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Elevated BMI and Antibodies to Citrullinated Proteins Interact to Increase Rheumatoid Arthritis Risk and Shorten Time to Diagnosis: a Nested Case-Control Study of Women in the Nurses' Health Studies

Sara K. Tedeschi, MD, MPH[1], **Jing Cui, PhD**[1], **Elizabeth V. Arkema, ScD**[2], **William H. Robinson, MD, PhD**[3], **Jeremy Sokolove, MD**[3], **Nithya Lingampalli, BS**[3], **Jeffrey A. Sparks, MD, MMSc**[1], **Elizabeth W. Karlson, MD**[1], and **Karen H. Costenbader, MD, MPH**[1]

[1]Brigham and Women's Hospital, Division of Rheumatology, Immunology and Allergy, Boston, MA, USA

[2]Karolinska Institutet, Department of Medicine Solna, Stockholm, Sweden

[3]Stanford University, Division of Immunology and Rheumatology, Palo Alto, CA, USA

Abstract

Objective—Overweight/obesity and anti-citrullinated protein antibodies (ACPA) increase RA risk. We investigated the relationship between body mass index (BMI) and ACPA, tested for an interaction between BMI and ACPA for RA risk, and examined effects of BMI and ACPA on time to RA diagnosis.

Design—Within the Nurses' Health Studies, blood samples were collected before diagnosis from medical record-confirmed incident RA cases and matched controls. Multiplex assays measured seven ACPA subtypes (biglycan, clusterin, enolase, fibrinogen, histone 2A, histone 2B, vimentin). Logistic regression analyses tested the association of BMI and ACPA and for a multiplicative interaction between BMI groups (≥25 vs. <25 kg/m^2) and ACPA positivity (≥2 vs. <2 subtypes), adjusting for age, smoking, alcohol use, and *HLA*-shared epitope. In case-only analyses, log-rank tests compared time from blood draw to RA onset by cross-classified BMI/ACPA status.

Results—Among 255 pre-RA cases and 778 matched controls, 15.7% vs. 2.1% ($p<0.001$) had ≥2 ACPA and 49.4% vs. 40.2% ($p<0.01$) were overweight/obese. Continuous BMI was not associated with presence of ≥2 ACPA (OR per kg/m^2 unit BMI: 1.03 [95% CI 0.97-1.09]). However, there was a multiplicative interaction between elevated BMI and presence of ≥2 ACPA for RA risk

Sara K. Tedeschi, MD, MPH, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115 USA, stedeschi1@partners.org, Telephone: +1-617-732-5325.

Contributors All authors have revised the manuscripts for important intellectual content and have approved this version of the article for publication. SKT, JC, EA, JAS, EWK, and KHC provided substantial contributions to study design and interpretation of data. SKT and JC performed data analyses. WHR, JS, and NL provided significant input on ACPA assay interpretation. SKT drafted the paper.

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(p=0.027). Women with BMI ≥25 kg/m^2 and ≥2 ACPA had OR 22.7 (95% CI [6.64-77.72]) for RA. Median time to RA differed by BMI/ACPA group (overall log-rank p<0.001) and was shortest among women with ≥2 ACPA and BMI ≥25 kg/m^2 (45.0 months, IQR [17.5-72.5]) and longest in women with <2 ACPA and BMI <25 kg/m^2 (125.0 months, IQR [72.0-161.0]) (pairwise log-rank p=0.002).

Conclusion—Elevated BMI and presence of ACPA interacted to increase RA risk. Time to RA onset was shortest among overweight/obese women with ≥2 ACPA.

Keywords

rheumatoid arthritis; autoantibodies; epidemiology; obesity; ACPA

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease that primarily affects synovial joints, causing pain, swelling, decreased function, bony erosions and ultimately joint deformity. During the pre-clinical phase, autoantibodies, cytokines, and chemokines may be elevated for up to 14 years prior to symptoms [1-7]. Among individuals with genetic risk factors, this loss of self-tolerance without clinically apparent disease may follow an exposure to one or more environmental factors [8, 9]. Synovitis develops subsequently, due to unknown factors. Given the accumulating evidence of this prolonged period of pre-clinical inflammation and autoimmunity, there is growing interest in potential environmental factors related to the induction of RA.

