Human Adenovirus Ad-36 Promotes Weight Gain in Male Rhesus and Marmoset Monkeys¹,²

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ABSTRACT Although obesity has multiple etiologies, an overlooked possibility is an infectious origin. We previously identified two viruses, SMAM-1, an avian adenovirus (Ad), and Ad-36, a human adenovirus, that produce a syndrome of visceral obesity, with paradoxically decreased serum cholesterol and triglycerides in chickens and mice. In the two studies presented in this paper, we used nonhuman primates to investigate the adiposity-promoting potential of Ad-36. In study 1, we observed spontaneously occurring Ad-36 antibodies in 15 male rhesus monkeys, and a significant longitudinal association of positive antibody status with weight gain and plasma cholesterol lowering during the 18 mo after viral antibody appearance. In study 2, which was a randomized controlled experiment, three male marmosets inoculated with Ad-36 had a threefold body weight gain, a greater fat gain and lower serum cholesterol relative to baseline (P <0.05) than three uninfected controls at 28 wk postinoculation. These studies illustrate that the adiposity-promoting effect of Ad-36 occurs in two nonhuman primate species and demonstrates the usefulness of nonhuman primates for further evaluation of Ad-36–induced adiposity. J. Nutr. 132: 3155–3160, 2002.

KEY WORDS: • cholesterol • adiposity • obesity • nonhuman primates • infection

Obesity has multiple etiologies, but infectious agents have been consistently overlooked as a possible origin of human obesity. Three animal viruses have been reported to cause obesity in nonprimate species, but have not been implicated in initiating or maintaining obesity in humans (1–3). We have now identified two additional viruses, SMAM-1, an avian adenovirus (Ad), and Ad-36, a human adenovirus, that produce obesity in animals (4–7). In 6 separate experiments, we have shown that these two adenoviruses produce a syndrome of visceral obesity, along with paradoxically decreased serum cholesterol and triglycerides in chickens and mice (4–8). A capillary electrophoresis assay designed to detect Ad-36 DNA (9) showed tropism of the virus for adipose tissue of the infected animals (6,7). Ad-36–induced adiposity in animals was hyperplastic and hypertrophic (10). Preliminary data showed a marked up-regulation of adipocyte differentiation induced by Ad-36 (11,12). In humans, serum antibodies to both SMAM-1 and to Ad-36 are associated with obesity and lower cholesterol and triglycerides levels (5,13). For ethical reasons, the definitive experiment of injecting humans with Ad-36 to determine a causal role for this virus in human obesity is not possible. Nonhuman primates are the best surrogates for human experiments. In this paper, we used two disparate nonhuman primate species as models in which to study the adiposity promoting potential of Ad-36.

MATERIALS AND METHODS

Study 1: spontaneously occurring antibodies to Ad-36 in rhesus monkeys. Adult male rhesus monkeys (Macaca mulatta) were screened for the presence of spontaneously occurring antibodies to Ad-36, to ascertain their association with longitudinal changes in body weight and cholesterol. Frozen plasma samples from adult male rhesus monkeys (n = 15) were obtained from the Wisconsin Regional...
Composition of diet offered to rhesus monkeys\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Energy, kJ (kcal)</th>
<th>Protein, g</th>
<th>Crude fat, g</th>
<th>Crude fiber, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16,828 (4020)</td>
<td>150</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Minerals: contains calcium, 7.9 g; phosphorus, 4.9 g; potassium, 3.6 g; magnesium, 1.2 g; sodium, 2.1 g; chloride 3.2 g; iodine, 0.8 mg; manganese, 7.4 mg.

\textsuperscript{2} Vitamins: contains riboflavin, 22 mg; niacin, 99 mg; pantothenic acid, 55 mg; folic acid, 2 mg; thiamin, 19 mg; biotin, 4 mg; choline, 1259 mg; cholecyscalcoluril, 34 mg; vitamin A, 40 mg; vitamin K, 50 mg; vitamin E, 227 mg; vitamin B-12, 30 \mu g; vitamin C, 1017 mg.

Tissue culture techniques. A549 cells (human lung carcinoma cells) obtained from American Type Culture Collection (ATCC, Manassas, VA) were used to grow Ad-36. Minimum essential medium Eagle (MEM) (Cat \# M-0643, Sigma Chemical, St. Louis, MO) with nonessential amino acids, Earle's salts, \gamma-glutamine, 10% fetal bovine serum and 2.9% NaHCO\(_3\) (v/v), pH 7.4, was used for growing the cells.

