Osteoarthritis and Cartilage 26 (2018) 276–284

Osteoarthritis and Cartilage

Differential involvement of synovial adipokines in pain and physical function in female patients with knee osteoarthritis. A cross-sectional study

J. Calvet † *, C. Orellana † ‡, N. Albiñana Giménez §, A. Berenguer-Llergo ||, A. Caixàs ¶, M. García-Manrique †, C. Galisteo Lencastre †, N. Navarro †, M. Larrosa †, J. Gratacós † ‡

† *Rheumatology Department, Parc Taulí University Hospital, I3PT Research Institute (UAB), 08208 Sabadell, Spain*
‡ *Departament de Medicina, Universitat Autónoma de Barcelona (UAB), 08003 Barcelona, Spain*
§ *I3PT Research Institute (UAB), 08208 Sabadell, Spain*
|| *Biostatistics and Bioinformatics Unit, Institute for Research in Biomedicine Barcelona (IRB Barcelona), 08028 Barcelona, Spain*
¶ *Endocrinology and Nutrition Department, Parc Taulí University Hospital, I3PT Research Institute (UAB), 08208 Sabadell, Spain*

ARTICLE INFO

Article history:
Received 26 June 2017
Accepted 16 November 2017

Keywords:
Cytokines
Interleukins
Osteoarthritis
Pain
Physical function
Synovial fluid

SUMMARY

Objective: Adipokines have been reported to play a role in the development, progression and severity of knee osteoarthritis but the influence of the different adipokines are not well known. The aim of this study was to evaluate the association between different synovial fluid adipokines with pain and disability knee osteoarthritis patients.

Methods: Cross-sectional study with systematic inclusion of 115 symptomatic primary knee osteoarthritis female patients with ultrasound-confirmed joint effusion. Age, physical exercise, symptoms duration and different anthropometric measurements were collected. Radiographic severity was evaluated according to Kellgren–Lawrence scale. Pain and disability were assessed by WOMAC-total, -pain, -function subscales and Knee injury and Osteoarthritis Outcome Score (KOOS) pain and function scales. Seven adipokines and three inflammatory markers were measured by ELISA in synovial fluid. Partial Correlation Coefficient (PCC) and corresponding 95% confidence interval were used as a measure of association.

Results: Leptin, osteopontin and inflammatory factors, especially TNF-alpha, were associated to pain and function. After adjustment for potential confounders including inflammatory factors and all adipokines, an association was found for adiponectin with pain (PCC 0.240 [0.012, 0.444]) and for resistin and visfatin with function (PCC 0.336 [0.117, 0.524] and −0.262 [−0.463, −0.036]). No other adipokines or inflammatory markers were statistically and independently associated. An association between physical exercise and pain and disability remained after adjustment, whereas an attenuation of the influence of anthropometric measurements was observed.

Conclusions: Different patterns of association between synovial fluid adipokines were observed regarding pain and disability in knee osteoarthritis patients. Specifically, adiponectin was associated to pain while resistin and visfatin were mainly related to function.

Introduction

Osteoarthritis (OA) is the most common joint condition, and knee involvement is the most prevalent[1] and usually associated with serious pain and disability[2]. The etiology of OA is multifactorial, classically related to mechanical and genetic factors, although anthropometric, metabolic and local inflammatory mechanisms have been recently implicated[3–5]. Together with age, obesity is the most important risk factor related to knee OA (KOA)[6,7]. In recent

* Address correspondence and reprint requests to: J. Calvet, c/Parc Taulí s/n, Edifici VII Centenari Rheumatology Department, 08208, Sabadell, Spain. Tel: 34-937231010x24065; fax: 34-937243549.

E-mail addresses: joan.calvet.fontova@gmail.com (J. Calvet), cristobalorellana1@gmail.com (C. Orellana), nalbinana@tauli.cat (N. Albiñana Giménez), antonio.berenguer@irbbarcelona.org (A. Berenguer-Llergo), acaixas@gmail.com (A. Caixàs), mariagarciamanrique@gmail.com (M. García-Manrique), cgalisteo@tauli.cat (C. Galisteo Lencastre), nnavarro@tauli.cat (N. Navarro), mlarrosa@tauli.cat (M. Larrosa), jgratacosmas@gmail.com (J. Gratacós).

https://doi.org/10.1016/j.joca.2017.11.010
1063-4584/

times, the relationship with metabolic factors and the presence of low-grade inflammation and its influence on the development of KOA have generated great interest[8]. In fact, a significant percentage of KOA patients presents with joint effusion, which could be considered as a marker of local inflammation. In this regard, patients with KOA and persisting joint effusion could represent a particular subset of KOA with special characteristics compared with those without effusion. These patients make up a group of particular interest to investigate metabolic or local inflammation in KOA[8].

Adipokines are proinflammatory molecules secreted systemically by adipose tissue but also locally in the synovial tissue and in the infrapatellar fat pad[9,10]. These cytokines have a wide range of physiological functions and have been suggested to be a link between obesity and OA[11,12]. The exact role of adipokines in KOA is not well known but a double mechanism has been hypothesized: via their link to obesity and low-grade systemic inflammation and, in a more straight forward manner, as a part of the local pathways leading to OA[13,14]. In this respect, different studies have reported a connection between the frequency and severity of KOA, usually measured by radiographic or histological damage, and the levels of adipokines such as leptin, adiponectin, resistin, visfatin and osteopontin, both in plasma and synovial fluid, with leptin, resistin and osteopontin being the most reported. However, many of the studies analyzing synovial fluid were performed on advanced disease patients undergoing prosthetic surgery, and were not focused on clinical severity or the inflammatory profile of KOA. Moreover, they usually evaluated these adipokines individually and without control for inflammatory markers or other adipokines present in synovial fluid[15–21].