Elevated body mass index (BMI) has been associated with increased RA risk in several past studies; some have observed a differential effect by sex, with elevated risk among women but not men [10-14]. Recent meta-analyses of BMI's effect on RA risk reported that BMI ≥25 kg/m^2 (the World Health Organization definition of overweight/obese) significantly increased RA risk by 15%, compared to BMI <25 kg/m^2; BMI ≥30 kg/m^2 (obesity) significantly increased RA risk by 21% to 31% compared to normal BMI [15-17]. Obesity may increase RA risk via systemic inflammation, as adipose tissue secretes pro-inflammatory adipokines [18]. Adipokines from visceral fat collections in particular are associated with elevated levels of systemic inflammation [19-21]. In many observational studies, overweight and obese individuals without RA have had higher levels of inflammatory biomarkers, including C-reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), than have people of normal weight suggesting independent associations between obesity and inflammation [18, 19, 22, 23].

Anti-citrullinated protein antibodies (ACPA) are autoantibodies targeted against proteins that have undergone post-translational modification of arginine to citrulline, catalyzed by peptidylarginine deiminase (PAD) enzymes [24]. ACPAs may be detectable in the serum more than ten years before the development of RA, and the number of distinct types of ACPAs increases in the years leading up to RA diagnosis, likely reflecting epitope spread [25-29]. ACPA positivity is strongly related to subsequent RA risk [30, 31]. In a nested case-control study in the Nurses' Health Studies, the presence of one or more ACPA in a blood

sample was associated with a five-fold increased risk of future RA [31]. ACPAs are associated with genetic and environmental RA risk factors, including the *HLA*-shared epitope (*HLA-SE*) and smoking, both in RA patients and asymptomatic individuals [31-35]. ACPAs to vimentin and enolase have been shown to synergistically interact with *HLA-DRB1* to increase risk of seropositive RA [35, 36].

As elevated BMI and ACPA are both associated with RA risk, particularly in women, and because BMI involves systemic inflammation, we aimed to determine the relationship between these two risk factors among asymptomatic women in a nested-case control study. We also investigated whether BMI was related to a genetic risk factor for RA, the *HLA-SE.* We then sought to investigate whether an interaction existed between elevated BMI and ACPA, or between elevated BMI and *HLA-SE*, such that the risk of RA was synergistically elevated in the presence of both risk factors. Lastly, among women who later developed RA, we examined the joint effects of BMI and ACPA on the time between blood sample collection and RA diagnosis (referred to as “time to RA”).

MATERIALS AND METHODS

Study design and population

We conducted a nested case-control study among women in the Nurses’ Health Studies, which are large prospective cohorts of female nurses living in the United States. The Nurses’ Health Study (NHS) enrolled 121,700 women aged 30-55 years living in 11 states at the time of enrollment in 1976. Nurses’ Health Study II (NHSII) enrolled 116,430 women aged 25-42 years living in 14 states at the time of enrollment in 1989. All participants completed questionnaires by mail at baseline and in follow-up every two years regarding the development of new diseases, lifestyle factors, and health behaviors.

Approximately one-fourth of subjects donated a one-time blood sample, collected between 1989-90 in the NHS (27%) and 1996-1999 in the NHSII (25%). Our nested case-control study included pre-RA cases and matched controls among women from the NHS/NHSII who had donated a blood sample. For this study, women were followed from cohort inception through May 30, 2012 (36 years in the NHS) and May 30, 2011 (22 years in the NHSII).

Identification of cases

Methods for RA case identification and validation have been previously reported [4, 37]. In brief, self-reported incident connective tissue disease after the blood draw was first elicited via biannual mailed questionnaires. Self-reporters then completed the Connective Tissue Screening Questionnaire [38]. If positive, medical records were obtained and reviewed in detail by two rheumatologists for the 1987 American College of Rheumatology (ACR) classification criteria for RA [39], including documentation of rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP) by commercial assay at the time of diagnosis. Since some women were diagnosed with RA prior to the clinical use of the anti-CCP test, we defined seropositive as being positive for either RF or anti-CCP by medical record

review. The month and year of diagnosis were recorded based on medical record review. Women with prevalent RA at the blood draw were not included in the study.

