

# Human adenovirus 36 improves insulin sensitivity and lipid profiles and increases inflammatory markers in Wistar rats

Fatemeh Shirani <sup>1</sup>, Ali Teimoori,<sup>2</sup> Andrew J McAinch,<sup>3,4</sup> Mohammad Rashno,<sup>5</sup> Seyed Mahmoud Latifi,<sup>6</sup> Majid Karandish<sup>7</sup>

<sup>1</sup>Department of Nutrition, School of Allied Medical Sciences, and Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran  
<sup>2</sup>School of Medicine, Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran  
<sup>3</sup>Institute for Health and Sport, Victoria University, Melbourne, Victoria, Australia  
<sup>4</sup>Australian Institute for Musculoskeletal Science (AIMSS), Victoria University, Melbourne, Victoria, Australia  
<sup>5</sup>Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran  
<sup>6</sup>Diabetes Research Center and Department of Statistics and Epidemiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran  
<sup>7</sup>Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

**Correspondence to** Professor Majid Karandish, Ahvaz, Iran (the Islamic Republic of); mkarandish@yahoo.com

Accepted 12 March 2020  
 Published Online First 16 April 2020



© American Federation for Medical Research 2020. No commercial re-use. See rights and permissions. Published by BMJ.

**To cite:** Shirani F, Teimoori A, McAinch AJ, et al. *J Investig Med* 2020;**68**:980–984.

## ABSTRACT

Human adenovirus 36 (Ad-36) causes obesity with increased adiposity, in contrast, Ad-36 infection reduces glucose and lipid metabolism; the results, however, are not consistent. In the current study, the effects of Ad-36 infection on glucose and lipid profile and inflammatory markers in Wistar rats were investigated. Sixty male Wistar rats were randomly divided into infected and control groups. Ad-36 virus suspension was injected in the experimental group rats. Blood samples were collected in the beginning and after 12 weeks in both groups. After 12 weeks, a significant improvement was observed in fasting blood glucose, fasting serum insulin, insulin sensitivity, serum triglycerides and total cholesterol in the infected group compared with the non-infected groups. There were no significant differences in inflammatory biomarkers including tumor necrosis factor- $\alpha$ , interleukin 6 and monocyte chemoattractant protein-1 levels between infected and control groups. This study showed that Ad-36 had favorable effects on glycemic and lipid control in infected rats, but inflammatory biomarker levels were similar for 2 groups. Ad-36 infections could potentially be a new way to develop novel antidiabetic and antihyperlipidemic therapeutic agents.

## INTRODUCTION

Obesity, as excess body fat accumulation, is a major public health epidemic in the world. Nearly 30% of adults are either obese or overweight. Obesity has multiple etiologies including a complex interaction among genetics, hormones, behavior, and the environment.<sup>1</sup> In addition, several viruses have been identified as possible promoters of obesity in animals and human.<sup>2</sup>

Adenovirus 36 (Ad-36), a human adenovirus, is known to be associated with weight gain in animals and it is considered as a possible risk factor for obesity in humans.<sup>3–6</sup> Some cross-sectional studies showed that Ad-36 infection was linked with greater adiposity and better metabolic control.<sup>3 7–9</sup> Ad-36 may increase adiposity in mice, chickens, and monkeys and paradoxically improves glycemic control and reduces serum triglycerides and cholesterol.<sup>10–12</sup>

## Significance of this study

### What is already known about this subject?

- ▶ Several pathogens are linked to obesity in animals and humans.
- ▶ Several studies over the past 30 years have indicated adenovirus 36 (Ad-36) infection is linked with greater adiposity.
- ▶ Ad-36 infection is associated with better metabolic control in animal models.

### What are the new findings?

- ▶ Our study showed that rats infected with Ad-36 had better glycemic control and enhanced insulin sensitivity.
- ▶ Serum triglycerides and total cholesterol concentrations were significantly lower in the Ad-36-infected group.
- ▶ Inflammatory biomarker levels were similar for the infected and control groups.

