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The Association between Anti-Nuclear Antibodies and Obesity is Likely Mediated by Abdominal Adiposity and Systemic Inflammation

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Abstract

Background: Obesity and abdominal adiposity have been associated with inflammation as have the presence of anti-nuclear antibodies (ANAs). It was recently reported that there is a decreased likelihood of ANAs in the obese general population. To examine this relationship we used data from adult participants in the National Health and Nutrition and Examination Survey 1999-2004.

Methods: Participants were excluded if they reported a history of arthritis other than osteoarthritis, thyroid or liver disease, or steroid use so as to rule out a history of possible prior autoimmune disease. We strictly defined a positive ANA as a titer ≥ 1:160. Overweight and obesity were classified using traditional BMI criteria. High and low C-reactive protein (CRP) were defined using the 75th percentile cutpoint as ≥0.42 and <0.42 mg/dL, respectively. Dual-energy X-ray absorptiometry (DEXA) was used to determine body composition. Logistic regression models were created to examine associations with ANA status.

Results: 2552 participants were included in our analyses. Obese participants were older (p<0.001), more likely to be men (p=0.004) and to have comorbidities, and had higher levels of CRP (<0.001). After multivariable adjustment, obesity was associated with a decreased odds of having ANAs (OR 0.78, 95%CI 0.62-0.99). However when adding log-transformed CRP into our model, this association was no longer significant (OR 0.85, 95%CI 0.62-1.15), and there was evidence of effect modification by CRP (p=0.12). Among participants with low CRP, obesity was again associated with a reduced likelihood of ANA positivity (OR 0.69, 95%CI 0.48-0.99), but a trend was seen in the opposite direction in those with high CRP (OR 1.77, 95%CI 0.81-3.88). When looking at the 1143 obese and overweight participants with low CRP, ANA positivity was associated with a higher prevalence of cardiovascular disease (p=0.02) and higher % total body fat (p=0.007), trunk fat (p=0.02), and non-trunk fat (p=0.004). This association, however, was not found in the high CRP group.

Conclusion: In the general population the association of obesity with ANA is modified by the presence of systemic inflammation as measured by CRP, where the inverse association previously found is eliminated when controlling for CRP. This inverse relationship remains among obese participants with low CRP, when these obese and overweight participants are ANA positive; it is associated with greater total body and trunk fat. It is possible that body composition is driving autoimmunity in the general population even in the absence of systemic inflammation.

Keywords: Obesity; Autoimmunity; Inflammation; Abdominal obesity; Visceral adipose tissue; Antinuclear antibodies

Introduction

There has been a growing body of literature discussing the link between obesity and inflammation. Obesity has been described as a state of low-grade, chronic inflammation. This inflammatory state is initiated by metabolic triggers like excess nutrition and mediated by metabolically-active cells such as adipocytes capable of producing cytokines, including TNF-α. Production of these cytokines leads to the activation of the inflammatory cascade [1-4].

Given the well-established relationship between autoimmunity and inflammation, the association of obesity with inflammation has led to the question of a potential link between obesity and autoimmunity [5,6]. Recently Crowson et al. have shown an association between obesity and the development of rheumatoid arthritis, with inflammation as the common factor between the two conditions [7]. A relationship between obesity and autoimmunity has also been found in multiple other conditions [8]. Given that anti-nuclear antibodies (ANAs) are correlated and are also pathologic in multiple autoimmune and rheumatic diseases, steps have been taken to look for the presence of these auto-antibodies in obese patients [9,10].

Satoh et al. examined key correlates, including obesity, that contribute to the prevalence of ANA in the general population through a cross-sectional analysis of individuals from the National Health and Nutrition Examination Survey 1999-2004 (NHANES) [11]. They found that ANAs were less common in overweight and obese individuals, a seemingly counterintuitive finding as one would expect increased levels of autoimmune markers related to inflammation in this group.

Gonzalez et al. have also previously described an inverse relationship between ANA and obesity in women [12].

Due to these findings, we sought to explore the interplay of obesity, inflammation and autoimmunity in the general population. We examined the NHANES cohort to further investigate the association between ANA and obesity as well as to identify key mediators in this relationship. In order to uncover previously unidentified factors in this relationship, obesity and body composition were examined using other measures beyond the Body-Mass Index (BMI), including measures of skeletal muscle mass and the distribution of body fat.

Methods

Study population

NHANES 1999-2004 was a nationally representative survey of the non-institutionalized civilian population in the United States [13]. A stratified, multistage, probability sampling design was used to select participants. The NHANES protocol was approved by the National Center for Health Statistics ethics review board and written informed consent was obtained from all participants. Overall, 3,564 adults aged ≥ 20 years completed the interview and examination components and had ANA measurements. In order to exclude individuals with possible autoimmune disease, we excluded participants who reported diagnoses of rheumatoid arthritis or arthritis other than osteoarthritis (n=278); steroid use within the previous month (n=39); thyroid disease or the use of thyroid medication (n=274); or liver disease (n=92). After excluding participants with missing body composition or other covariate data (n=329), 2,552 participants were available for analysis.

