Are Baseline High Molecular Weight Adiponectin Levels Associated with Radiographic Progression in Rheumatoid Arthritis and Osteoarthritis?

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ABSTRACT. ***Objective.*** To investigate whether high molecular weight adiponectin (hmwAPN) mediates the associations of total adiponectin (totAPN) with radiographic progression in rheumatoid arthritis (RA) and hand osteoarthritis (HOA).

Methods. Associations between baseline hmwAPN or totAPN levels with radiographic progression were determined using multivariate linear regression or generalized estimated equations.

Results. In patients with RA, totAPN associated positively, whereas in patients with HOA it associated negatively with radiographic progression. In contrast, hmwAPN did not associate significantly with radiographic progression in either cohort.

Conclusion. Our data indicate that the differential effects associated between totAPN and radiographic progression in either RA or HOA are not mediated by hmwAPN. (J Rheumatol First Release April 1 2014; doi:10.3899/jrheum.130888)

Key Indexing Terms:
RADIOGRAPHIC PROGRESSION TOTAL ADIPONECTIN HAND OSTEOARTHRITIS
HIGH MOLECULAR WEIGHT ADIPONECTIN RHEUMATOID ARTHRITIS

Obesity has been associated with altered radiographic progression in rheumatoid arthritis (RA) and hand osteoarthritis (HOA)[1,2]. While the underlying mechanisms of these associations remain unclear, it is believed that adipose tissue secreted factors (adipokines) could play an important role in systemic effects of obesity. Therefore, several studies have investigated the association of adipokines with disease progression in RA and OA. Some adipokines, including adiponectin, have been shown to influence joint damage. In patients with RA, total adiponectin (totAPN) levels in serum associated positively with radiographic progression, suggesting a predisposing effect on disease[3]. Intriguingly, high totAPN levels in the serum of patients with HOA were associated with reduced relative risk for disease progression[1], indicating a protective effect.

Adiponectin is a pleiotropic adipokine that consists of several isoforms in circulation: a trimeric low molecular weight adiponectin; a hexameric middle molecular weight adiponectin, and a multimeric high molecular weight adiponectin (hmwAPN). In addition, although its presence in serum has been questioned, a globular form of adiponectin exists, resulting from proteolytic cleavage of totAPN[4]. Of the different adiponectin isoforms described, hmwAPN emerges as one of the most biologically active isoforms in circulation[5]. Although both proinflammatory and antiinflammatory actions have been attributed to this isoform, its role in disease progression in RA and in HOA remains unknown[6,7]. Here, we explored the possibility that the association of totAPN with disease progression in RA and HOA is primarily mediated by hmwAPN, indicating that the association of totAPN with radiographic progression could be dependent on hmwAPN.

MATERIALS AND METHODS

Patients selected from the Leiden Early Arthritis Cohort (EAC) and the Genetics, ARthrosis and Progression (GARP) study were included in our study[8,9]. Both studies were approved by the Medical Ethical Committee of the Leiden University Medical Center. The 324 patients with RA selected from the EAC fulfilled the American College of Rheumatology (ACR) 1987 revised criteria for the classification of RA within the first year of followup (n = 324) and presented to the Leiden EAC between 1993 and 2002[3]. This study included 324 patients in total, whereas previously

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samples of 253 patients were tested[3]. Because of limited availability of serum, plasma was used in our study. Yearly obtained radiographs of hands and feet were scored according to the Sharp/van der Heijde method by 1 experienced scorer (MvdL) who was blinded for each patient's autoantibody status, treatment, and clinical outcome[10]. The intrareader variability described by the intraclass correlation coefficient (ICC) was 0.97 for the radiographic progression rate. As described, treatment strategy was considered a possible confounder and was corrected for in subsequent analyses[3,8].

Of the 384 total patients included in the GARP study, 344 fulfilled the ACR criteria for clinical HOA, or had a Kellgren-Lawrence score ≥ 2 in ≥ 1 hand joint. Radiographs of the hands were available at baseline and after a mean of 6.1 years for 227 of these patients. This study included 227 patients in total, whereas previously samples from 164 patients were tested[1]. Thirty-two hand joints were scored for joint space narrowing (JSN) by a team of 2 experienced scorers (IW/JB) who were blinded for patient characteristics as described[1]. The intrareader variability was good (ICC 0.87). Progression was defined as the difference between the sum of JSN scores at followup and at baseline that was above the smallest detectable change of 1.5[1].

