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The ratio adipsin/MCP-1 is strongly associated with structural changes and CRP/MCP-1 with symptoms in obese knee osteoarthritis subjects: data from the Osteoarthritis Initiative

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SUMMARY

Objective: There is a need to identify reliable biomarkers that can predict knee osteoarthritis (OA) progression. We investigated a panel of adipokines and some related inflammatory factors alone and their ratios for their associative value at assessing cartilage volume loss over time and symptoms in obese [High body mass index (BMI)] and non-obese (Low BMI) OA subjects.

Design: Human OA serum was from the Osteoarthritis Initiative Progression subcohort. Baseline levels of adiponectin (high and low molecular weight forms), adipsin, chemerin, leptin, visfatin, C-reactive protein (CRP), interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) were evaluated with specific assays. Cartilage volume was assessed at baseline and 48 months by quantitative magnetic resonance imaging (MRI), and symptoms using baseline Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores. Data were analysed by linear regression with confounding factors at baseline, followed by multiple comparison adjustment.

Results: The levels of the nine biomarkers and their ratios (36) were studied. Among High BMI subjects, only the ratio adipsin/MCP-1 was associated with cartilage volume loss over time in the lateral compartment [β, −2.95; 95% confidence interval (CI), −4.42, −1.49; $P = 0.010$], whereas MCP-1 was associated with WOMAC pain (−1.74; −2.75, −0.73; $P = 0.030$) and the ratio CRP/MCP-1 with WOMAC pain (0.76; 0.37, 1.14; $P = 0.023$), function (2.43; 1.20, 3.67; $P = 0.020$) and total (3.29; 1.58, 5.00; $P = 0.027$). No associations were found for biomarkers or ratios in Low BMI OA.

Conclusion: In this study, the ratio adipsin/MCP-1 was found to be associated with the knee structural changes and that of CRP/MCP-1 with symptoms in obese OA subjects. Our data further underline the relevance of ratios as biomarkers to a stronger association to OA progression and symptoms.

Introduction

Osteoarthritis (OA) is a most common and slowly progressive arthritis disease and a major cause of pain and disability worldwide. Patient diagnosis based on the guidelines currently available to clinicians occurs late, most often at a severe stage of the disease and often when joint destruction has reached an irreversible stage.

No treatment is currently recognized to cure the disease or slow its progression. The development of new disease modifying OA drugs (DMOADs) or therapies that will reduce the pace of joint tissue damage is clearly lagging behind other disease fields, and major progress is urgently needed. There are still a number of hurdles to be overcome, the most important being the identification of patients who would benefit most from such drugs, including individuals at an early stage of OA, those with progressive OA, and

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those at high risk of rapid disease progression. In short, current diagnostic procedures fall short of providing the healthcare industry with all the tools needed to develop new and effective DMOAD therapies.

Efficient and reliable means of screening at-risk OA patients is mandatory and the development of serum biomarkers is an avenue toward this goal. Biomarkers that are sensitive enough to detect early knee structure changes or patients at high risk of progressive OA are not yet available. Therefore, there is a great need to find new informative biomarkers to identify OA progressors.

As obesity is recognized as one of the major risk factors for the development and progression of OA, it appears logical to explore the factors that could be implicated in inducing the disease in such individuals. Evidence suggests that in OA patients with high body mass index (BMI), mechanical factors are not the only factors linked to the disease[1,2]. It is believed that the pathogenic evolution can be the result of a number of abnormal metabolic factors triggering and enhancing catabolism/inflammation in OA tissues. One such family of factors is the adipose tissue-derived mediators, adipokines and related proinflammatory factors. They represent a family of proteins considered key players in the complex network of mediators involved in the pathophysiology of OA. Although some have been found associated with the disease progression and/or clinical symptoms, inconsistencies have been reported[3–9]. Among the many possible explanations for such discrepancies are the different populations studied, sample sizes, outcomes, and methodologies. However, with regard to one particular adipokine, adiponectin, this could reflect the fact that in the circulation it is found in three forms, each of which could have different effects (pro- and anti-inflammatory)[10–12], and, in arthritis, adiponectin evaluation has generally been done on its total levels[5,13–16].

Given that knee structural alterations most often do not explain all knee pain, the associations between factors and structural changes may not necessarily translate to their association with joint pain. Although some inflammatory biomarkers have been reported to be associated with knee pain, there is still controversy, and more studies are necessary to identify biomarkers related to knee symptoms.

Since the literature suggests that metabolic disturbances could precede and induce joint structural changes and pain[17–19], adipokines could very well contribute to a “toxic environment” leading to a state of chronic inflammation in OA. Thus, its members could potentially serve as reliable predictors of OA. Moreover, while the analysis of an individual biomarker is undoubtedly useful, relationships between biomarkers are increasingly recognized, and for some diseases, biomarker ratios demonstrated better prediction/association assessment than individual biomarkers in general, including some adipokines[20–26]. As the exact roles and interactions of each serum marker in the specified outcomes have not all been previously identified, this prompted us to hypothesize the benefit of adipokines and related inflammatory factors as biomarkers of OA progression.

The aims of this study are first to identify the associations between serum levels of a panel of adipokines and some related inflammatory factors and OA knee structural changes and symptoms in an OA cohort stratified as obese and non-obese subjects. The second aim was to investigate, in an exploratory fashion, the associations between the ratios of the studied adipokines and related inflammatory factors and the abovementioned OA features.

Methods

Study population

The participants were selected from the Progression subcohort of the Osteoarthritis Initiative (OAI) database (https://oai.nih.gov). Participants had symptomatic radiographic OA and had undergone magnetic resonance imaging (MRI) at baseline and 48 months of follow-up for the most symptomatic knee, based on the highest Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score at baseline. Five hundred and eighty-three (583) participants were selected for this study and were further divided according to their BMI: obese (BMI $\geq$ 30 kg/m^2, High BMI) and non-obese (BMI < 30 kg/m^2, Low BMI).

Serum measurement of biomarkers

Baseline serum samples were obtained from the OAI. Samples were received aliquoted and frozen. Upon reception, they were stored at −80°C and thawed to 4°C before use. In brief, morning blood specimens were collected after an overnight fast using a uniform protocol. Additional details on specimen collection and processing methods can be found in the OAI operations manuals. The Institutional Ethics Committee Board of the University of Montreal Hospital Research Centre approved the use of the serum.