Matched controls

For each case, we matched three controls within the same cohort who donated a blood sample and had not reported RA or other rheumatic disease at the time of case identification. Matching factors at time of blood draw were age (±1 year), menopausal status and post-menopausal hormone use, time of blood collection, and fasting status, as in prior work [40, 41]. Women were excluded from being controls if they had self-reported RA that was not confirmed upon medical record review, or if they had self-reported another connective tissue disease, whether confirmed or not, at the time of blood draw. Women who reported low back pain or osteoarthritis, without another connective tissue disease, were allowed to be controls.

Laboratory methods

Anti-citrullinated protein antibodies (ACPA)—The development of multiplex ACPA assays was previously described in detail [25]. In brief, synovium-specific protein antigens (citrullinated peptides and native non-citrullinated peptides) were conjugated to spectrally-distinct beads using the BioPlex multiplex assay platform (Bio-Rad Laboratories, Hercules, CA, USA) for analysis on the Luminex 200 instrument (Luminex, Austin, TX, USA) [26]. Pre-established control serum samples with high, medium, low, or no reactivity were performed on each plate as internal controls.

Serum from each pre-RA case and matched controls was added to the bead mix, and the reactivity of ACPAs was measured in raw fluorescent intensity units. Fifteen antibodies against citrullinated proteins, all of which passed quality control, were included in this study. Background reactivity to eight non-citrullinated native proteins was also measured among pre-RA cases and controls. Inter-batch coefficients of variation were 2.2% to 9.1%. We defined a positive assay as having raw fluorescent intensity units ≥3 standard deviations (SD) above the mean fluorescent intensity units among controls, as previously described [31].

The 15 ACPAs analyzed were grouped into seven ACPA subtypes by proteins: biglycan (1 epitope), clusterin (2 epitopes), enolase (1 epitope), fibrinogen (5 epitopes), histone 2A (2 epitopes), histone 2B (2 epitopes), and vimentin (2 epitopes). Presence of ACPA against one or more epitope(s) within a given subtype was deemed positive for that subtype. For each subject we summed the number of different ACPA subtypes present, ranging from zero to seven. We first studied the relationship between BMI and number of ACPA subtypes as a continuous count. Our analyses then included ACPA as a binary variable, <2 ACPA subtypes present vs. ≥2 subtypes present, to permit us to investigate an interaction between elevated BMI and presence of ACPA on RA risk. We chose ≥2 ACPA subtypes to define positivity since the prevalence of ACPAs in controls using this cutoff was similar to the 1-2% prevalence of ACPA observed in healthy subjects in other population-based studies [42, 43].

HLA-shared epitope—Classical *HLA-SE* alleles were tested by the American Red Cross as previously described [31, 41]. Shared epitopes alleles tested were: *HLA-DRB1**0401,

*0404, *0405, *0408, *0101, *0102, *1001 or *09. For each woman, *HLA-SE* was recorded as absent (zero alleles), present (one or two alleles), or missing.

Exposures and covariates

BMI was calculated in kg/m^2 using self-reported weight from the questionnaire cycle immediately prior to the blood draw and height from the enrollment questionnaire. We dichotomized BMI <25 vs. ≥25 kg/m^2 based on the World Health Organization definition of normal vs. overweight/obese [15], as well as literature supporting this as a clinically important cutoff for RA risk [10, 11, 17, 44, 45]. Subjects were also cross-classified into four groups according to their BMI (<25 vs. ≥25 kg/m^2) and ACPA status (<2 vs. ≥2 subtypes).

Covariates selected *a priori* for inclusion in multivariable models were smoking (continuous pack-years) and cumulative average alcohol use (continuous grams/day) from the questionnaire cycle prior to blood draw, as well as *HLA-SE* (absent, present, or missing), given that each of these has been shown to be related to ACPA-positive RA [12, 32, 46-50]. In 1988 (NHS) and 1989 (NHSII), participants were asked whether they had a physical examination in the past two years. This variable was included as an indicator of healthcare utilization, as increased healthcare utilization could potentially lead to earlier RA diagnosis. As our conditional logistic regression models were conditioned on the matching factors, they were not additionally adjusted for matched covariates.

Statistical methods

Characteristics of pre-RA cases and matched controls at the blood draw, including the prevalence of ACPA subtypes, were described and compared using univariable conditional logistic regression models to account for the matched design. Characteristics of pre-RA cases at RA diagnosis were summarized using descriptive statistics.

