

REVIEW



## Role of adenoviruses in obesity

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### SUMMARY

Five human adenovirus subtypes, Ad5, Ad9, Ad31, Ad36, and Ad37, and a non-human adenovirus, SMAM1, are linked to increased adiposity *in vitro* or *in vivo*. Experimental infection with Ad5, Ad36, and Ad37 produced excess adiposity or weight gain in animals. Ad9 and Ad31 increase fat storage in tissue culture but are not associated with animal or human obesity. Ad36 is the most extensively studied adipogenic adenovirus and is correlated with some measure of overweight/obesity in humans from multiple countries. The correlation is strongest and most consistent in children, but some studies have been negative in both children and adults. About 30% of overweight/obese children and adults and about 15–20% of lean individuals have Ad36 antibodies in epidemiologic studies. The mechanisms of action of Ad36 are due to the early gene 4, open reading frame 1 (E4-ORF1). Blocking E4-ORF1 with siRNA prevents the effects of Ad36, and transfection of lentivirus with E4-ORF1 reproduces the Ad36 effects. Increased adiposity is caused by stimulation of at least three pathways by Ad36. Cell membrane glucose receptors are increased via the Ras pathway, leading to increased intracellular glucose. Fatty acid synthase is increased, which converts the glucose to fatty acids. Finally, peroxisome proliferator-activated receptor- $\gamma$  is increased, resulting in differentiation of adult stem cells into adipocytes. Conclusions: several adenoviruses increase adiposity in animals and are associated with obesity in humans. There are critical gaps in the literature needing further investigation including evaluation of other adenovirus subtypes and better research designs to improve the strength of causal inferences. Copyright © 2015 John Wiley & Sons, Ltd.

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### INTRODUCTION

*Adenoviridae* are a large family of viruses with seven human species (A–G) that include over 60 subtypes, many of which are known to cause human disease such as conjunctivitis, respiratory disease, and/or gastrointestinal disease. The metabolic effects of adenoviruses have gained more interest since the 1990s when SMAM1 was shown to cause adiposity in chickens and antibody to SMAM1 was associated with human obesity [1,2]. Since then, the metabolic effects of a total of nine adenoviruses have been investigated in cells, animal studies, and/or in human epidemiologic studies. Because of several recent publications investigating additional subtypes (Ad8 and Ad9) and identifying novel associations (Ad5 and Ad31), there are currently no reviews that provide

a panoptic view of the relationship between all investigated adenoviruses and obesity. Thus, we sought to review and synthesize the current evidence. We also discuss the E4-ORF1 protein of Adenovirus 36 (Ad36), sometimes referred to as a dUTPase. This protein is necessary and sufficient for the adipogenic effect of Ad36 [3], so we compare overall amino acid homology of this protein among adenoviruses. Finally, we discuss directions for future research including candidate serotypes and the implications for research design.

The nine adenoviruses that have been investigated for metabolic effects thus far include four species D types (Ad8, Ad9, Ad36, and Ad37), two species C types (Ad2 and Ad5), one species A type (Ad31), and two non-human adenoviruses (chick embryo lethal orphan virus and SMAM1) (Table 1).

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### ADENOVIRUS SPECIES D

Adenovirus 36 was the first human adenovirus to be associated with human adiposity and has the

**Table 1. A summary of current synthesized evidence**

Adenovirus		Adipogenic evidence				E4-ORF1 homology (score out of 253)**	Prevalence in obesity studies (%) <sup>††</sup>
Species	Type	<i>In vitro</i>	In animals	OR child	OR adult		
A	Ad31	Yes	No (chickens)	Untested	1.4 (0.3–7.8)*	127	31–73
C	Ad2	No	No (chickens)	Untested	0.8 (0.3–1.8)	108	78
	Ad5	Yes/no <sup>†</sup>	Yes (mice)	5.5 (1.6–23.9)	0.2 (0.0–1.1)	108	18
D	Ad8	No <sup>†</sup>	Untested	1.7 (0.5–7.0)*	Untested	122	11
	Ad9	Yes	Untested	Untested	Untested	239	Untested
	Ad36	Yes <sup>†</sup>	Yes (multiple)	2.0 (1.3–2.9)	1.4 (1.0–2.2)*	253	5–74
	Ad37	Yes	+/- Chickens <sup>‡</sup>	Untested	0.3 (0.0–3.6)*	122	3–18
Non-human	SMAM1	Untested	Yes (chickens)	Untested	BMI +4.8	Unknown	24
	CELO	Untested	No (chickens)	Untested	Untested	Unknown	Untested

OR, odds ratio; BMI, body mass index; CELO, chick embryo lethal orphan virus.

\*These findings are not statistically significant. Additionally, there was significant heterogeneity among the studies reporting the results for the association between Ad31, Ad36, and Ad37 and obesity among adults. Heterogeneity is not known for Ad8, as there has only been one study in children.

<sup>†</sup>Most *in vitro* studies have used 3T3-L1 cells, but Ad5, Ad8, and Ad36 have been tested in Colo-320 cells. Ad36 is adipogenic in both. Ad8 was only tested in Colo-320 cells and was not adipogenic. Ad5 was adipogenic in Colo-320 cells but not in 3T3-L1 cells. Additionally, Ad5 has also been shown to have metabolic (glycolytic enzyme upregulation) effects of its E4-ORF1 protein in epithelial cells.