In a previous work, our group reported an association between synovial levels of resistin and visfatin and an overall clinical severity score such as the Lequesne index after proper control for potential confounders[22]. The main objective of this study was to evaluate the different associations of synovial fluid adipokines with clinical severity when considering separately pain and disability in KOA women with joint effusion.

Patients and methods

Study design and subjects

Cross-sectional design study with systematic inclusion of 115 female patients aged 50–85 visited at our hospital in a monographic OA medical consultation for symptomatic primary KOA according to ACR criteria[23] and who showed significant joint effusion on physical examination confirmed by ultrasound ($\geq$4 mm on midline suprapatellar line). Symptomatic OA was defined as pain intensity $\geq$4 on a 10-cm visual analogical scale despite the use of prescribed analgesic drugs for at least 3 months. Only patients reporting persisting knee effusion or with documented effusion in several consultations were considered as eligible. Patients with secondary OA were excluded, as well as those with a history of trauma, meniscal injury, inflammatory rheumatic or septic conditions, previous knee surgery or any other condition which could interfere with pain perception, systemic glucocorticoids therapy in the last 6 months, intraarticular glucocorticoid in the last 3 months or hyaluronic acid injection in the last 6 months. Recruitment period comprised October 2013 to June 2015. We included only female patients in order to homogenize the sample, as differences exist between men and women related to pain perception and also regarding anthropometric measures, fat content and distribution that might influence the adipokine profile[24,25]. This study was approved by the Local Ethical Committee at the Hospital Universitari Parc Taulí, Sabadell. All patients included were verbally informed and signed informed consent.

Assessments

The following information was collected from all patients: age, KOA symptoms duration and physical exercise (never, occasional (less than 150 min per week) or regular). Anthropometric measurements included: weight (kg), body mass index (BMI, kg/cm^2), waist circumference (WC, cm), and percentage of body fat measured by bioelectric impedanciometry (TANITA BC-418MA bio logica, Tecnología Médica, Barcelona) following standard protocol. Metabolic syndrome (MetS) was defined in accordance with the modified criteria of the National Cholesterol Education Program-Adult Treatment Panel III[26]. Fasting blood analyses to assess MetS components were carried out. Radiographic severity was evaluated by antero-posterior knee X-ray examination in standing position performed over the last 18 months and graded according to Kellgren–Lawrence scale[27]. Two rheumatologists (JC, CO) evaluated X-rays independently. Clinical severity was assessed by two validated scores, Western Ontario & McMaster Universities Osteoarthritis Index (WOMAC) and Knee injury and Osteoarthritis Outcome Score (KOOS). The WOMAC-total scale (0–96) as well as the pain (0–20) and function (0–68) subscales were evaluated[28]. KOOS score for evaluation of pain (0–100) and function (0–100) were also completed[29]. It must be noticed that, as opposed to the WOMAC scale, low KOOS values indicate a worse score regarding pain and disability while high values correspond to lower clinical severity.

Joint aspiration was performed during the visit and at the same time of day in all patients for proper evaluation of synovial adipokines. The mean and median of synovial fluid aspired were 13.5 and 9 ml respectively, and a minimum of 2 ml was considered to include the patient. Synovial fluid was analyzed to ensure non-inflammatory fluid (cell count <2000 cells) and absence of microcrystals. Synovial samples were stored at −80°C. Seven adipokines and three inflammatory markers were measured by ELISA following manufacturer recommendations for synovial fluid dilutions (see Supplementary material for detailed description). Due to technical reasons related to ELISA technology (configuration of plates used), none of these markers could be assessed at the same time for all patients. In order to control for technical variability the rounds of measurement were considered as an adjustment factor in the statistical analyses (Supplementary material, Section 2.5).

Statistical analysis

Summary of clinical data and laboratory parameters and their association with WOMAC or KOOS scales were performed using non-parametric methods. Medians, interquartile ranges and Spearman correlation (r) were used for continuous measures, while frequencies and Mann–Whitney or Kruskal–Wallis tests were applied to categorical variables.

Linear regression models were used to assess the association with clinical severity while controlling by potential confounders. For doing so, four different models were fitted to obtained estimations adjusted by: 1) basal covariates: age at recruitment, KL, physical exercise and duration of the OA symptoms; 2) basal covariates and anthropometric and metabolic parameters: BMI and MetS; 3) basal covariates, anthropometric-metabolic factors and all inflammatory markers; 4) finally, association between each adipokine and clinical severity was estimated while controlling by the covariates listed above as well as for the rest of adipokines. For doing so, a unique model was fitted including all the previous confounders and all the adipokines. Linear models were fitted with suitable transformation of explanatory variables when necessary, in order to fit the models assumptions (see Supplementary material, Section 2.3).

For interpretation purposes, Partial Correlation Coefficient (PCC) was used as a measure of association for continuous variables (see Supplementary material, Section 2.4). Briefly, PCC is a measure of association for two continuous variables that controls for the effect of additional variables that are considered as confounders. Its values range from −1 to +1 and its interpretation is completely analogous to that of the Pearsons' correlation coefficient.