We investigated the cross-sectional relationship between continuous BMI (kg/m^2) and number of ACPA subtypes (zero to seven) using Spearman's correlation coefficient. We also tested for a relationship between BMI and ACPA positivity (≥2 subtypes) using logistic regression models to calculate odds ratios (OR) and 95% confidence intervals (CI) for ≥2 ACPA, adjusting for age at blood draw, smoking, alcohol intake and *HLA-SE*. We performed multivariable conditional logistic regression models to estimate ORs and 95% CIs for the risk of RA, including BMI, ≥2 ACPA, and a multiplicative interaction between BMI (≥25 vs. <25 kg/m^2) and ACPA (≥2 vs. <2 subtypes), adjusting for smoking, alcohol intake, *HLA-SE*, and physical exam in the past two years.

To test whether elevated BMI was related to the presence of *HLA-SE*, we used linear regression models to estimate the age-adjusted and then multivariable-adjusted effect (β coefficient [standard error]) of ≥1 *HLA-SE* allele on BMI in kg/m^2. Logistic regression models calculated OR (95% CI) for BMI ≥25 kg/m^2 based on *HLA-SE* status (≥1 vs. 0 alleles), adjusting for age at blood draw, smoking, alcohol intake and ACPA. Conditional logistic regression models then tested for a multiplicative interaction between BMI (≥25 vs. <25 kg/m^2) and *HLA-SE* (≥1 vs. 0 alleles) for RA risk, adjusting for smoking, alcohol intake, ACPA, and physical exam in the past 2 years.

In pre-RA case-only analyses, we evaluated the median time to RA diagnosis in each of four cross-classified BMI/ACPA groups. Overall and pairwise log-rank tests were used to assess differences in the time between the blood draw and RA diagnosis across these groups.

Analyses were performed using SAS (v 9.3, Cary, NC, USA). We considered a two-sided p value of <0.05 as statistically significant in all analyses. All aspects of this study were approved by the Partners HealthCare Institutional Review Board.

RESULTS

Pre-RA cases and matched controls

Two hundred and fifty-five pre-RA cases (166 in NHS, 89 in NHSII) were matched to 778 controls on characteristics at the blood draw. Both pre-RA cases and controls were predominantly White, but the two groups had a few expected differences (**Table 1**). At the time of blood draw, ≥2 ACPA were present in 15.7% of pre-RA cases and 2.1% of controls (p<0.001). Nearly 50% of pre-RA cases were overweight/obese, compared to 40.2% of controls (p<0.01). Among pre-RA cases, median BMI was 22.3 kg/m^2 (IQR 21.0-23.8) among those with BMI <25 kg/m^2, and 28.2 kg/m^2 (IQR 26.3-31.0) among those with BMI ≥25 kg/m^2. Among controls, median BMI was 22.3 kg/m^2 (IQR 20.9-23.5) among those with BMI <25 kg/m^2, and 28.2 kg/m^2 (IQR 26.2-30.9) among those with BMI ≥25 kg/m^2. Pre-RA cases had a greater number of pack-years of smoking (p=0.01) and less alcohol use (p<0.01) compared to controls. One or more *HLA-SE* alleles were present in 56.0% of pre-RA cases and 42.5% of controls (p<0.001). Each of the seven ACPA subtypes was observed more often in pre-RA cases than in controls at the time of blood draw (**Table 2**). Among all subjects, antibodies against citrullinated (cit) proteins were detected more often than antibodies against non-citrullinated (non-cit) proteins: vimentin (5.2% cit vs. 1.6% non-cit), fibrinogen (7.7% cit vs. 1.1% non-cit), histone 2B (3.4% cit vs. 1.7% non-cit), and histone 2A (2.4% cit vs. 1.7% non-cit).

The mean age for cases at RA diagnosis was 60.3 years (SD 9.9) (**Table 3**). Mean duration between blood draw and diagnosis (time to RA) was 106.9 months (SD 63.0) among all cases, though cases with BMI <25 kg/m^2 had a longer mean time to RA (118.1 months [SD 63.6]) and those with BMI ≥25 kg/m^2 had a shorter mean time to RA (95.4 months [SD 60.4]). RA cases otherwise had similar characteristics of their RA presentation, regardless of BMI category. Approximately two-thirds were seropositive by chart review at the time of diagnosis. The vast majority had symmetric arthritis involving the hands.