Virus growth. Ad-36 was obtained from the ATCC. The virus was plaque purified as previously described (6,7) and grown in A549 cells. A working stock of virus was prepared as previously described (6,7) and was titrated on A549 cells, divided into aliquots and stored at 

Serum neutralization test for detecting antibodies. Rhesus plasma and marmoset serum were tested for the presence of Ad-36 antibodies. The assay used A549 cells and was conducted as a constant-virus-decreasing-serum method, as previously described (6,7). The absence of cytopathic effect (CPE) of the virus on A549 cells in the presence of the test serum is considered an indication of effective neutralization of the virus and the serum is considered to have antibodies against the virus. Samples were considered antibody-positive if the serum titer was \(\geq 1:8\). A few rhesus samples demonstrated cell-toxicity up to 1:16 dilutions. For these samples, a more stringent criterion of titer (\(\geq 1:32\)) was used to denote antibody positivity.

Cholesterol assay. Fasting total cholesterol was determined in duplicate with a cholesterol-oxidase-peroxidase method (Cat \# 352-500P, Sigma Chemical) using 10 \mu l of serum.

Development of a nested PCR assay for detection of Ad-36 DNA. Four primers were designed to unique regions of the Ad-36 fiber protein gene for use in nested PCR detection of viral DNA. Sequences of primers were as follows: outer forward primer (5'-GTCTGGAAAACCTGAGTGCTGATA), outer reverse primer (5'-ATTCCCAATCTCAAATTGATAATAGG), inner forward primer (5'-TTAATCCTGAAAAGGAAATAGG), inner reverse primer (5'-GTTTGTATTGTTGCTTATAGG). DNA was isolated using a QIAamp Tissue Kit (Cat \# 29304; Qiagen, Valencia, CA). Negative PCR controls were water and DNA from uninfected A549 cells. Positive PCR control was DNA from Ad-36 infected A549 cells. DNA was denatured for 2 min at 95°C and subjected to 35 cycles of PCR (94°C for 1 min, 55°C for 1 min, 72°C for 2 min) followed by incubation at 72°C for 5 min. PCR products were visualized on a 1% agarose gel with a size marker. Nested PCR products from positive control (Ad-36 DNA) and infected marmoset brain tissue were sequenced to confirm amplification of targeted region of the gene.

Virus isolation. Adenovirus infection in marmosets was confirmed by fecal samples (wk 4 and 9 after Ad-36 inoculation) and growing the virus on cell cultures as described earlier (7).

Composition of the canned diet offered to marmosets\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Energy, kJ (kcal)</th>
<th>Protein, g</th>
<th>Fat, g</th>
<th>Ash, g</th>
<th>Fiber, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6,907 (4020)</td>
<td>93.3</td>
<td>32</td>
<td>24</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Minerals: contains calcium, 3.3 g; phosphorus, 2.4 g; sodium, 2.2 g; potassium, 3.3 g; magnesium, 0.5 g; iron, 54 mg; zinc, 56 mg; copper, 5.3 mg; iodine, 0.8 mg; manganese, 7.4 mg.

\textsuperscript{2} Vitamins: contains vitamin A, 42 mg; cholicacidferol, 0.235 mg; vitamin E, 94 mg; thiamine, 40.0 mg; riboflavin, 8.0 mg; pyridoxine, 5.2 mg; niacin, 55.0 mg; pantothenic acid, 25.0 mg; biotin, 0.28 mg; folate acid, 0.2 mg; choline, 587 mg.
plasma did not demonstrate an annual pattern. Of 15 monkeys, 8 were seronegative at baseline. Only these monkeys were included in the statistical analysis to compare the body weights and cholesterol levels before and after the first appearance of Ad-36 antibodies. The remaining 7 monkeys were seropositive for Ad-36 antibodies at baseline and were excluded from statistical analysis.

Before separate univariate tests were conducted, a multivariate test (see Methods) was conducted that indicated a clear effect of Ad-36 status on the combination of weight and cholesterol ($P = 0.005$).

The monkeys were completely free of antibodies from $-18$ to $-6$ mo before the first positive sample; this was designated as the “baseline” period, whereas the period from $+6$ to $+18$ mo after seroconversion was designated as the “postinfection” period. Body weight and plasma cholesterol level changes for 18 mo before and 18 mo after the first appearance of Ad-36 antibody are presented in Figures 1 and 2. Time point “0 mo” denotes the first antibody positive serum sample for each monkey.

