Published in final edited form as:
J Diabetes. 2014 November ; 6(6): 614–616. doi:10.1111/1753-0407.12178.

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

Nikhil V. Dhurandhar[1], **Emily J. Dhurandhar**[2], **Donald K. Ingram**[1], **Kelli Vaughan**[3,4], and **Julie A. Mattison**[3]

[1]Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, LA 70808

[2]University of Alabama, Birmingham, AL, 35205

[3]Translational Gerontology Branch, National Institute on Aging, NIH, Baltimore, MD 21224

[4]SoBran, Inc., Burtonsville, MD 20866, USA

To the Editor

Experimental infection of a 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 indicated that the *E4orf1* (early gene 4, open reading frame 1) gene of Ad36 is necessary and sufficient to improve cellular glucose disposal[4,5]. Consequentially, E4orf1 protein offers an excellent template to develop anti-diabetic drugs[4]. Considering the human relevance of a rhesus monkey model for pre-clinical drug development, we determined the associations of natural Ad36 infection with changes in glycemic control in rhesus monkeys.

Methods

For this study, serum samples were obtained from 20 male rhesus monkeys (*Macaca mulatta*) (age 7 – 13 y) enrolled in an ongoing study described earlier[6]. Briefly, all monkeys received a diet high in fat and sugar (42% kcal from fat and 27% kcal from sucrose; HFS) and were randomized into two experimental groups, HFS diet (N=10) or HFS with resveratrol treatment (RESV, N=10). The resveratrol dose was 80mg/d for 0 – 12 months, and 480mg/d 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 serum

Author for Correspondence and reprint request: Nikhil V. Dhurandhar, PhD., Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808, Tel: 225 763-2741; Fax: 225 763-3030, Nikhil.Dhurandhar@PBRC.EDU.

Competing interest declaration:
NVD: The following Patents are granted or have been applied for: Patent number 6,127,113: Viral obesity methods and compositions. Patent number 6,664,050: Viral obesity methods and compositions. Patent number US 8,008,436B2, dated August 30, 2011: Adenovirus 36 E4orf1 gene and protein and their uses. Provisional patent filed: Adenovirus Ad36 E4orf1 protein for prevention and treatment of non-alcoholic fatty liver disease, July 2010. Provisional patent filed: Enhanced glycemic control using Ad36E4orf1 and AKT1 Inhibitor. January 2012.

Other authors declare no conflict of interest.

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 one year in animals fed a HFS diet. Here, body fat was determined by dual-energy x-ray absorptiometry (Lunar, DPX-α X-Ray Bone Densitometer, Madison, WI), and fasting blood glucose was determined with an Ascensia Breeze 2 Blood Glucose Monitor (Bayer HealthCare LLC., Mishawaka, IN). For the current analysis, samples obtained at 12 months will serve as baseline measure.

Change data (24 months – baseline) were log transformed (base-10) 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 subjects were removed from all analyses due to extreme values

Results

At baseline, there was no difference in the HFS and RESV groups for body weight, body fat, or fasting glucose (Table 1). In the HFS group, four animals 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 animals 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 one year time period. Also, body fat for those monkeys identified as Ad36 POS increased from 4.80±1.11 kg at baseline to 6.03±1.32 kg (Mean±SEM) one year later. Those monkeys that remained negative throughout the one year testing period also had a non-significant increase in body fat (from 4.02±0.75 kg to 4.80±0.67 kg). Although the POS group gained fat, the magnitude of change in body fat was not statistically different between the POS and NEG groups.

For change in fasting glucose, there was a statistically significant main effect for Ad36 status [$F(1, 13) = 6.93$, $p = 0.021$] with POS animals having a greater change in fasting glucose (decrease of over 15%) over the one year period (Figure 1A). There was a significant experimental group by 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 (Figure 1B).

Comments

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. This 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 this study include a small sample size treatment due to RESV, and the absence of data indicating the precise timing of Ad36 seroconversion. While 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 to test the anti-diabetic 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.

