

Burden of Infection and Fat Mass in Healthy Middle-aged Men

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Abstract

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Objective: Our aim was to study the effect of exposure to four infections on fat mass.

Research Methods and Procedures: This was a cross-sectional study of healthy middle-aged men from the general population ($n = 74$). Each study subject's serum was tested for specific IgG class antibodies against herpes virus simplex-1 (HSV-1), HSV-2, enteroviruses, and *Chlamydia pneumoniae* through the use of quantitative in vitro enzyme-linked immunosorbent assays (ELISAs). A total pathogen burden score based on these seropositivities [Quantitative Seropositivity Index (QSI)] was constructed. Fat mass was measured by bioelectrical impedance.

Results: We observed significant relationships between the HSV-1 titer and fat mass and percentage fat mass. The associations were stronger when considering the infection burden. The QSI was significantly associated with fat mass ($r = 0.30$, $p = 0.009$) and percentage fat mass ($r = 0.27$, $p = 0.01$). Those subjects in the highest tertile of fat mass showed significantly higher QSI (259.5 ± 74.1 vs. 206.9 ± 78.2 , $p = 0.007$). In subjects that were seropositive for *Enteroviruses*, the relationship between the QSI and fat mass was strengthened ($r = 0.51$, $p = 0.02$). In a multivariate regression analysis, the QSI, independently of age and C-reactive protein, contributed to 9% of fat mass variance.

Discussion: Pathogen burden showed an association with

fat mass. Subjects with increased fat mass could be more susceptible to develop multiple infections resulting in a chronic low-grade inflammation. We can not exclude that exposure to multiple infections leads to increased fat mass.

Key words: fat mass, inflammation, cytokines, insulin action

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Introduction

The prevalence of obesity has risen dramatically worldwide (1), posing a serious health hazard, which contributes to the increased morbidity and mortality in Western societies. Obesity is determined by the interaction between predisposing genetic and environmental aspects, but at present, the gene–gene and gene–environment interactions contributing to the development of this complex disease are not fully understood. Impressive evidence has accumulated over the past decade that the development of obesity is linked to inflammatory pathways (1–5). The origin of obesity-related inflammation has been attributed to increased fat mass and concomitant insulin resistance, but the ultimate cause is unknown (1–7).

Exposures to different pathogens undoubtedly trigger and amplify inflammatory signals (5–11). In the link of inflammation to obesity, it is of note that several animal viruses are reported to increase adiposity when injected into animals (12–14). Canine distemper virus, Rous-associated virus type 7, and Borna virus produce obesity in mice, chickens, and rats in the context of hypothalamic damage or immune suppression (12–14). Animals experimentally infected with SMAM-1, an avian adeno-virus, or two human adenoviruses, adenovirus type 36 (Ad-36)¹ and Ad-37, developed adiposity (16). Interestingly, SMAM-1 and Ad-36 were associated with obesity in humans (16). Subjects positive for Ad-36 antibodies had significantly higher BMIs com-

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¹ Nonstandard abbreviations: Ad-36, adenovirus type 36; HSV, herpes simplex virus; ELISA, enzyme-linked immunosorbent assay; QSI, Quantitative Seropositivity Index.

pared with seronegative subjects. Prevalence of Ad-36 antibodies was almost 3-fold higher in obese vs. non-obese individuals (16).

On the other hand, obesity is frequently accompanied by related metabolic perturbations such as dyslipidemia, hypertension, and insulin resistance, which constitute risk factors for cardiovascular disease. In the last several years, pathogen burden (the number of exposures to a panel of pathogens) has been identified as an important factor influencing both inflammation and atherosclerosis (17–19). Together with conventional risk factors, pathogen burden imposed an additional independent risk for the presence and severity of cardiovascular disease (21–23). In this context, other studies have described associations between the exposure to pathogens associated with increased cardiovascular risk and the development of obesity. At least two studies disclosed increased prevalence of *Chlamydia pneumoniae* seropositivity in subjects with obesity (24,25). *C. pneumoniae* seropositivity has also been linked to the so-called metabolic syndrome and dyslipidemia (26–29) and to type 2 diabetes (30). Type 2 diabetes and hypertension have also been reported to be linked to herpes simplex virus type 1 IgG (HSV-1) (31,32), HSV-2 IgG seropositivity (33), and cytomegalovirus (34).