Six adipokines and three related inflammatory factors were tested. These included adiponectin in its high (H) and low (L) molecular weight (MW) forms, adipsin, chemerin, leptin, visfatin, C-reactive protein (CRP), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1). Of note, IL-8 and MCP-1 were included as, in addition to being associated with OA, they have also been linked to some of the adipokine pathological pathways[27,28]. All biomarkers were determined at baseline with specific assays according to the manufacturers' specifications. The HMW and LMW forms of adiponectin were determined by ELISA (Alpco, Salem, NH, USA) according to the manufacturer's specifications (dilution 1/5151), and the data analysed using the Thermo Multiscan Spectrum apparatus and the SkanIt software (Thermo Fisher Scientific, Waltham, MA, USA). Adipsin, CRP (both at 1/3000 dilution, Milliplex MAP kit, EMD Millipore Corporation, Billerica, MA, USA), leptin, chemerin, IL-8, MCP-1 (each at 1/2 dilution, Luminex assay, R&D systems, Minneapolis, MN, USA) and visfatin (undiluted, Bio-Plex Pro Assay, Bio-Rad Laboratories, Mississauga, ON, Canada) were quantitated by Luminex technology using the LiquiChip 200 apparatus (Qiagen, Toronto, ON, Canada), and the data analysis performed with the LiquiChip Analyzer software (Qiagen). For each biomarker, an 8-point standard curve and appropriate controls were included, and samples were done in duplicate.

The minimum detectable doses were for HMW and LMW adiponectin, 19 pg/ml; adipsin, 44 pg/ml; chemerin, 69 pg/ml; CRP, 1 pg/ml; leptin, 10.2 pg/ml; IL-8, 1.8 pg/ml; MCP-1, 9.9 pg/ml; and visfatin, 37.5 pg/ml.

Clinical evaluation at baseline

Clinical and radiographic data were obtained from the OAI database (https://oai.nih.gov).

MRI cartilage assessment

Knee MRI acquisitions were performed at the four OAI clinical centers at the OAI baseline and 48-month follow-up using 3T apparatus (Magnetom Trio, Siemens, Erlangen, Germany). MR images were acquired using DESS imaging, as defined by the OAI protocol. Cartilage volume was measured using the automatic human knee cartilage segmentation as previously described and validated[29,30]. Cartilage volume was analysed in the entire knee (global knee, comprising femur and plateaus) and subregions including the medial and lateral compartments. An *ad hoc*

measure was also done for the femur and plateau in the lateral compartment. The change in knee cartilage volume over time was obtained by subtracting the baseline volume from the 48-month follow-up volume, divided by the baseline volume, and calculated as a percentage for each of the studied knee regions as described[29,30].

Statistical analysis

Values are expressed as mean ± standard deviation (SD) or as the median ± interquartile range (first–third quartile), as indicated. Differences between groups were assessed using the Student's *t*-test or Mann–Whitney test (non-normal distribution) for continuous variables, and the chi-squared test or Fisher's exact test for categorical variables. As this was an exploratory study and we did not know which of the biomarkers alone or their ratios would be associated with the outcome (cartilage volume loss or WOMAC score) or whether differences would emerge between the Low and High BMI groups, we investigated for each group separately (Low BMI and High BMI), the biomarkers alone and their ratios. The cartilage volume (baseline and change at 48 months) as well as nine biomarker levels and their ratios (36) were compared between Low and High BMI groups using a linear model (employing natural log [ln] transformation to reduce the effect of skew) adjusted for age and gender at baseline. The relationships between baseline biomarker (level and ratio) and percentage of cartilage volume loss at 48 months or WOMAC score at baseline were evaluated using a linear regression (employing ln). To reduce bias, we adjusted for covariates including confounding factors and excluding mediators and colliders which induce adjustment bias instead of reducing confounding bias. Adjustments were done for baseline age, gender, BMI (the three main risk factors for OA, in addition BMI is associated with some adipokines), Kellgren–Lawrence (KL) grade (for disease evolution), diabetes and hypertension (which could impact the adipokines), and for the cartilage volume loss, the cartilage volume of the global knee, medial compartment or lateral compartment where appropriate (cartilage volume at baseline could impact the extent of the cartilage loss over time). Magnitude of association is expressed as β coefficient (representing the degree of change in the outcome variable for every 1 unit of change in the associated variable) and 95% confidence interval (CI). As the β coefficient should be statistically significant to be interpreted as associated to the outcome, statistical analyses were done and further adjusted for multiple comparisons using the Bonferroni method applied for biomarker levels and ratios separately. The families of tests consisted of looking for any significant relationship between the nine biomarker levels tested for three cartilage volume loss outcomes and four WOMAC outcomes separately. Such testing was also performed for the 36 biomarker ratios. To test the null hypothesis, the family-wise error rate (FWER) of 5% was used; therefore, an adjusted *P*-value $\leq$0.050 was considered statistically significant. All tests were two-sided. All statistical analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC, USA).

Results

Subjects' characteristics

Comparison between baseline characteristics of subjects with Low BMI (non-obese; $n = 307$) and High BMI (obese; $n = 276$) (Table I) showed that the latter were slightly younger, had higher WOMAC pain, function, stiffness and total scores, higher prevalence of diabetes, smaller joint space width, and greater cartilage volume changes (loss) at 48 months in the global knee and the medial compartment. Notably, and as reported[31], the OAI patients' characteristics at baseline, even those with High BMI, represented an OA population at a moderate stage of the disease. Compared with OA patients from clinical trials (severe disease), individuals in this cohort had lower WOMAC scores and KL grades, higher joint space width, and greater cartilage volume at baseline. The cartilage volume loss at 48 months was similar to that observed in OA patients at 24 months in a previous clinical trial[5].

Compared to the baseline biomarker levels of the Low BMI, the High BMI (Supplementary data Table S1) group had significantly higher levels, except for IL-8 and MCP-1, which exhibited a numerical trend toward significance, and both forms of adiponectin (HMW and LMW), which were lower. All biomarker ratios were significantly different between Low and High BMI groups, except for four of them: the ratio of adipsin to chemerin (adipsin/chemerin), CRP/visfatin, IL-8/MCP-1 and leptin/visfatin.