### How might these results change the focus of research or clinical practice?

- ▶ Investigating Ad-36-induced obesity in a rat model may lead to finding a therapeutic agent or even a vaccine or antiviral drugs for Ad-36-induced obesity; it may also help treat obesity and metabolic indices more effectively at least in a subgroup of population.

The only study to investigate the link between Ad-36 infection, obesity and insulin sensitivity in rats, reported that the changes in body weight did not significantly differ in the infected group by the control group after 12 weeks postinoculation. However, the mean body weight of the infected rats was significantly higher than that in the control group after 30 weeks postinoculation.<sup>13</sup> Inconsistent with other animal models,<sup>11 12 14</sup> Ad-36 did not reduce serum glucose concentration in the infected rats in this study.<sup>13</sup> Fasting serum insulin levels were significantly lower and the homeostatic model assessment of insulin resistance (HOMA-IR) indicated significantly higher insulin sensitivity for the infected.<sup>13</sup> Total serum cholesterol levels were similar for the 2 Ad-36-infected and

control groups but serum triglyceride levels were significantly greater in the infected compared with the control rats.<sup>13</sup>

Obesity and proinflammatory cytokine are closely correlated.<sup>15</sup> Ad-36 infection was associated with inflammatory biomarkers and thereby might contribute to chronic low-grade inflammation; however, the results are not consistent.<sup>16–18</sup> Na and Nam<sup>17</sup> reported that Ad-36 infection increased production of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and monocyte chemoattractant protein-1 (MCP-1), and consequently increased adiposity in mice.

Even though the association of Ad-36 and adiposity has been investigated in a series of animal studies, only 1 study has examined the relationship between Ad-36 and obesity in rats,<sup>13</sup> more animal and human research is needed to establish the contribution of Ad-36 in human obesity. For ethical reasons, experimental animal models are used to investigate the probable association between Ad-36 and obesity.<sup>5, 19</sup> The rat model behaves more like the human disease and provides a higher weight and adequate quantities of adipose tissue to be used in various mechanistic pathways representing the obesity process and human metabolism. Rats are good research models for investigating the metabolic and biochemical modulations induced by Ad-36.<sup>13, 20</sup> Therefore, investigating Ad-36-induced obesity and inflammation-related metabolic diseases in rats, as a standardized physiological and toxicological model, may lead to finding a therapeutic agent for preventing or treating Ad-36-induced obesity and metabolic disorders at least in a subgroup of the population. Therefore, the aim of the present study is to investigate the effect of Ad-36 infection on glucose, lipid metabolism and inflammatory markers in Wistar rats.

## MATERIALS AND METHODS

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 present study reports preliminary results of the effect of Ad-36 infection on glucose, lipid metabolism and inflammatory markers in Wistar rats. The study design and details have been previously described elsewhere.<sup>21</sup>

### Animal care

Sixty male Wistar rats (8 weeks old) from Laboratory Animal Unit of Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) were used for the experiment weighing 170–240 g. After 1 week of acclimatization period, rats were randomly divided into 2 groups (infected and control). Rats were housed in separate rooms under biosafety level 2 containment. Ad-36 virus suspension ( $5 \times 10^5$  50% cell culture infective dose (CCID50)) was injected in the left hindpaw of the infected group rats as previously mentioned.<sup>21</sup> All rats had free access to standard rat chow and drinking water and were weighed weekly by means of a digital scale (Sartorius 1413 MP 8/8-1, USA).