All of these findings suggest that inflammation could be behind the link between pathogen burden and obesity. In other words, burden of infection could be simultaneously associated with chronic low-grade inflammation and obesity (1–7). For that reason, we aimed to study the effect of exposure to four infections that had been previously associated with human atherosclerotic disease (18,20–22,35) on obesity phenotypes in apparently healthy middle-aged men.

Research Methods and Procedures

Inclusion and Exclusion Criteria

Seventy-four consecutive, unselected (except for inclusion criteria, see below) white subjects, participants in an ongoing epidemiological study of risk factors for cardiovascular disease in Northern Spain, were included in the study. Subjects were randomly localized from a census and were invited to participate. The participation rate was 71%. Smokers were defined as any person consuming at least one cigarette a day in the previous 6 months. A food frequency questionnaire was obtained from all subjects. None of the subjects were taking any medication or had any evidence of metabolic disease other than obesity. All subjects reported that their body weight had been stable for ≥ 3 months before the study. Inclusion criteria were 1) BMI < 40 kg/m², 2) absence of any systemic disease, 3) absence of clinical symptoms and signs of infection in the previous month by structured questionnaire to the patient, and 4) hepatitis C virus antibody seronegative.

Informed consent was obtained from all subjects. The local Ethics Committee approved the study.

Measurements

The subjects' waist was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteus region. The waist-to-hip ratio was calculated. Fat mass and fat-free mass were calculated using bioelectric impedance (BC Analyzer; Holtain, Cambridge, UK). Blood pressure was measured in the supine position on the right arm after a 10-minute rest; a standard sphygmomanometer of appropriate cuff size was used, and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-minute intervals. Patients were requested to withhold alcohol and caffeine for ≥ 12 hours before the different tests.

Analytical Methods

Blood samples were drawn from each subject after an overnight fasting period. Serum was centrifuged at 4000g for 10 minutes, immediately divided into aliquots, and frozen at -80°C until analysis. Each study subject's serum was tested for specific IgG class antibodies against HSV-1, HSV-2, enteroviruses, and *Chlamydia pneumoniae* through the use of quantitative in vitro ELISAs. In the IgG ELISA for HSV-1 (Euroimmun; Medizinische Labordiagnostika, Lübeck, Germany), HSV-2 (Euroimmun; Medizinische Labordiagnostika), and chlamydia (Euroimmun; Medizinische Labordiagnostika), a value of >18 relative units/mL was considered positive, and 16 to 18 was indeterminate according to the manufacturer's instructions. The anti-chlamydia antibody test is based on broad-reactive chlamydial inclusions. In the IgG ELISA for enteroviruses (Virion Serion, Würzburg, Germany), a value of >100 relative units/mL was considered positive, and 80 to 100 was indeterminate according to the manufacturer's instructions. Enteroviruses as such are a highly cross-reactive group, so that even without combining epitopes from an echovirus type and from a coxsackievirus type, most enteroviruses with either one of them would probably be detected. Intra- and inter-assay coefficients of variation for all these assays were $<6.1\%$ and 8.3% , respectively.

Two pathogens scores were constructed; in the semiquantitative seropositivity score, 4 to 12 was assigned according to the individual subject's seropositivity to each pathogen (1 for seronegative, 2 for indeterminate, and 3 for seropositive). In the Quantitative Seropositivity Index (QSI), the arithmetical sum of the individual titers of each pathogen was calculated. The enterovirus titer was multiplied by 0.18 to reflect a compatible range of burden compared with the other seropositivities. It should be recognized that QSI was made up for this study. The idea was that a higher QSI reflects a higher integrated response (exposure) to different pathogens.