In all cases, estimations obtained for adipokines and inflammatory markers were adjusted by measurement round (see Supplementary material, Section 2.5). Due to the high correlation observed between adiponectin and omentin (PCC 0.792), only one of them was included at a time in the linear model. Associations were assessed in the linear models using the corresponding F and Wald tests. Tests were performed at 5% significance level. All statistical analyses were conducted using R (see Supplementary material for detailed description).

Results

One hundred and fifteen women were included (Table I). A majority of the patients did occasional or regular exercise. The prevalence of MetS was 40.9%. The median BMI, WC and percentage of body fat were in the high range (30.5 kg/m^2, 100.5 cm and 41.8, respectively). The KL grades 2 and 3 were predominant in this cohort (41.7% each) while only 3.5% had a grade 4. The median for clinical severity were in the high range of pain and disability of the different tests. Because of the presence of strong batch measurements effects, reference values or cut-off points for inflammatory markers or adipokines could not be established, but corrected values are shown in Table I.

Table II shows the association between WOMAC scales and the demographic variables, radiographic and anthropometric measurements, inflammatory factors and adipokines in synovial fluid. BMI and WC were significantly and proportionally associated to all WOMAC scales, showing a similar correlation coefficient of mild magnitude. Among the inflammatory markers under study, TNF-alpha showed the strongest association with all WOMAC scales after controlling by measurement batch (PCC 0.328; PCC 0.305, PCC 0.318 regarding total, pain and function scales, respectively). Correlations estimated for IL-6 were lower in magnitude (55–67% of the observed for TNF-alpha) and did not reach statistical significance with WOMAC-function. Regarding hs-CRP, only a significant association with WOMAC-pain was observed (PCC 0.220). WOMAC-total and WOMAC-function significant associations of a moderate magnitude were found for synovial fluid leptin, resistin and osteopontin, while leptin and osteopontin were associated to WOMAC-pain.

Tables III–V show the association between WOMAC-pain, WOMAC-function and WOMAC-total and each adipokine for the four previously described different settings. This sequential analysis allowed for the evaluation of the relationship between each adipokine and OA severity while assessing the effect of confounders in these associations.

A statistically significant correlation with WOMAC-pain was found for leptin and osteopontin (PCC 0.199 and 0.232, respectively). Nevertheless, these associations became markedly attenuated after control for basal covariates, anthropometric, metabolic factors and inflammatory markers (PCC −0.023 and 0.185, respectively). Notably, BMI and MetS adjustment was enough to explain virtually all the effect observed for leptin in the univariate setting. On the other hand, an association between WOMAC-pain and adiponectin became apparent only after adjustment by confounders, especially anthropometric factors and the rest of adipokines (complete model; PCC 0.240, Table III, Fig. 1(A)). Out of the covariates in the complete model, only physical exercise retained a significant association (decrease of 2.7 points in WOMAC-pain in patients doing regular exercise vs never). A moderate correlation was also observed for BMI and TNF-alpha, although they did not reach statistical significance (PCC 0.214 [−0.015, 0.422] and 0.197 [−0.032, 0.408], respectively; Supplementary Tables S1, S2). All these results were consistent with those observed for the KOOS pain questionnaire (KOOS Supplementary Tables S3–S5).

Leptin, resistin and osteopontin were significantly associated to WOMAC-function (PCC of 0.218, 0.313 and 0.276, respectively). However, only resistin preserved this association in terms of magnitude and statistical significance after control for all confounders (complete model: PCC 0.336 (0.117, 0.524), Table IV, Fig. 1(B)). Similarly to the case of WOMAC-pain, the effects of leptin and osteopontin on disability were highly attenuated after metabolic-anthropometric and inflammatory factors were considered in the model for adjustment (67% and 37% decrease, respectively), and the contribution of BMI and MetS to the attenuation of these effects was much higher for leptin than for osteopontin (43% and 9%, respectively). In addition, a negative correlation was found for visfatin and disability in the complete model, although it was of a higher magnitude than that observed for WOMAC-pain (33% increase) and statistically significant (PCC −0.262 [−0.463, 0.036], Table IV, Fig. 1(C)). Regarding the rest of covariates considered in the analyses, none of them reached statistical significance even though physical exercise (decrease of 6.5 points in patients doing

Table I
Demographic variables, cardiovascular risk factors, radiographic and clinical severity, anthropometric measurements, inflammatory markers and adipokines levels in synovial fluid

Variables	Category		Median (IQR)/N (%)
Age			68.8 (11.1)
KOA symptoms duration (months)			50.0 (73.0)
Physical exercise	Never		53 (46.1%)
	Occasional		28 (24.3%)
	Regular		34 (29.6%)
Radiographic severity	KL	1	15 (13.1%)
		2	48 (41.7%)
		3	48 (41.7%)
		4	4 (3.5%)
Anthropometric measurements	Weight (kg)		72.2 (13.5)
	BMI (Kg/m^2)		30.5 (6.4)
	WC (cm)		100.5 (14.5)
	% Body fat		41.8 (6.5)
	MetS		47 (40.9%)
Clinical severity	WOMAC pain		11.0 (3.5)
	WOMAC function		37.0 (13.5)
	WOMAC total		52.0 (20.0)
	KOOS pain		42.0 (17.0)
	KOOS function		46.0 (19.0)
Inflammatory markers in SF*	IL-6 pg/ml		106.0 (302.6)
	TNF-alpha pg/ml		10.2 (8.0)
	hs-CRP mg/ml		0.91 (0.76)
Adipokines in SF*	Leptin pg/ml		42,079.4 (29,566.0)
	Adiponectin ng/ml		1734.8 (1352.5)
	Resistin pg/ml		2225.7 (2205.8)
	Visfatin ng/ml		1.53 (1.18)
	Osteopontin ng/ml		57.7 (83.2)
	Omentin pg/ml		3396.0 (3550.4)
	Chemerin ng/ml		103.5 (84.2)