**Weight.** Body weight changed little during the baseline period, decreasing by $-0.3\%$ ($0.04 \pm 1.5$ kg; $P = 0.87$). In contrast, the body weight increased by $-10\%$ in 6 mo and by $-15\%$ ($1.7 \pm 0.8$ kg; $P < 0.03$) at 18 mo during the “postinfection” period (Fig. 1). In the full statistical model, the effect of Ad-36 antibody status was significantly associated with weight gain ($F = 5.47; \text{df} = 1.96; P = 0.021$). The parameter estimate for the effect of Ad-36 antibody status was $0.81$ kg, indicating that the generation of Ad-36 antibodies was associated with an increase in body weight of $0.81$ kg.

**Cholesterol.** Plasma cholesterol levels were stable during the baseline period, but decreased by $-23\%$ ($P = 0.06$) during the 6 mo immediately after the appearance of Ad-36 antibodies and remained low for at least 18 mo during the postinfection period (Fig. 2). In the full statistical model, the effect of Ad-36 antibody status was significant ($F = 5.33; \text{df} = 1.96; P = 0.023$). The parameter estimate for the effect of Ad-36 antibody status was $0.49$ mmol/L, indicating that the generation of Ad-36 antibodies was associated with a decrease in

![FIGURE 1](https://academic.oup.com/jn/article-abstract/132/10/3155/4687126)

**RESULTS**

**Study 1:** spontaneously occurring antibodies to Ad-36 in rhesus monkeys. During the 90-mo period, all 15 monkeys showed Ad-36 antibodies at some point in time. Because blood samples were obtained at 6-mo intervals, the exact date of seroconversion during the preceding 6 mo of the first positive sample is unknown. The appearance of antibodies in the

![FIGURE 2](https://academic.oup.com/jn/article-abstract/132/10/3155/4687126)
plasma levels of cholesterol by 0.49 mmol/L. Thus, when controlled for age, and after the first appearance of Ad-36 antibody, the rhesus monkeys had greater body weights and lower cholesterol levels.

**Study 2: infection of marmosets with Ad-36.** Body weights of both groups did not differ at the time of inoculation (336.4 ± 19.8 g vs. 346.3 ± 27.8 g, *P* = 0.65; for the Control and the Ad-36–infected groups, respectively). The serum neutralization assay showed an absence of Ad-36 antibodies in the control group at all times during the experiment. Two of the three Ad-36–inoculated marmosets were antibody positive at both 10 and 17 wk postinoculation. Ad-36 could not be isolated from the fecal samples of the control group at any time during the study. However, the infective virus was isolated at both 4 and 9 wk postinoculation from the fecal samples of the two Ad-36 inoculated marmosets that had detectable Ad-36 antibodies. The nested PCR assay, however, detected Ad-36 DNA in the adipose tissue, liver, skeletal muscle, lung and the brain samples of all three Ad-36 inoculated marmosets, but not in any monkey in the control group. The nested PCR assay results for the adipose tissue of the two groups are presented in Figure 3. Isolation of Ad-36 virus from fecal samples and the presence of viral DNA in the tissue of all infected males demonstrated that marmosets were readily infected with Ad-36.

At 28 wk after inoculation, the Ad-36 group had greater body weight gain (41.4 ± 11.2 g vs. 10.8 ± 13.4 g, *P* = 0.039; [Fig. 4](#)) and total body fat gain (36.3 ± 6.1 g vs. 23.0 ± 3.0 g, *P* = 0.013) than controls. The mean weight gain of 41.4 g represented a 12% increase in the Ad-36 group compared with a 3.2% increase in the control group, a threefold difference. The Ad-36 group tended to have more visceral fat than controls (7.3 ± 2.5 g vs. 4.4 ± 0.9 g, *P* = 0.089). Despite the gain in fat mass and body weight, serum cholesterol levels were lower in the Ad-36 group than in controls (change from baseline: −0.79 ± 0.28 mmol/L vs. 0.10 ± 0.08 mmol/L, *P* = 0.006; [Fig. 5](#)). The change in serum triglycerides from baseline tended to be greater in infected marmosets that in controls (−0.52 ± 0.23 mmol/L vs. 0.14 ± 0.53 mmol/L, *P* = 0.122).

