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RESEARCH LETTER

Natural infection of human adenovirus 36 in rhesus monkeys is associated with a reduction in fasting glucose

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Experimental infection of the human adenovirus Ad36 increases adiposity, yet improves glycemic control in rodents.[1] In humans, natural Ad36 infection is cross-sectionally and temporally associated with adiposity and better glycemic control.[1–3] In vitro studies indicate that the early gene 4, open reading frame 1 (*E4orf1*) gene of Ad36 is necessary and sufficient to improve cellular glucose disposal.[4,5] Consequentially, E4orf1 protein offers an excellent template to develop antidiabetic drugs.[4] Considering the human relevance of a rhesus monkey model for preclinical drug development, we determined the associations of natural Ad36 infection with changes in glycemic control in rhesus monkeys.

For this study, serum samples were obtained from 20 male rhesus monkeys (*Macaca mulatta*; 7–13 years of age) enrolled in an ongoing study described earlier.[6] Briefly, all rhesus monkeys received a diet high in fat and sugar (42% kcal from fat, 27% kcal from sucrose; HFS) and were randomized to two experimental groups (n = 10 in each group), one on the HFS diet and the other on the HFS diet with resveratrol treatment (RESV). The resveratrol dose was 80 mg/day for 0–12 months and 480 mg/day for 13–24 months. All procedures were approved by the Animal Care and Use Committee of the National Institute of Aging Intramural Program. Serum samples obtained at 12 months were screened for neutralizing antibodies to Ad36 by a serum neutralization assay.[7] A general linear model approach was used to examine the association of resveratrol treatment and Ad36 infection on body weight, total body fat, and fasting glucose over 1 year in rhesus monkeys fed the HFS diet. Here, body fat was determined by dual-energy X-ray absorptiometry (DPX-α X-ray Bone Densitometer; Lunar, Madison, WI, USA) and fasting blood glucose was determined with an Ascensia Breeze 2 Blood Glucose Monitor (Bayer HealthCare, Mishawaka, IN, USA). For the present analysis, samples obtained at 12 months served as baseline measures.

Change data (values at 24 months – values at baseline) were log transformed to increase normality and homoscedasticity. Statistical analyses included separate two-way analysis of covariance for each dependent variable, with Ad36 status and resveratrol as fixed factors and baseline measures as covariates. Data from two rhesus monkeys were removed from all analyses because of extreme values.

At baseline, there were no differences in HFS and RESV groups for body weight, body fat, or fasting glucose (Table 1). In the HFS group, four rhesus monkeys were Ad36 seropositive (POS) and four were negative (NEG). In the RESV group, five were POS. At baseline, Ad36 status was not significantly associated with body weight, body fat, or fasting glucose. Documentation of food consumption by individual rhesus monkeys throughout the study indicated that there was no difference in average consumption between groups on a per kg body weight basis. When examining the descriptive data, we found that there was a slight increase in body weight across all groups during the 1-year time period, but the difference did not reach statistical significance. In addition, body fat for those rhesus monkeys identified as Ad36 POS increased from a mean ± SD) of 4.80 ± 1.11 kg at baseline to 6.03 ± 1.32 kg 1 year later. Those rhesus monkeys that remained Ad36 NEG throughout the 1-year testing period also had a non-significant increase in body fat (from 4.02 ± 0.75 to 4.80 ± 0.67 kg). Although the Ad36 POS group gained fat, the magnitude of the change in body fat was not significantly different between the Ad36 POS and NEG groups.

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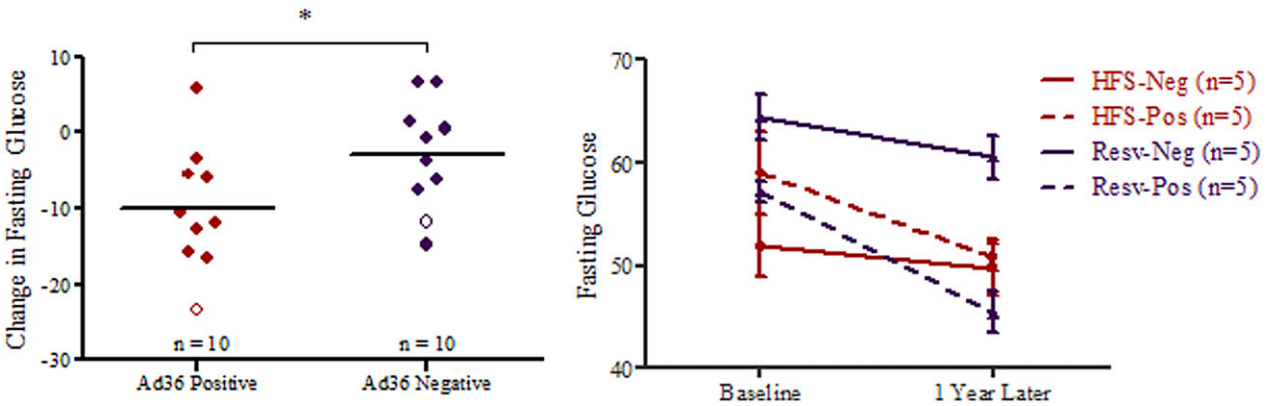