<sup>‡</sup>Ad37-infected chickens had more adiposity than other chickens. Ad37-infected chickens also weighed more than Ad2-infected chickens, but body weight was not different than uninfected chickens.

\*\*Score per <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.

<sup>††</sup>Prevalence as noted in references cited in text [60–62].

largest evidence base including the most animal models (chickens, rats, mice, and monkeys) [4–7], numerous human cross-sectional studies [8,9], three longitudinal studies [10–12], and even an association among sero-discordant twins [13]. Furthermore, the adipogenic mechanisms for Ad36 have been extensively investigated and are derived from the early gene 4, open reading frame 1 (E4-ORF1) [3], a gene that is entirely conserved after approximately 12 serial *in vitro* passages [14]. The viral mRNA is necessary for the effect regardless of DNA replication [15]. Blocking E4-ORF1 with siRNA prevents the effects of Ad36, and transfection of lentivirus with E4-ORF1 reproduces the Ad36 effects [3,16]. The E4-ORF1 gene creates a 125-amino acid protein by the same name, E4-ORF1 [3,16], which is known to have conserved sites important for functional binding [17]. The adipogenesis occurs via at least three molecular pathways. First, the protein increases cell membrane glucose receptors (Glut4 and Glut1) via the Ras pathway, upregulating Phosphatidylinositol 3-kinase and leading to increased intracellular

glucose [16,18,19]. For this pathway, mutation of a specific protein binding domain (i.e., PDZ binding domain) prevents the activation of Ras [16]. PDZ was named based on the three proteins a 95kda protein involved in post-synaptic density named PSD-95, a Drosophila discs large protein named Dlg and the zonula occludens 1 protein (ZO1). Secondly, enzymatic activity of acetyl Co-A carboxylase-1 and fatty acid synthase is increased, which converts the glucose to fatty acids [20]. Thirdly, peroxisome proliferator-activated receptor- $\gamma$  is increased, resulting in differentiation of adult stem cells into adipocytes [21,22].

Aside from the acute adipogenesis, the virus could also affect the maintenance of obesity with lowered fat oxidation [23] and altered hormone expression [20,24]. Leptin is an appetite hormone that normally increases after lipid accumulation, but adipocyte secretion of leptin (normalized to lipid content) is lower in adenovirus 36-infected adipocytes [20]. Additionally, Ad-36 (unlike E4-ORF1) stimulates an inflammatory response [25], and monocyte

chemoattractant protein 1 (MCP-1) shows an important role in maintaining obesity in mice [24], while an anti-inflammatory supplement (Mulberry extract containing resveratrol) reduces MCP-1 and reduces body fat in infected mice [26]. An inflammatory pathway for obesity maintenance is coherent with other basic science demonstrating the causal role of low-grade inflammation mediated via MCP-1 and proliferator-activated receptor- $\gamma$  in the development of obesity in mice [27].

Despite this mounting evidence, there has also been some seemingly conflicting data including a study in monkeys showing no association with body weight [28] and some cross-sectional studies that have not shown an adipogenic association in humans [9,29]. Two meta-analyses both supported a persistent association between human obesity and Ad36 (most recent, odds ratio [OR]=1.6, 95% confidence interval [CI] 1.1–2.3), but both also showed significant heterogeneity that was not attributable to chance alone (most recent,  $I^2=77%$ ,  $p<0.001$ ) [8,9]. The most recent meta-analysis (February 2014) also performed meta-regression and found a significant association and 0% heterogeneity among the four studies that only included children (OR=2.0, 95% CI 1.3–2.9). Three more recent studies among children not included in this meta-analysis also found an association between obesity and Ad36 (OR=3.3, 95% CI 1.1–11.0; OR=1.5, 95% CI 1.1–2.0; OR=2.6,  $p=0.01$ ) [30–32]. Two additional studies among children were not included because they reported a mean body mass index (BMI) among children (as compared with obesity as a dichotomous outcome), but these two also showed an association between Ad36 and higher mean BMI [33,34]. Preliminary data indicate children with Ad36 infection are less successful in losing weight, which is also suggestive of an age interaction because adults with Ad36 infection appear more successful in losing weight after being placed on a diet [34–36]. The relationship is likely more complex than simply based on age alone because a longitudinal study found a stronger association with adiposity in older subjects with infection [10]. Instead, age could be a surrogate of coinfection with other adenoviruses, time since seroconversion, or other factors related to age.

Another complexity with adenovirus 36 is the associated metabolic outcomes beyond body fat that are sometimes paradoxical when compared with obesity from other causes. For instance, some

human or non-human primate studies have shown lower serum insulin [10], lower serum glucose [28], and less diabetes [37], which is plausible based on the higher expression of the glucose receptors [16]. Similarly, human studies have shown less fatty liver disease among infected individuals [35,38,39]. Additionally, a favorable serum lipid profile has sometimes been observed in studies of both infected animals and humans [5,13], but this is not consistent [8,40–42]. Such heterogeneity between studies could be attributable to differences in the populations studied (e.g. age, race, or geography) [13,34,41].

