# Using rats as a research model to investigate the effect of human adenovirus 36 on weight gain

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**Original Article**

## Abstract

**BACKGROUND:** Recent evidence has shown a positive correlation between obesity and viral infections with a particular emphasis on the human adenovirus-36 (Ad-36). Ad-36 is the first human virus that may increase adiposity in animals, and it is considered as a possible risk factor for obesity in humans; however, the results were not consistent across all the studies. The present study was conducted to examine the influence of Ad-36 infection on obesity in a rat model.

**METHODS:** Eight-week-old male Wistar rats weighing 170-240 gram (g), were randomly divided into two groups, infection group (48 rats) and a control group (12 rats). The rats in the infection group were infected with human Ad-36. All rats were given free access to a normal chow diet and water. They were weighed weekly.

**RESULTS:** The mean ± standard deviation (SD) body weights were 229.0 ± 25.9 g and 232.3 ± 16.6 g in the infection and control groups, respectively at the time of infection. The mean ± SD body weight of the infection group (304.0 ± 39.0 g) was higher than the control group (301.0 ± 36.5 g) at 12 weeks post-infection (P = 0.82). Although two groups had approximately same food intakes, the mean change in body weight was greater in the infection group than the control group (75.8 ± 27.9 g vs. 70.8 ± 24.5 g) but it was not significant (P = 0.57).

**CONCLUSION:** We did not find a statistically significant association between weight gain and Ad-36 infection in the rat model. It seems that longer follow-up duration is needed to develop a significant weight gain in the infected rats. Rats can be used as a good animal model for further investigations about Ad-36-induced obesity, provided not to rely merely on weight measurements. Evaluating body composition or histopathological assessments are suggested.

**Keywords:** Adenovirus Infections, Weight Gain, Wistar Rats

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## Introduction

Obesity is defined as excessive fat accumulation in the adipose tissue that may be associated with increased risk of chronic diseases, and impose a significant economic burden to the health care system. Over 600 million adults were obese in 2014, and it is predicted that 51% of the world's population will be obese by 2030.[1-3]

Obesity is a multifactorial disease that develops from a complex of interactions among genetic, metabolic, behavioral, as well as environmental factors.[1-4] Moreover, infection by certain pathogens may be considered as possible causes of obesity.[4,5] Dhurandhar coined the term infectobesity -obesity of infectious origin- in 2001.[6] Infectobesity is receiving a considerable attention. Several pathogens have been identified as the causes of obesity in animal models.[5-8]

Recent evidence has shown a positive correlation between obesity and adenoviruses, with a particular emphasis on the human adenovirus-36 (Ad-36) that has a direct effect on adipose tissue.[9-12]

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Human adenoviruses are implicated in infections of the upper respiratory and gastrointestinal tracts and in conjunctivitis.[13-16] The first study of Ad-36 infection in adult humans in the United States by Atkinson et al. showed that the antibody-positive obese or nonobese subjects were heavier compared with their antibody negative counterparts. About 30% of obese and 11% of non-obese had been infected with Ad-36. In addition, the obese adults had a higher prevalence of serum neutralizing antibodies to Ad-36 than the lean adults.[17]

Similarly, antibody-positive twins were heavier [body mass index: 26.1 vs 24.5 kg/m$^2$ ($P < 0.04$)] and fatter [body fat percentage was 29.6 vs. 27.5 ($P < 0.04$)] than their co-twins who did not have Ad-36 antibodies.[17] These results have been confirmed by other studies, while some studies failed to confirm these findings.[18-19] Pasarica et al. have reported that rats infected with Ad-36 by 30 weeks post-inoculation, had a significant increase in body weight and body fat.[20]

Although the link between Ad-36 infection and obesity has been investigated in animal models, only one study has examined the relationship between Ad-36 and obesity in rats.[20] Obviously, rat is a good animal model for investigating human infectobesity and the metabolic and biochemical modulations induced by Ad-36 in adipose tissue. Rat provides a higher body weight and adequate quantities of adipose tissue for use in various mechanistic pathways representing the obesity process.[20,21]

Finding a therapeutic agent for Ad-36-induced obesity may help to treat obesity more effectively at least in a subgroup of population.[10,15] To the best of our knowledge, there is no Food and Drug Administration (FDA) approved vaccine or clinical drug for Ad-36-induced obesity. Therefore, a research project was designed and carried out to investigate "the effects of the alcoholic green tea extract and conjugated linoleic acid on body weight, metabolic indices and inflammatory markers in Ad-36 infected rats". The purpose of the present study was to describe study design and to report the preliminary results.