Relationship between BMI and ACPA positivity

We did not observe a significant association between continuous BMI and continuous number of ACPA subtypes among all nested case-control subjects (r=−0.015, p=0.63). We also did not detect a significant association between increasing BMI and the presence of ≥2 ACPA in age-adjusted (OR 1.04, 95%CI [0.98-1.10]) or multivariable-adjusted (OR 1.03, 95%CI [0.97-1.09]) models among all subjects (**Table 4**). However, among pre-RA cases only we detected a significant association between continuous BMI and ACPA positivity in

age-adjusted (OR 1.09, 95%CI [1.02-1.16]) and multivariable-adjusted (OR 1.07, 95%CI [1.00-1.15]) models.

Relationship between BMI, ACPA, and RA risk

Women with ≥2 ACPA had an eight-fold increased RA risk, compared to women with <2 ACPA, in the conditional logistic regression model (OR 8.05, 95% CI [4.43-14.66]). The odds of RA were greater than six-fold elevated (OR 6.65, 95% CI [3.56-12.43]) in the presence of ≥2 ACPA in a model additionally adjusting for smoking, alcohol use, BMI, *HLA-SE*, and physical exam in the past two years. BMI ≥25 kg/m^2 (vs. <25) was also associated with increased RA risk: age-adjusted OR 1.49 (95% CI 1.11-1.99) and multivariable-adjusted OR 1.35 (95% CI 0.99-1.84).

In stratified models, women with BMI ≥25 kg/m^2 and ≥2 ACPA had 23-fold increased odds of RA compared to the reference group of women with BMI <25 kg/m^2 and <2 ACPA (OR 22.72, 95%CI [6.64-77.72]) (**Figure 1**). We observed three-fold increased odds for RA among women with BMI <25 kg/m^2 and ≥2 ACPA, compared to the reference group (OR 3.44, 95%CI [1.53-7.74]). We observed a significant multiplicative interaction between BMI and ACPA for RA risk (p for interaction 0.027).

Relationship between BMI and *HLA-SE* positivity

Among 916 subjects with *HLA-SE* measured, the presence of ≥1 *HLA-SE* allele was not related to increasing BMI in kg/m^2 in age-adjusted (β 0.28 [standard error 0.30], p=0.35) or multivariable-adjusted (β 0.22 [standard error 0.30], p=0.45) linear regression models. Similarly, we observed no relationship between the presence of ≥1 *HLA-SE* allele (OR 0.98, 95% CI [0.75-1.27]) and BMI ≥25 kg/m^2 in the age-adjusted logistic regression model. In a multivariable-adjusted model, ≥1 *HLA-SE* allele was associated with an OR 0.96 (95%CI 0.74-1.26) for BMI ≥25 kg/m^2. Among pre-RA cases only (n=248 with *HLA-SE* measured) we did not detect a significant association between presence of ≥1 *HLA-SE* allele and continuous BMI in age-adjusted (β 1.13 [standard error 0.61], p=0.07) or multivariable-adjusted (β 0.85 [standard error 0.61], p=0.16) linear regression models.

Relationship between BMI, *HLA-SE* and RA risk

The presence of *HLA-SE* significantly increased RA risk, with OR 1.75 (95% CI 1.30-2.37) for RA in age-adjusted and OR 1.53 (95% CI 1.11-2.12) in multivariable-adjusted conditional logistic regression models. However, we did not observe a multiplicative interaction between BMI and *HLA-SE* in RA risk (p for interaction 0.92).

Time to RA diagnosis among pre-RA cases

Among the 255 pre-RA cases, time to RA diagnosis differed by BMI/ACPA group (overall log-rank p<0.001) (**Table 5**). Women with BMI ≥25 kg/m^2 and ≥2 ACPA progressed to diagnosis the fastest with a median time to RA 45.0 months (IQR [17.5-72.5]). The longest time to RA was among women with BMI <25 kg/m^2 and <2 ACPA, with a median of 125.0 months (IQR [72.0-161.0]).

In pairwise comparisons of time to RA between each of these groups, we observed a significant difference between women with <2 ACPA and BMI <25 kg/m^2 and those with ≥2 ACPA and BMI ≥25 kg/m^2 (pairwise log-rank p=0.002). Among women with ≥2 ACPA, time to RA diagnosis was also significantly different based on BMI <25 vs. ≥25 kg/m^2 (pairwise log-rank p=0.001).

