Leptin and adiponectin as predictors of disease activity in rheumatoid arthritis

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Abstract

Objective

To assess whether baseline levels of leptin and adiponectin predict disease activity or response to treatment in patients with RA at 6 months, 1 and 2 years of follow-up.

Methods

A consecutive cohort of patients, classified according to the 2010 ACR/EULAR RA criteria, was evaluated at baseline, 6 months, 1 and 2 years. All were treated with steroids and/or DMARDs. None received biologics. Blood was taken at a baseline to determine plasma anti-CCP, leptin and adiponectin. The relationship between leptin, adiponectin, DAS28 and changes in DAS28 was assessed by multivariable linear and logistic regression from baseline to follow-up.

Results

127 patients completed 6 months, 91 one year and 52 two years of follow-up. All were female, mean age 45 years (18–70), time since onset of disease 7.5 years (0–36). A U-shaped relationship between DAS28 and leptin baseline levels was seen. Adjusting for different factors, leptin levels at baseline predicted higher DAS28 at 6 months and, in patients who were not overweight or obese, predicted disease activity at 6 months, 1 and 2 years. In patients who were not overweight or obese, baseline leptin was able to predict response to treatment at 6 and 12 months.

Conclusion

In the short term, baseline leptin levels predict disease activity in all RA patients and response to treatment in RA patients with normal weight.

Key words

rheumatoid arthritis, adiponectin, leptin, response to treatment, prognosis.

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Introduction

In rheumatoid arthritis (RA), cytokines play a fundamental role in inflammation and joint destruction, originating in synoviocytes and leucocytes infiltrating synovial tissue (1, 2). Adipose tissue synthesises and releases highly bioactive substances known as adipocytokines (*e.g.* leptin, adiponectin, resistin, etc.) (3). Adipocytokines exert multiple regulating effects on local and systemic inflammation, and autoimmune processes, such as RA, can be modified by changes in their levels (4, 5). Hence, there is a great deal of interest in understanding the relationship between adipocytokines and RA clinical activity, but results have been heterogeneous and controversial (6-9).

Different studies show an association between increased leptin levels and severe disease or poor RA prognosis (7, 8, 10). There is also evidence that the rate of synovial to serum leptin correlates with RA duration and activity (11). A two-year follow-up of RA patients showed that changes in leptin levels correlate with modifications in disease activity (12); nevertheless, these associations have not been confirmed in other studies (6, 13), and serum leptin even inversely correlates with radiological joint damage (14).

Moreover, high levels of adiponectin have also been associated with increased RA disease activity (7, 15) and recent *in vitro* studies have confirmed an inflammatory effect (16, 17). However, its serum levels do not seem to change during the course of anti-TNF treatment in RA (9, 18).

Obesity has been consistently associated with less radiographic damage in RA joints (19), and patients with RA and low levels of visceral fat have higher levels of adiponectin. Similar studies in RA also associate obesity with worse disease activity (20), less response to treatment and comorbidities (21), however, the relationship between obesity and RA is more complex than what is attributable to adipocytokines (5, 22). In this respect, when comparing leptin and adiponectin between patients and healthy controls, elevations of both leptin and adiponectin in RA seem independent of BMI (23). Recent evidence shows that obese patients with RA have higher DAS scores than non-obese patients, irrespective of disease activity (24). However, it is unknown if higher disease activity in obese patients is associated with leptin or adiponectin.

No previous studies have evaluated whether leptin or adiponectin baseline levels are able to predict clinical activity or patient response to treatment in a clinical setting. Therefore, we assessed the association between baseline serum levels of adipocytokines and response to disease-modifying treatment in patients with RA after 6 months, one and two years of follow-up, and evaluated whether such a relationship was modified by the patient's body mass index (BMI).