Laboratory assessments. Baseline plasma (RA) and serum (OA) samples were stored at –80°C. Concentrations of total adiponectin (μg/ml) were measured using the Bio-Plex Pro Human Diabetes kit (Bio-Rad, range 33–500,000 pg/ml), the Bio-Plex array reader, and Bio-Plex software, following the manufacturers' instructions. Concentrations of hmwAPN (μg/ml) were measured by ELISA (Merck Millipore, range 3.125–200 ng/ml), according to the manufacturer's instructions. Differences in levels of total and hmwAPN could be due to different experimental techniques used to quantify them.

Statistical analyses. Body mass index (BMI) was normally distributed in the study populations. Correlations between BMI and adiponectin levels were calculated using Spearman's rank correlation test. A correlation coefficient (r) below 0.2 was considered very weak; between 0.2 and 0.4 as weak; between 0.4 and 0.7 as moderate, and above 0.7 as strong.

In patients with RA, associations between adiponectin levels and radiological progression rates over 4 years were tested using a multivariate linear regression model, as described[3]. All analyses were adjusted for age, sex, and treatment strategy. Analyses were adjusted for BMI when an association between adiponectin and BMI was found. Sharp/van der Heijde scores were logarithmically transformed to meet the assumptions of linear regression. The estimates were recalculated to reflect the relationship between radiographic progression and adiponectin levels; the estimates (with 95% CI) represent the relative change in the rate of joint destruction over 4 years, corresponding to a change of 10 μg/ml in adiponectin levels[3].

In patients with HOA, the relative risk of progression was estimated (with 95% CI) using tertiles of adiponectin levels in generalized estimating equations with robust variance estimators to account for family effects, as described[1]. Corrections were made for age and sex and when necessary, for BMI.

The Statistical Package for the Social Sciences (SPSS) version 20.0 was used to analyze the data.

RESULTS

Study populations. Baseline patient characteristics, totAPN, and hmwAPN are depicted in Figure 1A.

TotAPN and hmwAPN levels correlated negatively with BMI in both cohorts (RA totAPN: r = –0.19; p = 0.002/hmwAPN r = –0.16; p = 0.009; HOA totAPN r = –0.18; p = 0.008/hmwAPN r = –0.32; p < 0.001). Because adipose tissue is the primary source of adiponectin, and to investigate whether the association of adiponectin with progression is dependent on BMI, we have additionally corrected for BMI in some analyses. In addition, hmwAPN levels correlated with totAPN levels in patients with RA (Figure 1B) and in patients with HOA (Figure 1C), indicating that systemically, there is no selective increase of hmwAPN in patients with higher totAPN levels.

Association of totAPN and hmwAPN with radiographic progression in RA. First we investigated whether baseline totAPN and hmwAPN could predict radiographic progression over a period of 4 years in patients with RA. BMI associated negatively with radiographic progression over 4 years (estimate 0.98; 95% CI 0.95–1.00; p = 0.04). Confirming and expanding our previous finding[3], totAPN associated with progression over 4 years (basic model[3], Figure 2A). A graphic representation of radiographic progression in patients divided according to tertiles of adiponectin concentrations is presented in Figures 2B and 2C. Similar results were obtained when JSN was used as determinant for progression (data not shown). These associations of totAPN with progression were all independent of BMI (Figure 2A).

In contrast, hmwAPN was not significantly associated with radiographic progression, although we did observe a trend for association (Figure 2A). This trend was, however, lost upon additionally correcting for BMI (Figure 2A).

Association of totAPN and hmwAPN with radiographic progression in OA. Next, we investigated whether totAPN and hmwAPN are associated with radiographic progression in patients with HOA over 6 years. Some samples were unavailable for testing and are indicated as missing values. BMI was not associated with progression (OR 0.99; 95% CI 0.94–1.05). Confirming and expanding published data[1], totAPN was inversely associated with progression (Figure 3A). This association remained significant after correcting for BMI (Figure 3A). In addition, totAPN levels were lower in progressive than in nonprogressive patients (Figure 3B). hmwAPN, however, was not associated with progression, nor did levels differ between progressive and nonprogressive patients (Figures 3A and 3C).

DISCUSSION

We investigated whether circulating levels of hmwAPN could mediate the association of totAPN with disease progression in RA and HOA. We have shown that hmwAPN is not significantly associated with radiographic progression, while confirming that totAPN levels are. hmwAPN, therefore, does not appear to be the main contributing isoform to the associations of totAPN with radiographic progression in RA and HOA.

The association between totAPN in serum and progression has been reported[3,11]. In our present study we showed a similar association with plasma totAPN levels. Notably, totAPN levels in plasma were lower than in serum when paired samples were tested (data not shown).