Data collection

Information on education, smoking status, and comorbidities was obtained by self-report. Race/ethnicity was self-identified. Smoking was classified as never, former, or current smoker. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, physician diagnosis, and/or antihypertensive medication use [14]. A participant was considered to have diabetes mellitus if he or she reported a physician diagnosis while not pregnant or the current use of insulin or oral hypoglycemic medications, or had a glycohemoglobin level ≥ 6.5%. Cardiovascular Disease (CVD) was defined by self-report of a physician diagnosis of congestive heart failure, coronary heart disease, angina, myocardial infarction, or stroke.

Body-Mass Index (BMI, kg/m^2) was categorized as underweight (<18), normal weight (18-24.9), overweight (25-29.9), and obese (≥ 30). Waist Circumference (WC) was available for 1,644 participants and was used to define abdominal obesity as WC ≥ 88 cm for women and ≥ 102 cm for men [15]. Total body fat, trunk fat, and Lean Body Mass (LBM) were assessed using whole-body Dual-Energy X-Ray Absorptiometry (DXA). Due to the pattern of non-response, missing and invalid data were multiply imputed. Details of the DXA protocol, quality control analyses, and the multiple imputation procedure are available (http://www.cdc.gov/nchs/nhanes/dxx/dxa.htm). Percent total body fat was calculated as 100 × total body fat/total mass, and % trunk and non-trunk fat were calculated as 100 × trunk fat/trunk mass and 100 × non-trunk fat/non-trunk mass, respectively. Truncal body fat ratio was calculated as trunk fat/non-trunk fat. Appendicular Skeletal Muscle Mass Index (ASMI) was calculated as the sum of lean mass for the arms and legs (kg)/height^2 (m^2) [16]. ASMI is a measure of relative muscle mass that is analogous to BMI as a measure of relative adiposity.

Total cholesterol and triglycerides were measured using coupled enzymatic reactions, HDL cholesterol was measured using a heparin-manganese precipitation method, and LDL cholesterol was calculated from these values. C - reactive protein (CRP) was quantified by latex-enhanced nephelometry. High and low CRP were defined using the 75th percentile cutpoint in our cohort as ≥ 0.42 and <0.42 mg/dL, respectively. Fasting glucose and insulin were used to calculate homeostasis model assessment of insulin resistance (HOMA-IR), a model for estimating insulin resistance [17]. Glucose was measured using the glucose hexokinase method and insulin was measured by radioimmunoassay. HOMA-IR ≥ 2.5 was categorized as insulin resistant. Serum triglycerides, HDL and LDL cholesterol, and HOMA-IR were examined in the 730 participants with available data for each of these parameters.

Outcome variables

ANA testing was performed in a subsample of participants from 1999-2004 using the HEp-2 cell immunofluorescence assay (INOVA Diagnostics, San Diego, CA). In samples with a ≥ 3+ nuclear and/or cytoplasmic immunofluorescence pattern, ANA titers were determined by serial dilution. Full details of the protocol are available at http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/SSANA_C.html. We selected a stringent and highly specific definition of ANA positivity, defining a positive ANA as a titer ≥ 1:160.

Statistical analysis

All analyses used NHANES-appropriate sampling weights and accounted for the complex multistage cluster design using the "survey" command in Stata 11.2 (Stata Corporation, College Station, TX, USA). Specifically, the svyset command was used to specify the survey design characteristics, and all estimation commands were preceded by the svy prefix. A p-value <0.05 was considered statistically significant. The distributions of participant characteristics were examined by categories of BMI. Logistic regression models were created to examine the association of BMI categories with ANA positivity, first without adjustment for additional covariates and then including age, sex, and race/ethnicity as covariates. Multivariable models were then created including variables determined a priori to be potential confounders of the association of BMI with ANA positivity, including smoking status, education (as a marker of socioeconomic status), diagnosis of diabetes mellitus, hypertension, CVD, and total cholesterol. Finally, log-transformed CRP was added to the multivariable models to separately examine the effect of systemic inflammation.

We tested the possibility of effect modification by inflammation of the association of BMI with ANA positivity by first including a multiplicative interaction term of log-transformed CRP and BMI categories in the multivariable model. We determined a priori that a p-value <0.20 would be considered suggestive of interaction. We then examined associations of BMI categories with ANA positivity in the high and low CRP subgroups, separately. Based on these results, we next examined the distributions of participant characteristics including body composition by ANA status in overweight and obese participants only, and in the overweight and obese subgroup by high and low CRP status, separately. We further explored the importance of body composition by categorizing participants into sex-specific quartiles of

truncal body fat ratio and examining the association with ANA positivity in the high and low CRP subgroups, separately.

Results

Of the 2552 participants, 933 were overweight and 743 were obese (Table 1).