Materials and methods

Population and sample

The patients were part of a cohort of more than 600 consecutive RA patients from the Rheumatology Outpatient Clinic of the Hospital General de Cuernavaca, in Morelos, Mexico. Patients were recruited from March 2011 to September 2012 according to the following inclusion criteria: 18 years of age or older at inclusion, at least 6 months of follow-up, signed informed consent, treatment with prednisone at a dose equal to or less than 10 mg/day (or its equivalent) and stable disease-modifying anti-rheumatic drug (DMARD) therapy, with none of the patients receiving biologic therapy. Patients were excluded if compliance to treatment was in doubt, or if they were pregnant. Follow-up was carried out at six months, one and two years. RA was diagnosed according to the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 (25) criteria and we defined early onset RA as patients with less than one year since disease onset.

The Hospital Ethics Committee approved the protocol. After obtaining informed consent, the patient underwent a clinical evaluation by a rheumatologist in each visit; venous blood samples were withdrawn at baseline and plasma samples were obtained the same day of collection and stored at -75°C.

Treatment

Treatment received by patients was divided as follows: Those patients treated only with non-steroidal anti-inflammatory drugs (NSAID) and/or prednisone were classified as not having received disease modifying anti-rheumatic drugs (no DMARD); those treated with a single DMARD were classified as monotherapy (mono) and those receiving more than one DMARD (including cloroquine) were classified as combination therapy (combination).

Outcomes

Disease activity was measured at baseline and follow-up, using the 28 joint Disease Activity Score (DAS28), calculated using Erythrocyte Sedimentation Rate (ESR). Disease activity at baseline was divided into low (DAS28≤3.2), medium (3.2<DAS28≤5.1) and high (DAS28>5.1). Response to treatment was defined by the EULAR response criteria (26). This instrument is based on both the total DAS28 and changes in the score through time (ΔDAS28), classifying response to treatment into 3 categories: good response (DAS28 improvement >1.2 units and a score of <2.4), moderate response (improvement of 0.4–1.2 and a score of 2.4–3.7) and no response (less than 0.4 improvement and a score over 3.7). At subsequent visits, DAS28 was evaluated, also tracking the type of treatment the patient received and changes it had undergone. Treatment modifications were performed based on the physicians' criteria in a case-dependent manner.

Measurements

Leptin and adiponectin were determined at baseline through indirect ELISA (12, 23), using human recombinant leptin (PeproTech Inc.) and adiponectin (R&D Systems) for the calibration curve. All assays were performed in triplicate and reported as means and standard deviations. Additionally, clinical and laboratory test data was collected: body mass index, tender joint count (TJC), swollen joint count (SJC), ESR, C-reactive protein (CRP; qualitative), RF (nephelometry; quantitative determinations were not available for all patients), anti-CCP (chemiluminescence ELISA; quantitative determinations were not available for all patients) and medications used by the patient.

Statistical analysis.

Prior to statistical analysis, we imputed the missing values of DAS28 with the mean DAS28 between the prior and the following visit, if possible (n=3). We used a similar approach for imputing missing dates (n=2).

We used multiple linear regression to estimate the mean difference (95% CI) in DAS28 and ΔDAS28 per each increase of 1 ng^2/ml^2 and 100 ng/ml in baseline levels of leptin (modelled as a second-order polynomial) and adiponectin, respectively. Along with those crude models, we tested four prediction models defined *a priori*: Basic, adjusted for age (continuous); Metabolic, with further adjustment for BMI (continuous); and Rheumatic, with two versions: 1) age adjusted (continuous), BMI (continuous), methotrexate therapy at inclusion (yes/no), RF (positive/negative) and time in years since onset of disease, and 2) with further adjustment for anti-CCP antibody titers on a logarithmic scale. The latter model had a poor fit and was eventually removed from the results. We fitted all crude and prediction models for each time point: baseline, 6 months, 1 year and 2 years. We also performed the analyses for the whole observation period using repeated-measures multiple linear regression models with additional adjustments for the exact visit date.