Association between baseline biomarker levels/ratios and cartilage volume loss (%) at 48 months

In the High BMI group (Table IIA), positive associations (i.e., higher biomarker value is associated with less cartilage volume loss) were found for adiponectin LMW in the medial compartment (β [95% CI] 2.15 [0.03, 4.28]; $P = 0.046$) and MCP-1 in the global knee and the lateral compartment (2.01 [0.54, 3.48]; $P = 0.008$ and 2.27 [0.54, 4.00]; $P = 0.010$, respectively). A negative association (i.e., higher biomarker value is associated with greater cartilage volume loss) was found for adipsin in the lateral compartment (−3.82 [−6.41, −1.24]; $P = 0.004$). However, after adjusting for multiple comparisons, none of these associations reached statistical significance. Associations between biomarker levels and cartilage volume loss over time in the Low BMI group (Table IIB) was found only for the leptin in the global knee (−0.82 [−1.59, −0.06]; $P = 0.035$), which was not sustained after adjusting for multiple comparisons.

Evaluation of the biomarker ratios in the High BMI group (Table III; Supplementary data Table S2) revealed that nine (Table III) had negative associations with cartilage volume loss over time in either one or two of the studied cartilage regions. After adjusting for multiple comparisons, only the adipsin/MCP-1 ratio reached statistical significance in the lateral compartment (−2.95 [−4.42, −1.49]; $P = 0.010$). Of note, the β coefficient for the adipsin/MCP-1 ratio means that an increase of 1 unit of the ln transformed ratio is associated with an increase of cartilage volume loss of 2.95%; thus, in terms of the absolute ratio (non-transformed) this means that when the ratio doubles, the increase is 2.05%. An *ad hoc* evaluation of the association within the lateral compartment subregions, femur and plateau, revealed that both were statistically significant after adjusting for multiple comparisons (femur: −2.41 [−3.81, −1.01]; $P = 0.0008$; plateau: −4.66 [−7.45, −1.88]; $P = 0.001$). For the Low BMI group, data showed that the two ratios, leptin/adiponectin LMW and leptin/visfatin in one or two knee regions were associated, however, this was not sustained after adjusting for multiple comparison (Supplementary data Table S3).

Association between baseline biomarker levels/ratios and WOMAC scores at baseline

With regard to the High BMI subjects (Table IVA), analysis of the linear regression displayed negative coefficients (i.e., higher values associated with lower WOMAC score, hence less symptoms) between all the WOMAC subscales and MCP-1, whereas positive coefficients (i.e., higher baseline values associated with greater

Table I
Baseline characteristics and cartilage volume and loss at 48 months

	Low BMI ($n = 307$)	High BMI ($n = 276$)	*P*-value
Gender, male, % (n)	60% (185)	58% (159)	0.516*
Age, years	62 ± 9	59 ± 8	**0.0002**
BMI, kg/m^2	26.5 ± 2.3	33.9 ± 3.1	**<0.0001**
Abdominal circumference (cm)	97.9 ± 8.9	114.4 ± 10.2	**<0.0001**
Target knee, % (n)			
Right	55% (169)	53% (146)	
Left	45% (138)	47% (130)	0.603*
Diabetes % (n)	6% (17)[a]	14% (38)[b]	**0.0005**
Hypertension % (n)	40% (122)	47% (130)	0.073
Symptom duration % (n)‡			
<1 year	14% (35)[c]	18% (45)[d]	
2–5 years	40% (100)	41% (99)	
≥5 years	46% (115)	41% (100)	0.328
WOMAC (median [IR])			
Pain (0–20)	3.0 (1, 6)	4.0 (2, 8)	**0.0002**
Function (0–68)	10.0 (2, 18)[e]	15.9 (7, 24)	**<0.0001**
Stiffness (0–8)	2.0 (1, 3)	3.0 (2, 4)	**<0.0001**
Total (0–96)	15.0 (6, 26)[e]	23.9 (11, 36)	**<0.0001**
Kellgren–Lawrence grade, % (n)			
0–1	22% (68)	16% (45)	
2	34% (103)	37% (102)	
3	32% (97)	34% (94)	
4	13% (39)	13% (35)	0.346*
Joint space width, mm	4.02 ± 1.71[f]	3.66 ± 1.78[f]	**0.012**
Cartilage volume at baseline, mm^3			
Global knee	13,292 ± 3574	13,453 ± 3412	0.579
Medial compartment	6216 ± 2044	6290 ± 1828	0.643
Lateral compartment	7076 ± 1825	7162 ± 1922	0.577
Percentage of cartilage volume changes at 48 months			
Global knee	−5.3 ± 4.7	−6.0 ± 5.7	**0.031**†
Medial compartment	−5.1 ± 5.8	−6.5 ± 8.1	**0.004**†
Lateral compartment	−5.7 ± 5.9	−5.8 ± 6.4	0.591†

Data are presented as mean ± standard deviation (SD) or as indicated.
Continuous variables were compared using the Student's *t* test/Mann–Whitney test; *P*-values in bold indicate statistical significance.
BMI, body mass index; IR, interquartile range; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
Missing values: a) 4; b) 8; c) 57; d) 32; e) 1; f) 2.
* Proportions were compared using the chi-squared test/Fisher's exact test.
† Linear model adjusted for age and gender.
‡ For the symptom duration in the OAI database, the individuals had to answer the following question; "Have you ever had knee pain or stiffness on most days for at least 1 month in your right/left knee? By most days, we mean more than half the days of a month". If the answer was yes, the individual answered the following question: "And how many years ago did this pain, aching, or stiffness start?".

WOMAC score) were found between CRP and WOMAC pain, function and total scores. Interestingly, only the MCP-1 WOMAC pain subscale reached statistical significance after adjusting for multiple comparisons (−1.74 [−2.75, −0.73]; $P = 0.030$). In the Low BMI subjects (Table IVB), although linear regression showed an association of adipsin, chemerin, CRP and visfatin with at least two of the WOMAC subscales, none reached significance after adjustment for multiple comparisons.