	Body Mass Index (kg/m^2)				
Characteristic	**Underweight**	**Normal**	**Overweight**	**Obese**	**P**
	(<18)	(18-24.9)	(25-29.9)	(≥ 30)	
Number	26	850	933	743	
Age (years)	36.8 (3.2)	41.7 (0.6)	45.8 (0.6)	45.1 (0.7)	<0.001
Women (%)	65.6 (9.1)	56.5 (1.9)	38.8 (2.3)	48.0 (2.2)	0.004
Race/Ethnicity (%)					<0.001
Non-Hispanic White	67.6 (10.1)	74.4 (2.2)	72.1 (2.1)	67.8 (2.6)	
Mexican American	5.3 (3.4)	5.9 (0.7)	8.8 (1.3)	8.0 (1.3)	
Non-Hispanic Black	13.7 (7.1)	7.5 (1.0)	7.8 (1.0)	14.7 (1.8)	
Less than high-school diploma (%)	22.0 (7.3)	18.6 (1.5)	18.2 (1.4)	20.8 (1.7)	0.47
Smoking (%)					<0.001
Never	44.9 (11.6)	53.8 (1.8)	48.5 (1.7)	50.3 (2.2)	
Former	7.8 (4.7)	18.4 (1.5)	26.2 (1.6)	28.1 (2.2)	
Current	47.3 (11.6)	27.9 (1.7)	25.4 (2.0)	21.6 (1.7)	
Hypertension (%)	27.2 (11.1)	23.2 (1.8)	37.8 (2.3)	54.1 (2.0)	<0.001
Diabetes mellitus (%)	0	3.0 (0.7)	6.9 (1.0)	12.3 (1.1)	<0.001
Cardiovascular disease (%)	1.6 (1.6)	3.9 (0.7)	7.0 (0.9)	9.5 (1.4)	<0.001
ANA positive (%)	12.8 (7.0)	16.1 (1.6)	12.7 (1.6)	12.4 (1.0)	0.01
Total cholesterol (mg/dL)	189.3 (6.3)	194.5 (1.6)	209.1 (1.9)	209.3 (2.0)	<0.001
CRP ≥ 0.42 mg/dL (%)	7.7 (4.7)	12.5 (1.4)	19.8 (1.4)	42.5 (2.4)	<0.001

Abbreviations: ANA: Anti-nuclear antibody; CRP: C-reactive protein. Data are expressed as mean (SE) or percent (SE).

Table 1: Participant characteristics by categories of Body-Mass Index.

Participants with higher BMI were older (p<0.001) and more likely to be men (p=0.004). Overall, participants in the higher weight categories had higher rates of hypertension (p<0.001), diabetes (p<0.001) and cardiovascular disease (p<0.001). Interestingly, while the overweight and obese participants were less likely to be ANA positive compared to those with normal BMI (p=0.01), they were substantially more likely to have a high CRP level (p<0.001). For further results please refer to Table 1.

Using a simple logistic regression model we confirmed the result found by Satoh et al. that obese individuals are less likely to be ANA positive [11]. Obesity was associated with a significantly decreased risk of ANA positivity (OR: 0.74, 95%CI (0.59-0.93)). This association remained after adjustment for multiple covariates including age, sex, race/ethnicity and multiple comorbidities as detailed in (Table 2).

	Odds Ratio (95% CI)			
		Normal weight	**Overweight**	**Obese**
		Ref	0.76 (0.55-1.04)	0.74 (0.59-0.93)
	Underweight	Ref	0.85 (0.62-1.18)	0.76 (0.60-0.96)
Model 1	0.77 (0.21-2.77)			
		Ref	0.87 (0.64-1.20)	0.78 (0.62-0.99)
Model 2	0.73 (0.20-2.72)			
		Ref	0.91 (0.66-1.26)	0.85 (0.62-1.15)

Abbreviations: CI: Confidence Interval. Bold values indicate p<0.05.
Body-mass index (BMI) cutoffs: Underweight BMI<18 kg/m^2; Normal weight BMI 18-24.9 kg/m^2; Overweight BMI 25-29.9 kg/m^2; Obese BMI ≥ 30 kg/m^2.
Model 1: unadjusted, Model 2: adjusted for age, sex, race/ethnicity.
Model 3: adjusted for age, sex, race/ethnicity, smoking status, education level, diagnosis of diabetes mellitus, hypertension, cardiovascular disease, and total cholesterol, Model 4: adjusted for age, sex, race/ethnicity, smoking status, education level, diagnosis of diabetes mellitus, hypertension, cardiovascular disease, total cholesterol, and log-transformed C-reactive protein

Table 2: Odds ratio of positive antinuclear antibodies by body-mass index in 2,552 participants of NHANES 1999-2004.

However the association between ANA positivity and obesity was no longer significant after adjustment for log-transformed CRP (OR: 0.85, 95%CI (0.62-1.15)). Furthermore, the p-value for interaction (p=0.12) by CRP was suggestive of an effect modification in the association of BMI with ANA positivity.

To further evaluate the role of inflammation in the association between obesity and ANA positivity, we stratified our logistic regression models based on CRP level (Table 3).

Among the 1676 overweight and obese participants (Table 4), those who were ANA positive were more likely to be female (p<0.001).

Otherwise, ANA positive and ANA negative participants were similar in terms of demographic characteristics and comorbidities, with the exception of a higher prevalence of CVD in the ANA positive group (12.6% *vs.* 7.5%, p=0.02). However, overweight and obese ANA positive and negative participants differed in terms of body habitus. Although the groups were comparable in terms of BMI, there was a trend toward higher waist circumference in the ANA positive group, and percentages of total body fat (p=0.007), trunk fat (p=0.02) and non-trunk fat (p=0.004) were each higher among ANA positive versus

ANA negative participants. In addition, ANA positive participants had lower ASMI compared to the ANA negative group (7.88 *vs.* 8.35, p<0.001).