We evaluated the potential modifying effect of BMI by stratifying the analysis for the normal weight (BMI <25 kg/m^2) and overweight/obesity (BMI>= 25 kg/m^2) categories. Additionally, we also estimated the odds ratios (95% CI) for the relationship between adipocytokines and two clinical endpoints, *i.e.* remission (defined as DAS28 <2.6) and poor-response to treatment (as defined by EULAR criteria). The analytical approach was the same as aforementioned, with the only difference being the use of multiple logistic regressions for estimations. The *p*-values for changes in DAS28, ΔDAS28 and remission over time from baseline were obtained from repeated-measures linear or logistic regression, as appropriate. We used the Stata 13 (Statacorp, TX, USA) statistical software package for analysis. The level of statistical significance was 0.05 (two-tailed).

Results

Of over 600 cohort subjects, 213 patients had the follow-up time needed for eligibility into the study; of them, 127 female patients were included. As described in Table I, on average, patients were middle aged (mean age 45 years; SD 10.7) and overweight (mean BMI of 27 kg/m^2; SD 4.5). The mean time since onset of disease was 7.5 years, 88.2% and 72.8% of patients were RF and antiCCP positive, respectively, although antiCCP was only determined in 81 subjects. Mean titers were 574.1 UI for RF and 134.8 UI for anti-CCP. Mean baseline DAS28 showed moderate activity (4.5, SD 1.4). Most patients (43.3%) had moderate disease activity scores at baseline. On average, baseline levels were 0.55 ng/ml for leptin and 142.54 ng/ml for adiponectin. Other

Table I. Baseline characteristics of patients included.

Characteristic	Mean (SD)
Age, y	45.0 (10.7)
BMI, kg/m^2	26.9 (4.5)
Normal weight, n (%)	42 (33.1)
Overweight, n (%)	61 (48.0)
Obesity, n (%)	24 (18.9)
Time since RA onset, y	7.5 (8.0)
DAS28	4.5 (1.4)
Disease activity	
Low, DAS28≤3.2, n (%)	29 (22.8)
Medium, 3.2<DAS28≤5.1, n (%)	55 (43.3)
High, DAS28>5.1, n (%)	43 (33.9)
Leptin, ng/ml	0.55 (0.56)
Adiponectin, ng/ml	142.54 (93.22)
Anti-CCP antibodies*, UI	134.8 (106.6)
Positive anti-CCP antibodies*, n (%)	59 (72.8)
RF*, UI	574.1 (789.2)
Positive RF, n (%)	112 (88.2)
MTX prescription at inclusion, n (%)	110 (86.6)
Steroid prescription at inclusion, n (%)	119 (93.7)
Type of treatment	
No DMARD, n (%)	15 (11.8)
Mono, n (%)	3 (2.4)
Combination, n (%)	109 (85.8)

Data are mean (SD), except where indicated.
*Data available for n=81.

Table II. Disease activity and response to treament at 6 months, 1 year and 2 years.

Variables	6 months n=127	1 year n=91	2 year n=52	Any time n=270
Time from last visit, months	6.0 (1.9)	6.6 (3.0)	13.0 (2.9)	7.5 (3.7)
DAS28*	3.8 (1.3)	3.7 (1.3)	3.7 (1.1)	3.7 (1.2)
ΔDAS28*	0.7 (1.6)	0.7 (1.9)	1.1 (1.8)	0.8 (1.7)
Remission*, n (%)	23 (18.1)	22 (24.2)	7 (13.5)	52 (19.3)
Response to treatment				
Good, n (%)	22 (17.3)	19 (20.9)	13 (25.0)	54 (20.0)
Moderate, n (%)	40 (31.5)	27 (29.7)	18 (34.6)	85 (31.5)
None, n (%)	65 (51.2)	45 (49.5)	21 (40.4)	131 (48.5)

Data are mean (SD), except where indicated. *p<0.001 for changes over time from baseline.

characteristics of patients are shown in Table I.