### Virus growth

Human Ad-36 was purchased from the American Type Culture Collection (Catalog No VR-1610; Manassas, VA, USA). In order to confirm the hexon gene, PCR was

performed and subsequently, sequencing was carried out by chain termination using ABI 3730XL DNA Analyzers (Bioneer, Korea). A549 cells, a human lung carcinoma cell line, was obtained from the Razi Vaccine and Serum Research Institute (Tehran, Iran) to grow Ad-36 was used. The virus was cultured as previously described.<sup>21</sup> Briefly, in preparation of virus seeding, the culture of Ad-36 with multiplicity of infection of 0.01 in confluent A549 cells was performed and 72 hours after infection the cytopathic effect was completed. Infection cells were frozen, thawed, and then centrifuged into  $5000 \times g$  and supernatants were collected. Virus titration was performed by CCID50 assay. Ad-36 virus suspension ( $5 \times 10^5$  CCID50) was injected in the left hindpaw of the experimental group rats immediately after the first blood sampling. Blood sera of rats were collected on the 14th day after virus injection, and infection was confirmed by neutralization assay.

### Biochemical assays

Venous blood samples were taken from all rats after 12–14 hours of overnight fasting just before injection of Ad-36 virus suspension in infected group. The rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg).<sup>22</sup> Two rats in the infected group died 2 days after the first phase of blood sampling and a rat in the control group died in the second phase of blood sampling (12 weeks). The blood samples were centrifuged at  $3000 \times g$  for 10 minutes and stored at  $-70^\circ\text{C}$  for further analysis. Fasting serum glucose was measured using glucose-oxidize (Pars Azmoon, Tehran, Iran). Autoanalyzer (BT3000) and a commercially available kit (Pars Azmoon) were used to determine triglyceride and total cholesterol. A commercially available rat ELISA kit (Hangzhou Eastbiopharm, Torrance, USA) was used for the measurements of insulin, TNF- $\alpha$ , interleukin 6 (IL-6) and MCP-1. A homeostasis model assessment index was calculated by multiplying the glucose level (mM) with the insulin level (U/mL) and dividing the product by 2430.<sup>23</sup>

### Statistical analysis

Statistical analyses were performed using SPSS software for Windows (V.20, IBM). The normal distribution of variables was checked with the Kolmogorov-Smirnov test. Paired t-test was used to compare the baseline with endpoint values. Data from the infection and control groups were compared with independent samples t-test (at the baseline) and analysis of covariance (at the endpoint values), adjusted by baseline covariate. The values are presented as means  $\pm$  SD. A p value of 5% or lower was considered statistically significant.

## RESULTS

The mean  $\pm$  SD of body weight was not significantly different between the infected and control groups at the baseline ( $192.8 \pm 16.3$  g vs  $195.3 \pm 9.0$  g,  $p=0.82$ ) and 12 weeks after infection ( $304.0 \pm 39.0$  g vs  $301.0 \pm 36.5$  g,  $p=0.82$ ). There were no significant differences in glucose, lipid profiles and inflammatory markers between groups at baseline. After 12 weeks, fasting blood glucose ( $95.3 \pm 17.4$  vs  $107.5 \pm 13.6$  mg/dL,  $p=0.02$ ) and fasting serum insulin ( $55.2 \pm 21.4$  vs  $73.6 \pm 24.6$   $\mu\text{U/mL}$ ,  $p=0.04$ ) were significantly lower in the