The analysis of the biomarker ratios and WOMAC scores in the High BMI group (Table V; Supplementary data Table S4) also showed associations (Table V), mostly with those in which CRP and MCP-1 were included, with the exception of the chemerin/adiponectin HMW ratio, for which association was found for WOMAC pain. After adjustment for multiple comparisons, only the CRP/MCP-1 ratio remained statistically significant and was associated with greater scores in WOMAC pain (0.76 [0.37, 1.14]; $P = 0.023$), function (2.43 [1.20, 3.67]; $P = 0.020$) and total (3.29 [1.58, 5.00]; $P = 0.027$). The β coefficient represents for example for WOMAC pain score an increase of 0.76 for each increase of 1 unit of the ln transformed ratio, i.e., when the absolute ratio doubles, the increase is 0.53. In the Low BMI group, although some biomarker ratios were associated in at least one of the WOMAC subscales (Table VI; Supplementary data Table S5), none were statistically significant after adjustment for multiple comparisons.

Discussion

Serum biomarkers that are sensitive enough to accurately and reliably monitor early knee structure changes or OA progression are not yet available. This study investigated a number of adipokines and related inflammatory factors as potential biomarkers, which were chosen as they were previously documented to be associated with both OA and adipokines, are known to trigger different signalling pathways leading to the modulation of pro-inflammatory/catabolic factors contributing to degradation of the cartilage, and some were found to be associated, to some extent, with OA progression[3,5,7,9].

To our knowledge, this is the first study to simultaneously examine a panel of serum adipokines/inflammatory factors and their associations with OA cartilage volume loss assessed by MRI and with the symptoms. After adjustment for multiple comparisons, in obese subjects, an increase in the ratio adipsin/MCP-1 was associated with lateral compartment knee cartilage volume loss over time in which every time the ratio doubles, it is associated with an increase of cartilage volume loss of 2.05%. Although, this

Table II
Association between baseline biomarker levels and % of cartilage volume loss at 48 months in High BMI ($n = 268$*) and Low BMI ($n = 303$*) OA groups

	Adiponectin (HMW)	Adiponectin (LMW)	Adipsin	Chemerin	CRP	IL-8	Leptin	MCP-1	Visfatin
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
A. High BMI group									
Global knee	0.76 (−0.13, 1.66)	0.82 (−0.73, 2.37)	−1.47 (−3.69, 0.76)	−0.37 (−2.20, 1.46)	−0.52 (−1.16, 0.12)	0.88 (−0.55, 2.31)	0.17 (−0.90, 1.24)	2.01 (0.54, 3.48)	0.17 (−0.19, 0.54)
P-value	*0.092*	*0.300*	*0.196*	*0.690*	*0.112*	*0.226*	*0.753*	***0.008***	*0.354*
Medial compartment	1.23 (−0.01, 2.46)	2.15 (0.03, 4.28)	0.49 (−2.59, 3.57)	−0.21 (−2.74, 2.33)	−0.32 (−1.21, 0.57)	0.80 (−1.18, 2.78)	0.60 (−0.87, 2.07)	1.48 (−0.57, 3.53)	0.02 (−0.48, 0.52)
P-value	*0.051*	***0.046***	*0.755*	*0.872*	*0.478*	*0.425*	*0.421*	*0.156*	*0.929*
Lateral compartment	0.40 (−0.65, 1.45)	−0.01 (−1.82, 1.81)	−3.82 (−6.41, −1.24)	−0.62 (−2.77, 1.53)	−0.74 (−1.49, 0.01)	0.68 (−1.00, 2.37)	0.03 (−1.23, 1.28)	2.27 (0.54, 4.00)	0.28 (−0.15, 0.70)
P-value	*0.451*	*0.993*	***0.004***	*0.570*	*0.052*	*0.424*	*0.967*	***0.010***	*0.203*
B. Low BMI group									
Global knee	−0.01 (−0.84, 0.81)	0.68 (−0.53, 1.89)	−0.51 (−2.36, 1.35)	−0.06 (−1.30, 1.17)	−0.32 (−0.78, 0.14)	−0.87 (−2.18, 0.43)	−0.82 (−1.59, −0.06)	−0.10 (−1.33, 1.14)	−0.01 (−0.20, 0.18)
P-value	*0.978*	*0.269*	*0.591*	*0.921*	*0.173*	*0.190*	***0.035***	*0.879*	*0.928*
Medial compartment	0.24 (−0.77, 1.25)	0.73 (−0.75, 2.21)	0.31 (−1.97, 2.59)	−0.19 (−1.71, 1.33)	−0.29 (−0.86, 0.28)	−1.33 (−2.93, 0.28)	−0.87 (−1.81, 0.06)	0.39 (−1.12, 1.90)	0.04 (−0.20, 0.27)
P-value	*0.640*	*0.334*	*0.789*	*0.808*	*0.312*	*0.105*	*0.068*	*0.614*	*0.766*
Lateral compartment	−0.46 (−1.51, 0.60)	0.27 (−1.28, 1.83)	−1.13 (−3.50, 1.24)	0.29 (−1.29, 1.87)	−0.31 (−0.90, 0.29)	−0.35 (−2.02, 1.32)	−0.56 (−1.54, 0.42)	−0.62 (−2.20, 0.95)	−0.07 (−0.32, 0.17)
P-value	*0.395*	*0.730*	*0.349*	*0.721*	*0.312*	*0.678*	*0.263*	*0.438*	*0.552*

Linear regression of biomarker (ln) at baseline and cartilage volume loss at 48 months adjusted for age, gender, BMI, Kellgren–Lawrence grade, diabetes, hypertension, and cartilage volume at baseline in the corresponding region (global knee, medial compartment, lateral compartment). *P*-values in bold indicate statistical significance. Negative coefficients indicate that higher baseline biomarker values are associated with greater cartilage volume loss.

BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HMW, high molecular weight; IL-8, interleukin-8; LMW, low molecular weight; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis.

* Data on diabetes missing for 8 High BMI and 4 Low BMI individuals (refer to Table I).