Odds Ratio (95% CI)				
Underweight		**Normal weight**		**Obese**
Participants with CRP<0.42 mg/dL (n=1,899)				
Model 1	0.58 (0.12-2.85)	Ref	0.67 (0.48-0.95)	0.63 (0.45-0.88)
Model 2	0.53 (0.10-2.77)	Ref	0.78 (0.55-1.10)	0.71 (0.50-0.99)
Model 3	0.53 (0.10-2.90)	Ref	0.78 (0.55-1.11)	0.69 (0.48-0.99)
Participants with CRP ≥ 0.42 mg/dL (n=653)				
Model 1	6.39 (0.87-47.08)	Ref	1.73 (0.84-3.58)	1.61 (0.77-3.34)
Model 2	6.71 (0.94-48.07)	Ref	1.88 (0.84-4.20)	1.56 (0.70-3.45)
Model 3	6.19 (0.95-40.42)	Ref	2.06 (0.94-4.51)	1.77 (0.81-3.88)

Abbreviations: CI: Confidence Interval; CRP: C-reactive protein. Bold values indicate p<0.05.
Body-mass index (BMI) cutoffs: Underweight BMI<18 kg/m^2; Normal weight BMI 18-24.9 kg/m^2; Overweight BMI 25-29.9 kg/m^2; Obese BMI ≥ 30 kg/m^2.
*p for interaction=0.12 for effect modification by CRP of association of BMI with antinuclear antibody status.
Model 1: Unadjusted.
Model 2: Adjusted for age, sex and race/ethnicity.
Model 3: Adjusted for age, sex, race/ethnicity, smoking status, education level and diagnosis of diabetes mellitus, hypertension, cardiovascular disease, and total cholesterol.

Table 3: Odds ratio of positive antinuclear antibodies by body-mass index and C-reactive protein concentration in 2,552 participants of NHANES 1999-2004.

Characteristic	ANA Positive	ANA Negative	P
Number	220	1456	
Age (years)	47.9 (1.5)	45.2 (0.5)	0.1
Women (%)	60.9 (4.8)	40.2 (1.8)	<0.001
Race/Ethnicity (%)			0.48
Non-Hispanic White	68.6 (4.0)	70.5 (1.9)	
Mexican American	6.6 (1.4)	8.7 (1.2)	
Non-Hispanic Black	12.0 (2.8)	10.7 (1.1)	
Less than high-school diploma (%)	18.9 (3.1)	19.4 (1.0)	0.88
Smoking (%)			0.23
Never	52.2 (4.2)	48.8 (1.4)	
Former	29.5 (3.0)	26.7 (1.3)	
Current	18.4 (3.4)	24.5 (1.4)	
Body-mass index (kg/m2)	30.5 (0.3)	30.8 (0.2)	0.32
Hypertension (%)	51.2 (4.2)	44.0 (1.9)	0.11
Diabetes mellitus (%)	7.4 (1.8)	9.5 (0.9)	0.34
Cardiovascular disease (%)	12.6 (2.2)	7.5 (0.9)	0.02
CRP ≥ 0.42 mg/dL (%)	32.9 (3.3)	29.3 (1.4)	0.34
Total cholesterol (mg/dL)	208.2 (3.1)	209.4 (1.5)	0.74
HDL cholesterol (mg/dL)*	47.5 (1.4)	48.7 (0.7)	0.57
LDL cholesterol (mg/dL)*	131.3 (4.4)	130.6 (2.2)	0.77
Triglycerides (mg/dL)*	154.0 (14.2)	146.7 (3.0)	0.73
HOMA-IR ≥ 2.5 (%)*	54.7 (6.2)	54.6 (2.1)	0.83
Abdominal obesity (%)†	76.0 (4.2)	68.2 (1.1)	0.11
% Total body fat	37.8 (0.8)	35.6 (0.3)	0.007
% Trunk fat	38.2 (0.7)	36.4 (0.3)	0.02
% Non-trunk fat	37.4 (0.9)	34.6 (0.3)	0.004
ASMI	7.88 (0.12)	8.35 (0.05)	<0.001

Abbreviations: HOMA-IR: Homeostasis Model Assessment –Insulin Resistance; CRP: C-Reactive Protein; ASMI: Appendicular Skeletal Muscle Index. Data are expressed as mean (SE) or percent (SE).
*Analyses performed in 730 participants with available data.
†Waist circumference measurements available in 1,644 participants.

Table 4: Characteristics by antinuclear antibody status in 1,676 overweight and obese participants of NHANES 1999-2004.

Among obese and overweight participants with high CRP, there was no significant difference in fat distribution between the ANA positive and ANA negative participants (Table 5).

However, in those with low CRP, there was a significant difference in body habitus between the ANA groups. ANA positive participants had higher percentages of abdominal obesity (p=0.04), total body fat (p=0.006) trunk fat (p=0.009), and non-trunk fat (p=0.005). In addition to the differences seen in fat distribution between the groups, the ASMI was significantly lower in the ANA positive patients regardless of their CRP level.