All patients received a similar treatment strategy, which included prednisone at doses of 10 mg or less/daily (or its equivalent) and one or more of the following DMARDs: azathioprine (AZA), cloroquine (CLQ), prednisone (PDN), leflunomide (LFN), methotrexate (MTX), sulphasalazine (SSZ). At baseline, 85.8% of patients were receiving combination therapy. 11.8% did not receive any DMARDs and only 2.4% received methotrexate monotherapy. There was no significant variation in treatment strategy at 6 months and at 1 and 2 years of follow-up.

As observed in Table II, 127 patients were followed for 6 months, 91 were followed for one year and 52 for 2 years. The only difference between those who completed two years of follow-up and those who did not was the mean baseline leptin concentration (0.42 *vs*. 0.65 ng/ml, respectively, p=0.02). The mean DAS28 score improved with time, 3.8 at 6 months and 3.7 at one and two years of follow-up. Overall change in the DAS28 score was 0.8. At 6 months, 18.1% had achieved remission, 20.9% reached remission at one year and 13.5% at two years. Most patients (51.2%) showed no response to treatment at 6 months, but this improved at one year, with patients steadily improving over time (49.5% were non responders at one year and 40.4% were non responders at two years). Overall, patients showed a moderate or good response (51.5%) to treatment.

Higher baseline adiponectin was associated to higher disease activity only at baseline, irrespective of the statistical model employed, but no association was seen with response to treatment over time. We observed a U-shaped relationship between leptin and DAS28 at 6 months in each of the 4 analysis models; however, these associations disappeared at 1 or 2 years of follow-up. Similar to what was seen with adiponectin, baseline leptin was not capable of predicting response to treatment over any time period (Table III). Patients with higher leptin levels, followed through time, improved with a greater frequency.

Table IV shows a stratified analysis of the relationship between leptin and dis-

Table III. Association between adipocytokines and disease activity and response to treatment at baseline, 6 months, 1 year and 2 years.

Variables	Crude	Model 1	Model 2	Model 3
Adiponectine, per 100 ng/ml				
Disease activity				
DAS28 at baseline	**0.27 (0.01, 0.54)**	**0.27 (0.00, 0.54)**	**0.29 (0.02, 0.56)**	**0.29 (0.01, 0.56)**
DAS28 at 6m	0.10 (-0.14, 0.33)	0.09 (-0.14, 0.33)	0.13 (-0.11, 0.37)	0.13 (-0.11, 0.37)
DAS28 at 1y	-0.04 (-0.32, 0.23)	-0.05 (-0.32, 0.23)	-0.03 (-0.31, 0.25)	-0.02 (-0.30, 0.26)
DAS28 at 2y	0.05 (-0.28, 0.38)	0.07 (-0.26, 0.40)	0.08 (-0.25, 0.42)	0.06 (-0.29, 0.40)
DAS28 at any time*	0.13 (-0.04, 0.30)	0.12 (-0.04, 0.29)	0.15 (-0.02, 0.32)	0.14 (-0.03, 0.31)
Response to treatment				
ΔDAS28 at 6m	0.18 (-0.12, 0.48)	0.18 (-0.13, 0.48)	0.16 (-0.15, 0.47)	0.16 (-0.15, 0.47)
ΔDAS28 at 1y	0.24 (-0.16, 0.64)	0.23 (-0.17, 0.63)	0.24 (-0.16, 0.65)	0.21 (-0.20, 0.63)
ΔDAS28 at 2y	-0.14 (-0.68, 0.39)	-0.13 (-0.67, 0.41)	-0.14 (-0.69, 0.41)	-0.16 (-0.72, 0.39)
ΔDAS28 at any time*	0.19 (-0.10, 0.49)	0.19 (-0.10, 0.49)	0.18 (-0.12, 0.48)	0.17 (-0.13, 0.47)
Leptin, per 1 ng²/ml² (quadratic term)				
Disease activity				
DAS28 at baseline	0.23 (-0.11, 0.57)	0.24 (-0.10, 0.58)	0.24 (-0.10, 0.58)	0.23 (-0.11, 0.57)
DAS28 at 6m	**0.44 (0.16, 0.73)**	**0.45 (0.16, 0.74)**	**0.45 (0.17, 0.74)**	**0.47 (0.19, 0.76)**
DAS28 at 1y	0.35 (-0.21, 0.91)	0.37 (-0.20, 0.93)	0.44 (-0.14, 1.02)	0.51 (-0.08, 1.09)
DAS28 at 2y	0.70 (-1.19, 2.60)	0.65 (-1.21, 2.51)	0.64 (-1.22, 2.50)	0.32 (-1.65, 2.30)
DAS28 at any time*	**0.32 (0.10, 0.55)**	**0.33 (0.11, 0.56)**	**0.34 (0.12, 0.56)**	**0.35 (0.13, 0.57)**
Response to treatment				
ΔDAS28 at 6m	-0.22 (-0.59, 0.16)	-0.21 (-0.59, 0.17)	-0.21 (-0.59, 0.17)	-0.24 (-0.63, 0.15)
ΔDAS28 at 1y	-0.32 (-1.15, 0.50)	-0.28 (-1.11, 0.55)	-0.26 (-1.12, 0.59)	-0.36 (-1.24, 0.51)
ΔDAS28at 2y	-0.06 (-3.12, 3.01)	-0.08 (-3.17, 3.02)	-0.07 (-3.20, 3.05)	0.07 (-3.19, 3.32)
ΔDAS28 at any time*	-0.16 (-0.55, 0.23)	-0.15 (-0.55, 0.24)	-0.16 (-0.55, 0.24)	-0.19 (-0.58, 0.21)