Most other D species have  $\geq 84%$  homology with Ad36 at E4-ORF1 [17]. Adenovirus 37, on the other hand, is an exception, as it has a frame shift in E4-ORF1 leading to only 47% homology [17]. There is evidence of an adipogenic effect of Ad37 in cells [43], but the evidence in other models appears to point more toward visceral fat than obesity. For instance, Ad37-infected chickens developed more visceral and total body fat than Ad31, Ad2, or control chickens [43]. The Ad37-infected chickens also weighed more than Ad2-infected chickens, but the Ad37 chickens were not heavier than the uninfected control chickens [43]. A human cross-sectional study also showed an association between Ad37 and non-alcoholic fatty liver disease, but not obesity (OR=0.9, 95% CI 0.3–2.3), in a population with 18% prevalence of Ad37 [44]. Another study assessed Ad37 and obesity but did not report full results because there were only five subjects out of 198 testing positive for Ad37 (prevalence  $<3%$ ) [13]. Based on reporting of other results in this paper, assuming 148 subjects with obesity and 50 without, there was an inverse association (OR=0.1, 95% CI 0.0–0.8) with obesity. Combining these studies shows significant heterogeneity not explained by chance alone (OR=0.3, 95% CI 0.0–3.6,  $I^2=76%$ ,  $p=0.04$ ).

Ad8 and Ad9 have had only minimal testing for an adipogenic effect. The E4-ORF1 protein of Ad9 has 94% homology with Ad36 as compared with 84% for Ad8, but each has 100% similarity at the functional sites [17]. Ad9 is adipogenic in 3T3-L1 cells [45]. By contrast, Ad8 appears to not be adipogenic in Colo-320 cells [46]. Further, Ad8 was evaluated for an association with childhood obesity in Turkey, and it was not statistically significant (OR=1.7, 95% CI 0.5–7.0) [30]. Definitive interpretation, however, remains tentative because the sample size was small ( $n=120$ ), the prevalence

was low (6%), and the point estimate for the association is comparable to the estimate of association for Ad36 and childhood obesity (OR=2.0) from the most recent meta-analysis [9].

### ADENOVIRUS SPECIES C

Adenovirus 2 has the strongest evidence *against* an adipogenic effect. Ad2 and Ad5 are the only adenoviruses known to have no adipogenic effect in 3T3-L1 cells [43,47]. As noted in the previous text, the study in Ad2-infected chickens showed lower body weight (as compared with Ad37-infected chickens [43]). Additionally, epidemiologic investigation has shown no relationship between Ad2 and adult obesity in the United States [13].

Adenovirus 5 appears to have 100% homology at E4-ORF1 with Ad2 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), but the current evidence for an adipogenic effect of Ad5 is more complex than Ad2. Although a study from Poland reported Ad5 is not adipogenic in the 3T3-L1 cells (data were not shown) [47], it was adipogenic in a study reporting data in Colo-320 cells [46]. Further, molecular testing shows the Ad5 E4-ORF1 protein has a metabolic effect in cultured epithelial cells through activation of Myc, which enhances host cell glycolysis [48]. This type of cellular signaling resembles the molecular studies of Ad36's activity, which activates the Ras pathway [18] (upstream from Myc). Further, although Ad5 has not been studied in chickens, the virus is adipogenic in mice [49], and one epidemiologic study in children showed it was associated with obesity (OR=5.5, 95% CI 1.6–23.9) [30]. By contrast, a study among adults showed an inverse association (OR=0.2,  $p=0.04$  [13]). Combining these studies in a random effects model leaves no association (OR=1.2, 95% CI 0.1–27.5;  $I^2=91%$ ,  $p=0.01$ ). The heterogeneity between these studies is not due to chance alone. The differences in the populations might be related to testing method or geography, but the results were much different, and the seroprevalence was reasonably similar (11% and 18%). An alternative explanation is that the difference in association between children and adults mirrors the meta-regression of Ad36 data, which shows a stronger association with obesity in studies among children [9]. As noted in the previous text, age could be a surrogate of coinfection with other adenoviruses, time since seroconversion, or other factors related to age.

### ADENOVIRUS SPECIES A

Adenovirus A species have about 49% homology with Ad36 at E4-ORF1. The only A species that has been tested for an adipogenic effect is Ad31. Two studies have shown it is adipogenic in cells [43,47]. Among chickens, however, the virus is not adipogenic [43]. The evidence from human epidemiologic studies is also mixed, with one study [47] showing an association (OR=3.5, 95% CI 1.2–10.3) and one showing no association (OR=0.6, 95% CI 0.2–1.4) with obesity [13]. Combining these two studies in a random effects model shows no association between Ad31 and obesity but also demonstrates statistically significant heterogeneity (OR=1.4, 95% CI 0.3–7.8;  $I^2=87%$ ,  $p=0.006$ ). The heterogeneity is not due to chance alone and may be related to differences in testing or geography (USA versus Poland), as the difference in seroprevalence between the studies was large (73% vs. 31%, respectively).