Table III
Association between baseline biomarker ratios and % of cartilage volume loss at 48 months in the High BMI group ($n = 268$*)

	Adipsin/ Adiponectin (HMW)	Adipsin/IL-8	Adipsin/MCP-1	Chemerin/MCP-1	CRP/Adiponectin (HMW)	CRP/Chemerin	CRP/IL-8	CRP/MCP-1	CRP/Visfatin
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Global knee	−0.92 (−1.78, −0.07)	−1.12 (−2.36, 0.12)	−1.99 (−3.25, −0.72)	−1.29 (−2.41, −0.17)	−0.53 (−1.01, −0.04)	−0.53 (−1.17, 0.11)	−0.58 (−1.16, 0.004)	−0.71 (−1.28, −0.14)	−0.25 (−0.57, 0.06)
P-value	***0.034***	*0.077*	***0.002***	***0.024***	***0.033***	*0.103*	*0.051*	***0.014***	*0.113*
Medial compartment	−1.06 (−2.24, 0.13)	−0.45 (−2.18, 1.27)	−0.95 (−2.72, 0.83)	−0.95 (−2.51, 0.61)	−0.55 (−1.22, 0.12)	−0.33 (−1.22, 0.56)	−0.40 (−1.21, 0.41)	−0.48 (−1.27, 0.32)	−0.09 (−0.53, 0.34)
P-value	*0.080*	*0.605*	*0.295*	*0.233*	*0.109*	*0.466*	*0.327*	*0.237*	*0.670*
Lateral compartment	−0.94 (−1.94, 0.07)	−1.70 (−3.14, −0.25)	−2.95 (−4.42, −1.49)	−1.51 (−2.82, −0.20)	−0.55 (−1.12, 0.02)	−0.75 (−1.50, 0.003)	−0.73 (−1.41, −0.04)	−0.93 (−1.59, −0.26)	−0.39 (−0.75, −0.02)
P-value	*0.067*	***0.022***	***0.0001***	***0.024***	*0.059*	***0.049***	***0.037***	***0.006***	***0.039***
†*P-value*			***0.010***						

Linear regression of biomarker ratio (ln) at baseline and cartilage volume loss at 48 months adjusted for age, gender, BMI, Kellgren–Lawrence grade, diabetes, hypertension and cartilage volume at baseline in the corresponding region (global knee, medial compartment, lateral compartment). *P*-values in bold indicate statistical significance. Negative coefficients indicate that higher baseline biomarker ratio values are associated with greater cartilage volume loss.

BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HMW, high molecular weight; IL-8, interleukin-8; LMW, low molecular weight; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis.

* Data on diabetes missing for 8 High BMI individuals (refer to Table I).

† Adjusted *P*-value by the Bonferroni procedure.

Table IV
Association between biomarker levels and WOMAC score at baseline in High BMI ($n = 268$*) and Low BMI (303*) OA groups

	Adiponectin (HMW)	Adiponectin (LMW)	Adipsin	Chemerin	CRP	IL-8	Leptin	MCP-1	Visfatin
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
A. High BMI group									
WOMAC									
Pain	−0.59 (−1.21, 0.03)	−0.56 (−1.63, 0.50)	−0.11 (−1.66, 1.44)	0.63 (−0.64, 1.90)	0.62 (0.18, 1.06)	−0.02 (−1.02, 0.97)	0.11 (−0.63, 0.85)	−1.74 (−2.75, −0.73)	−0.09 (−0.34, 0.17)
P-value	*0.062*	*0.300*	*0.889*	*0.327*	***0.006***	*0.962*	*0.778*	***0.001***	*0.501*
†*P-value*								***0.030***	
Function	−1.12 (−3.10, 0.87)	−0.66 (−4.06, 2.74)	−0.50 (−5.47, 4.47)	2.78 (−1.27, 6.84)	2.22 (0.83, 3.62)	−0.26 (−3.45, 2.92)	0.91 (−1.45, 3.27)	−4.41 (−7.66, −1.17)	−0.67 (−1.46, 0.13)
P-value	*0.269*	*0.702*	*0.843*	*0.177*	***0.002***	*0.871*	*0.448*	***0.008***	*0.101*
Stiffness	0.05 (−0.23, 0.32)	0.16 (−0.31, 0.62)	−0.17 (−0.85, 0.50)	0.21 (−0.34, 0.77)	0.05 (−0.15, 0.24)	−0.35 (−0.78, 0.09)	0.09 (−0.23, 0.42)	−0.47 (−0.92, −0.03)	−0.01 (−0.12, 0.10)
P-value	*0.746*	*0.509*	*0.616*	*0.454*	*0.647*	*0.117*	*0.570*	***0.037***	*0.806*
Total	−1.46 (−4.24, 1.32)	−1.22 (−5.93, 3.49)	−0.59 (−7.47, 6.30)	3.56 (−2.05, 9.17)	2.88 (0.95, 4.82)	−0.53 (−4.95, 3.90)	0.97 (−2.30, 4.24)	−6.65 (−11.12, −2.17)	−0.77 (−1.88, 0.33)
P-value	*0.301*	*0.610*	*0.867*	*0.212*	***0.004***	*0.815*	*0.560*	***0.004***	*0.171*
B. Low BMI group									
WOMAC									
Pain	0.06 (−0.54, 0.65)	−0.32 (−1.20, 0.55)	1.54 (0.20, 2.88)	0.83 (−0.06, 1.71)	0.20 (−0.14, 0.53)	−0.64 (−1.58, 0.31)	0.14 (−0.41, 0.69	−0.33 (−1.22, 0.57)	0.15 (0.02, 0.29)
P-value	*0.848*	*0.470*	***0.024***	*0.068*	*0.250*	*0.187*	*0.615*	*0.474*	***0.027***
Function	0.09 (−1.78, 1.96)	−1.44 (−4.19, 1.32)	4.48 (0.26, 8.69)	3.53 (0.72, 6.34)	1.19 (0.13, 2.24)	−0.34 (−3.34, 2.66)	0.48 (−1.26, 2.21)	−0.11 (−2.94, 2.71)	0.46 (0.03, 0.89)
P-value	*0.927*	*0.306*	***0.037***	***0.014***	***0.028***	*0.823*	*0.588*	*0.936*	***0.037***
Stiffness	0.01 (−0.27, 0.29)	−0.26 (−0.67, 0.15)	0.29 (−0.34, 0.93)	0.03 (−0.40, 0.45)	0.06 (−0.10, 0.22)	−0.28 (−0.72, 0.16)	0.11 (−0.15, 0.36)	−0.21 (−0.63, 0.21)	0.02 (−0.04, 0.09)
P-value	*0.926*	*0.213*	*0.367*	*0.899*	*0.465*	*0.213*	*0.417*	*0.325*	*0.485*
Total	0.13 (−2.42, 2.68)	−2.08 (−5.83, 1.66)	6.28 (0.55, 12.01)	4.37 (0.55, 8.19)	1.47 (0.04, 2.91)	−1.15 (−5.23, 2.93)	0.74 (−1.60, 3.09)	−0.55 (−4.40, 3.30)	0.63 (0.04, 1.22)
P-value	*0.919*	*0.274*	***0.032***	***0.025***	***0.045***	*0.581*	*0.533*	*0.778*	***0.038***

Linear regression of biomarker (ln) at baseline and WOMAC adjusted for age, gender, BMI, Kellgren–Lawrence grade, diabetes and hypertension. *P*-values in bold indicate statistical significance. Positive coefficients indicate that higher baseline biomarker values are associated with greater WOMAC score.

BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HMW, high molecular weight; IL-8, interleukin-8; LMW, low molecular weight; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

* Data on diabetes missing for 8 High BMI and 4 Low BMI individuals (refer to Table I).

† Adjusted *P*-value by the Bonferroni procedure separately for the High BMI and Low BMI groups.

percentage might appear low, it is likely to be clinically meaningful as it was reported that every 1% increase in the rate of cartilage volume loss was associated with a 20% increase in the risk of knee replacement[32]. Furthermore, this study also showed that the CRP/MCP-1 ratio was associated with the symptoms, in which, for the WOMAC pain score for example, when the ratio doubles an increase of 0.53 is found. In this population, from the baseline value (Table I), this corresponded to an increase of 13%. Again, this finding is clinically relevant as Angst *et al.*[33] reported that the minimal clinically important differences (MCID) for worsening conditions for WOMAC pain is 14%.

None of the individual biomarkers or their ratios demonstrated associations in non-obese OA subjects after adjusting for multiple comparisons. This was not surprising as metabolic changes related to obesity may have an additional etiological influence on OA susceptibility and progression. Importantly, this study also revealed that the evaluation of biomarker ratios provides better association assessment compared to that obtained with individual biomarkers, in agreement with studies investigating adipokines and conducted in different diseases[20–26].

The association of MCP-1 with lower levels of cartilage volume loss and symptoms in the obese OA subjects, even with the confounding factors diabetes and hypertension, is intriguing. It is well known that this proinflammatory factor is a potent chemokine and a chemoattractant of monocytes/macrophages[34–36]. However, although some studies reported that MCP-1 in OA could correlate with greater pain and/or disease progression[37–39], another showed that neither the synovial fluid nor the serum MCP-1 levels correlated with severity of radiographic changes in OA[39]. Moreover, most studies investigating the role of MCP-1 were done using synovial fluid, and not serum as in our study. This is important as this factor is believed to act mainly as a local factor in arthritic tissues[40]. Although the paucity of data available on the relationship between the serum level of MCP-1 and OA cartilage loss has not yet allowed for any definite conclusion regarding the findings of the present study, one can speculate that, in patients with greater levels of inflammation associated with more pain and/or cartilage degradation, the "local consumption" of MCP-1 is greatly enhanced, reducing the extent of release of such "local factor" into the systemic circulation. Thus, it could be that in the serum, levels of this factor relate to the intensity of the disease process, and not its mechanism of action on the articular tissues.

With regard to adipsin, the present findings confirm and extend our previous observations of its association with cartilage volume loss in the lateral compartment of the knee in OA patients[5,31]. Adipsin is an integral component of the alternative complement pathway, which is well known to be involved in mediating inflammation in arthritis[41–43]. Because its key role in articular tissues is the activation of the alternative complement pathway[31], it is tempting to speculate that the association could be through a systemic inflammatory cascade. Here, the finding may indicate that in the population of patients with higher BMI, adipsin may play a more predominant role than many other potential factors that are involved in cartilage loss and, more particularly in the lateral compartment where there is a larger volume of cartilage (than in the medial compartment), which could be more liable to exposure to such a factor. Moreover, as in the previous publications[5,31], although the exact reason for a factor being associated with a specific knee cartilage compartment/region is at present unknown, such favoured knee locations were also observed for other products/biomarkers[44–47].

CRP was suggested to reflect a systemic inflammatory state, which is known to be associated with adiposity[48]. Our data corroborate this statement, as the association of this factor with symptoms was found only in the High BMI OA patients. Our data also agree with a recent meta-analysis[49] reporting no association between this factor and OA structural progression, but only with symptoms. The association of the CRP/MCP-1 ratio with the WOMAC pain, function and total subscales is supportive of the importance of CRP in association with OA symptoms. However, it should be noted that the significance of CRP alone was not sustained after adjustment for multiple comparisons, but remained only when normalized with MCP-1. The positive association of CRP and the negative one of MCP-1 is also reinforced by their ratios with the other factors, the impact of each of them still being strong. For instance, the ratio adipsin/CRP was significantly and inversely associated with pain indicating that patients with lower CRP levels have less severe pain, while ratios significantly and positively associated with pain (e.g., adipsin/MCP-1) indicate that subjects with lower levels of MCP-1 experience greater levels of pain.

Our results showed no statistical significance after adjustment for multiple comparisons in either the High or Low BMI group with cartilage loss or symptoms for adiponectin (HMW and LMW), chemerin, leptin, visfatin or IL-8. Some of these findings are surprising as, for instance, there is a belief that an increased level of leptin is associated with cartilage degradation and pain. However, this notion remains controversial; some studies reporting a positive correlation[5,7,50,51] and others not[3,8,9,13]. This discrepancy appears to be related to the fact that some studies did not adjust for important covariates such as gender and BMI. Moreover, heterogeneity among the studies' cohorts is also of concern. In the present cohort, patients presented a moderate stage of OA (Table I) as compared to those from clinical trials.

The association between OA progression and adiponectin, an adipokine that could have both pro- and anti-inflammatory properties depending on its MW[10–12], is still unclear. Most studies that investigated the association between adiponectin and OA were done by quantifying its total levels[5,13–16]. Some studies reported either no association between adiponectin and cartilage loss, progression, or incidence of radiographic knee OA, an inverse correlation with disease severity, or an association with reduced medial tibial cartilage volume loss in obese patients[5,9,13,16]. Although we found no evidence of association between the HMW form of adiponectin and knee structure or symptoms in either High or Low BMI subjects, in the High BMI OA group, an inverse association was found between the LMW form of adiponectin and cartilage volume loss in the medial compartment, which was not statistically significant after correcting for multiple comparisons. These findings concur with a previous study in knee OA patients[5].