**DISCUSSION**

Although 50 types of human adenoviruses are deposited with the ATCC, Ad-36 is the first reported to cause obesity in animals (6,7). Ad-36 is serologically different from at least 47 of the other 49 human adenoviruses (15–22); it was first isolated in 1978 in Germany from the feces of a diabetic girl suffering from enteritis (17). Relative antigenic uniqueness was one of the main reasons for selecting Ad-36 to test for the adiposity-promoting effect.

The concept of virus-induced obesity is of greater importance if shown to be relevant to human obesity. Although we have demonstrated the adipogenic and hypolipidemic effects of a human virus in animals such as chickens and mice, we can not conclusively extrapolate the results, without verification, to human obesity. Differences in lipid metabolism between lower animals and primates preclude such a direct extrapolation. For instance, the energy metabolism of chickens is based on free fatty acids, not glucose, and insulin is of minor importance. Such metabolic differences clearly warranted the use of a higher model to establish the relevance of the findings to human obesity. In addition, because of ethical reasons, humans can not be infected experimentally with Ad-36 to verify its adipogenic effect directly. Therefore, we decided to use nonhuman primate species as the best way of determining the relevance of Ad-36 in human obesity.
In our rhesus monkey study, the fact that all of the 15 monkeys had Ad-36 antibodies at some time during the 90-mo period suggests a coevemic epidemic of Ad-36 infection in the rhesus monkey colony at WRPRC. The source of Ad-36 infection in monkeys is not yet known. However, transmission from humans is a possibility. Although most adenoviruses are species specific in their replication cycle, we have recovered infectious Ad-36 virus from Ad-36–infected chickens (6,7) and have detected naturally occurring antibodies to Ad-36 in chickens as well as rats (unpublished data). The ability of Ad-36 to infect and replicate in widely disparate vertebrate species is in itself unusual. Therefore, the presence of naturally occurring antibodies in rhesus monkeys to a human adenovirus is not very surprising.

Serum neutralization is considered the “gold standard” to specifically detect neutralizing antibodies. In addition to the previously reported minimal antigenic cross-reactivity of Ad-36 (16,–22), we confirmed that Ad-36 does not cross-react with other human adenoviruses such as Ad-2, Ad-31 or Ad-37 (unpublished data). Therefore, it is unlikely that the antibodies detected in the rhesus monkey plasma that neutralized Ad-36 were antibodies to other human adenoviruses. Antibodies to simian adenovirus(es) that may cross-react with Ad-36 are a possibility, although cross-reactivity between a simian adenovirus and Ad-36 has not been reported.

In rhesus monkeys, a positive correlation of age with body weight and serum cholesterol would have been expected. The increase in body weight and drop in cholesterol during the postinfection period that persisted even after controlling for age supports our hypothesis that these changes were associated with the appearance of Ad-36 antibodies. A comparison of body weight and plasma cholesterol changes between antibody positive and antibody-free monkeys would have been optimal to eliminate the effect of age on these variables. However, all 15 rhesus monkeys developed Ad-36 antibodies at different times during the study period, thus precluding such a comparison. Nevertheless, the following measures ensure that the changes in body weight and cholesterol observed were not age related.

The age of the monkeys ranged from 8 to 14 y. The age of the first appearance of antibodies varied among the monkeys. Regardless of the age of seroconversion, there was little change in body weights or plasma cholesterol in the 18 mo before the first appearance of antibodies. Thus, although the period before the onset of antibodies represents different ages for individual monkeys, their body weights were stable during this period. Such a stabilization of body weight is expected in adult rhesus monkeys who have completed their growth. Also, an age-related decline in plasma cholesterol is not expected in freely fed rhesus monkeys. In effect, each monkey acted as his own control for body weight and cholesterol comparisons before and after the seroconversion. As stated earlier, we further ascertained the age effect by controlling statistically for age, and found a clear effect of seroconversion on body weight and cholesterol that was independent of age. These measures demonstrate that the observed changes were not age related.

In the marmoset experiment, the persistence of Ad-36 DNA in various tissues of all marmosets 6 mo postinfection is an important finding, demonstrating the spread of Ad-36 in their bodies, even though one marmoset failed to generate detectable levels of antibodies. In addition, the presence of Ad-36 DNA in the adipose tissue is particularly intriguing because our preliminary data showed Ad-36 induced up-regulation of 

**LITERATURE CITED**

type 7 induces a syndrome in chickens characterized by stunting and obesity.