### OTHER ADENOVIRUS TESTING

Human adenoviruses overall may be associated with obesity. One study tested for antibody against the common human adenovirus protein and showed an association with adiposity indices among subjects within body weight categories [50]. Two non-human adenoviruses have also been tested. In one experiment, chickens were infected with an avian adenovirus (chick embryo lethal orphan virus) but did not develop increased adipose tissue [4]. Another non-human adenovirus, SMAM1, as noted in the previous text, was the first adenovirus identified to have an adipogenic effect in experimentally infected chickens [1]. Additionally, although it is considered a non-human adenovirus, it had a robust association with BMI among 52 adults evaluated in India (BMI +4.6,  $p<0.001$ ) [2].

### DIRECTIONS FOR FUTURE STUDIES

Establishing causation has been a longstanding goal of scientific inquiry. Historically, leaders of causal models such as Koch and Bradford-Hill were evaluating single agent–single disease connections. Today, there are many causal questions relating to multifactorial diseases that are complex and more challenging to study. Specifically, as it relates to the connection between viruses and chronic diseases, there are often cofactors involved as has been observed in the relationship between viruses and cancer [51]. Two potential cofactors for adenovirus and obesity could be herpes simplex virus

(HSV) and adeno-associated virus (AAV). Herpes simplex virus 1 (HSV-1) infection is known to be associated with obesity by itself in three studies [52–54], including one that showed the total burden of infection is also associated with adiposity [53]. Like HSV-1 antibody, herpes virus entry mediator genetic polymorphisms were also associated with higher BMI [54], suggesting infection comes before excess weight. Likewise, synthetic herpes simplex DNA (HSV60) has been shown to enhance proliferation of adipocytes [55]. Interestingly, AAV provides another link between herpes viruses and adenoviruses. While coinfection with either adenoviruses or herpes viruses enables/promotes AAV replication, AAV coinfection dampens adenovirus replication [56] and modulates host DNA damage response in setting of HSV1 infection [57]. Further molecular studies of HSV60, E4-ORF1-related contents, or other important gene products could provide further insight [16,55].

New methods of inquiry, such as those that are intermediary between observational and experimental studies (e.g. quasi-experiment, packet randomization, and instrumental variables), could investigate these questions and allow stronger causal inferences [58]. Ultimately, well-controlled randomized trials of vaccines will be required to determine the effectiveness of this intervention for addressing viral adiposity. In fact, one study in mice has already shown that a vaccine against Ad36 was capable of preventing virus-induced obesity in experimentally infected animals [59].

Even purely observational studies could add substantial information by looking at new

adenoviruses and temporality of seroconversion and outcomes. The importance of clearly defining the onset of a new infection was demonstrated in two contrasting studies in monkeys. In one study, rhesus monkey infection status had no relationship with body weight 1 year later, but it was unknown when the monkeys became infected [28]. In another study that tracked serostatus and weight every 6 months, there was a temporal relationship between timing of infection and timing of weight gain [5]. The same type of serial assessment of serostatus is necessary in humans and has not yet been performed. Additionally, most studies have only evaluated a single type of adenovirus. One study adjusted for infection with Ad8, Ad36, and Ad5, along with other serum markers, and found Ad5 was the only adenovirus to have a persistent association with adiposity [30].

To adjust for infection with other adenoviruses, a more comprehensive list of the most prevalent adipogenic adenoviruses will be necessary. We identified three population-based seroepidemiologic studies investigating the prevalence of antibody to various adenoviruses (Table 2) [60–62]. It appears as though a species C adenovirus (Ad6) and two species D adenoviruses (Ad26 and Ad48) are known to have high prevalence of infection in some populations and have not yet been studied for their relationship with obesity. In Table 2, we show the homology of these viruses with the E4-ORF1 protein in Ad36 and Ad5, as this protein appears to be metabolically active in both Ad5 and Ad36 [18,48].

**Table 2. Adenoviruses with known prevalence based on nationwide population-based studies**

Adenovirus		Observed prevalence range (%) <sup>†</sup>		E4-ORF1 homology with Ad36* (score out of 253)	E4-ORF1 homology with Ad5* (score out of 257)
Species	Type	Children	Adults		
B	Ad35	14	3–22	118	110
C	Ad5	66	37–91	108	257
	Ad6	Unknown	46–79	108	257
D	Ad26	26	9–88	Unknown	Unknown
	Ad36	Unknown	12–88	253	108
	Ad48	7	3–51	122	52.8

\*Score per <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.

<sup>†</sup>Prevalence as noted in references cited in text [60–62].

The picture is likely more complex than simply the relationship between different adenoviruses, however. For instance, the human gut microbiome has also shown the capacity to transmit obesity to germ-free mice, but the associations between human obesity and specific bacterial components of the microbiome differ between studies [63]. In other words, it is not known what component of the gut microbiome is responsible for these obesogenic effects that are transmissible from humans to mice.