**Table 1** Glucose, lipid profile and inflammatory markers in the Ad-36-infected rats versus control\*†

Variables		Infected	Control	P value
Fasting blood glucose (mg/dL)	At the time of infection	91.5±17.2	94.4±15.3	0.621‡
	12 weeks postinfection	95.3±17.4	107.5±13.6	0.030§
	P1¶	0.135	0.017	
Fasting serum insulin (µU/mL)	At the time of infection	68.1±29.3	74.3±23.3	0.642‡
	12 weeks postinfection	55.2±21.3	73.6±24.6	0.017§
	P1	0.004	0.773	
HOMA-IR index	At the time of infection	2.6±1.4	2.8±0.69	0.621‡
	12 weeks postinfection	2.1±1.3	3.2±0.87	0.003§
	P1	0.04	0.06	
Triglycerides (mg/dL)	At the time of infection	66.4±25.3	59.1±15.3	0.360‡
	12 weeks postinfection	51.4±16.1	60.2±10.8	0.012§
	P1	0.000	0.803	
Cholesterol (mg/dL)	At the time of infection	56.7±8.9	51.1±13.6	0.141‡
	12 weeks postinfection	46.5±8.9	53.6±14.8	0.001‡
	P1	0.000	0.111	
TNF-α (ng/L)	At the time of infection	18.1±5.8	19.5±4.5	0.442‡
	12 weeks postinfection	25.7±6.8	20.7±6.6	0.627‡
	P1	0.000	0.420	
IL-6 (ng/L)	At the time of infection	16.6±4.5	16.7±4.6	0.949‡
	12 weeks postinfection	20.6±3.6	18.4±5.4	0.085§
	P1	0.000§	0.102	
MCP-1 (ng/L)	At the time of infection	26.3±7.5	24.1±5.1	0.376‡
	12 weeks postinfection	37.8±14.8	26.6±9.5	0.892§
	P1	0.000§	0.517	

\*Values are means±SD.

†Data at 12 weeks are for 11 rats from the control group and 46 rats from the infection group.

‡Independent samples t-test (p&lt;0.05: significant difference).

§Analysis of covariance (ANCOVA) (p&lt;0.05: significant difference), adjusted by baseline covariate.

¶Paired samples t-test (p&lt;0.05: significant difference).

HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α.

infected group compared with the non-infected group. The HOMA-IR index indicated significantly improved insulin sensitivity in the infected group. Serum triglycerides and total cholesterol concentrations were significantly lower in the infected group than the non-infected group after 12 weeks of infection. There was no significant change in TNF-α, IL-6 and MCP-1 levels in both groups (table 1).

## 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. After 12 weeks, a significant improvement was observed in fasting blood glucose, fasting serum insulin, insulin sensitivity, serum triglycerides, and total cholesterol in the infected group compared with the control group. There were no significant differences in inflammatory biomarkers including TNF-α, IL-6 and MCP-1 levels between infected and non-infected groups.

Obesity is a complex condition with many causal contributors leading to adverse metabolic effect on blood glucose, lipid profile, and so on, and increasing incidence of many chronic diseases. Genetic inheritance, overeating and underactivity are considered as the main etiological factors of obesity. Moreover, a subset of obesity may be caused

by specific infection. In the past 30 years, infectoobesity, obesity of infectious origin, has identified 10 pathogens that associate with obesity in animal models. Adenoviruses are the first infective agents that have been reported to cause obesity in both animal models and naturally infected humans. Adenoviruses infection is common in the general population and causes respiratory diseases, gastroenteritis, conjunctivitis and cystitis. Ad-36, a human adenovirus, was the first human adipogenic virus reported. Previously, studies on Ad-36 have shown a significant increase in body weight in mice (7%), marmosets (12%), but not in chickens.<sup>10–12</sup>