Data are coefficients (95% CI) from multiple linear regression. Bold type indicates p<0.05. Model 1. Basic. Adjusted for age. Model 2. Metabolic. Model 1 + BMI. Model 3. RA. Model 2 + MTX at inclusion, Rheumatoid Factor and Time from onset of RA.
*Data are coefficients (95% CI) from repeated-measures multiple linear regression with additional adjustment for exact date of visit.

Table IV. Association between leptin (quadratic term) and disease activity and response to treatment at baseline, 6m, 1y and 2y by BMI categories.

Variables	Crude	Model 1	Model 2
Normal weight, n=42			
Disease activity			
DAS28 at baseline	-0.46 (-1.24, 0.32)	-0.51 (-1.31, 0.29)	-0.48 (-1.33, 0.38)
DAS28 at 6m	0.43 (-0.26, 1.12)	0.48 (-0.23, 1.19)	**0.69 (0.02, 1.37)**
DAS28 at 1y	**1.10 (0.07, 2.13)**	**1.24 (0.18, 2.29)**	**1.76 (0.54, 2.98)**
DAS28 at 2y	**3.45 (1.18, 5.72)**	**3.58 (1.31, 5.84)**	**3.40 (0.32, 6.48)**
DAS28 at any time*	0.18 (-0.35, 0.71)	0.18 (-0.37, 0.74)	0.31 (-0.27, 0.88)
Response to treatment			
ΔDAS28 at 6m	**-0.89 (-1.69, -0.10)**	**-0.99 (-1.79, -0.18)**	**-1.17 (-2.00, -0.35)**
ΔDAS28 at 1y	-1.40 (-2.90, 0.11)	-1.49 (-3.07, 0.09)	**-2.02 (-3.78, -0.25)**
ΔDAS28 at 2y	-3.39 (-10.03, 3.25)	-3.72 (-10.39, 2.95)	-5.13 (-13.77, 3.51)
ΔDAS28 at any time*	**-0.93 (-1.78, -0.09)**	**-1.04 (-1.90, -0.19)**	**-1.18 (-2.09, -0.27)**
Overweight or obesity, n=85			
Disease activity			
DAS28 at baseline	**0.41 (0.04, 0.79)**	**0.41 (0.05, 0.78)**	**0.39 (0.01, 0.77)**
DAS28 at 6m	**0.46 (0.14, 0.78)**	**0.46 (0.14, 0.78)**	**0.43 (0.10, 0.76)**
DAS28 at 1y	0.11 (-1.46, 1.69)	0.09 (-1.52, 1.70)	0.10 (-1.63, 1.84)
DAS28 at 2y	0.20 (-2.48, 2.88)	0.15 (-2.36, 2.65)	0.33 (-2.41, 3.08)
DAS28 at any time*	**0.36 (0.12, 0.61)**	**0.36 (0.12, 0.61)**	**0.35 (0.11, 0.60)**
Response to treatment			
ΔDAS28 at 6m	-0.05 (-0.49, 0.39)	-0.05 (-0.49, 0.39)	-0.04 (-0.49, 0.41)
ΔDAS28 at 1y	1.81 (-0.47, 4.09)	1.58 (-0.73, 3.89)	1.62 (-0.89, 4.14)
ΔDAS28 at 2y	0.79 (-3.07, 4.64)	0.79 (-3.13, 4.72)	0.55 (-3.66, 4.77)
ΔDAS28 at any time*	0.06 (-0.38, 0.50)	0.06 (-0.38, 0.50)	0.04 (-0.42, 0.49)

Data are coefficients (95% CI) from multiple linear regression. Bold type indicates $p<0.05$. Model 1. Basic. Adjusted for age. Model 2. RA. Model 1 + MTX at inclusion, Rheumatoid Factor and Time from onset of RA. *Data are coefficients (95% CI) from repeated-measures multiple linear regression with additional adjustment for exact date of visit.