## CONCLUSIONS

The overall importance of understanding the relationship between infectious disease and human metabolism is a compelling reason for further investigation. Despite advancements, the vast majority (>70%) of those with obesity are unable to maintain substantial long-term weight loss (>10%) even with an intensive lifestyle intervention [64]. Although the multifactorial etiology of obesity has been recognized [65,66], its current treatment strategies are seldom cause-specific (unlike other multifactorial diseases) [67]. If certain microbes contribute to obesity, this subgroup of obesity may benefit from prevention or treatment strategies directed to these microbes, particularly because differential response to diet [35] and exercise [36] among those with Ad36 infection has already been shown. Among all the putative adipogenic pathogens, Ad36 has been studied most extensively and offers a pattern for how to investigate microbial contribution to obesity [68]. Human adenoviruses include many serotypes, which may have shared as well as unique adipogenic properties. Of the serotypes screened for their adipogenic effect, using either *in vitro* or animal studies, a majority of adenoviruses show some evidence indicating an adipogenic effect. Comprehensively identifying adipogenic adenovirus serotypes may help in determining their (individual or combined) role in human obesity. The importance of developing a stronger evidence base in this area is clear when considering the high prevalence of human adenoviral infections and the 35% prevalence of obesity in the US population [62,69]. Moreover, studies that examine adiposity-related outcome variables in both human and animal populations should include an inquiry about the infection status with adipogenic adenoviruses to minimize the potentially confounding influence of these infections. The number of adipogenic

adenovirus serotypes identified by research groups worldwide in a relatively short period underscores the overall importance of understanding the relationship between infectious disease and human obesity and provides a compelling reason for further investigation.

## METHODS

We performed an updated literature summary on the relationship between adenoviruses and obesity. The association between adenovirus 36 and obesity has already been systematically reviewed twice in recent years [8,9]. Additionally, most of the other adenoviruses have minimal literature ("Adenovirus X" AND "obesity" searched in May 2015 in PubMed returns no additional relevant studies for Ad2, Ad5, Ad8, Ad9, Ad31, or Ad37). Where necessary, for consistent data reporting, chi-square analysis was performed using raw numbers reported in prior studies using Stata v. 12.0 (StataCorp LP, College Station, TX, USA) and  $p < 0.05$  was considered statistically significant. Two reports of an investigation of the association between obesity and an adenovirus were found for Ad5, Ad31, and Ad37, which have not been previously synthesized. For these studies, the results were combined with random effects meta-analysis and heterogeneity was assessed using  $I^2$ .

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## CONFLICT OF INTEREST

JDV has nothing to declare. RLA declares he is the owner of Obetech, LLC, a company that provides assays for adenoviruses that produce obesity and has patents for diagnostic assays and vaccines in the area of virus-induced obesity. NVD declares several patents in viral obesity and adenovirus 36 including uses for E1A, E4-ORF1 gene and protein, and AKT1 inhibitor. He also declares ongoing grant support from Vital Health Interventions for determining anti-diabetic properties of E4-ORF1 protein.