Given the association of truncal fat with ANA positivity, we repeated the analyses presented in Table 3 using quartiles of truncal body fat ratio instead of BMI categories. With the inclusion of this variable into the models, the association between ANA positivity and obesity is no longer statistically significant in those with a low CRP. Therefore the relationship seen between ANA positivity and obesity is in fact partially explained by the presence of truncal obesity (Table 6).

Characteristic	ANA Positive	ANA Negative	P
Participants with CRP<0.42 mg/dL (n=1,143)			
Body Mass Index (kg/m2)	29.7 (0.3)	29.8 (0.2)	0.77

Smoking (%)			0.18
Never	52.9 (4.9)	49.8 (1.8)	
Former	31.7 (3.4)	27.1 (1.6)	
Current	15.4 (3.8)	23.1 (1.6)	
Hypertension (%)	51.9 (5.2)	39.1 (2.1)	0.03
Diabetes mellitus (%)	6.6 (1.8)	8.6 (1.0)	0.43
Cardiovascular disease (%)	13.3 (3.2)	7.0 (1.0)	0.03
Total cholesterol (mg/dL)	209.2 (4.4)	209.2 (1.5)	0.99
HDL cholesterol (mg/dL)*	48.2 (1.7)	48.7 (0.8)	0.79
LDL cholesterol (mg/dL)*	130.9 (4.7)	131.5 (2.3)	0.92
Triglycerides (mg/dL)*	156.6 (18.4)	141.7 (3.5)	0.42
HOMA-IR ≥ 2.5 (%)*	48.7 (10.3)	49.7 (2.5)	0.92
Abdominal obesity (%)†	72.6 (5.0)	60.7 (2.3)	0.04
% Total body fat	36.4 (0.9)	33.8 (0.3)	0.006
% Trunk fat	36.7 (0.8)	34.6 (0.3)	0.009
% Non-trunk fat	36.0 (1.1)	32.9 (0.4)	0.005
ASMI	7.89 (0.1)	8.34 (0.06)	0.005
Participants with CRP ≥ 0.42 mg/dL (n=533)			
Body Mass Index (kg/m2)	32.2 (0.7)	33.5 (0.4)	0.09
Smoking (%)			0.81
Never	50.7 (6.6)	46.6 (2.7)	
Former	24.8 (6.4)	25.7 (2.6)	
Current	24.5 (5.3)	27.7 (2.1)	
Hypertension (%)	49.9 (6.8)	55.8 (3.0)	0.42
Diabetes mellitus (%)	8.9 (3.0)	11.9 (2.0)	0.45
Cardiovascular disease (%)	11.1 (3.1)	8.6 (1.6)	0.47
Total cholesterol (mg/dL)	206.2 (4.3)	209.9 (2.8)	0.48
HDL cholesterol (mg/dL) §	46.0 (2.1)	47.7 (1.2)	0.49
LDL cholesterol (mg/dL) §	124.2 (7.3)	127.7 (3.1)	0.69
Triglycerides (mg/dL) §	143.1 (16.1)	157.9 (6.7)	0.43
HOMA-IR ≥ 2.5 (%)§	65.0 (11.4)	67.6 (4.5)	0.85
Abdominal obesity (%)‡	82.6 (6.2)	85.9 (2.3)	0.58
% Total body fat	40.8 (1.2)	39.9 (0.5)	0.44
% Trunk fat	41.2 (1.2)	40.7 (0.5)	0.7
% Non-trunk fat	40.3 (1.2)	38.9 (0.5)	0.27
ASMI	7.86 (0.18)	8.37 (0.11)	0.02

Abbreviations: HOMA-IR: Homeostasis Model Assessment–Insulin Resistance; CRP: C-Reactive Protein; ASMI: Appendicular Skeletal Muscle Index.

Data are expressed as mean (SE) or percent (SE).
*Analyses performed in 466 participants with available data.
†Waist circumference measurements available in 1,123 participants.
§Analyses performed in 243 participants with available data.
‡Waist circumference measurements available in 521 participants.

Table 5: Characteristics by antinuclear antibody status and C-reactive protein concentration in 1,676 overweight and obese participants of NHANES 1999-2004.

Odds Ratio (95% CI)				
	Quartile 1	**Quartile** 2	**Quartile** 3	**Quartile 4**
Participants with CRP<0.42 mg/dL (n=1,899)				
Model 1	Ref	0.84 (0.53-1.31)	0.69 (0.40-1.18)	0.85 (0.58-1.26)
Model 2	Ref	0.80 (0.49-1.29)	0.66 (0.38-1.15)	0.84 (0.53-1.32)
Model 3	Ref	0.79 (0.49-1.28)	0.67 (0.39-1.17)	0.87 (0.53-1.44)
Participants with CRP ≥ 0.42 mg/dL (n=653)				
Model 1	Ref	1.64 (0.49-5.49)	1.65 (0.57-4.83)	1.74 (0.51-5.88)
Model 2	Ref	1.66 (0.48-5.77)	1.58 (0.53-4.67)	1.60 (0.48-5.39)
Model 3	Ref	1.74 (0.51-5.91)	1.70 (0.59-4.95)	1.81 (0.54-6.03)

Abbreviations: CI: Confidence Interval; CRP: C-Reactive Protein. Bold values indicate p<0.05. *p for interaction=0.09 for effect modification by CRP of association of truncal body fat ratio with antinuclear antibody status.
Model 1: Unadjusted.
Model 2: Adjusted for age, sex, race/ethnicity.
Model 3: Adjusted for age, sex, race/ethnicity, smoking status, education level, diagnosis of diabetes mellitus, hypertension, cardiovascular disease, and total cholesterol.