ease activity and response to treatment, by BMI categories. In women with normal weight, after multivariable adjustment, we observed a U-shaped relationship between the levels of leptin and disease activity at 6 months, 1 and 2 years of follow-up as well as to treatment response at 6 months and 1 year. Patients in the overweight/obese group showed no association between baseline adipocytokine levels and treatment response. Analysis of the relationship between adipocytokines and remission and poor response to treatment yielded no results (data not shown).

Discussion

Baseline DAS28 in our patients reflected moderate to high disease activity (4.5) and this changed, albeit moderately, over time, reaching a moderate level of activity after two years of follow-up. A similar phenomenon has been observed in other studies where the most important clinical effect was reached during the first six months of follow-up but with very limited change after that (20).

Associations between clinical activity or therapeutic response with leptin and adiponectin was analysed using 4 multiple linear regression models, each one adjusting for different variables analysing the ability of these hormones to predict treatment response at 6 months, one and two years. We observed that neither leptin nor adiponectin significantly predicted therapeutic response and only high and low baseline levels of leptin were associated with DAS28 at 6 months and 2 years, upon quadratic analysis. These results confirm previous observations suggesting a relationship between leptin levels and inflammatory disease activity (8, 10, 12). However, recent studies also suggest that other parameters such as age, race, geography, BMI and disease duration could modify leptin (27, 28), limiting its use for monitoring RA. Recent studies from our group show that recombinant leptin *in vitro* is capable of inducing increased cell proliferation, activation and pro-inflammatory cytokine secretion in $CD4^+$ T cells from RA patients compared to healthy donor cells (unpublished data), confirming observations in T lymphocytes (29) or synovial fibroblasts of RA patients (30).

We observed that baseline adiponectin was not associated with clinical activity at any time during follow-up. This is in line with other studies where 6-month infliximab treatment did not show any effect over adiponectin (9) or where body composition was more relevant than RA clinical activity in explaining changes in adiponectin (31). However, more studies must be carried out to explain their biological effects (16, 17).