## Materials and Methods

Eight-week-old male Wistar rats from Laboratory Animal Unit of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) were used for the experiment weighing 170-240 g. Six rats per cage were housed in a temperature-controlled (20–22 °C) animal room with a 12-hour light-dark cycle. After one-week acclimatization period, rats were randomly divided into two groups, infection group (48 rats) and control group (12 rats). Infection and control groups were housed in separate rooms under biosafety level 2 containment. They had free access to drinking water and standard rat chow for 1 week while adjusting to their new environment. During the experimental period, the rats had free access to a normal chow diet and water. Food disappearance was recorded for each of the cages, but we were not able to measure the amount of food spillage. However, visual inspection indicated that spillage patterns were similar in all groups. At the end of the study, the average food intake per day per rat was calculated. All rats were weighed weekly by means of a digital scale (Sartorius 1413 MP 8/8-1, USA).

A549 cells, a human lung carcinoma tissue culture line purchased from the Razi Vaccine and Serum Research Institute (Tehran-Iran) were used to grow Ad-36. Human Ad-36 was obtained from the American Type Culture Collection (Catalog no. VR-1610; American Type Culture Collection, Manassas, VA 20108 USA). In order to confirm the hexon gene, polymerase chain reaction (PCR) was performed and subsequently, sequencing was carried out by chain termination, using ABI 3730XL DNA Analyzers (Bioneer, Korea).

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% of heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml of penicillin, 100 µg/ml of streptomycin and incubated at 37 °C, 5% $CO_2$. In preparation of virus seeding, culture of Ad-36 with multiplicity of infection (MOI) 0.01 in confluent A549 cells was performed and 72-hour post-infection cytopathic effect was completed. Infection cells were frozen, thawed, and then centrifuged into 5000 g and supernatant were collected. Virus titration was performed by 50% cell culture infective dose ($CCID_{50}$) assay. $5 \times 10^5$ $CCID_{50}$ of Ad-36 virus suspension was injected in the left hind paw of the experimental group rats. On the 14$^{th}$ day after virus injection, blood sera of rats were collected and infection was confirmed by neutralization assay.

This experiment was performed in accordance with the National Research Council (US) Committee for Guide for the Care and Use of Laboratory Animals, 8$^{th}$ edition, and the guidelines provided by the ethical committee of experimental animal care at Ahvaz Jundishapur University of Medical Sciences.

The data were analyzed using SPSS Software (version 20, IBM Corporation, Armonk, NY, USA). One-sample Kolmogorov-Smirnov test was used to

test normal distribution of the variables. Paired t-test was used to compare the baseline with the endpoint values. Data from the infection and control groups were compared with independent samples t-test. The values are presented as means ± standard deviation (SD). P-value of 5% or lower was considered statistically significant.

## Results

At the beginning of the study, the mean ± SD of bodyweight for infection group was 192.8 ± 16.3 g and in the control group was 195.3 ± 9.0 g (P = 0.48). At the time of infection, the mean body weights were 229.0 ± 25.9 g and 232.3 ± 16.6 g in the infection and the control groups, respectively (P = 0.60). Obviously, a significant weight increase was observed in the infection (from 36.3 ± 17.7 g to 75.8 ± 27.9 g) and control (from 37.0 ± 12.8 to 70.8 ± 24.5 g) groups after 12 weeks (P < 0.01). At 12 weeks after infection, while the two study groups had approximately equal food intakes and food disappearance, the mean body weight of the infection group was higher than the control group (304.0 ± 39.0 g vs. 301.0 ± 36.5 g, P = 0.82, Table 1). In addition, the mean change in body weight was greater in the infection group than the control group; but the between groups difference were not statistically significant (75.8 ± 27.9 g vs. 70.8 ± 24.5 g, P = 0.57).