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## REFERENCES

- Dhurandhar NV, Kulkarni P, Ajinkya SM, Sherikar A. Effect of adenovirus infection on adiposity in chicken. *Veterinary Microbiology* 1992; **31**(2–3): 101–107.
- Dhurandhar NV, Kulkarni PR, Ajinkya SM, Sherikar AA, Atkinson RL. Association of adenovirus infection with human obesity. *Obesity Research* 1997; **5**(5): 464–469.
- Rogers PM, Fusinski KA, Rathod MA, et al. Human adenovirus Ad-36 induces adipogenesis via its E4 orf-1 gene. *International Journal of Obesity* 2008; **32**(3): 397–406. DOI:10.1038/sj.ijo.0803748.
- Dhurandhar NV, Israel BA, Kolesar JM, Mayhew GF, Cook ME, Atkinson RL. Increased adiposity in animals due to a human virus. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* 2000; **24**(8): 989–996.
- Dhurandhar NV, Whigham LD, Abbott DH, et al. Human adenovirus Ad-36 promotes weight gain in male rhesus and marmoset monkeys. *The Journal of Nutrition* 2002; **132**(10): 3155–3160.
- Dhurandhar NV, Israel BA, Kolesar JM, Mayhew G, Cook ME, Atkinson RL. Transmissibility of adenovirus-induced adiposity in a chicken model. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity* 2001; **25**(7): 990–996. DOI:10.1038/sj.ijo.0801668.
- Pasarica M, Shin AC, Yu M, et al. Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. *Obesity (Silver Spring, Md)* 2006; **14**(11): 1905–1913. DOI:10.1038/oby.2006.222.
- Yamada T, Hara K, Kadowaki T. Association of adenovirus 36 infection with obesity and metabolic markers in humans: a meta-analysis of observational studies. *PloS One* 2012; **7**(7e42031): . DOI:10.1371/journal.pone.0042031.
- Shang Q, Wang H, Song Y, et al. Serological data analyses show that adenovirus 36 infection is associated with obesity: a meta-analysis involving 5739 subjects. *Obesity (Silver Spring, Md)* 2014; **22**(3): 895–900. DOI:10.1002/oby.20533.
- Lin WY, Dubuisson O, Rubicz R, et al. Long-term changes in adiposity and glycaemic control are associated with past adenovirus infection. *Diabetes Care* 2013; **36**(3): 701–707. DOI:10.2337/dc12-1089.
- Voss JD, Burnett DG, Olsen CH, Haverkos HW, Atkinson RL. Adenovirus 36 antibodies associated with clinical diagnosis of overweight/obesity but not BMI gain: a military cohort study. *The Journal of Clinical Endocrinology and Metabolism* 2014; **99**(9): E1708–E1712. DOI:10.1210/jc.2014-1863.
- Sabin MA, Burgner D, Atkinson RL, et al. Longitudinal investigation of adenovirus 36 seropositivity and human obesity: the Cardiovascular Risk in Young Finns Study. *International Journal of Obesity* 2015. doi:10.1038/ijo.2015.108. [Epub ahead of print].
- Atkinson RL, Dhurandhar NV, Allison DB, et al. Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *International Journal of Obesity* 2005; **29**(3): 281–286. DOI:10.1038/sj.ijo.0802830.
- Nam JH, Na HN, Atkinson RL, Dhurandhar NV. Genomic stability of adipogenic human adenovirus 36. *International Journal of Obesity* 2014; **38**(2): 321–324. DOI:10.1038/ijo.2013.67.
- Rathod MA, Rogers PM, Vangipuram SD, McAllister EJ, Dhurandhar NV. Adipogenic cascade can be induced without adipogenic media by a human adenovirus. *Obesity (Silver Spring, Md)* 2009; **17**(4): 657–664. DOI:10.1038/oby.2008.630.
- 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): . DOI:10.1371/journal.pone.0023394.
- Arnold J, Janoska M, Kajon AE, et al. Genomic characterization of human adenovirus 36, a putative obesity agent. *Virus Research* 2010; **149**(2): 152–161. DOI:10.1016/j.virusres.2010.01.011.
- Dhurandhar NV. Insulin sparing action of adenovirus 36 and its E4orf1 protein. *Journal of Diabetes and its Complications* 2013; **27**(2): 191–199. DOI:10.1016/j.jdiacomp.2012.09.006.
- Dubuisson O, Dhurandhar EJ, Krishnapuram R, et al. PPARgamma-independent increase in glucose uptake and adiponectin abundance in fat cells. *Endocrinology* 2011; **152**(10): 3648–3660. DOI:10.1210/en.2011-0225.
- Vangipuram SD, Yu M, Tian J, et al. Adipogenic human adenovirus-36 reduces leptin expression and secretion and increases glucose uptake by fat cells. *International Journal of Obesity* 2007; **31**(1): 87–96. DOI:10.1038/sj.ijo.0803366.
- Pasarica M, Mashtalir N, McAllister EJ, et al. Adipogenic human adenovirus Ad-36 induces commitment, differentiation, and lipid accumulation in human adipose-derived stem cells. *Stem Cells (Dayton, Ohio)* 2008; **26**(4): 969–978. DOI:10.1634/stemcells.2007-0868.
- Na HN, Kim H, Nam JH. Novel genes and cellular pathways related to infection with adenovirus-36 as an obesity agent in human mesenchymal stem cells. *International Journal of Obesity* 2012; **36**(2): 195–200. DOI:10.1038/ijo.2011.89.
- Wang ZQ, Yu Y, Zhang XH, Floyd EZ, Cefalu WT. Human adenovirus 36 decreases fatty acid oxidation and increases de novo lipogenesis in primary cultured human skeletal muscle cells by promoting Cidec/FSP27 expression. *International Journal of Obesity* 2010; **34**(9): 1355–1364. DOI:10.1038/ijo.2010.77.
- Na HN, Nam JH. Adenovirus 36 as an obesity agent maintains the obesity state by increasing MCP-1 and inducing inflammation. *The Journal of Infectious Diseases* 2012; **205**(6): 914–922. DOI:10.1093/infdis/jir864.
- Na HN, Dubuisson O, Hegde V, Nam JH, Dhurandhar NV. Human adenovirus Ad36 and its E4orf1 gene enhance cellular glucose uptake even in the presence of inflammatory cytokines. *Biochimie* 2014. pii: S0300-9084(14)00334-4. doi: 10.1016/j.biochi.2014.11.005. [Epub ahead of print].
- Na HN, Park S, Jeon HJ, Kim HB, Nam JH. Reduction of adenovirus 36-induced obesity and inflammation by mulberry extract. *Microbiology and Immunology* 2014; **58**(5): 303–306. DOI:10.1111/1348-0421.12146.

27. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, *et al.* Macrophage-specific PPAR $\gamma$  controls alternative activation and improves insulin resistance. *Nature* 2007; **447**(7148): 1116–1120. DOI:10.1038/nature05894.
28. Broderick MP, Hansen CJ, Irvine M, *et al.* Adenovirus 36 seropositivity is strongly associated with race and gender, but not obesity, among US military personnel. *International Journal of Obesity* 2010; **34**(2): 302–308. DOI:10.1038/ijo.2009.224.
29. Cakmakliogullari EK, Sanlidag T, Ersoy B, Akcali S, Var A, Cicek C. Are human adenovirus-5 and 36 associated with obesity in children? *Journal of Investigative Medicine: The Official Publication of the American Federation for Clinical Research* 2014; **62**(5): 821–824. doi:10.231/JIM.0000000000000084.
30. Aldhoon-Hainerova I, Zamrazilova H, Atkinson RL, *et al.* Clinical and laboratory characteristics of 1179 Czech adolescents evaluated for antibodies to human adenovirus 36. *International Journal of Obesity* 2014; **38**(2): 285–291. DOI:10.1038/ijo.2013.72.
31. Parra-Rojas I, Del Moral-Hernandez O, Salgado-Bernabe AB, Guzman-Guzman IP, Salgado-Goytia L, Munoz-Valle JF. Adenovirus-36 seropositivity and its relation with obesity and metabolic profile in children. *International Journal of Endocrinology* 2013; **2013**: 463194. DOI:10.1155/2013/463194.
32. Tosh AK, Broy-Aschenbrenner A, El Khatib J, Ge B. Adenovirus-36 antibody status & BMI comparison among obese Missouri adolescents. *Missouri Medicine* 2012; **109**(5): 402–403.
33. Vander Wal JS, Huelsing J, Dubuisson O, Dhurandhar NV. An observational study of the association between adenovirus 36 antibody status and weight loss among youth. *Obesity Facts* 2013; **6**(3): 269–78. DOI:10.1159/000353109.
34. Trovato GM, Martines GF, Trovato FM, *et al.* Adenovirus-36 seropositivity enhances effects of nutritional intervention on obesity, bright liver, and insulin resistance. *Digestive Diseases and Sciences* 2012; **57**(2): 535–544. DOI:10.1007/s10620-011-1903-8.
35. Na HN, Hong YM, Ye MB, Park S, Kim IB, Nam JH. Adenovirus 36 attenuates weight loss from exercise but improves glycemic control by increasing mitochondrial activity in the liver. *PloS One* 2014; **9**(12 e114534): . DOI:10.1371/journal.pone.0114534.
36. Dhurandhar NV, Dhurandhar EJ, Ingram DK, Vaughan K, Mattison JA. Natural infection of human adenovirus 36 in rhesus monkeys is associated with a reduction in fasting glucose 36. *Journal of Diabetes* 2014; **6**(6): 614–616. DOI:10.1111/1753-0407.12178.
37. Almgren M, Atkinson RL, Hilding A, *et al.* Human adenovirus-36 is uncommon in type 2 diabetes and is associated with increased insulin sensitivity in adults in Sweden. *Annals of Medicine* 2014; **46**(7): 539–546.
38. Trovato FM, Catalano D, Garozzo A, Martines GF, Pirri C, Trovato GM. ADV36 adipogenic adenovirus in human liver disease. *World Journal of Gastroenterology: WJG* 2014; **20**(40): 14706–14716. DOI:10.3748/wjg.v20.i40.14706.
39. Trovato GM, Martines GF, Garozzo A, *et al.* Ad36 adipogenic adenovirus in human non-alcoholic fatty liver disease. *Liver International: Official Journal of the International Association for the Study of the Liver* 2010; **30**(2): 184–190. DOI:10.1111/j.1478-3231.2009.02127.x.
40. Trovato GM, Castro A, Tonzuso A, *et al.* Human obesity relationship with Ad36 adenovirus and insulin resistance. *International Journal of Obesity* 2009; **33**(12): 1402–1409. DOI:10.1038/ijo.2009.196.
41. Na HN, Hong YM, Kim J, Kim HK, Jo I, Nam JH. Association between human adenovirus-36 and lipid disorders in Korean schoolchildren. *International Journal of Obesity* 2010; **34**(1): 89–93. DOI:10.1038/ijo.2009.207.
42. Na HN, Kim J, Lee HS, *et al.* Association of human adenovirus-36 in overweight Korean adults. *International Journal of Obesity* 2012; **36**(2): 281–285. DOI:10.1038/ijo.2011.102.
43. Whigham LD, Israel BA, Atkinson RL. Adipogenic potential of multiple human adenoviruses in vivo and in vitro in animals. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 2006; **290**(1): R190–R194. DOI:10.1152/ajpregu.00479.2005.
44. Trovato GM, Martines GF, Pirri C, *et al.* Obesity-independent association of human adenovirus Ad37 seropositivity with nonalcoholic fatty liver disease. *Journal of Clinical Gastroenterology* 2012; **46**(6): e46–e54. DOI:10.1097/MCG.0b013e31824b225c.
45. Bil-Lula I, Sochocka M, Zatonska K, Szuba A, Sawicki G, Wozniak M. Adenovirus type 9 enhances differentiation and decreases cytokine release from preadipocytes. *Journal of Medical Virology* 2015; **87**(2): 230–239. DOI:10.1002/jmv.24009.
46. Sanlidag T, Akcali S, Vatansver S, *et al.* Investigation of adipogenic effects of human adenovirus serotypes 36 and 5 in a Colo-320 cell line. *Future Virology* 2013; **8**(6): 617–622.
47. Bil-Lula I, Stapor S, Sochocka M, *et al.* Infectobesity in the Polish Population—Evaluation of an Association between Adenoviruses Type 5, 31, 36 and Human Obesity. *International Journal of Virology and Molecular Biology* 2014; **3**(1): 1–8.
48. Thai M, Graham NA, Braas D, *et al.* Adenovirus E4ORF1-induced MYC activation promotes host cell anabolic glucose metabolism and virus replication. *Cell Metabolism* 2014; **19**(4): 694–701. DOI:10.1016/j.cmet.2014.03.009.
49. So PW, Herlihy AH, Bell JD. Adiposity induced by adenovirus 5 inoculation. *International Journal of Obesity* 2005; **29**(6): 603–606. DOI:10.1038/sj.ijo.0802917.
50. Bil-Lula I, Stapor S, Krzywonos-Zawadzka A, Wozniak M. Is there any link between visceral obesity and adenovirus infections in the Polish population? *Advances in Clinical and Experimental Medicine: Official Organ Wroclaw Medical University* 2014; **23**(3): 415–422.
51. Haverkos HW. Viruses, chemicals and co-carcinogenesis. *Oncogene* 2004; **23**(38): 6492–6499. DOI:10.1038/sj.onc.1207822.
52. Karjala Z, Neal D, Rohrer J. Association between HSV1 seropositivity and obesity: data from the National Health and Nutritional Examination Survey, 2007–2008. *PloS One* 2011; **6**(5 e19092): . DOI:10.1371/journal.pone.0019092.
53. Fernandez-Real JM, Ferri MJ, Vendrell J, Ricart W. Burden of infection and fat mass in healthy middle-aged men. *Obesity (Silver Spring, Md)* 2007; **15**(1): 245–252. DOI:10.1038/oby.2007.541.
54. Bassols J, Moreno JM, Ortega F, Ricart W, Fernandez-Real JM. Characterization of