Serum glucose concentrations were measured in duplicate by the glucose oxidase method with the use of a

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Beckman Glucose Analyser II (Beckman Instruments, Brea, CA). The coefficient of variation was 1.9%. Hemoglobin A_{1c} was measured by high-performance liquid chromatography by means of a fully automated glycated hemoglobin analyzer system (L-9100; Hitachi, Peoria, IL). Normal range among 774 subjects with normal glucose tolerance was $4.71 \pm 0.46\%$. Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase.

Serum C-reactive protein (ultrasensitive assay; Beckman, Fullerton, CA) was determined by routine laboratory test, with intra- and inter-assay coefficients of variation <4%. The lower limit of detection was 0.02 mg/L.

Statistical Methods

Descriptive results of continuous variables are expressed as mean (standard deviation) if normally distributed or as median and interquartile range. Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test, and variables were given a log-transformation if necessary. These parameters (triglycerides, seropositivities) were analyzed on a log scale and tested for significance on that scale. The anti-log trans-

Table 1. Anthropometric and biochemical variables of the study subjects

Variable	
<i>N</i>	74
Age (years)	52.7 (11)
Smokers, <i>n</i> (%)	19 (25.7%)
BMI (kg/m ²)	28.3 (3.2)
Waist-to-hip ratio (%)	0.95 (0.06)
Fat mass (kg)	11.4 (6.5–19.7)
Percent fat mass (%)	14.8 (8.7–21.8)
Systolic blood pressure (mm Hg)	128.7 (16)
Diastolic blood pressure (mm Hg)	81.3 (10)
Fasting glucose (mg/dL)	99.2 (9)
Hemoglobin A _{1c} (%)	4.7 (0.4)
Total cholesterol (mg/dL)	215 (40)
Low-density lipoprotein-cholesterol (mg/dL)	148 (87)
High-density lipoprotein-cholesterol (mg/dL)	53.4 (12.2)
Triglycerides (mg/dL)*	86 (59–129)
C-reactive protein (mg/liter)*	0.21 (0.1–0.4)

* Expressed as median and interquartile range.

Table 2. Burden of infection variables of the study subjects

Pathogen	Titer
HSV-1 serologic status*	134.5 (101.5 to 150)
Negative	7 (9.5%)
Indeterminate	1 (1.4%)
Positive	66 (89.2%)
HSV-2 serologic status*	9.5 (6.7 to 13)
Negative	71 (95.9%)
Indeterminate	0 (0%)
Positive	3 (4.1%)
Enterovirus serologic status*	39 (20.7 to 82)
Negative	54 (73%)
Indeterminate	8 (10.8%)
Positive	12 (16.2%)
<i>C. pneumoniae</i> serologic status*	19.5 (8 to 46)
Negative	33 (44.6%)
Indeterminate	6 (8.1%)
Positive	35 (47.3%)
Seropositivity Score	
0–6	30 (40.6%)
7–8	30 (40.5%)
9–10	14 (18.9%)
QSI*	221 (171.7 to 274.5)

Results are expressed as median (interquartile range) or as absolute value (percentage).

In the QSI, the arithmetical sum of the individual titers of each pathogen was calculated. The enterovirus titer was multiplied by 0.18 to reflect a compatible range of burden compared with the other seropositivities.

formed values of the means are reported in the tables. Differences between groups were tested by χ^2 test for categorical variables and by ANOVAs test for continuous variables. Relation between variables was tested using Pearson's test. The statistical power of the relationship between QSI and fat mass was 78%. Multivariate regression analysis was performed, including BMI, age, C-reactive protein, and the sum of titers for each pathogen as continuous variables for the dependent variable fat mass. A value of $p \leq 0.05$ was considered significant. Computations were carried out with SPSS version 11.0 (SPSS, Inc., Chicago, IL).

Results

Anthropometric and biochemical characteristics of the subjects and serological status are shown in Tables 1 and 2.