Regarding chemerin, our data agree with a previous study in which higher concentrations of chemerin in the serum were not associated with knee OA severity, although a positive association was found for the synovial fluid[52]. This could also explain the discrepancy between the current findings on visfatin and data from previous studies, in which visfatin levels in synovial fluid of OA patients were positively linked to cartilage degradation markers[53] or inversely correlated with clinical severity[4].

The findings regarding IL-8, a pro-inflammatory mediator[54,55], concur with previous studies reporting the lack of association between its synovial fluid/serum levels and WOMAC scores and radiographic OA[56,57].

This study like any other ones has a number of potential limitations. It was performed using a cohort from the United States (the OAI), therefore limiting the generalization of the findings. Such work should also be undertaken with populations from different countries and of different ethnic origins. Moreover, the cross-sectional nature of the study did not allow causality to be

Table V
High BMI OA group: Association between baseline biomarker ratios and WOMAC score at baseline ($n = 268^{*}$)

WOMAC	Adiponectin (LMW)/MCP-1 β (95% CI)	Adipsin/CRP β (95% CI)	Adipsin/MCP-1 β (95% CI)	Chemerin/ Adiponectin (HMW) β (95% CI)	Chemerin/MCP-1 β (95% CI)	CRP/Adiponectin (HMW) β (95% CI)
Pain	0.67 (−0.10, 1.43)	−0.62 (−1.06, −0.18)	1.27 (0.39, 2.15)	0.60 (0.05, 1.16)	1.26 (0.49, 2.03)	0.54 (0.20, 0.87)
P-value	0.086	***0.006***	***0.005***	***0.033***	***0.001***	***0.002***
†*P*-value						
Function	2.09 (−0.34, 4.51)	−2.23 (−3.62, −0.85)	3.16 (0.33, 6.00)	1.46 (−0.33, 3.24)	3.64 (1.18, 6.11)	1.62 (0.56, 2.69)
P-value	*0.092*	***0.002***	***0.029***	0.109	***0.004***	***0.003***
†*P*-value						
Stiffness	0.34 (0.01, 0.67)	−0.06 (−0.25, 0.13)	0.30 (−0.09, 0.69)	0.004 (−0.24, 0.25)	0.35 (0.01, 0.69)	0.01 (−0.14, 0.16)
P-value	**0.043**	*0.550*	*0.131*	*0.972*	***0.043***	*0.862*
Total	3.04 (−0.32, 6.40)	−2.89 (−4.81, −0.97)	4.83 (0.92, 8.74)	1.90 (−0.59, 4.39)	5.25 (1.84, 8.66)	2.11 (0.63, 3.60)
P-value	*0.076*	***0.003***	***0.016***	*0.135*	***0.003***	***0.005***
†*P*-value						

Linear regression of biomarker ratio (ln) at baseline and WOMAC adjusted for age, gender, BMI, Kellgren–Lawrence grade, diabetes and hypertension. *P*-values in bold indicate statistical significance. Positive coefficients indicate that higher baseline values of biomarker ratios are associated with greater WOMAC score.
BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HMW, high molecular weight; IL-8, interleukin-8; LMW, low molecular weight; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
[*] Data on diabetes missing for 8 High BMI individuals (refer to Table I).
[†] Adjusted *P*-value by the Bonferroni procedure.

established. Although the β coefficient compares the strength of the effect of the independent variable to the dependent variable in which the higher the absolute value the stronger the effect, to reject the null hypothesis, our conclusions are based on statistical significance after adjusting for multiple comparisons using the *P*-value. To counteract type I errors (false positive results), we corrected for multiple comparisons using the Bonferroni method which increases the probability of the type II error rate. However, in our study, this increased probability was not a crucial issue as we were interested in knowing which biomarkers/ratios were most associated with our study outcomes. As the Bonferroni method is very conservative, this gives us confidence in the validity of our findings.

In the linear regression analysis, we did not include disease symptom duration, as the individuals had to answer the question "Have you ever had knee pain or stiffness on most days for at least 1 month in your right/left knee? By most days, we mean more than half the days of a month", and indicate an approximation of the start of the symptoms (Table I). One could therefore question the reliability of the answer. Moreover, as about 12% and 19% of the individuals (Table I) did not answer the question, we would have lost these individuals in the analysis. However, as the KL grade was added in the analysis, this corrected for the disease evolution. Another limitation could be that, as the study sample size was relatively modest, it is possible that with a larger sample size we may have detected more significant associations that would have remained after multiple comparisons. It would have been interesting to be able to also test the synovial fluid from the studied individuals and compare the data to those of the serum. However, synovial fluid was not available. Study subjects had moderate OA from a symptomatic and imaging point of view at baseline. It would be interesting for future research to contrast our findings, on the one hand, with participants at an earlier stage of the disease and, on the other hand, with OA patients with more severe disease. The former work could be done for instance with the OAI Incidence subcohort, in which participants at baseline are without symptomatic knee OA, but have specific characteristics that represent an increased risk of developing symptomatic knee OA over time. Moreover, future work could also be done with the biomarker ratios found in this study to explore the response of a medication that influences these serum adipokine/inflammatory factors in OA and/or reduces knee structure alterations.

Finally, this study was seeking to identify biomarker associations and although the findings need to be confirmed by conclusive studies in larger patient populations, it may also warrant further investigation as potential predictors of disease progression. In this

Table VI
Low BMI OA group: Association between baseline biomarker ratios and WOMAC score at baseline ($n = 303^{*}$)

WOMAC	Adipsin/Adiponectin (LMW)	Adipsin/IL-8	Adipsin/MCP-1	Adipsin/Visfatin	Chemerin/Adiponectin (LMW)	Chemerin/IL-8	Chemerin/MCP-1
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Pain	0.79 (0.01, 1.58)	0.95 (0.19, 1.72)	0.81 (0.01, 1.61)	−0.14 (−0.28, −0.002	0.60 (−0.03, 1.22)	0.85 (0.16, 1.54)	0.67 (−0.02, 1.36)
P-value	**0.048**	***0.015***	***0.047***	***0.048***	*0.061*	***0.016***	*0.059*
Function	2.72 (0.24, 5.19)	1.76 (−0.68, 4.20)	1.70 (−0.84, 4.23)	−0.42 (−0.85, 0.02)	2.53 (0.56, 4.51)	2.31 (0.10, 4.53)	2.24 (0.03, 4.45)
P-value	***0.031***	*0.157*	*0.188*	*0.061*	***0.012***	***0.041***	***0.047***
Stiffness	0.31 (−0.06, 0.69)	0.31 (−0.06, 0.67)	0.27 (−0.10, 0.65)	−0.02 (−0.09, 0.05)	0.15 (−0.15, 0.45)	0.16 (−0.17, 0.49)	0.14 (−0.19, 0.47)
P-value	0.098	*0.096*	*0.155*	*0.543*	*0.314*	*0.350*	*0.405*
Total	3.87 (0.50, 7.23)	2.94 (−0.38, 6.26)	2.70 (−0.75, 6.14)	−0.56 (−1.16, 0.03)	3.31 (0.63, 5.99)	3.26 (0.24, 6.27)	2.98 (−0.02, 5.99)
P-value	***0.024***	*0.082*	*0.124*	*0.062*	***0.016***	***0.034***	*0.052*