## Discussion

In this experimental study, no statistically significant association was found between weight gain and Ad-36 infection in male Wistar rats at 12 weeks after Ad-36 infection. Pasarica et al. performed a similar study on Ad-36 infected rats.[20] which, to our knowledge, is the only study that had monitored infected Wistar rats for a long time (12 and 30 weeks). This study showed that body weight and adiposity were significantly increased in the infected vs. control rats at 30 weeks after Ad-36 infection.[20] Ad-36-induced adiposity and significant weight gain had been documented in mice, rats, and marmosets.[18-20] In agreement with Pasarica et al.,[20] the body weights did not differ significantly at 12 weeks after infection in our experiment. It seems that longer follow-up duration, approximately six months, is required to increase significant weight gain in Ad-36 infected rats.

Obesity is a major modifiable risk factor for many chronic diseases and as a critical medical condition that can lead to serious health consequences, including shortened life span and increased morbidity, as well as health care costs. Many people are trying to lose weight by dietary restraint, increased physical activity and healthy lifestyle behaviors.[22-24] These approaches may be difficult, ineffective or inappropriate in the vast majority of overweight and obese individuals. In addition, these weight loss strategies are often ineffective in the long term and may have unintended consequences, particularly higher risk of repeated cycles of weight loss and weight regain.[24-25] Obviously, few individuals successfully achieve long-term results from weight loss strategies. Therefore, prevention strategies or cause-specific treatment could be determined according to various contributing factors to obesity. Although a combination of several factors may lead to obesity, a subtype of obesity may be caused by infections.[10, 22-27] Over the past thirty years, ten pathogens have been reported to induce obesity in animals, but their contribution to human obesity has not yet been fully understood.[28] Overall, viral infections have been implicated as a possible cause of obesity in human. Adenoviruses are the first adipogenic agents that may cause obesity in both animal models and naturally infected humans.[29-32] The adenoviruses are common pathogens of humans and cause a wide range of illnesses and symptoms such as common cold, enteritis, acute upper respiratory tract infections, or conjunctivitis.[14]

**Table 1.** Body weights and food intake in the adenovirus-36 (Ad-36) infected rats vs. the control group[*†]

| Variables | Infected | Control | P |
|---|---|---|---|
| Body weight (g) | | | |
| Baseline | 192.8 ± 16.3 | 195.30 ± 9.00 | 0.48 |
| At the time of infection | 229.0 ± 25.9 | 232.30 ± 16.60 | 0.60 |
| 12 weeks post-infection | 304.0 ± 39.0 | 301.00 ± 36.50 | 0.82 |
| Change[‡] | 75.8 ± 27.8 | 70.80 ± 24.50 | 0.57 |
| Food intake/d (g) | | | |
| Baseline | 22.8 ± 1.0 | 22.60 ± 0.56 | 0.52 |
| At the time of infection | 23.1 ± 1.1 | 22.80 ± 0.58 | 0.36 |
| 12 weeks post-infection | 25.9 ± 1.1 | 25.20 ± 1.60 | 0.26 |

[*] Values are means ± standard deviation; [†] Data at 12 weeks are for 11 rats from the control group and 46 rats from the infection group; [‡] Weight changes from the time of infection to 12 week post-infection

The presence of serum antibodies to adenoviruses is common in the general population, which makes them promising candidates for studying their potential role in human obesity with a particular emphasis on the Ad-36.[13,29] Clearly, Ad-36 is the first human virus associated with increased adiposity and significant weight gain in animals.[33] In addition, results from epidemiological studies have shown that Ad-36 infection might be associated with obesity in both children and adults.[29,34-40] However, more animal and human researches are needed to establish the contribution of Ad-36 in human adiposity. For ethical reasons, humans cannot be infected experimentally with Ad-36 to study the virus induced adiposity; therefore, animal models are used to determine the relevance of Ad-36 induced human obesity.[7,10] Rats are good research models for investigating human obesity and the metabolic and biochemical modulations induced by Ad-36. Rat's body provides a higher weight and adequate quantities of adipose tissue to be used in various mechanistic pathways representing the obesity process. Rat has become a standardized physiological and toxicological model because it behaves more similar to human.[20,41] Therefore, investigating Ad-36-induced obesity in rat model may lead to finding a therapeutic agent for preventing or treating Ad-36-induced obesity.

## Conclusion

In the present study, Ad-36 had no statistically significant effect on weight gain in infected rats. Further studies with longer duration of follow-up are suggested. As a model of medical research, rat has many advantages over other animal models for further investigations about Ad-36-induced obesity. In addition, it is strongly recommended not to rely merely on weight measurement; body composition and or histopathological assessments would be accompany weight measurement.

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## Conflict of Interests

Authors have no conflict of interests.

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