In a rat model,<sup>13</sup> after 12 weeks postinfection, the body weight was not different between the infected and non-infected groups; however, after 30 weeks postinoculation, the body weight of the infected group was significantly greater than the control group. In agreement with Pascarica *et al*,<sup>13</sup> the body weight of rats in our study did not differ significantly after 12 weeks of infection.<sup>21</sup> Based on animal experiments, development of a significant weight gain in rodent and marmoset experimental models seems to require a longer follow-up duration, approximately 6 months.<sup>13 21</sup> In spite of no alteration in body weight in the current study, we observed a significant increase in insulin sensitivity in the infected rats compared with uninfected counterparts. The previous rat study showed that Ad-36-infected rats had an increase in insulin sensitivity and glucose uptake.<sup>13</sup> Furthermore, they have reported similar findings in an in vitro study with Ad-36-infected primary adipocytes of rats demonstrating an increased glucose use.<sup>13</sup> Recent studies suggest that Ad-36 gene mediated glucose disposal through the Ras/phosphoinositide 3-kinase (PI3K) pathway to improve glucose uptake, independently from insulin activation.<sup>24 25</sup> It has been reported that Ad-36 increases cellular glucose uptake via a Ras/PI3K pathway in mice fed a high-fat diet.<sup>26</sup> Ad-36 predicts better glycemic control by stimulating glucose uptake in skeletal muscle, adipose tissue and suppressing hepatic glucose production in the mice model.<sup>26</sup> Wang *et al*<sup>25</sup> confirmed an increase in glucose transporter type 1 (GLUT1) and type 4 (GLUT4) gene expression mediated by Ras-activated PI3K pathway in Ad-36-infected diabetic and non-diabetic human skeletal muscle cells. Several studies have focused on the virus E4 open reading frame 1 (E4orf1) protein as the mediator of Ad-36-induced glucose uptake.<sup>25 27 28</sup> E4orf1, a viral gene expressed early during Ad-36 infection, is a 125-amino acid peptide. E4orf1 protein of Ad-36 is necessary for Ad-36 to induce adipogenesis and improve glycemic control. The E4orf1 protein is responsible for increasing glucose uptake due to the translocation of GLUT4 via the Ras-PI3K pathway. E4orf1-induced PI3K activation is also responsible for the increased expression of GLUT1 and GLUT4.<sup>29 30</sup> In vitro, Ad-36 significantly increases glucose uptake into 3T3-L1 preadipocyte via E4orf1 protein.<sup>28</sup> Moreover, E4orf1 is expressed in adipose tissue or liver in Ad-36-infected animals and enhanced viral replication.<sup>24</sup> Yoon *et al*<sup>31</sup> showed that in the experimental infected high-fat, diet-fed and streptozotocin-injected mice adipocyte targeting sequence, E4orf1 improved the ability to eliminate excess glucose from the blood and ameliorated liver function in disease models of type 2 and type 1 diabetes, respectively. These findings suggest a strong effect of E4orf1 on glucose disposal. Several gaps in knowledge

still exist about the development of vaccines to prevent Ad-36-induced obesity and antidiabetic agents based on the insulin sparing effect of the E4orf1 protein.<sup>28–31</sup> Similar to the results in previous animal models, we found that Ad-36 infection significantly reduces serum cholesterol and triglyceride concentrations in Wistar rats. A reduction of total cholesterol was found in hamsters, monkeys and non-human primates after infection with Ad-36.<sup>10 12 29</sup> In the study performed on Ad-36-infected Wistar rats for a long time (12 and 30 weeks), serum triglyceride levels were significantly greater for the infected group and serum cholesterol levels were similar for the 2 infected and control groups.<sup>13</sup> The infection with Ad-36 increases adiposity in rats without concomitant hypolipidemia. Our study showed that serum lipids were decreased, without any significant change in body weight in the Ad-36-infected rats. This may suggest that the adipogenic and hypolipidemic effects of Ad-36 are mediated through separate pathways. The mechanisms of changes in lipid metabolism related to Ad-36 have not yet been fully elucidated.

In addition, Ad-36 could cause low-grade inflammation by increasing the infiltration of macrophages and the number of macrophages in adipose tissue via production of MCP-1, TNF- $\alpha$  and IL-6.<sup>5 32</sup> MCP-1 plays a key role in stimulating macrophage migration into adipose tissue.<sup>17</sup> The correlation between Ad-36-induced obesity and inflammatory cytokines have been reported from a few epidemiological and cellular studies.<sup>16–18</sup> Na *et al*<sup>33</sup> showed that in the experimental infected mice model, Ad-36 infection stimulated an inflammatory state through activation of nuclear factor kappa-light-chain-enhancer of activated B cells, and consequently increasing MCP-1 levels, which in turn induces the infiltration of macrophages into adipocytes. MCP-1 signaling has a direct role in the development of Ad-36-induced obesity. The mechanism of change in inflammation markers and attributable to Ad-36 is still not fully understood. There are some limitations in this study. First, only male rats were included in the current study. It is because the menstrual cycle was found to be a confounding factor in female rats. Therefore, these results should be generalized cautiously to males. Lack of food intake measurements was an additional limitation. A major limitation of our study is that the number of infected and control groups was not equal and it was affected by the power of statistical analysis. Finally, we only focused on the blood sampling and we did not assess the fat pad and adipose tissue. Further investigations on viruses contributing to obesity, inflammation markers and favorable effects of Ad-36 on metabolic consequences of obesity are required.