Linear regression of biomarker ratio (ln) at baseline and WOMAC adjusted for age, gender, BMI, Kellgren–Lawrence grade, diabetes and hypertension. *P*-values in bold indicate statistical significance. Positive coefficients indicate that higher baseline values of biomarker ratios are associated with greater WOMAC score.
BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HMW, high molecular weight; IL-8, interleukin-8; LMW, low molecular weight; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
[*] Data on diabetes missing for 4 Low BMI individuals (refer to Table I).

CRP/Adiponectin (LMW) β (95% CI)	CRP/Chemerin β (95% CI)	CRP/IL-8 β (95% CI)	CRP/MCP-1 β (95% CI)	CRP/Visfatin β (95% CI)	IL-8/MCP-1 β (95% CI)	Leptin/CRP β (95% CI)	Leptin/MCP-1 β (95%CI)
0.57 (0.18, 0.96)	0.51 (0.07, 0.95)	0.50 (0.10, 0.91)	0.76 (0.37, 1.14)	0.21 (0.003, 0.43)	1.23 (0.37, 2.08)	−0.45 (-0.84, −0.06)	0.67 (0.07, 1.28)
0.005	***0.024***	***0.014***	***0.0002***	*0.053*	***0.005***	***0.024***	***0.029***
			0.023				
1.84 (0.60, 3.09)	1.81 (0.40, 3.22)	1.86 (0.58, 3.13)	2.43 (1.20, 3.67)	1.04 (0.35, 1.73)	2.97 (0.21, 5.73)	−1.46 (−2.70, −0.22)	2.15 (0.23, 4.07)
0.004	***0.012***	***0.005***	***0.0001***	***0.003***	***0.035***	***0.021***	***0.029***
			0.020				
0.01 (−0.16, 0.19)	0.03 (−0.17, 0.22)	0.09 (−0.09, 0.27)	0.11 (−0.07, 0.28)	0.02 (−0.07, 0.12)	0.08 (−0.30, 0.46)	−0.01 (−0.18, 0.16)	0.23 (−0.04, 0.49)
0.872	*0.798*	*0.308*	0.226	*0.661*	*0.692*	*0.920*	*0.090*
2.44 (0.72, 4.16)	2.34 (0.39, 4.30)	2.43 (0.66, 4.21)	3.29 (1.58, 5.00)	1.28 (0.32, 2.23)	4.41 (0.59, 8.22)	−1.95 (−3.67, −0.24)	2.97 (0.31, 5.64)
0.006	***0.019***	***0.007***	***0.0002***	***0.009***	***0.024***	***0.025***	***0.029***
			0.027				

line of thought, new and novel approaches including artificial intelligence methodologies are emerging and could be of great help in the future in developing predictive models.

In conclusion, this study is the first to demonstrate the strength of association between the adipsin/MCP-1 ratio with structural knee OA changes and CRP/MCP-1 with symptoms in obese OA subjects. This study also highlights the relevance and added value of using ratios of serum biomarkers rather than individual ones to a stronger association to OA progression.

Author contributions

All authors contributed substantially to the conception and design of the work. JMP, FA, GT and JPP contributed to data collection and assembly. JRT, MD and JPR were involved in the statistical analysis. JMP, GT and JPP drafted the article. All authors contributed to the analysis and interpretation of the data, critically reviewed the manuscript for important intellectual content, and approved the final article. All authors had full access to all the data and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of interest

J. Martel-Pelletier and J.P. Pelletier are shareholders in ArthroLab Inc. F. Abram is an employee of ArthroLab Inc. M. Dorais and J.P. Raynauld are consultants for ArthroLab Inc. G. Tardif and J. Rousseau Trépanier have no conflicts of interest.

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CRP/Adiponectin (LMW)	CRP/IL-8	CRP/MCP-1	IL-8/Visfatin	Visfatin/Adiponectin (HMW)	Visfatin/Adiponectin (LMW)	Visfatin/MCP-1
β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
0.20 (−0.10, 0.51)	0.26 (−0.07, 0.58)	0.22 (−0.10, 0.54)	−0.17 (−0.31, −0.03)	0.14 (0.01, 0.27)	0.15 (0.02, 0.29)	0.16 (0.03, 0.30)
0.190	*0.118*	*0.172*	***0.015***	***0.038***	***0.024***	***0.020***
1.16 (0.20, 2.12)	1.10 (0.09, 2.11)	1.07 (0.08, 2.06)	−0.47 (−0.91, −0.04)	0.42 (0.04, 0.84)	0.47 (0.05, 0.89)	0.47 (0.03, 0.91)
0.018	***0.033***	***0.035***	***0.034***	***0.048***	***0.029***	***0.035***
0.08 (−0.07, 0.22)	0.08 (−0.07, 0.23)	0.08 (−0.07, 0.23)	−0.03 (−0.10, 0.03)	0.02 (−0.04, 0.08)	0.03 (−0.04, 0.09)	0.03 (−0.04, 0.09)
0.279	*0.295*	0.310	*0.351*	*0.516*	*0.384*	*0.391*
1.48 (0.17, 2.79)	1.45 (0.08, 2.82)	1.38 (0.03, 2.73)	−0.66 (−0.25, −0.06)	0.57 (0.003, 1.14)	0.64 (0.07, 1.21)	0.65 (0.06, 1.25)
0.027	***0.039***	***0.046***	***0.030***	***0.049***	***0.029***	***0.032***

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Supplementary data

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