Medians and interquartilic ranges (**IQR**) were used to describe continuous variables; categorical data were summarized using absolute frequencies (N) and percentages (%); **MetS**: Metabolic Syndrome; **KL**: Kellgren–Lawrence scale; **BMI**: Body Mass Index; **WC**: Waist Circumference; **% Body Fat**: Body Fat percentage; **WOMAC**: Western Ontario & McMaster Universities Osteoarthritis Index; **KOOS**: Knee injury and Osteoarthritis Outcome Score; **SF**: Synovial Fluid; **IL-6**: (Interleukine 6); **TNF-alpha**: Tumor Necrosis Factor-alpha and **hs-CRP**: High sensitivity C-reactive protein.

* Levels of inflammatory markers and adipokines in synovial fluid were corrected a-priori by measure round.

Table II
Associations between WOMAC-scores and demographic, radiographic, cardiovascular risk factors, anthropometric measurements, inflammatory markers and adipokines in synovial fluid

	Category	WOMAC-total	WOMAC-pain	WOMAC-function
		Medians/Correlation [95%CI]	Medians/Correlation [95%CI]	Medians/Correlation [95%CI]
Age		0.031 [−0.174, 0.213]	0.054 [−0.147, 0.248]	0.026 [−0.166, 0.205]
KOA symptoms duration (months)		−0.038 [−0.198, 0.142]	0.031 [−0.144, 0.219]	−0.055 [−0.225, 0.121]
Physical exercise	Never	52.0 [48.0, 59.0]	11.0 [10.0, 12.0]	37.0 [35.0, 42.0]
	Occasional	49.5 [45.0, 63.0]	10.0 [9.0, 12.0]	36.0 [32.0, 45.0]
	Regular	52.0 [42.0, 57.0]	9.5 [8.0, 12.0]	37.5 [31.0, 41.0]
Radiographic severity (KL)	1	49.0 [36.0, 60.0]	11.0 [7.0, 13.0]	35.0 [27.0, 42.0]
	2	50.5 [44.0, 56.0]	10.0 [9.0, 11.0]	37.0 [33.0, 40.0]
	3–4	52.5 [48.0, 59.0]	11.0 [10.0, 12.0]	37.5 [35.5, 43.0]
Weight (Kg)		0.148 [−0.027, 0.329]	0.189 [0.011, 0.365]	0.132 [−0.068, 0.297]
BMI (Kg/m^2)		0.201 [0.021, 0.364]	0.227 [0.048, 0.385]	0.188 [0.015, 0.366]
WC		0.210 [0.028, 0.363]	0.246 [0.079, 0.401]	0.196 [0.001, 0.370]
% Body Fat		0.154 [−0.023, 0.330]	0.200 [0.017, 0.357]	0.142 [−0.030, 0.329]
MetS	Yes	52.0 [48.00, 60.0]	11.0 [10.0, 12.0]	39.0 [34.0, 42.0]
	No	50.5 [48.0, 56.0]	10.0 [10.0, 11.0]	37.0 [34.0, 40.0]
IL-6		0.195 [0.005, 0.371]	0.203 [0.014, 0.378]	0.174 [−0.017, 0.352]
TNF-alpha		0.328 [0.138, 0.495]	0.305 [0.112, 0.475]	0.318 [0.127, 0.487]
hs-CRP		0.095 [−0.095, 0.279]	0.220 [0.033, 0.393]	0.056 [−0.134, 0.243]
Leptin		0.279 [0.090, 0.448]	0.258 [0.068, 0.430]	0.283 [0.095, 0.452]
Adiponectin		0.040 [−0.150, 0.227]	0.080 [−0.111, 0.265]	0.014 [−0.176, 0.202]
Resistin		0.292 [0.109, 0.455]	0.136 [−0.054, 0.317]	0.328 [0.149, 0.487]
Visfatin		0.090 [−0.101, 0.274]	0.107 [−0.084, 0.290]	0.078 [−0.112, 0.263]
Osteopontin		0.269 [0.084, 0.436]	0.208 [0.020, 0.382]	0.277 [0.093, 0.443]
Omentin		0.088 [−0.106, 0.275]	0.109 [−0.085, 0.294]	0.049 [−0.144, 0.238]
Quemerin		0.054 [−0.140, 0.244]	0.037 [−0.156, 0.228]	0.055 [−0.139, 0.245]

Correlations and medians/means by group and their corresponding intervals at 95% confidence (95%CI) are shown. For **KL** (Kellgren Lawrence scale, 1, 2, 3 + 4), grade 3 and 4 were combined in one category, as only four patients were recorded as grade 4. Medians are used as summary in the rest of categorical variables. Partial Correlation Coefficient after adjustment by measure round was used to assess associations for **IL-6** (Interleukine 6), **TNF-alpha** (Tumor Necrosis Factor-alpha) and **hs-CRP** (High sensitivity C-reactive protein). Spearman correlation coefficient is shown for the rest of continuous measures. **MetS**: metabolic syndrome; **% Body Fat**: body fat percentage; **BMI**: Body Mass Index; **WC**: Waist Circumference.

