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Lack of Evidence for the Role of Human Adenovirus-36 in Obesity in a European Cohort

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Adenovirus infection has been shown to increase adiposity in chickens, mice, and nonhuman primates. Adenovirus type 36 (Ad-36) DNA was detected in adipose tissues in these animal trials. In the United States, Ad-36 significantly correlates with obesity as illustrated by an Ad-36 seroprevalence of 30% in obese individuals and 11% in nonobese individuals. We investigated the possibility of a similar correlation of Ad-36 in Dutch and Belgian persons. In total, 509 serum samples were analyzed for Ad-36 antibodies using a serum neutralization assay. In addition, PCR was used to detect adenoviral DNA in visceral adipose tissue of 31 severely obese surgical patients. Our results indicated an overall Ad-36 seroprevalence of 5.5% increasing with age. BMI of Ad-36 seropositive humans was not significantly different from seronegative humans. No adenoviral DNA could be found using PCR on visceral adipose tissue. In conclusion, this first Ad-36 study in the Netherlands and in Belgium indicates that Ad-36 does not play a role as a direct cause of BMI increase and obesity in humans in Western Europe.

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In 2001, the possibility of an infectious component in the etiology of obesity was raised by Dhurandhar introducing the term "infectobesity" (1). In particular, support for Adenovirus type 36 (Ad-36) as a contributor to the obesity epidemic was found in chickens, mice and nonhuman primates (1–6). In 2005, a significant (relative risk = 2.7, $P < 0.001$) association between obesity and the presence of Ad-36 neutralizing antibodies in human serum was described in the United States by Atkinson *et al.* (7). Recently, subjects harboring Ad-36 DNA in their adipose tissue due to natural infection were described (8). To the best of our knowledge, very limited Ad-36 data in humans outside the United States are available, e.g., in Korean children as described by Atkinson *et al.* (9). Therefore our study explored this possible association in the Dutch and Belgian population data.

METHODS AND PROCEDURES

In total, 509 serum samples of four groups of patients were analyzed for Ad-36 antibodies, including 128 Dutch health-care students, 131 Dutch obese patients (BMI range 27–40 kg/m^2), 127 Belgian twin-members and 123 Belgian blood donors. The selected twin-members included 62 subjects with overweight (BMI >25 kg/m^2) and 65 lean subjects (BMI <21.5 kg/m^2). The presence of antibodies to Ad-36 in serum was determined using the "constant virus-decreasing serum" method and, according to Atkinson, the cutoff titer used to determine positivity was 1:8 (7).

PCR was used to determine whether adenoviral DNA was present in visceral adipose tissue of 31 severely obese surgical patients of the Netherlands. After an in-house preliminary comparison of three different ways to extract a highest yield of total DNA from human visceral adipose tissue (data not shown), DNA was extracted from 10 mg adipose tissue using the MagnaLyser, Proteinase K, MagnaPure LC instrument and the total DNA extraction kit (Roche Diagnostics, Almere, the Netherlands). Prior to isolation, the adipose tissue was spiked with known concentrations of an extraction and amplification control. The real-time PCR used for quantification of Adenoviral DNA was based on primers described by Echavarria *et al.* (10), and detection of Ad-36 DNA was confirmed by testing reference Ad-36 ATCC strain VR-913 (data not shown). The quality of the assay was assured by positive and negative controls as well as a test on amplification inhibition in each sample by an external amplification control. The analytical sensitivity of the entire assay was determined as <5.0 adenoviral DNA copies per PCR.

Statistical analysis including logistic regression analysis was performed using SPSS, version 15 (SPSS, Chicago, IL).

The study was approved by the local ethics committee.