DISCUSSION

In this case-control study nested within the NHS/NHSII, being overweight or obese was more common among pre-RA cases than matched controls. However, we observed no cross-sectional association between elevated BMI and the presence of ≥2 ACPA among all women. Within the pre-RA cases, elevated BMI was associated with ≥2 ACPA in a multivariable-adjusted model. We detected a multiplicative interaction between elevated BMI and ≥2 ACPA associated with RA risk among all participants, with a 23-fold risk of RA if both risk factors occurred together. These findings suggest that the presence of both elevated BMI and ACPA has a synergistic effect on RA risk. Moreover, among the pre-RA cases, we observed that women with both risk factors progressed to clinical development of RA the most rapidly.

We were interested to investigate elevated BMI, a potentially modifiable risk factor, since this may stimulate the development of ACPAs as smoking is thought to do, perhaps due to citrullination in the inflammatory milieu of adipose tissue [51]. This hypothesis was based upon reports of a murine model in which expression of PAD enzymes was detected in macrophage collections within mammary gland adipose tissue [52]. PAD enzymes and citrullinated histones have been found in adipose breast tissue from obese women as well [53]. In our study, the lack of a cross-sectional relationship between BMI and presence of ACPA among all subjects makes it less likely that excess adipose tissue promotes the development of ACPA, although we did observe an association between BMI and ACPA among pre-RA cases. Among seropositive (anti-CCP and/or RF) patients with early arthralgias, those with elevated BMI were more likely to develop classifiable RA than those with normal BMI [54]. It is possible but unlikely that the relationship between elevated BMI and the development of ACPA differs at other time points prior to RA.

A possible explanation for the multiplicative interaction between BMI and ACPA is that, in the context of already having ACPA, overweight and obesity foster systemic inflammation which hastens RA pathogenesis. In murine models, for example, immunization with neutrophil-derived citrullinated histones has been shown to be arthritogenic, but only in the setting of underlying inflammation [55]. Given the nested case-control study design, we were unable to conduct a mediation analysis to further investigate this hypothesis.

We did not find an association between the *HLA-SE* and elevated BMI. We also did not observe an interaction between the presence of ≥1 *HLA-SE* allele and elevated BMI upon RA risk. This suggests that elevated BMI does not affect RA in the same way as smoking, which has been shown to increase the risk of RA in particular among those carrying *HLA-SE* alleles [35, 41, 56].

In analyses including pre-RA cases only, both BMI and ACPA status were associated with decreased time to diagnosis, and women with BMI ≥25 kg/m^2 and ≥2 ACPA had the shortest interval between blood draw and diagnosis. Deane et al. developed a model predicting time from blood draw to RA among pre-RA subjects from a predominantly male military population [7]. In this model, increasing cytokine/chemokine count and age (by decade) were inversely related to time to RA onset. Our observation of shorter time to diagnosis among ACPA positive women with BMI ≥25 kg/m^2 may be explained by higher cytokine/chemokine levels in the setting of obesity, which could trigger or accelerate RA pathogenesis in those who already have ACPAs. We investigated the possibility that increased healthcare utilization among obese women could have led to surveillance bias, in which their RA was diagnosed at earlier stages, but we found no evidence that heavier women had increased use of routine physical exams, and adjustment for this healthcare utilization did not affect our results.

Two or more ACPA subtypes were present in 15.7% of pre-RA cases and 2.1% of controls, with a positive assay defined as previously published [31]. These percentages are similar to other recent RA nested case-control studies, including the prospective European cohort study (EPIC), in which the prevalence of each of three ACPA subtypes was 6-18% among pre-RA cases and 2% among controls [42].

We were unable to perform subgroup analyses due to small numbers of women with each individual ACPA subtype. Since many women were diagnosed prior to the widespread clinical availability of the anti-CCP test, we also could not perform stratified analyses based on this test. We had relatively small numbers of women in each BMI category, and thus our confidence intervals were wide. While we did not adjust for inflammatory cytokine concentrations, inflammatory cytokines likely are mediators on the causal pathway between elevated BMI and RA onset. Another potential limitation is that these analyses were not controlled for all pre- RA comorbidities and medications, which could conceivably be related to both obesity and risk of ACPA-positive RA. Additionally, our study included only U.S. women, the vast majority of whom were White with mean age 60 years at RA diagnosis. It is unlikely that the biologic mechanisms of RA differ in other female populations, although differences by sex, age, and socioeconomic status are possible.