Table 6: Odds ratio of positive antinuclear antibodies by truncal body fat ratio and C-reactive protein concentration in 2,552 participants of NHANES 1999-2004.

Discussion

We have found that the relationship between obesity and autoimmunity in the general population is partly mediated by systemic inflammation. We first confirmed the previously reported, yet seemingly counterintuitive association of obesity with a lesser likelihood of ANA positivity. However, this association was no longer significant after accounting for levels of CRP. The relationship of obesity with autoimmunity was modified by the presence of systemic inflammation, as measured by serum CRP. Specifically, among participants in the highest quartile of CRP, being overweight or obese was associated with an increased risk of ANA positivity. Although not statistically significant, this trend was in sharp contradistinction to our results among participants with lower levels of serum CRP, in whom the opposite association was found. Furthermore, among overweight and obese participants with low serum CRP, ANA positivity was

associated with a greater prevalence of not only cardiovascular disease and hypertension but also higher percentages of total body fat and abdominal obesity. These data suggest an important interplay of inflammation, body composition, and autoimmunity in the general population.

It is a well-acknowledged that obesity is a chronic inflammatory state where this inflammation plays a key role in the metabolic syndrome and in the relationship between obesity and cardiovascular disease. While general obesity is associated with the metabolic syndrome, abdominal/visceral adiposity appears to be the driving force behind this association [1,18]. Within obese populations there are two major constituents, those with high rates of the metabolic disturbances that constitute the metabolic syndrome– hypertension, hyperlipidemia and insulin resistance-, and those "healthy obese" without such derangements. A major difference between the two groups is body habitus where those with the metabolic syndrome have higher rates of abdominal adiposity [19]. Central adiposity has been shown to be associated with higher rates of C-reactive protein (CRP), a common measure of systemic inflammation, in all age groups as well as in people with normal BMI who have higher abdominal fat deposits [20-22].

As has been previously reported, in our study obese participants had elevated levels of inflammation as measured by CRP at baseline [20,21]. Therefore when evaluating the association between obesity and ANA positivity it is imperative to consider the role of inflammation given that it is typically inflammatory conditions such as lupus that induce ANA positivity. As to be expected, in those obese participants with CRP levels higher than the median, obesity was associated with an increased risk of being ANA positive. While the result was not statistically significant it is possible that this is due to the small sample size in this group. However we see that in the low CRP group there is still decreased risk of being ANA positive if obese.

In order to determine what could potentially be driving this relationship we evaluated the individual characteristics of the ANA positive and negative participants within the overweight and obese as stratified by CRP levels. Overall in the high CRP group there was no difference between the ANA positive and negative participants with regards to the presence of various co-morbidities or body habitus. However in the low CRP group the ANA positive participants were more likely to be hypertensive, have cardiovascular disease and have higher percentages not only of total body fat and truncal obesity. Therefore we postulate that even in the setting of low systemic inflammation, these ANA positive, overweight and obese participants are likely in an inflammatory state that is causing them to be ANA positive.

It has been shown through animal models as well has human samples that Visceral Adipose Tissue (VAT) is metabolically active and produces inflammation through various cytokines, chemokines, and adipokines [1,2]. These molecules, which include TNF-alpha, IL-6, IL-8, and MCP-1, activate other inflammatory mediators including macrophages, T cells and B cells, and other adipocytes to further the inflammatory cascade [22-25]. Activated adipose tissue may then play a role in the development of autoimmunity through the hormones leptin and adiponectin [26-28]. As an inflammatory hormone, leptin promotes T-cell activation and B-cell class switching, a step that is important in antibody development [29,30]. Adiponectin, an anti-inflammatory hormone, promotes the release of anti-inflammatory cytokines such as IL-1 and IL-10 but is inhibited by TNF-alpha and IL-6 [31,32]. Leptin also inhibits the apoptosis of antigen-specific T cells, while adiponectin facilitates the clearance of apoptotic bodies. Treating auto-immune prone mince with adiponectin decreases ANA levels. [31] Therefore, within the inflammatory milieu of obesity, low levels of adiponectin are likely insufficient to blunt the inflammatory response to leptin. Hence, increased cellular debris from inflammation could act as antigenic targets leading to the production of ANAs by B cells, possibly within the VAT itself. Therefore, obesity, through inflammation, could lead to autoimmunity [32].