RESULTS

An overall Ad-36 seroprevalence of 5.5% was found in 509 persons. In different patient subgroups, seroprevalence ranged

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from 8.9% in blood donors (age: 41 ± 10 years (mean ± s.d.)), 6.1% in selected obese persons (42 ± 6 years), 3.9% in health-care students (19 ± 3 years) and 3.1% in twins (27 ± 4 years). With increasing age, seroprevalence increased from 4.2, 5.3, 7.5 to 10.3% in patients aged ≤30, 31–40, 41–50, >50 years, respectively. Ad-36 seroprevalence was not different between men and women. Except one very high titer (1,024), other positive Ad-36 titers were within the range 8–64. BMI values in Ad-36 seropositive humans (with a BMI of 28.2 ± 5.8 kg/m^2) were in complete overlap with those in seronegative humans (BMI of 26.7 ± 5.9 kg/m^2). Similarly, in individuals with BMI of 16–20 (n = 49), 21–25 (n = 130), 26–30 (n = 102), 31–35 (n = 74), 36–40 (n = 24), and 41–45 kg/m^2 (n = 7), the corresponding Ad-36 seroprevalence was 4.1, 3.1, 4.9, 6.8, 4.2, and 0% respectively. These differences were statistically not significant (P > 0.05). In addition, no adenoviral DNA could be detected using PCR on DNA extracted from adipose tissue of 31 severely obese surgical patients.

DISCUSSION

In this study of >500 individuals of the Netherlands and Belgium, no significant association between Ad-36 seropositivity and obesity was found. The overall Ad-36 seroprevalence of 5.5% (3.9% and 5.7% in nonobese and obese subjects, respectively) that we found is much lower than previously described seroprevalences in the United States, e.g., seroprevalences of 11% in nonobese subjects and 30% in obese subjects (7). An Ad-36 seroprevalence lower than in the United States has also been demonstrated by Atkinson several years ago in an unpublished study, revealing an Ad-36 seroprevalence of about 8% in Belgian obese persons (R.L. Atkinson, personnel communication). The possible trend of increasing Ad-36 seroprevalence with age in the Netherlands and Belgium, ranging from <5% in subjects until 30 years to >10% in subjects over 50 years, is not unexpected and reflects the cumulative risk for infection and seropositivity with increasing age. In part, this is also reflected by lower Ad-36 seroprevalences in the youngest subgroups, i.e., twins and health-care students (Ad-36 seroprevalence of 3.1% and 3.9% and mean age of 27 and 19 year, respectively), compared to higher Ad-36 seroprevalences in relatively older groups of obese subjects and blood donors (Ad-36 seroprevalence of 6.1% and 8.9% and mean age of 42 and 41 year, respectively). In this study, also twin-members were included but the Ad-36 seroprevalence within these twin-members (3.1%) was too low for statistical analysis. Remarkably, a single extraordinary high titer of Ad-36 antibodies (1,024) was found in a lean twin member.

Unlike results obtained in animal studies (1–6), no adenoviral DNA could be detected in adipose tissue of our 31 severely obese surgical patients. In concordance with a low Ad-36 seroprevalence, our adenoviral DNA results therefore cannot confirm the prior association found between Ad-36 and obesity. However, the absence of adenoviral DNA in adipose tissue does not entirely rule out a potential obesity-inducing role of Ad-36 (4) and recent studies contribute to increase our knowledge about Ad-36 infection-induced effects including, e.g., inflammatory responses of (pre-) adipocytes (11), metabolically favorable remodelling of human adipose tissue (12) and enhanced glucose uptake (13).

We conclude that at this moment, at least in the Netherlands and in Belgium, no significant correlation is found between Ad-36 seropositivity and BMI. In addition, we obtained no indication that Ad-36 DNA is present in visceral adipose tissue of severely obese subjects. Therefore, we expect it to be unlikely that Ad-36 currently plays a role as a direct cause of BMI increase and obesity in humans in Western Europe. Nonetheless, future Ad-36 research may focus on an etiological role, if any, of the virus in lipid and/or glycemic disorders.

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DISCLOSURE

The authors declared no conflict of interest.

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