Table III
Association between adipokines and WOMAC-pain index in four different settings

	Effects adjusted by basal covariates	Effects adjusted by anthropometric factors	Effects adjusted by anthropometric and inflammatory factors	Complete model*
	PCC [95%CI]	PCC [95%CI]	PCC [95%CI]	PCC [95%CI]
Leptin	0.199 [0.000, 0.382]	0.076 [−0.127, 0.272]	−0.032 [−0.246, 0.185]	−0.018 [−0.246, 0.211]
Adiponectin	0.105 [−0.090, 0.293]	0.188 [−0.008, 0.369]	0.137 [−0.076, 0.338]	0.240 [0.012, 0.444]
Resistin	0.102 [−0.094, 0.289]	0.084 [−0.113, 0.275]	−0.011 [−0.221, 0.200]	0.045 [−0.185, 0.271]
Visfatin	0.045 [−0.149, 0.237]	0.040 [−0.157, 0.233]	−0.126 [−0.328, 0.087]	−0.176 [−0.389, 0.055]
Osteopontin	0.232 [0.041, 0.407]	0.203 [0.008, 0.383]	0.140 [−0.073, 0.341]	0.151 [−0.080, 0.367]
Omentin	0.144 [−0.054, 0.331]	0.237 [0.041, 0.416]	0.194 [−0.020, 0.391]	0.195 [−0.035, 0.406]
Chemerin	0.058 [−0.141, 0.253]	0.096 [−0.106, 0.290]	0.092 [−0.127, 0.301]	0.203 [−0.027, 0.412]

Effects adjusted by basal covariates: estimation of adipokines effects were assessed adjusting their effects by measurement batch and by potential confounders: age, OA evolution time, KL grade and physical exercise; **effects adjusted by anthropometric factors**: adipokines associations were additionally assessed adjusting their effects by BMI and Metabolic syndrome; **effects adjusted by anthropometric and inflammatory factors**: estimation of adipokines effects were additionally adjusted by inflammatory factors; **complete model**: effects were simultaneously estimated using a single model including previous confounders and all adipokines except omentin (due to a high collinearity observed with adiponectin, PCC = 0.792).

* Multivariant association for omentin was assessed in a analogous model in which adiponectin was excluded. Partial Correlation Coefficient (PCC) after adjustment by measure round was used to assess associations between WOMAC-pain and adipokines.

regular exercise vs never, $P = 0.066$), KL degree (increase of 2.8 points for every KL grade, $P = 0.0552$) and TNF-alpha (PCC 0.190 [−0.040, 0.401]) showed an association with a statistically significance below 10% in the complete model (Supplementary Tables S6, S7). Again, these results were consistent in significance and magnitude when another functional questionnaire was evaluated (KOOS-function, Supplementary Tables S8–S10).

The results of WOMAC-total showed a similar pattern to that observed of WOMAC-function: although leptin, osteopontin and resistin were associated to this general score (PCC 0.213, 0.271 and 0.270, respectively), only resistin kept its statistical significance and magnitude after control for potential confounders. Also, an inverse association with visfatin emerged in the complete model, where BMI, MetS, inflammatory factors as well as the rest of adipokines were considered (PCC −0.252 [−0.455, −0.025]). Although its correlation increased after adjustment for confounders, adiponectin did not reach statistical significance, probably due to the fact that the disability component dominates over the pain perception component of the questionnaire (Table V). The covariate that retained a significant association with WOMAC-total in the complete model was physical exercise (decrease of 10 points for patients doing regular exercise vs never; $P = 0.045$). Although a non-significant association was observed, TNF-alpha (PCC 0.204 [−0.026, 0.413]) and KL degree (3.3 point difference between degrees; Supplementary Tables S11, S12) retained a trend toward association. These results were consistent with our observations in

Table IV
Association between adipokines and WOMAC-function index in four different settings

	Effects adjusted by basal covariates	Effects adjusted by anthropometric factors	Effects adjusted by anthropometric and inflammatory factors	Complete model*
	PCC [95%CI]	PCC [95%CI]	PCC [95%CI]	PCC [95%CI]
Leptin	0.218 [0.020, 0.399]	0.126 [−0.076, 0.318]	0.074 [−0.144, 0.285]	0.078 [−0.153, 0.301]
Adiponectin	0.020 [−0.174, 0.213]	0.085 [−0.112, 0.276]	0.018 [−0.194, 0.227]	0.104 [−0.128, 0.325]
Resistin	0.313 [0.127, 0.478]	0.308 [0.120, 0.475]	0.234 [0.025, 0.424]	0.336 [0.117, 0.524]
Visfatin	0.019 [−0.175, 0.212]	0.013 [−0.183, 0.208]	−0.153 [−0.352, 0.060]	−0.262 [−0.463, −0.036]
Osteopontin	0.276 [0.087, 0.446]	0.252 [0.060, 0.427]	0.174 [−0.038, 0.371]	0.166 [−0.065, 0.380]
Omentin	0.078 [−0.120, 0.270]	0.152 [−0.048, 0.340]	0.096 [−0.119, 0.303]	0.146 [−0.086, 0.362]
Chemerin	0.038 [−0.161, 0.233]	0.070 [−0.131, 0.266]	0.013 [−0.203, 0.228]	−0.027 [−0.254, 0.203]

Effects adjusted by basal covariates: estimation of adipokines effects were assessed adjusting their effects by measurement batch and by potential confounders: age, OA evolution time, KL grade and physical exercise; **effects adjusted by anthropometric factors**: adipokines associations were additionally assessed adjusting their effects by BMI and Metabolic syndrome; **effects adjusted by anthropometric and inflammatory factors**: estimation of adipokines effects were additionally adjusted by inflammatory factors; **complete model**: effects were simultaneously estimated using a single model including previous confounders and all adipokines except omentin (due to a high collinearity observed with adiponectin, PCC = 0.792).