Obesity has been associated with higher disease activity as well as with a higher frequency of comorbidities in RA, suggesting its participation in RA treatment response (20, 24). Interestingly, when we stratified the analysis by BMI categories, we observed that leptin was able to significantly predict short and medium term (at 6 and 12 months) response to treatment only in non-overweight/non-obese patients (n=42). In the model adjusted for MTX treatment, RF and time since onset of disease, we were able to predict treatment response at one year. At the same time, in the non-overweight/non-obese group of RA patient's, baseline levels of leptin were associated with clinical activity at 6, 12 and 24 months. In comparison, overweight/obese RA patients did not show any association to clinical activity or treatment response, suggesting that the effect of leptin is masked by the influence of body mass, as recently reported (20, 24). Adiposity may influence leptin-endothelial activation, favoring atherosclerosis and cardiovascular disease in RA (32, 33), explaining the lack of association between leptin and the clinical effects observed in patients. Additionally, although we did not find an association between age and leptin (data not shown), most of our patients were in an age group potentially influenced by adiposity (33).

To the best of our knowledge, no previous studies have assessed whether adipocytokines function as prognostic factors for treatment response, although preliminary analyses performed in this group of patients, and studies published by other groups, have shown that high levels of leptin are associ-

ated with greater disease activity (8, 10, 23); leptin increases on par with disease activity when followed through time (10) and its changes are associated with levels of other cytokines (12) or inflammatory parameters (ESR, C-reactive protein) (8, 34).

Besides secreting adipocytokines, adipose tissue is the source of other molecules such as TNF-α and IL-6, which play a role on inflammation (3, 35, 36). Several studies have suggested that adipocytokines, including leptin, are related to RA clinical activity (13, 17, 19, 23); our findings suggest that leptin's inflammatory effect is significant only in patients who are not overweight or obese, and that other factors influence the biological role of leptin on endothelial or inflammatory cells in response to RA treatment.

It is worth noting that not all patients started treatment at the time of baseline sampling. This study was not only useful in determining whether or not adipocytokines predict the effectiveness of treatment, but also whether or not the patient responded to new or previously established treatment in relation to serum levels of adipocytokines. There are some studies that have attempted to elucidate the role that certain treatments play on the serum levels of adipocytokines, with inconclusive results (14-16, 18), but which generally point to a lack of treatment effect on the levels of adipocytokines. Our results suggest that patients with higher levels of leptin and adiponectin at their initial visit might have more disease activity but this is not a determining prognostic factor regarding response to therapy, unlike what happens with acute phase reactants (37).

An important limitation of our study is the fact that leptin and adiponectin were measured in a single moment in time, which differed between individuals. By avoiding measurements over time we assumed that the temporal variations (1, 2 years, etc.) were not relevant to the therapeutic response. Therapeutic response was measured at several points in time, which also differed among individuals. The change in disease activity, presented as ΔDAS28, is probably more complex than initially changing over time and in relation to circumstances that also vary over time. Another important limitation of our study was the substantial drop-out rate over time, limiting the statistical power for evaluating changes in disease activity in the long term.

Based on a treat-to-target strategy, treatment success translates as maintaining low DAS28 scores. Therefore, the time patients have been treated (measuring individual therapies is inaccurate as not all patients were equally treated throughout their disease) would be an important variable. In patients treated for less time a greater change in DAS28 at 6 months would be expected, but in the following measurements, the change would be considerably reduced. In patients with longer treatment periods we assume that changes in DAS28 would be very low, as treatment is appropriate and change would be practically absent. Since some of the patients in the study population had been treated for significant time periods (over a year), this may explain why there were no major changes in DAS28.

We conclude that, in the short term, baseline leptin levels were able to predict clinical activity in all RA patients and treatment response at 6 or 12 months in the group of non-overweight, non-obese RA patients, suggesting that body mass may modify the relationship between leptin and inflammation in RA patients.

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