We observed significant relationships between the HSV-1 titer and fat mass and percentage fat mass (Table 3)

T1/T2

T3

Table 3. Linear association analysis among obesity phenotypes and pathogen seropositivities in the study subjects

	Correlations										
	BMI	Waist	Waist-to-hip ratio	Fat mass	Percent fat mass	HSV-1 titer	HSV-2 titer	Arterio-virus titer	Chlamydia titer	Semi-quantitative score	QSI index
BMI											
Pearson correlation	1	0.869*	0.480*	0.634*	0.592*	0.227	0.137	-0.027	0.132	0.217	0.222
Sig. (two-tailed)		0.000	0.000	0.000	0.000	0.052	0.246	0.817	0.261	0.063	0.058
N	74	74	74	74	74	74	74	74	74	74	74
Waist											
Pearson correlation	0.869*	1	0.682*	0.588*	0.546*	0.217	0.169	-0.081	0.087	0.215	0.174
Sig. (two-tailed)	0.000		0.000	0.000	0.000	0.065	0.153	0.497	0.463	0.068	0.141
N	74	74	74	74	74	74	74	74	74	74	74
Waist-to-hip ratio											
Pearson correlation	0.480*	0.682*	1	0.312*	0.311*	0.024	-0.070	-0.105	0.108	0.031	-0.021
Sig. (two-tailed)	0.000	0.000		0.007	0.007	0.841	0.559	0.374	0.363	0.795	0.863
N	74	74	74	73	74	74	74	74	74	74	74
Fat mass											
Pearson correlation	0.634*	0.588*	0.312*	1	0.925*	0.242†	-0.001	0.155	0.125	0.211	0.303*
Sig. (two-tailed)	0.000	0.000	0.007		0.000	0.038	0.997	0.187	0.287	0.071	0.009
N	74	74	74	74	74	74	74	74	74	74	74
Percent fat mass											
Pearson correlation	0.592*	0.546*	0.311*	0.925*	1	0.229	-0.041	0.150	0.105	0.167	0.272†
Sig. (two-tailed)	0.000	0.000	0.007	0.000		0.050	0.728	0.203	0.375	0.154	0.019
N	74	74	74	74	74	74	74	74	74	74	74
HSV-1 titer											
Pearson correlation	0.227	0.217	0.024	0.242†	0.229	1	-0.027	0.045	-0.134	0.244†	0.634*
Sig. (two-tailed)	0.052	0.065	0.841	0.038	0.050		0.820	0.703	0.254	0.036	0.000
N	74	74	74	74	74	74	74	74	74	74	74
HSV-2 titer											
Pearson correlation	0.137	0.169	-0.070	-0.001	-0.041	-0.027	1	-0.035	0.004	0.327*	0.210
Sig. (two-tailed)	0.246	0.153	0.559	0.997	0.728	0.820		0.765	0.971	0.005	0.073
N	74	74	74	74	74	74	74	74	74	74	74
Enterovirus titer											
Pearson correlation	-0.027	-0.081*	-0.105	0.155	0.150	0.045	-0.035	1	0.085	0.453*	0.646*
Sig. (two-tailed)	0.817	0.497	0.374	0.187	0.203	0.703	0.765		0.471	0.001	0.000
N	74	74	74	74	74	74	74	74	74	74	74
Chlamydia titer											
Pearson correlation	0.132	0.087	0.108	0.125	0.105	-0.134	0.004	0.085	1	0.442*	0.360*
Sig. (two-tailed)	0.261	0.463	0.363	0.287	0.375	0.254	0.971	0.471		0.000	0.002
N	74	74	74	74	74	74	74	74	74	74	74
Semi-quantitative score											
Pearson correlation	0.217	0.215	0.031	0.211	0.167	0.244†	0.327*	0.453*	0.442*	1	0.687*
Sig. (two-tailed)	0.063	0.068	0.795	0.071	0.154	0.036	0.005	0.000	0.000		0.000
N	74	74	74	74	74	74	74	74	74	74	74
QSI											
Pearson correlation	0.222	0.174	-0.021	0.303*	0.272†	0.634*	0.210	0.646*	0.360*	0.687*	1
Sig. (two-tailed)	0.058	0.141	0.863	0.009	0.019	0.000	0.073	0.000	0.002	0.000	
N	74	74	74	74	74	74	74	74	74	74	74

* Correlation is significant at the 0.01 level (two-tailed).