* Multivariant association for omentin was assessed in a analogous model in which adiponectin was excluded. Partial Correlation Coefficient (PCC) after adjustment by measure round was used to assess associations between WOMAC-pain and adipokines.

Table V
Association between adipokines and WOMAC-total index in four different settings

	Effects adjusted by basal covariates	Effects adjusted by anthropometric factors	Effects adjusted by anthropometric and inflammatory factors	Complete model*
	PCC [95%CI]	PCC [95%CI]	PCC [95%CI]	PCC [95%CI]
Leptin	0.213 [0.015, 0.395]	0.117 [−0.086, 0.310]	0.052 [−0.166, 0.264]	0.056 [−0.175, 0.281]
Adiponectin	0.052 [−0.143, 0.243]	0.121 [−0.076, 0.309]	0.050 [−0.162, 0.258]	0.144 [−0.088, 0.360]
Resistin	0.270 [0.080, 0.440]	0.261 [0.069, 0.434]	0.177 [−0.035, 0.373]	0.275 [0.049, 0.473]
Visfatin	0.030 [−0.164, 0.222]	0.023 [−0.173, 0.217]	−0.150 [−0.350, 0.063]	−0.252 [−0.455, −0.025]
Osteopontin	0.271 [0.081, 0.441]	0.245 [0.053, 0.421]	0.166 [−0.046, 0.364]	0.162 [−0.069, 0.376]
Omentin*	0.120 [−0.078, 0.309]	0.201 [0.003, 0.384]	0.145 [−0.071, 0.347]	0.185 [−0.046, 0.396]
Chemerin	0.044 [−0.155, 0.239]	0.077 [−0.125, 0.272]	0.032 [−0.185, 0.246]	0.032 [−0.197, 0.259]

Effects adjusted by basal covariates: estimation of adipokines effects were assessed adjusting their effects by measurement batch and by potential confounders: age, OA evolution time, KL grade and physical exercise; **effects adjusted by anthropometric factors**: adipokines associations were additionally assessed adjusting their effects by BMI and Metabolic syndrome; **effects adjusted by anthropometric and inflammatory factors**: estimation of adipokines effects were additionally adjusted by inflammatory factors; **complete model**: effects were simultaneously estimated using a single model including previous confounders and all adipokines except omentin (due to a high collinearity observed with adiponectin, PCC = 0.792).

* Multivariant association for omentin was assessed in a analogous model in which adiponectin was excluded. Partial Correlation Coefficient (PCC) after adjustment by measure round was used to assess associations between WOMAC-pain and adipokines.

a previous work that evaluated the association between these adipokines and general clinical severity as measured by the Lequesne index (20).

BMI and MetS were used as a measures of the influence of the anthropometric and metabolic factors on pain and disability to obtain the previous estimations. However and for the sake of robustness, we replicated all the previous models using alternative obesity and metabolic status measures using BMI and WC alone, and combinations of them like BMI plus WC and BMI plus MetS plus % of body fat. The results and conclusions obtained from these analyses resulted quite congruent regardless of the adjustment parameters used (See Supplementary Tables S13–S22 for details).

Discussion

A different association of some adipokines regarding pain and function was detected in this study when those two components of clinical severity were evaluated separately. Specifically and after proper adjustment by potential confounders, adiponectin were found to be positively correlated with pain, whereas resistin (directly) and visfatin (inversely) showed a significant association with disability. In addition, the correlation for leptin and ostepontin with both components of clinical severity seemed to be strongly explained by anthropometric, metabolic and inflammatory factors. Importantly, results were consistent regardless of the questionnaire used for severity assessment (WOMAC or KOOS) or the anthropometric and metabolic factors used for adjustment (BMI, WC, percentage of body fat and MetS). Strikingly, some associations between adipokines and clinical severity became apparent only after consideration of the rest of adipokines in the analyses. It was the case of the effects of adiponectin on pain perception and visfatin on function, which were in accordance with work previously reported by our group using the Lequesne index (20). These observations suggest the existence of a biologic interplay between different synovial fluid adipokines and highlight the need to analyze these compounds jointly rather than in an individually manner.

When evaluating WOMAC-total as a measure of global clinical severity, results were quite similar to those obtained in the analysis of function, i.e., a direct association for leptin, osteopontin and resistin and an inverse correlation with visfatin, the two later preserving statistical significance in the complete model. This similarity was not unexpected, due to the high weight of this outcome in the complete WOMAC questionnaire compared to the contribution of the pain component. In addition, these results were in accordance to our previous reported work with Lequesne index (20).