**Acknowledgements** We thank the Ahvaz Jundishapur University of Medical Sciences. The authors offer their special thanks to Elham Mosavi for her help in cell culture. The authors appreciate Mehdi Zarei and Mohammad Ebrahim Azemi for their kind advice and cooperation.

**Contributors** FS, MK and AT designed the research project. FS and AT performed the preparation of cell cultures, viral suspension and injection. FS and MR performed the sampling and data collection. FS, SML and MK analyzed and interpreted the data. FS wrote the draft of the paper. MK, AT and AJM read the final draft and commented on it. All authors read and approved the final manuscript.

**Funding** This paper is issued from the PhD thesis of FS, which was financially supported by grant (NRC 9204) from Vice-Chancellor for Research Affairs of Ahvaz Jundishapur University of Medical Sciences.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** The ethical committee of experimental animal care at Ahvaz Jundishapur University of Medical Sciences (NRC 9204) approved the study. The study was performed in accordance with the National Research Council (US) Committee for Guide for the Care and Use of Laboratory Animals, 8th Edition.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. Data are available by emailing MK (mkarandish@yahoo.com) and FS (shirani\_ir@yahoo.com).

#### ORCID iD

Fatemeh Shirani <http://orcid.org/0000-0001-5223-2530>

#### REFERENCES

- World Health Organization. Overweight. Available: <http://www.who.int/mediacentre/factsheets/fs311/en/> [Accessed 5 Jun 2015].
- Huo L, Lyons J, Magliano DJ. Infectious and environmental influences on the obesity epidemic. *Curr Obes Rep* 2016;5:375–82.
- Atkinson RL, Dhurandhar NV, Allison DB, *et al*. Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes* 2005;29:281–6.
- Dhurandhar NV, Bailey D, Thomas D. Interaction of obesity and infections. *Obes Rev* 2015;16:1017–29.
- Ponterio E, Gnessi L. Adenovirus 36 and obesity: an overview. *Viruses* 2015;7:3719–40.
- Villavicencio F, Valladares M. [The potential contribution of adenovirus 36 to the development of obesity]. *Rev Med Chil* 2017;145:1054–9.
- Trovato GM, Martines GF, Garozzo A, *et al*. Ad36 adipogenic adenovirus in human non-alcoholic fatty liver disease. *Liver Int* 2010;30:184–90.
- Almgren M, Atkinson R, He J, *et al*. Adenovirus-36 is associated with obesity in children and adults in Sweden as determined by rapid ELISA. *PLoS One* 2012;7:e41652.
- Karamese M, Altöparlak U, Turgut A, *et al*. The relationship between adenovirus-36 seropositivity, obesity and metabolic profile in Turkish children and adults. *Epidemiol Infect* 2015;143:3550–6.
- Dhurandhar NV, Israel BA, Kolesar JM, *et al*. Increased adiposity in animals due to a human virus. *Int J Obes Relat Metab Disord* 2000;24:989–96.
- Dhurandhar NV, Israel BA, Kolesar JM, *et al*. Transmissibility of adenovirus-induced adiposity in a chicken model. *Int J Obes Relat Metab Disord* 2001;25:990–6.
- Dhurandhar NV, Whigham LD, Abbott DH, *et al*. Human adenovirus Ad-36 promotes weight gain in male rhesus and marmoset monkeys. *J Nutr* 2002;132:3155–60.
- Pasarica M, Shin AC, Yu M, *et al*. Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. *Obesity* 2006;14:1905–13.
- Dhurandhar NV, Kulkarni PR, Ajinkya SM, *et al*. Association of adenovirus infection with human obesity. *Obes Res* 1997;5:464–9.
- Bastard J-P, Maachi M, Lagathu C, *et al*. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur Cytokine Netw* 2006;17:4–12.
- Bouwman JJM, Vissers FLJ, Bouter KP, *et al*. Infection-Induced inflammatory response of adipocytes in vitro. *Int J Obes* 2008;32:892–901.
- Na H-N, Nam J-H. Adenovirus 36 as an obesity agent maintains the obesity state by increasing MCP-1 and inducing inflammation. *J Infect Dis* 2012;205:914–22.
- Berger PK, Pollock NK, Laing EM, *et al*. Association of adenovirus 36 infection with adiposity and inflammatory-related markers in children. *J Clin Endocrinol Metab* 2014;99:3240–6.
- Genoni G, Prodam F, Marolda A, *et al*. Obesity and infection: two sides of one coin. *Eur J Pediatr* 2014;173:25–32.
- Iannaccone PM, Jacob HJ. Rats! *Dis Model Mech* 2009;2:206–10.
- Shirani F, Teimoori A, Rashno M, *et al*. Using rats as a research model to investigate the effect of human adenovirus 36 on weight gain. *ARYA Atheroscler* 2017;13:167–71.
- Wellington D, Mikaelian I, Singer L. Comparison of ketamine-xylazine and ketamine-dexmedetomidine anesthesia and intraperitoneal tolerance in rats. *J Am Assoc Lab Anim Sci* 2013;52:481–7.
- Cacho J, Sevillano J, de Castro J, *et al*. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab* 2008;295:E1269–76.