† Correlation is significant at the 0.05 level (two-tailed).

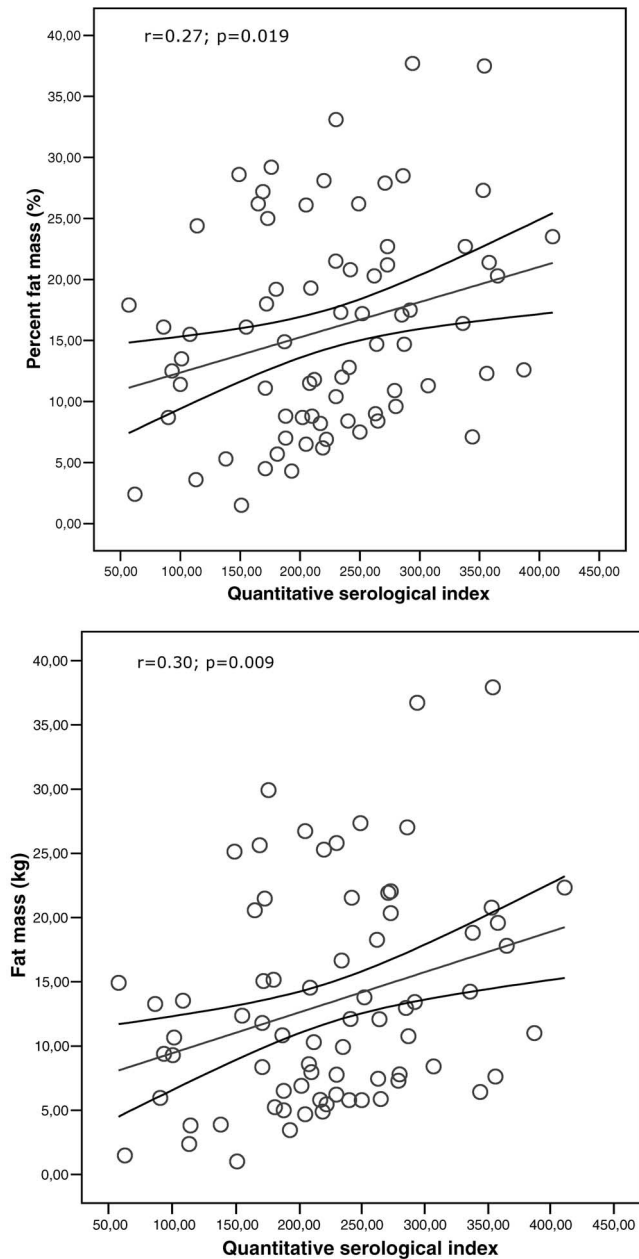


Figure 1: Linear association between QSI and percentage fat mass (top) and absolute fat mass (bottom) in study subjects.

and a tendency with BMI and waist diameter. The associations were stronger when considering the infection burden. The QSI was significantly associated with fat mass ($r = 0.30, p = 0.009$) and percentage fat mass ($r = 0.27, p = 0.01$).

When the subjects were divided into tertiles of fat mass, those subjects in the highest tertile of fat mass showed significantly higher QSI (259.5 ± 74.1 vs. $206.9 \pm 78.2, n = 24, p = 0.007$, Figure 2), and similar age (52.8 ± 11.6 vs. 52.6 ± 10.8 years, $p = 0.94$) than the remaining subjects ($n = 50$).