Acknowledgments

NVD has ongoing grant support from Vital Health Interventions for determining anti-diabetic properties of E4orf1 protein. This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

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(5):E779–E789. [PubMed: 21266671]
2. Dhurandhar NV. A framework for identification of infections that contribute to human obesity. Lancet Infect Dis. 2011; 11:963–969. [PubMed: 22115071]
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(3):701–707. [PubMed: 23160725]
4. Dhurandhar NV. Insulin sparing action of Adenovirus 36 and its E4orf1 protein. Journal of Diabetes and Its Complications. 2013; 27(2):191–199. [PubMed: 23246247]
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(8):e23394. [PubMed: 21886789]
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 metabolism. 2013 Oct 1; 18(4):533–545. [PubMed: 24093677]
7. Rogers PM, Mashtalir N, Rathod MA, et al. Metabolically favorable remodeling of human adipose tissue by human adenovirus type 36. Diabetes. 2008 Sep; 57(9):2321–2331. [PubMed: 18599527]
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 Oct; 132(10):3155–3160. [PubMed: 12368411]

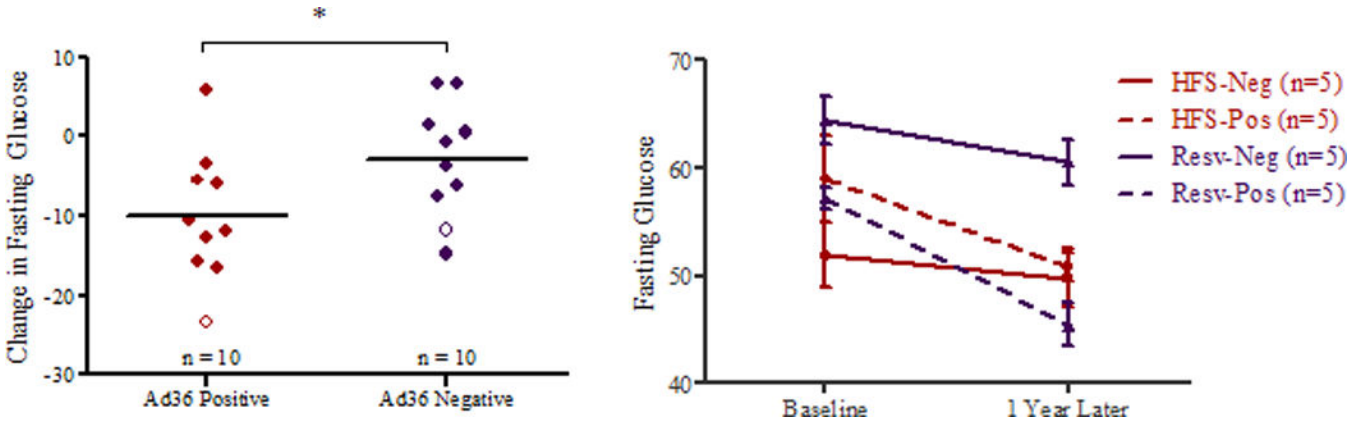

Figure 1.

Fasting glucose (mg/dL) values and Ad36 status of rhesus monkeys during year 1 – 2 of a HFS diet study where half of the animals were receiving daily resveratrol treatment. A two-way ANCOVA to evaluate changes in fasting glucose revealed a significant association for Ad36 status ($p = 0.021$) with Ad36 positive animals having greater change in fasting glucose over the one year time period (A) (removed data points are included in the graph ○). A significant experimental group by Ad36 status interaction was also found ($p < 0.001$) suggesting that fasting glucose levels are affected by Ad36 status based on resveratrol treatment group (B) (Mean ±SEM).

Table 1

Characteristics of monkeys receiving a high fat and sucrose diet for one year with or without resveratrol treatment.

	Mean ± SD	
	HFS	**RESV**
N	8	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

Note: There were no significant differences between groups in age, body weight, body fat, or fasting glucose after one year on a high fat diet.