To our knowledge, this is the first study investigating the association of circulating hmwAPN levels with disease progression in RA. The proinflammatory and antiinflam-

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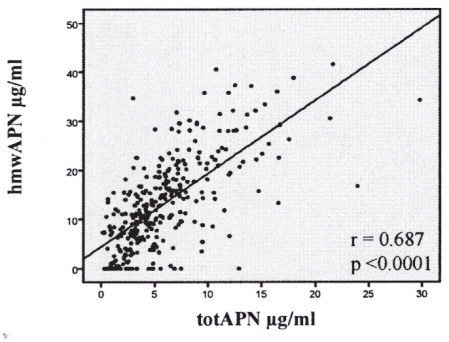
Characteristics	
RA patients (n = 324)	
Age mean (SD), years	56.9 (16.8)
Female, %	66.4
BMI mean (SD), Kg/m^2	25.6 (3.8)
non smoker, %	56.4
anti-CCP+, %	52.5
Adipokines (plasma)	
totAPN (µg/ml)	4.9 (2.9 - 7.4)
hmwAPN (µg/ml)	11.9 (6.4 - 18.7)
Total SvdH (0 - 448)	
after 1 yr	9 (4 - 21)
after 2 yrs	12 (5 -25)
after 3 yrs	6 (14 -31)
after 4yrs	7 (17 -34)
Hand OA patients (n = 227)	
Age mean (SD), years	59.1 (7.1)
Female, %	82.8
BMI mean (SD), Kg/m^2	27.2 (5.0)
Adipokines (serum)	
totAPN (µg/ml)	21.9 (15.5 - 34.0)
hmwAPN (µg/ml)	2.9 (1.7 - 5.1)
Hand OA	
Progressive (n = 62),%	27.3
non-progressive (n = 165),%	72.7

B



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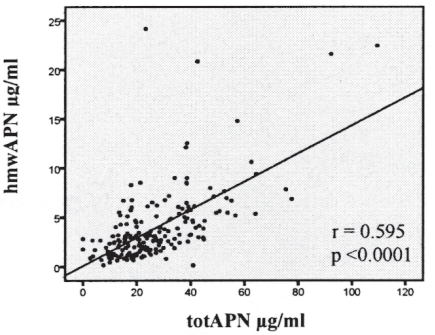

Figure 1. A. Patient characteristics of the Early Arthritis Cohort (EAC) and the Genetics ARthrosis and Progression (GARP) cohort. Spearman's rank test correlation coefficients between total adiponectin (totAPN) and high molecular weight adiponectin (hmwAPN) in plasma of EAC (B) and serum of GARP (C) patients. Unless otherwise specified, values depicted are medians (interquartile range). A p value < 0.05 was considered significant. BMI: body mass index; RA: rheumatoid arthritis; anti-CCP: anticyclic citrullinated peptide; SvdH: Sharp/van der Heijde scores; OA: osteoarthritis.

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		estimate (95% confidence interval)	P- value
basic model	totAPN	1.30 (1.10 - 1.52)	**0.002**
	hmwAPN	1.09 (0.99 - 1.19)	0.068
basic model + BMI	totAPN	1.26 (1.07 - 1.49)	**0.006**
	hmwAPN	1.05 (0.96 - 1.16)	0.300

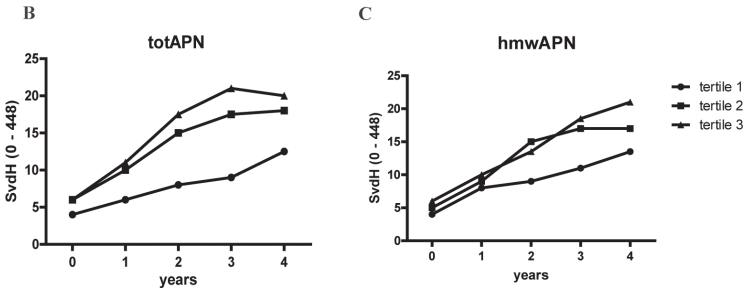

Figure 2. Associations of baseline total adiponectin (totAPN) and high molecular weight adiponectin (hmwAPN) levels with radiographic progression in patients with rheumatoid arthritis (RA). A. Repeated measurement analysis with Sharp/van der Heijde (SvdH) scores over 4 years as dependent variable in patients with RA (Early Arthritis Cohort). Analyses were corrected for age, sex, treatment strategy, and, body mass index (BMI). A p value ≤ 0.05 was considered significant. Concentrations of adiponectin are expressed in µg/ml. Median rates of joint destruction are depicted for the tertiles of totAPN (B) or hmwAPN (C).

matory effects of hmwAPN are still being debated[12,13] and could depend on the disease studied and the experimental setting. However, our results are in line with a study that indicated that all isoforms can exert potent proinflammatory effects on RA synovial fibroblasts and monocytes *in vitro*[6,14]. Although there was a trend toward an association between hmwAPN and progression of RA, the size of this association is considerably less than for totAPN, indicating a possible contribution of other isoforms to the observed association, or a possible modulatory effect of local environmental factors, which could result in a stronger local effect[6,14] than systemic effects of hmwAPN.