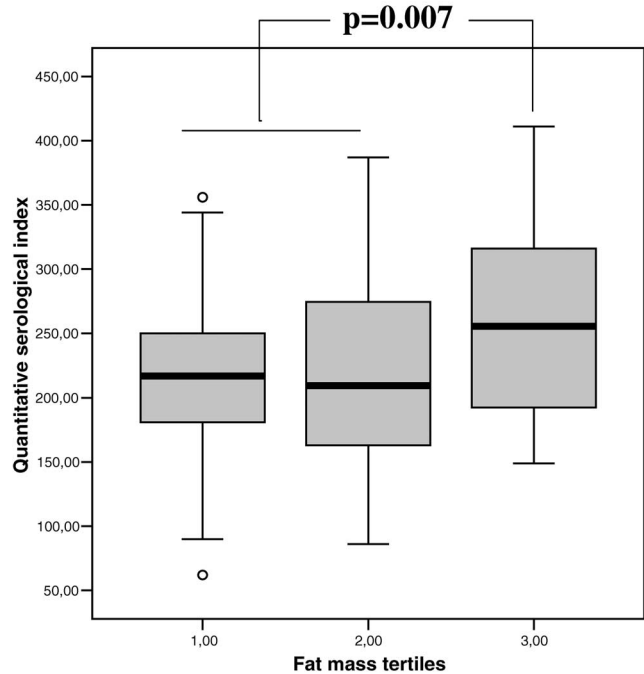


Figure 2: Box plot showing QSI according to fat mass tertiles. Each box shows the median, quartiles (25th and 75th percentiles), and extreme values within a category.

Furthermore, in those subjects that were seropositive for enteroviruses, the relationship between the QSI and fat mass (Figure 3) was strengthened. However, the low number of subjects seropositive for enteroviruses limits the power of this association.

Age and serum C-reactive protein were significantly and positively associated with several obesity phenotypes such as waist ($r = 0.40, p = 0.001$ and $r = 0.40, p = 0.001$, respectively). Age was also associated with BMI ($r = 0.27, p = 0.01$), and C-reactive protein with fat mass ($r = 0.26, p = 0.03$). For that reason, we constructed a multivariate regression analysis, in which the QSI, but not age or C-reactive protein, predicted 9% of fat mass variance.

Discussion

In this article, we described a significant association between burden of infection and fat mass and percentage fat mass. The association between HSV-1 titer and fat mass was weak but significant. However, it seems unlikely that one specific pathogen interacts with the development of obesity. This is supported by our findings that show a significant linear relationship between the number of infectious pathogens to which an individual has been exposed (a combined antibody response, expressed as QSI) and fat mass. This relationship was especially significant in subjects that were seropositive for enteroviruses.

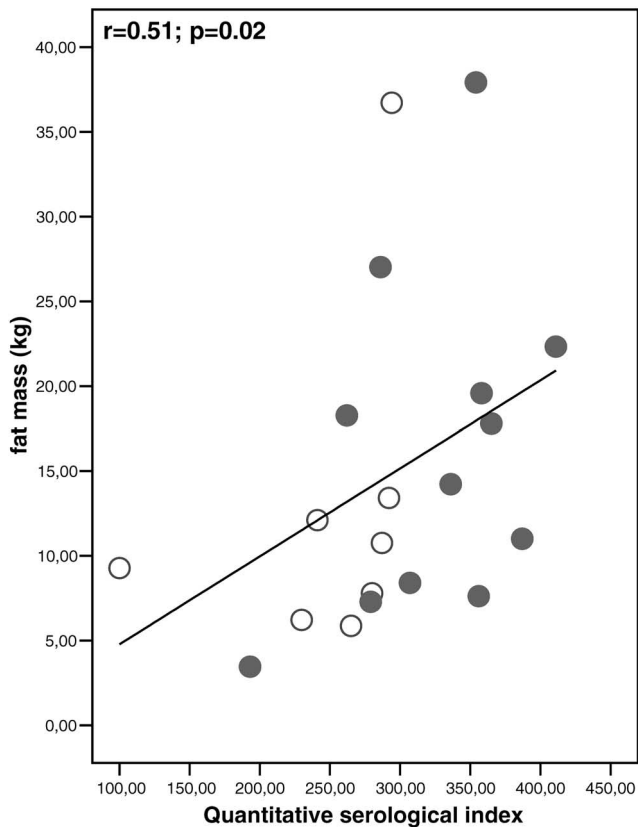


Figure 3: Linear association between QSI and absolute fat mass in subjects with indeterminate (○) or positive (●) titer for enterovirus. The enterovirus titer was multiplied by 0.18 to reflect a compatible range of burden compared with the other seropositivities.