Previous studies have linked pain in KOA evaluated by the WOMAC-scale to adiponectin in plasma and synovial fluid and to the adiponectin/leptin ratio[30,31]. In contrast, an inverse association

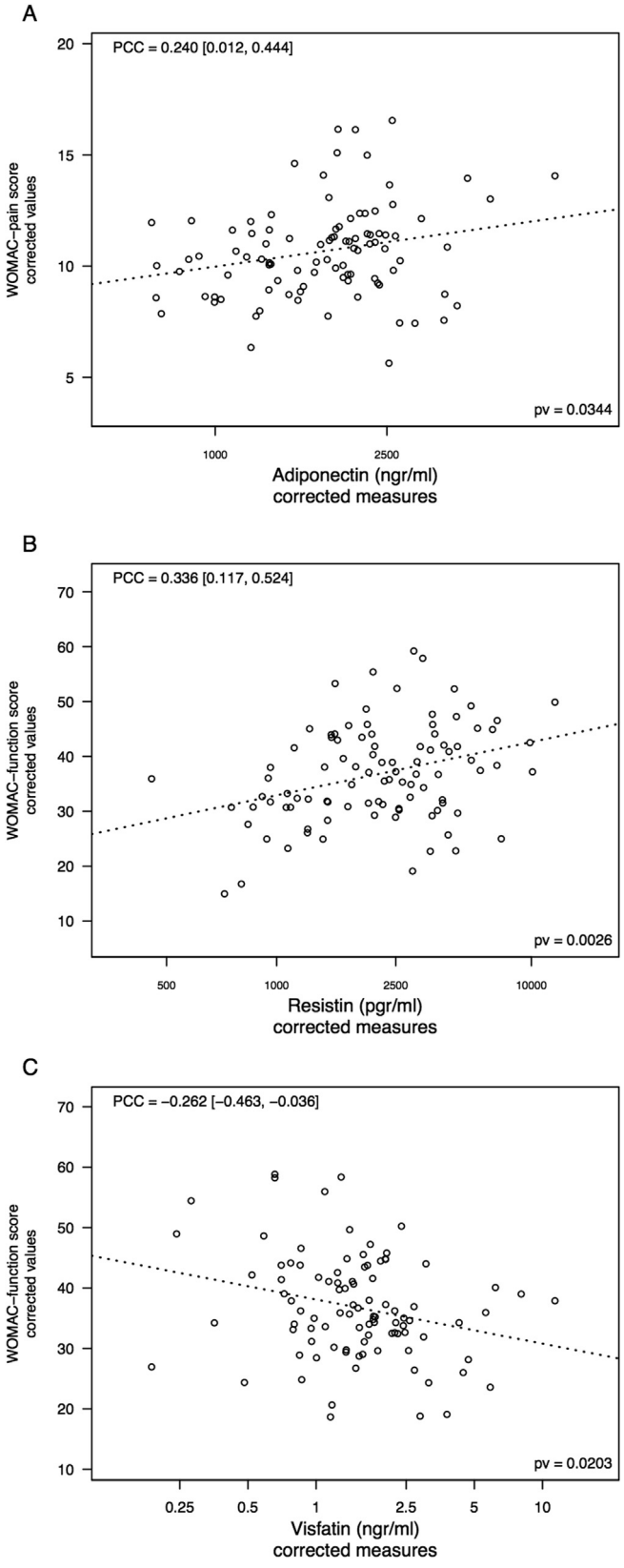

Fig. 1. Scatter plots showing significant correlations found between adipokines and knee osteoarthritis (KOA) pain and function independently of clinical, anthropometric, metabolic and inflammatory factors as well as the rest of adipokines. The figures show associations between adiponectin and WOMAC-pain (A), resistin and WOMAC-function (B) and vifatin and WOMAC-function (C). In all cases, values were corrected by confounders using the corresponding linear model. Adipokines values are drawn in the scale of the Tukey transformation applied in each case in order to fit the model assumptions. Labels in X-axes are shown in the original scale of the adipokines. **PCC**: Partial Correlation Coefficient; **95%CI**: PCC interval at 95% confidence; **pv**: association *P*-value according to a F-test derived from the linear model.

between plasma and synovial fluid adiponectin and radiographic severity has been described, pointing to an opposite direction in the association of adiponectin regarding clinical and radiological severity[32,33]. A remarkable, unless non-significant, correlation of visfatin with pain was observed in our sample. Visfatin has been linked with hip pain, but not with knee pain[31] in previous reports, but patients in these studies were KL grade 3 or 4 and undergoing joint arthroplasty. Some *in vitro* experiments have evaluated the relationship between visfatin and different pain mechanisms in OA like nerve growth factor and nicotinamide phosphoribosyltransferase enzyme activity[34,35]. This association could explain the effect of visfatin in pain, but to our knowledge its association with KOA pain has not been clinically evaluated, and it is possible that under the stimuli of different inflammatory factors visfatin may exert different local actions in KOA.

A prior study showed an association between the WOMAC-total score and the WOMAC subscales with synovial fluid resistin but not with plasma resistin levels, suggesting that the local effects of resistin might be more important than the systemic ones but, in contrast to our study, patients had advanced disease and it was not focused on people with joint effusion[18]. In this regard, our group may have a greater inflammatory local effect of resistin. Other authors have reported an association between resistin and IL-6 with severity scores of KOA[36] but there is a study evaluating the role of synovial IL-6 and TNF-alpha in patients with similar KOA stages as those in our sample and found no relation between IL-6 and WOMAC subscales of pain or disability[37]. Thus, the role of resistin in KOA clinical disability was not explained only by the possible association with IL-6, which might be more important in more advanced disease stages[38]. Previous studies have linked visfatin to cartilage degradation or pain in hip osteoarthritis but, to our knowledge there are no studies focused on the association of visfatin and KOA disability. The association with visfatin became evident only when controlled by TNF-alpha and the rest of adipokines in the analysis, especially resistin. The hypothetical mechanism behind this observation is not known, but this result suggests the existence of biological interrelations among these compounds regarding their role in KOA disability. Overall, these findings may provide new and promising hypotheses to explore in future research and to investigate how inflammation is activated or blocked by visfatin. It also points to visfatin as an important candidate as a marker of local adipokine activity related to KOA disability.