In conclusion, in this large nested case-control study with a wide range of follow-up after blood draw and prospective exposure data collected prior to blood draw and RA diagnosis, we found that BMI and ACPA interacted in predicting RA risk and together shortened the time to diagnosis. As our understanding of RA pathogenesis grows, these findings may provide important insights into prevention, screening, and treatment. Obesity is a potentially modifiable risk factor. Thus, the ability of weight loss interventions to reduce RA risk, particularly among individuals who are already ACPA positive, may be tested in the near future. Further investigation into the mechanisms underlying the interaction between BMI and ACPA in RA is needed.

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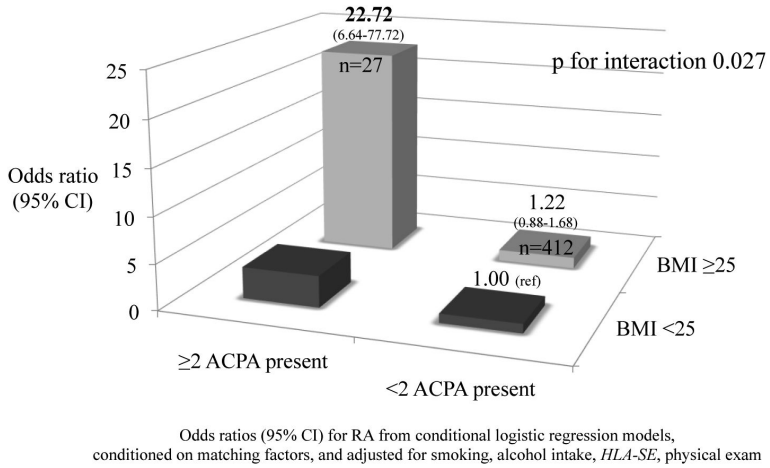

Odds ratios (95% CI) for RA from conditional logistic regression models, conditioned on matching factors, and adjusted for smoking, alcohol intake, *HLA-SE*, physical exam

Figure 1.

Multiplicative interaction between ACPA status (≥2 ACPA) and BMI ≥25 on risk of RA among 1033 women (NHS and NHSII) in a nested case-control study of RA

Table 1

Characteristics of 1033 women from the Nurses' Health Study and Nurses' Health Study II in a nested case-control study of rheumatoid arthritis, at the time of blood draw [1989-90 (NHS) or 1996-99 (NHSII)]

Characteristics	Pre-RA Cases (n=255)	Matched Controls (n=778)	p value
Age at blood draw, years	51.4 (8.0)	51.4 (8.0)	*matching factor*
White	250 (98.0%)	758 (97.4%)	0.45
≥2 ACPA subtypes present	40 (15.7%)	16 (2.1%)	<0.001
BMI ≥25	126 (49.4%)	313 (40.2%)	<0.01
Smoking, pack-years	12.3 (16.1)	9.4 (15.7)	0.01
Cumulative average alcohol, g/day	4.0 (5.3)	5.5 (8.6)	<0.01
≥1 HLA-SE allele present *	139 (56.0%)	284 (42.5%)	<0.001

Presented as mean (SD) or n (%). P values are from univariable conditional logistic regression models. ACPA: anti-citrullinated protein antibody. BMI: body mass index.

* HLA-SE: HLA-shared epitope (missing in 117 women). Presented as % of 248 pre-RA cases and 668 controls

Table 2

Prevalence of ACPA subtypes among 1033 women from the Nurses' Health Study and Nurses' Health Study II in a nested case-control study, at the time of blood draw [1989-90 (NHS) or 1996-99 (NHSII)]

ACPA subtype*	Pre-clinical RA Cases (n=255)	Matched Controls (n=778)	p value
Biglycan	12 (4.7%)	7 (0.9%)	<0.001
Clusterin	26 (10.2%)	13 (1.7%)	<0.001
Enolase	6 (2.4%)	4 (0.5%)	0.02
Fibrinogen	45 (17.7%)	34 (4.4%)	<0.001
Histone 2A	14 (5.5%)	11 (1.4%)	0.001
Histone 2B	22 (8.6%)	13 (1.7%)	<0.001
Vimentin	38 (14.9%)	16 (2.1%)	<0.001
≥1 ACPA	59 (23.1%)	59 (7.6%)	<0.001
≥2 ACPAs	40 (15.7%)	16 (2.1%)	<0.001

P values are from univariable conditional logistic regression models.