- herpes virus entry mediator as a factor linked to obesity. *Obesity (Silver Spring, Md)* 2010; **18**(2): 239–246. DOI:10.1038/oby.2009.250.
55. Yu L, Liu P, Liu Z, *et al.* p204-mediated innate antiviral responses in mouse adipose cells and their effects on cell functions. *Immunology and Cell Biology* 2015; **93**(2): 147–157. DOI:10.1038/icb.2014.83.
56. Weger S, Hammer E, Heilbronn R. Differential contribution of adeno-associated virus type 2 Rep protein expression and nucleic acid elements to inhibition of adenoviral replication in cis and in trans. *Journal of Virology* 2014; **88**(24): 14126–14137. DOI: 10.1128/jvi.02350-14.
57. Vogel R, Seyffert M, Strasser R, *et al.* Adeno-associated virus type 2 modulates the host DNA damage response induced by herpes simplex virus 1 during coinfection. *Journal of Virology* 2012; **86**(1): 143–155. DOI:10.1128/jvi.05694-11.
58. Pavela G, Wiener H, Fontaine KR, Fields DA, Voss JD, Allison DB. Packet randomized experiments for eliminating classes of confounders. *European Journal of Clinical Investigation* 2015; **45**(1): 45–55. DOI:10.1111/eci.12378.
59. Na HN, Nam JH. Proof-of-concept for a virus-induced obesity vaccine; vaccination against the obesity agent adenovirus 36. *International Journal of Obesity* 2014; **38**(11): 1470–1474. DOI:10.1038/ijo.2014.41.
60. Barouch DH, Kik SV, Weverling GJ, *et al.* International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations. *Vaccine* 2011; **29**(32): 5203–5209. DOI:10.1016/j.vaccine.2011.05.025.
61. Mast TC, Kierstead L, Gupta SB, *et al.* International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials. *Vaccine* 2010; **28**(4): 950–957. DOI:10.1016/j.vaccine.2009.10.145.
62. Nwanegbo E, Vardas E, Gao W, *et al.* Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of The Gambia, South Africa, and the United States. *Clinical and Diagnostic Laboratory Immunology* 2004; **11**(2): 351–357.
63. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Letters* 2014; **588**(22): 4223–4233. DOI:10.1016/j.febslet.2014.09.039.
64. Eight-year weight losses with an intensive lifestyle intervention: the look AHEAD study. *Obesity (Silver Spring, Md)* 2014; **22**(1): 5–13. DOI:10.1002/oby.20662.
65. McAllister EJ, Dhurandhar NV, Keith SW, *et al.* Ten putative contributors to the obesity epidemic. *Critical Reviews in Food Science and Nutrition* 2009; **49**(10): 868–913. DOI:10.1080/10408390903372599.
66. Keith SW, Redden DT, Katzmarzyk PT, *et al.* Putative contributors to the secular increase in obesity: exploring the roads less traveled. *International Journal of Obesity* 2006; **30**(11): 1585–1594. DOI:10.1038/sj.ijo.0803326.
67. Field AE, Camargo CA Jr, Ogino S. The merits of subtyping obesity: one size does not fit all. *Jama* 2013; **310**(20): 2147–2148. DOI:10.1001/jama.2013.281501.
68. Dhurandhar NV. A framework for identification of infections that contribute to human obesity. *The Lancet Infectious Diseases* 2011; **11**(12): 963–969. DOI:10.1016/s1473-3099(11)70274-2.
69. Ogden CL, Carroll MD, Flegal KM. Prevalence of obesity in the United States. *Jama* 2014; **312**(2): 189–190. DOI:10.1001/jama.2014.6228.