Our results showed a clear positive association between the inflammatory factors studied and pain and function disability, especially for TNF-alfa[37]. The probable effect of these molecules, mainly TNF-alpha, could be explained by two mechanisms. On one hand, because of systemic production associated with obesity[39] or, alternatively but not necessary exclusively, because of local production by synovial tissue with inflammatory features[40]. The association with TNF-alpha was attenuated when controlled by anthropometric measures and became non-statistically significant when controlled by all adipokines. These results suggest the existence of mechanisms shared with adipokines regarding local inflammation and its role on pain and disability[41] and could support the involvement of TNF-alpha in the pathogenesis of OA, but a recent review did not advise TNF-alpha blockers as a treatment option for OA because the lack of evidence different studies. However, research in a specific group of patient with an inflammatory profile was recommended[42].

Several studies have linked physical exercise to less pain and disability in KOA. Whether patients with less disability and pain are more prone to exercise or if exercise exerts a beneficial effect on KOA is difficult to ascertain. It has been suggested that this improvement in KOA symptoms could be related to a better muscle strength[43,44]. In our study physical exercise was associated with a decrease in pain and disability. One study pointed out a relationship between adiponectin and less muscular strength in midlife women[45]. Nevertheless, no statistical association was found between synovial adiponectin levels and physical exercise in our series of patients.

Pain and disability in KOA patients have been linked to different metabolic and anthropometric measurements[46–48]. In our study, neither anthropometric nor metabolic parameters were independently associated to pain or disability, although most of them were related in a univariate way. Analyzing the models, when controlling by inflammatory markers and all adipokines, the effect of anthropometric measures was attenuated, suggesting that anthropometric measures may explain the inflammatory systemic effect but not the local inflammatory effect of adipokines. It is known that the association of BMI in KOA pain is attenuated when controlled by leptin[49], but in our group of patients the effect of anthropometric measures was attenuated when controlling by the effect of all adipokines.

The main limitation of our study arises from its cross-sectional nature and therefore its inability to establish causality. Accordingly, conclusions can only be drawn in terms of associations. A selection bias toward higher disease severity could exist as all patients were referred from Primary Care or other specialists to our Rheumatology Unit and were systematically included. These results warrant replication in other groups of KOA, such as men, or patients with more heterogeneous levels of pain and ranges of anthropometric measures. In order to assess their possible systemic influence, it would be of interest to determine serum adipokine values. In this study, OA at other sites that could interfere with the evaluation of pain and disability was not adequately collected to be analyzed. There may be technical concerns regarding measures in synovial fluid which are inherent to ELISA technology; non-negligible effects associated to time of measurement were identified. For this purpose we corrected these measures by round in the statistical models to make values of adipokines and inflammatory markers totally comparable across samples. Although this correction resulted in reliable estimations of the association for these measures, it was not possible to establish either their real range of variability or meaningful cutoffs that could be extrapolated to other datasets.

A remarkable strength of this work is the homogeneity of this series of KOA patients regarding presence of synovial effusion, the availability of symptomatic (not only radiologic or diagnosed) information and the high frequency of low to moderate KOA stage (only 3.5% were KL grade 4). This homogeneity is relevant for the analysis as, in theory, should increase the statistical power to detect associations of a moderate magnitude. Another strength is the availability of patient information relevant to outcome measures, which makes this study singular among others as it allowed for a simultaneous analysis of a high number of clinical, anthropometric, metabolic and inflammatory factors and their relation to up to seven different adipokines. Also it is clinically relevant as results can be applied to patients with a more definite phenotype. Another important strength is consistency, as the results observed were practically the same using two validated questionnaires to assess pain and disability, which adds robustness to the results.

In conclusion, our results suggest a different pattern of association between synovial fluid adipokines and components of KOA severity, pointing to an association of adiponectin with pain and a link between resistin and visfatin with disability. Another remarkable observation of our study is the importance to evaluate the effects of different adipokines jointly due to the possible existence of common mechanisms regarding clinical severity, together with relevant anthropometric, metabolic and inflammatory factors. These results warrant replication in other groups of osteoarthritis patients and also evaluation of serum adipokines in order to assess their potential relationships.

Contributions

Joan Calvet (JC) and Cristobal Orellana (CO): participated in the conception and design of the study, acquisition of data and analysis and interpretation of the results; drafting the article and revising the article critically for important intellectual content and final approval of the version to be submitted.

Néstor Albiñana Giménez (NA): participated in the conception of the study, analysis of synovial fluid, revising the article critically for important intellectual content and final approval of the version to be submitted.

Antoni Berenguer-Llergo (AB): participated in the design of the study, in the analysis and interpretation of the results, revising the article critically for important intellectual content and final approval of the version to be submitted.

Assumpta Caixàs (AC): participated in the conception of the study, acquisition data, revising the article critically for important intellectual content and final approval of the version to be submitted.

Carlos Galisteo (CG) and María García-Manrique (MG): participated in acquisition data, interpretation of the results, revising the article critically for important intellectual content and final approval of the version to be submitted.

Noemí Navarro: participated in acquisition data, drafting the article and final approval of the version to be submitted.

Marta Larrosa (ML) and Jordi Gratacós (JG): participated in the conception and design of the study, interpretation of the results, revising the article critically for important intellectual content and final approval of the version to be submitted.

Conflict of interest

All the authors declared no financial nor personal disclosures for the study.

Funding sources

This study was partially funded by the 2015 Osteoarthritis Grant of Catalan Rheumatology Society and by 2015 Grant of Hospital Parc Tauli.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.joca.2017.11.010.

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