Figure 1 Fasting glucose and Ad36 status of rhesus monkeys during Years 1–2 of a high-fat, high-sugar (HFS) diet study in which half the animals were receiving daily resveratrol (Resv) treatment. (a) Two-way ANCOVA evaluating changes in fasting glucose revealed a significant association with Ad36 status ($P = 0.021$), with Ad36-positive rhesus monkeys having greater change in fasting glucose over the 1-year time period (the open symbols indicate data points removed from analysis). (b) A significant experimental group × Ad36 status interaction was also found ($P < 0.001$), suggesting that fasting glucose levels are affected by Ad36 status based on resveratrol treatment group. Data are the mean ± SEM. Neg, Ad36 negative; Pos, Ad36 positive.

Table 1 Characteristics of rhesus monkeys receiving a high-fat, high-sucrose diet for 1 year, with or without resveratrol treatment

	HFS (n = 8)	RESV (n = 10)
Age (years)	12.4 ± 1.9	12.0 ± 1.7
Body weight (kg)	16.3 ± 5.2	14.7 ± 3.7
Body fat (kg)	5.2 ± 3.1	3.8 ± 2.4
Fasting glucose (mg/dL)	53.2 ± 8.4	60.8 ± 5.2

Data are the mean ± SD.
Note, there were no significant differences between the resveratrol-treated (RESV) and untreated (HFS) groups in age, body weight, body fat, or fasting glucose after 1 year on the high-fat, high-sucrose diet.

For change in fasting glucose, there was a significant main effect for Ad36 status ($F_{(1,13)} = 6.93$; $P = 0.021$), with Ad36 POS rhesus monkeys having a greater change in fasting glucose (decrease >15%) over the 1-year period (Fig. 1a). There was a significant experimental group × Ad36 status disordinal interaction ($F_{(1,\ 13)} = 35.90$; $P < 0.001$), indicating that fasting glucose levels were linked to Ad36 status based on resveratrol treatment (Fig. 1b).

A previous study has shown that Ad36 infection is causatively and correlatively linked with gain in adiposity in marmosets and rhesus monkeys, respectively.[8] However, the relationship, if any, of Ad36 with changes in glycemic control is unknown in non-human primates. The present study indicates that Ad36 infection may be linked with improvement in glycemic control in rhesus monkeys, supporting the need for further scientific investigation in this area. The limitations of the present study include a small sample size and the absence of data indicating the precise timing of Ad36 seroconversion. Although serum neutralization is the gold standard method for determining neutralizing antibodies, seropositivity only indicates an association with the changes observed, and not causation. Nonetheless, the findings suggest that rhesus monkeys may be a suitable model in which to test the antidiabetic effects of Ad36 and E4orf1 in vivo. Finally, the presence of natural Ad36 infection and its association with phenotypic changes in rhesus monkeys suggests that screening rhesus monkeys for Ad36 seropositivity may be prudent to avoid potential confounding effects.

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Disclosure

NVD has had the following patents granted: US patent no. 6 127 113: Viral obesity methods and compositions; US patent no. 6 664 050: Viral obesity methods and compositions; US patent no. 8008,436B2: Adenovirus 36 E4orf1 gene and protein and their uses. NVD has applied for the following patents: Adenovirus Ad36 E4orf1 protein for prevention and treatment of non-alcoholic fatty liver disease, July 2010; Enhanced glycemic control using Ad36E4orf1 and AKT1 inhibitor, January 2012. NVD has ongoing grant support from Vital Health Interventions (Daton, OH, USA) for determining antidi-

abetic properties of E4orf1 protein. The other authors declare no conflicts of interest.

References

1. 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**: E779–789.
2. Dhurandhar NV. A framework for identification of infections that contribute to human obesity. *Lancet Infect Dis*. 2011; **11**: 963–9.
3. Lin W, Dubuisson O, Rubicz R et al. Long term changes in adiposity and glycemic control are associated with past adenovirus infection. *Diabetes Care*. 2013; **36**: 701–7.
4. Dhurandhar NV. Insulin sparing action of Adenovirus 36 and its E4orf1 protein. *J Diabetes Complicat.*. 2013; **27**: 191–9.
5. Dhurandhar EJ, Dubuisson O, Mashtalir N, Krishnapuram R, Hegde V, Dhurandhar NV. E4orf1: A novel ligand that improves glucose disposal in cell culture. *PLoS ONE*. 2011; **6**: e23394.
6. Jimenez-Gomez Y, Mattison JA, Pearson KJ et al. Resveratrol improves adipose insulin signaling and reduces the inflammatory response in adipose tissue of rhesus monkeys on high-fat, high-sugar diet. *Cell Metab*. 2013; **18**: 533–45.
7. Rogers PM, Mashtalir N, Rathod MA et al. Metabolically favorable remodeling of human adipose tissue by human adenovirus type 36. *Diabetes*. 2008; **57**: 2321–31.
8. 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.