The inverse association of adiponectin with radiographic progression in HOA is intriguing, because the different forms of adiponectin have proinflammatory effects on OA synoviocytes, and circulating adiponectin levels are associated with synovial inflammation in knee OA[14,15]. In addition, higher levels of adiponectin have previously been suggested in patients with erosive compared to nonerosive HOA[16]. These differences may lie in differences in study cohort or differences in determination of radiographic damage.

Because synovial inflammation has been associated with radiographic progression in knee OA[17], these data suggest a deleterious effect of adiponectin on disease progression rather than a protective effect. This could be caused by a different effect of synovial inflammation on radiographic progression in HOA compared to the knee or by a different effect of adiponectin on synoviocytes of the hand joint compared to the knee joint. The mechanisms underlying this discrepancy remain to be further investigated.

While our data await replication, there was a clear inverse association between totAPN and HOA progression and this association was not observed for hmwAPN, indicating that other isoforms could mediate the observed association.

Our study further substantiates the known associations of totAPN with radiographic progression in RA and HOA and indicates that these associations are not mediated by a selective effect of hmwAPN.

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	Serum conc (μg/ml)	non-progressive (n = 165)	progressive (n = 62)	Odds ratio (95% Confidence Interval)	Sig.
basic model	TotAPN (9 missing)				
	< 17.6	42	30	1 (reference)	
	17.6 - 28.8	59	14	0.36 (0.16 - 0.78)	**.010**
	>28.8	59	14	0.31 (0.13 - 0.70)	**.005**
	HmwAPN (23 missing)				
	<2.2	51	17	1 (reference)	
	2.2 - 4.1	53	15	.91 (0.42 - 1.96)	.804
	>4.1	52	16	0.92 (0.39 - 2.15)	.842
basic model + BMI	TotAPN				
	< 17.6			1 (reference)	
	17.6 - 28.8			0.34 (0.16 - 0.73)	**.006**
	>28.8			0.29 (0.13 - 0.66)	**.003**
	HmwAPN				
	<2.2			1 (reference)	
	2.2 - 4.1			0.91 (0.41 - 1.99)	.807
	>4.1			0.91 (0.38 - 2.15)	.820

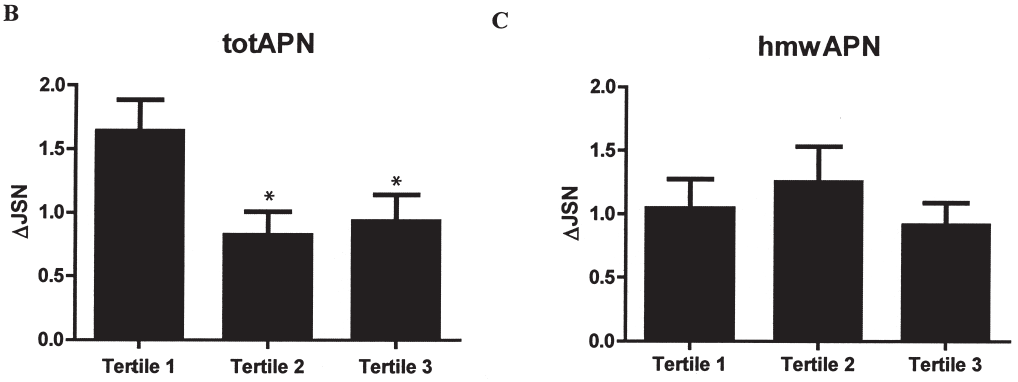

Figure 3. Associations of baseline total adiponectin (totAPN) and high molecular weight adiponectin (hmwAPN) levels with radiographic progression in patients with hand osteoarthritis (HOA). A. Generalized estimating equations analysis between tertiles of adiponectin in patients with HOA (Genetics ARthrosis and Progression cohort analyses) were corrected for age, sex, treatment strategy, and body mass index (BMI). Concentrations of adiponectin are expressed in μg/ml. Difference in sum of joint space narrowing (ΔJSN) between baseline and followup are depicted for tertiles of totAPN (B) and hmwAPN (C). Significance was calculated using Mann-Whitney U test. A p value < 0.05 was considered significant.

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