*For each ACPA subtype, antibodies against the following number of epitopes were tested. Subjects testing positive for antibody against one or more epitope were considered positive for that ACPA subtype: biglycan (1), clusterin (2), enolase (1), fibrinogen (5), histone 2A (2), histone 2B (2), vimentin (2)

Table 3

Characteristics of 255 rheumatoid arthritis cases from the Nurses' Health Study and Nurses' Health Study II in a nested case-control study, at the time of diagnosis [*]

	All cases (n=255)	BMI <25 kg/m^2 (n=129)	BMI ≥25 kg/m^2 (n=126)
Mean age at RA diagnosis, years	60.3 (9.9)	61.9 (9.4)	58.7 (10.2)
Mean time from blood draw to RA diagnosis, months	106.9 (63.0)	118.1 (63.6)	95.4 (60.4)
Seropositive (RF and/or CCP positive) [**]	158 (62.0%)	79 (61.2%)	79 (62.7%)
Hand arthritis	251 (98.4%)	126 (97.7%)	125 (99.2%)
Symmetric arthritis	250 (98.0%)	126 (97.7%)	124 (98.4%)
Morning stiffness >1 hour	194 (76.1%)	97 (75.2%)	97 (77.0%)
Erosions	53 (20.8%)	28 (21.7%)	25 (19.8%)
Rheumatoid nodules	24 (9.4%)	10 (7.8%)	14 (11.1%)
Self-reported physical exam in past 2 years [+]	105 (41.2%)	58 (45.0%)	47 (37.3%)

Presented as mean (SD) or %. RA: rheumatoid arthritis. RF: rheumatoid factor. CCP: cyclic citrullinated peptide antibody.

[*] All RA cases had at least 4 criteria of the 1987 ACR criteria present.

[**] Seropositivity was determined by medical record review at time of RA diagnosis. Some RA cases were diagnosed before the clinical use of CCP.

[+] Assessed in 1988 (NHS) and 1989 (NHSII)

Table 4

Logistic regression models for the presence of ≥2 ACPA according to continuous or categorical BMI among women from the Nurses' Health Study and Nurses' Health Study II in a nested case-control study of rheumatoid arthritis, at the time of blood draw

Predictor	Age-adjusted OR (95% CI)	Multivariable-adjusted OR (95% CI)*
Among all 1033 subjects		
Continuous BMI, kg/m^2	1.04 (0.98-1.10)	1.03 (0.97-1.09)
BMI categories		
<25 kg/m^2	1.00 (ref)	1.00 (ref)
≥25 kg/m^2	1.28 (0.75-2.19)	1.22 (0.70-2.11)
Among 255 pre-RA cases		
Continuous BMI, kg/m^2	1.09 (1.02-1.16)	1.07 (1.00-1.15)**
BMI categories		
<25 kg/m^2	1.00 (ref)	1.00 (ref)
≥25 kg/m^2	1.67 (0.84-3.32)	1.68 (0.81-3.51)

*Unconditional logistic regression models for ACPA positivity adjusted for age at blood draw, HLA-shared epitope, smoking, alcohol use

**p=0.049

Table 5

Time from blood draw to RA diagnosis among the 255 cases who developed RA within the nested case-control study, by cross-classified ACPA and BMI group

Group	N	Median age at blood draw, years (IQR)	Median age at RA diagnosis, years (IQR)	Median time from blood draw to RA diagnosis, months (IQR)	Pairwise log-rank p value*
A <2 ACPA, BMI <25	113	51.0 (47.0-58.0)	61.0 (55.0-69.0)	125.0 (72.0-161.0)	A vs. B 0.49 A vs. C 0.71 A vs. D **0.002**
B <2 ACPA, BMI ≥25	102	48.5 (45.0-58.0)	57.0 (52.0-68.0)	102.0 (62.0-152.0)	B vs. C 0.84 B vs. D 0.89
C ≥2 ACPA, BMI <25	16	51.5 (48.0-56.0)	60.0 (56.0-68.0)	107.5 (41.0-174.5)	C vs. D **0.001**
D ≥2 ACPA, BMI ≥25	24	49.0 (44.5-57.0)	54.5 (48.0-60.5)	45.0 (17.5-72.5)	

*P values are from pairwise log-rank tests comparing time to RA diagnosis between cross-classified ACPA and BMI groups