Environmental factors, such as nutrition, are associated with multiple epigenetic changes. It has been shown that the intake of a diet consisting of high amounts of saturated fats can lead to significant epigenetic changes in human adipose tissue where these changes are potentially genome wide and are influenced by weightloss [33-35]. These epigenetic chromosomal changes may also affect the generation of autoantibodies in the setting of obesity. It has been shown in both lupus and Sjogern's syndrome, two conditions characterized by the presence of ANAs and other auto-antibodies, that there are potentially pathologic epigenetic changes that are associated with auto-antibody production [36,37]. Given that epigenetic changes are implicated in the generation of autoimmunity, and that obesity has been linked to multiple epigenetic mechanisms, it is possible that nutritionally induced epigenetic changes are also driving autoimmunity in obese patients [38]. This could in fact potentially amplify whatever role, adipokines may play in autoimmunity. While the above proposed mechanisms are interesting, they remain speculative at this time.

If the ANA positive patients are part of the obese population in an inflammatory state that may potentially develop the metabolic syndrome, especially given their higher rates of hypertension and cardiovascular disease, those with low CRPs who are ANA negative may constitute a population of patients known as the "Metabolically Healthy but Obese" (MHO). MHO patients constitute approximately 20% of the obese population [29]. Overall the MHO population has similar BMIs however they tend to have lower amounts of visceral fat when compared to the obese at risk for the metabolic syndrome. Not only do MHOs have a different body habitus but these patients also have higher rates of insulin sensitivity, have high lean body mass and better lipid profiles [30]. This improved metabolic profile in the MHOs maybe secondary to the fact that they also have improved inflammatory profiles with lower levels of CRP and higher levels of adiponectin at baseline [32,33,39]. Because of their lower percentage of VAT, lower CRP and higher adiponectin levels, MHO patients are likely protected from developing the metabolic syndrome in the future.

Absent autoimmunity and relatively little systemic inflammation may therefore represent a new MHO subgroup. These characteristics were associated with lesser body fat and greater skeletal muscle mass among overweight and obese participants, despite no apparent difference in BMI. In contrast, among overweight and obese participants with high levels of CRP, there was no difference in adiposity based on ANA status. However, ANA positivity was still associated with lesser skeletal muscle mass and with a trend toward lower BMI. Taken together, these results suggest an important role of skeletal muscle in the interplay of obesity, inflammation, and autoimmunity that deserves further exploration. Indeed, a growing body of evidence suggests that skeletal muscle is a key regulator of metabolism, and that loss of muscle mass and impaired skeletal muscle function directly contribute to metabolic dysregulation and inflammation [40,41].

There are several potential limitations to our study. First, as this was a cross-sectional analysis, causality cannot be inferred. Also, we

are unable to determine whether ANA positivity predicts future development of the metabolic syndrome or of a connective tissue disease. Our ascertainment of the components of the metabolic syndrome was limited as most participants did not have measurements of insulin resistance. It is also possible that ANA positive participants had an undiagnosed connective tissue disease. However this would be unlikely given that we excluded participants who reported having arthritis that was not osteoarthritis, those with a thyroid condition or liver disease, and anyone reporting recent steroid use. Nevertheless there is a remote possibility. Lastly, the only inflammatory marker available to us was CRP, we do not know baseline leptin or adiponectin levels, nor do we have access to samples from these participants VATs to then better associate these levels with the presence or absence of ANAs.

Despite the limitations of this study we feel that this data adds to the body of literature regarding the association of obesity with inflammation. This data demonstrates that there could potentially be another mechanism for inflammation in humans where there in not only inflammatory milieu created but B cells are activated and are potentially generating ANA locally within the VAT. We feel that this exciting data generates many new avenues of inquiry into inflammation in humans with visceral adiposity.