- 24 Dhurandhar EJ, Dubuisson O, Mashtalir N, *et al.* E4orf1: a novel ligand that improves glucose disposal in cell culture. *PLoS One* 2011;6:e23394.
- 25 Wang ZQ, Cefalu WT, Zhang XH, *et al.* Human adenovirus type 36 enhances glucose uptake in diabetic and nondiabetic human skeletal muscle cells independent of insulin signaling. *Diabetes* 2008;57:1805–13.
- 26 Krishnapuram R, Dhurandhar EJ, Dubuisson O, *et al.* Template to improve glycemic control without reducing adiposity or dietary fat. *Am J Physiol Endocrinol Metab* 2011;300:E779–89.
- 27 Dhurandhar NV. Insulin sparing action of adenovirus 36 and its E4orf1 protein. *J Diabetes Complications* 2013;27:191–9.
- 28 Krishnapuram R, Dhurandhar EJ, Dubuisson O, *et al.* Doxycycline-regulated 3T3-L1 preadipocyte cell line with inducible, stable expression of adenoviral E4orf1 gene: a cell model to study insulin-independent glucose disposal. *PLoS One* 2013;8:e60651.
- 29 Kim J, Na H, Kim J-A, *et al.* What we know and what we need to know about adenovirus 36-induced obesity. *Int J Obes* 2020. doi:10.1038/s41366-020-0536-4. [Epub ahead of print: 15 Jan 2020].
- 30 Akheruzzaman M, Hegde V, Dhurandhar NV. Twenty-Five years of research about adipogenic adenoviruses: a systematic review. *Obes Rev* 2019;20:499–509.
- 31 Yoon I-S, Park S, Kim R-H, *et al.* Insulin-sparing and fungible effects of E4orf1 combined with an adipocyte-targeting sequence in mouse models of type 1 and type 2 diabetes. *Int J Obes* 2017;41:1601–5.
- 32 Kapila M, Khosla P, Dhurandhar NV. Novel short-term effects of adenovirus Ad-36 on hamster lipoproteins. *Int J Obes Relat Metab Disord* 2004;28:1521–7.
- 33 Na H-N, Kim H, Nam J-H. Novel genes and cellular pathways related to infection with adenovirus-36 as an obesity agent in human mesenchymal stem cells. *Int J Obes* 2012;36:195–200.