It could be argued that the association was modest, but the QSI contributed to 9% of fat mass variance after accounting for age and C-reactive protein. Furthermore, subjects in the upper tertile of fat mass had an increased burden of infection as reflected by the QSI (Figure 2). This finding suggests a threshold effect of fat mass above which subjects would be more vulnerable to chronic infection. The opposite cannot be excluded—a threshold of burden of infection above which the impact on fat mass is significant.

The importance of the findings of this study is highlighted by the prevalence of these infections. Almost ~73% of the population ≥ 12 years of age in the United States had antibodies to one or both types of HSV (36). The population prevalence of antibodies to *C. pneumoniae* is 40% to 55% in the northern hemisphere and >60% in underdeveloped countries (37).

On the other hand, reduction in lifetime exposure to infectious diseases has made an important contribution to the historical decline in old age mortality (38). Given the well-known increased rate of obesity in the last years, this would argue against the number of infections increasing and

causing obesity but favor the hypothesis of decreased immunity in obese people (39). However, an increased ability to buffer pathogens with increased fat mass cannot be excluded.

These observational data do not prove any causal association, but there are plausible biological explanations and other studies pointing in the same direction. Evidence is accumulating that Ad-36 plays a role in human obesity by stimulation of adipocyte differentiation (40). Increased seropositivity against two viruses (Ad-36 and SMAM-1) has been described in obese humans (16). Some studies have described associations between *C. pneumoniae* seropositivity, the metabolic syndrome, and dyslipidemia (26–28). At least two other studies disclosed increased prevalence of *C. pneumoniae* seropositivity in subjects with obesity (24,25) together with increased fasting insulin (25).

It is important to recognize that we report serologies against pathogens that are characterized by persistent infection. HSV-1, HSV-2, and Chlamydiae infections are characterized by recurrent disease (41–44). Whether this persistence or recurrence influences the formation of de novo fat tissue is unknown. Enteroviruses show some tropism for fat tissue (45). On the other hand, genetic factors that increase the risk of developing obesity might also determine the extent of antibody response to several pathogens. As we previously hypothesized, high cytokine responders may be at an advantage in an environment where infectious risk is prevalent but at a disadvantage where obesity and atherosclerosis dominate (4).

In this study, we used two measures of pathogen burden. Most published studies on pathogen burden use the number of seropositivities as a measure of exposure (18–22). The QSI might add information concerning persistence for the different pathogens. In this sense, we want to highlight that we report serologies against pathogens that are characterized by persistent infection. We have selected these four infections a priori based on their associations with cardiovascular disease (18,20–22,35).

Strengths of this research are the study of a homogenous sample of healthy men and the use of bioelectrical impedance.

This is a cross-sectional study that establishes an association but not causality. That obesity might cause increased susceptibility to infection cannot be excluded. The elevated exposure to HSV-1 in our sample of subjects contributed to increase the seropositivity score. The small number of individuals negative for HSV-1 and the small number positive for HSV-2 limit the power of the study to find significant associations with these serologies. However, of note is that all subjects were hepatitis C virus antibody seronegative. It should also be recognized that the subject population was from one specific area, so the findings may not be characteristic of all populations and areas.

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We conclude that, among apparently healthy men, HSV-1 seropositivity was modestly linked to fat mass and percentage fat mass, whereas a total pathogen burden based on HSV-1, HSV-2, enteroviruses, and *Chlamydia pneumoniae* IgG serostatus showed the strongest association with fat mass.

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