References

1. Gregor M, Hotamisligil G (2011) Inflammatory Mechanisms in Obesity. Annu Rev Immunol 29: 415-445.
2. Hotamisligil G, Arner P (1995) Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Investig 95: 2409-2415.
3. Hotamisligil G, Peraldi P (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-indused insulin resistance. Science 271: 665-668.
4. Hotamisligil G, Shargill N, Spiegelman B (1993) Adipose expression of tumor necrosis factor-alpha:direct role in obesity linked insulin resistance. Science 259: 87-91.
5. Lukens J, Dixit V, Kanneganti T (2011) Inflammasome activation in obesity-related inflammatory diseases and autoimmunity. Discov Med 12: 65-74.
6. Carbone F, La Rocca C, De Candia P, Procaccini C, Colamatteo A, et al. (2016) Metabolic control of immune tolerance in health and autoimmunity. Semin Immunol 28: 491-504.
7. Crowson C, Matteson E (2012) Obesity fuels the upsurge in rheumatoid arthritis. Arthritis Care Res.
8. Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y (2014) Obesity in autoimmune diseases: not a passive bystander. Autoimmun Rev 13: 981-1000.
9. Pisetsky DS (2017) Antinuclear antibody testing - misunderstood or misbegotten? Nat Rev Rheumatol.
10. Ludwig RJ, Vanhoorelbeke K, Leypoldt F, Kaya Z, Bieber K, et al. (2017) Mechanisms of Autoantibody-Induced Pathology. Front Immunol 8: 603.
11. Satoh M, Chan E, Ho L, Rose KM, Parks CG (2012) Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. Arthritis Rheum 64: 2319-2327.
12. Gonzalez D, Cabrera De Leon A, Rodriguez PMC, Coello SD, Gonzalez HA, et al. (2008) Inverse association between obesity and antinuclear antibodies in Women. J Rheumatol 35: 2449-2451.
13. http://www.cdc.gov/nchs/nhanes/about_nhanes.htm.
14. Muntner P, Woodward M, Mann DM, Shimbo D, Michos ED, et al. (2010) Comparison of the Framingham Heart Study hypertension model with blood pressure alone in the prediction of risk of hypertension: the Multi-Ethnic Study of Atherosclerosis. Hypertension 55: 1339-1345.
15. Bethesda (1998) Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. Obes Res 6 Suppl 2: 51S-209S.
16. Baumgartner RN, Koehler KM, Gallagher D, Romero L, Heymsfield SB, et al. (1998) Epidemiology of sarcopenia among the elderly in New Mexico. Am J Epidemio 147: 755-763.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419.
18. Mathieu P, Lemieux I, Despres JP (2010) Obesity, inflammation, and cardiovascular risk. Clin Pharmacol Ther 87: 407-416.
19. Primeau V, Coderre L, Karelis AD, Brochu M, Lavoie ME, et al. (2011) Characterizing the profile of obese patients who are metabolically healthy. Int J Obes (Lond) 35: 971-981.
20. Hermsdorff HH, Zulet MA, Puchau B, Martinez JA (2011) Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. Inflammation 34: 161-170.
21. Lapice E, Maione S, Patti L, Paola C, Angela AR, et al. (2009) Abdominal adiposity is associated with elevated C-reactive protein independent of BMI in healthy nonobese people. Diabetes Care 32: 1734-1736.
22. Brinkley TE, Hsu FC, Beavers KM, Church TS, Goodpaster BH, et al. (2012) Total and abdominal adiposity are associated with inflammation in older adults using a factor analysis approach. J Gerontol A Biol Sci Med Sci 67: 1099-1106.
23. Stofkova A (2009) Leptin and adiponectin: from energy and metabolic dysbalance to inflammation and autoimmunity. Endocr Regul 43: 157-168.
24. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, et al. (2009) CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 15: 914-920.
25. Lumeng CN, Bodzin JL, Saltiel AR (2007) Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117: 175-184.
26. Winer DA, Winer S, Shen L, Yantha J, Paltser G, et al. (2011) B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat Med 17: 610-617.
27. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, et al. (2003) Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112: 1796-1808.
28. Kim SJ, Choi Y, Choi YH, Park T (2012) Obesity activates toll-like receptor-mediated proinflammatory signaling cascades in the adipose tissue of mice. J Nutr Biochem 23: 113-122.
29. Karelis AD, St-Pierre DH, Conus F, Rabasa-Lhoret R, Poehlman ET (2004) Metabolic and body composition factors in subgroups of obesity: what do we know? J Clin Endocrinol Metab 89: 2569-2575.
30. Brochu M, Tchernof A, Dionne IJ, Sites CK, Eltabbakh GH, et al. (2001) What are the physical characteristics associated with a normal metabolic profile despite a high level of obesity in postmenopausal women? J Clin Endocrinol Metab 86: 1020-1025.
31. Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, et al. (2007) Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. J Clin Invest 117: 375-386.
32. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, et al. (2008) Identification and characterization of metabolically benign obesity in humans. Arch Intern Med 168: 1609-1616.
33. Karelis AD, Faraj M, Bastard JP, Pierre DH, Brochu M, et al. (2005) The metabolically healthy but obese individual presents a favorable inflammation profile. J Clin Endocrinol Metab 90: 4145-4150.
34. Perfilyev A, Dahlman I, Gillberg L, Fredrik R, David I, et al. (2017) Impact of polyunsaturated and saturated fat overfeeding on the DNA-methylation pattern in human adipose tissue: a randomized controlled trial. Am J Clin Nutr 105: 991-1000.

35. Macartney-Coxson D, Benton MC, Blick R, Stubbs RS, Hagan RD, et al. (2017) Genome-wide DNA methylation analysis reveals loci that distinguish different types of adipose tissue in obese individuals. Clin Epigenetics 9: 48.
36. Thabet Y, Canas F, Ghedira I, Youinou P, Mageed RA, et al. (2012) Altered patterns of epigenetic changes in systemic lupus erythematosus and auto-antibody production: is there a link?. J Autoimmun 39: 154-160.
37. Konsta OD, Thabet Y, Le Dantec C, Wesley HB, Athanasios GT, et al. (2014) The contribution of epigenetics in Sjogren's Syndrome. Front Genet 5: 71.
38. Brooks WH, Le Dantec C, Pers JO, Youinou P, Renaudineau Y (2010) Epigenetics and autoimmunity. J Autoimmun 34: J207-219.
39. Aguilar-Salinas CA, Garcia EG, Robles L, Riano D, Ruiz-Gomez DG, et al. (2008) High adiponectin concentrations are associated with the metabolically healthy obese phenotype. J Clin Endocrinol Metab 93: 4075-4079.
40. Basaria S, Bhasin S (2012) Targeting the skeletal muscle-metabolism axis in prostate-cancer therapy. N Engl J Med 367: 965-967.
41. Wolfe RR (2006) The underappreciated role of muscle in health and disease. Am J Clin Nutr 84: 475-482.