlation between ghrelin and insulin resistance and insulin sensitivity in AS patients undergoing infliximab therapy. However, in our series only 3 of the 30 patients were obese. Therefore, other mechanisms different from obesity may account for our findings. In this regard, a persistently mild chronic inflammatory response may play a major role for the results observed in our series. With respect to this, in a former study we observed that the anti-TNF-α blockade led to significant decrease of HOMA-IR and significant increase of QUICKI in AS patients (9).

In the present study, we also found a significant correlation between serum ghrelin and resistin levels prior to infliximab infusion. Resistin and ghrelin participate in the mechanisms of glucose homeostasis, and they seem to have in common a close association with inflammation, a fact that may involve them in the pathogenesis of chronic inflammatory rheumatic diseases (7, 26, 27). Despite seeing a significant correlation between ghrelin and resistin levels at time 0, we could not observe association between ghrelin and resistin levels after TNF-α inhibitor infusion. In keeping with that observation, we previously reported that TNF-α antagonist-infliximab therapy infusion did not influence resistin levels in this series of AS patients (10). This finding may be due to the low levels of inflammation observed in this series of AS patients, who were undergoing periodical therapy with infliximab, at the time of the study.

We previously reported a significant elevation of ghrelin serum concentration upon a single infliximab infusion in RA patients with severe disease (28). However, in AS patients we did not observe a significant increase of ghrelin upon infliximab infusion. As pointed out before, this disparate result may be due to the absence of severe disease and low inflammation levels at the time of the assessment in our series of patients of AS when compared with our cohort of RA who had severe active disease despite periodical treatment with infliximab.

To our knowledge, Toussirot *et al*. reported the only previous study performed to evaluate ghrelin levels in AS patients (29). They described higher levels of ghrelin in AS patients when compared to controls (29). Since ghrelin inhibits the production of inflammatory cytokines (8), the results reported by Toussirot *et al*. seem to be unexpected. In this regard, ghrelin levels were found decreased in patients with RA when compared to controls (30). Whether a different degree of severity of the inflammatory burden in RA and AS may account for these contradictory results needs further elucidation.

In conclusion, in AS patients undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab, we observed a link between insulin resistance and serum ghrelin concentration.

Table III. Differences in basal Ghrelin serum levels (time 0) according to categorical variables.

Variable	Category	Ghrelin: Mean±SD	*p*
Sex	Men	474.85 ± 404.38	0.151
	Women	696.16 ± 285.75	
Arterial hypertension	Yes	466.27 ± 306.50	0.368
	No	598.08 ± 426.37	
Dyslipidemia	Yes	606.94 ± 322.01	0.494
	No	504.79 ± 416.99	
Obesity	Yes	500.94 ± 576.83	0.842
	No	548.45 ± 368.38	
Current smoker	Yes	576.27 ± 370.69	0.705
	No	520.43 ± 397.71	
Hip involvement	Yes	348.81 ± 302.70	0.164
	No	594.33 ± 388.42	
Synovitis and/or enthesitis in other peripheral joints	Yes	561.49 ± 388.79	0.847
	No	532.56 ± 387.06	
Anterior uveitis	Yes	587.63 ± 429.08	0.782
	No	534.35 ± 379.70	
Syndesmophytes	Yes	549.28 ± 482.15	0.954
	No	540.51 ± 330.95	
HLA-B27	Positive	516.99 ± 403.47	0.382
	Negative	675.04 ± 395.58	

HLA: human leukocyte antigen; SD: standard deviation.

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