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Twenty-five years of research about adipogenic adenoviruses: A systematic review

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Summary

Infectious etiology is implicated in chronic diseases such as gastric ulcer or atherosclerosis. However, "infection" is a recent term in the field of obesity. Since the first report in 1982 of obesity due to infection, several microbes have been linked to obesity. Among the adipogenic microbes, avian adenovirus SMAM-1 and human adenovirus Ad36 have been studied most extensively for the past 25 years. Here, we present a systematic review of literature about SMAM-1 and Ad36. Reports from North America, Europe, and Asia reveal strong evidence that Ad36 causes obesity in animals and paradoxically improves glycemic control, and in vitro data provides mechanistic explanation. Considering that experimental Ad36 infection of humans is unlikely, its causative role in human obesity or glycemic control has not been demonstrated unequivocally. Nonetheless, most, but not all, observational studies in children and adults link Ad36 infection to obesity and improvement in glycemic control. The *E4orf1* gene of Ad36 was identified as responsible for better glycemic control. Overall, 25 years have considerably advanced knowledge about the role of infection in obesity. Potential translational benefits include the development of vaccines to prevent Ad36-induced obesity and drug development based on the E4orf1 protein to improve glycemic control.

KEYWORDS

Ad36, adiposity, E4orf1, glycemic control, infect, obesity

1 | INTRODUCTION

The American Medical Association recognized obesity as a disease in 2013, making it more effective to tackle this complex issue and associated comorbidities including diabetes.[1] Although the global obesity prevalence has nearly tripled since 1975,[2] its treatment or prevention has remained very challenging. Lifestyle modification approaches tend to produce 7% to 10% weight loss in 1 year[3] but regaining weight is a common concern, which makes longer-term weight loss maintenance very difficult.[4] Traditionally, the stigma of obesity commonly believes that excessive eating and reduced physical activity as the only causes of obesity, which has severely restricted the search for additional risk factors and causes.[5] Moreover, dietary and behavioral interventions are commonly used as a blanket treatment approach regardless of the potential cause of obesity. However, besides eating and physical activity, there are several putative factors such as infection, epigenetic changes, increased maternal age, endocrine disruptions, and intrauterine effects that might contribute to obesity.[6] The expression of obesity and its prevention or treatment approaches may vary depending on the contributing factors. For example, in individuals for whom a poor quality or quantity of sleep is linked with obesity,[7] it would be important to address sleep for comprehensive and effective treatment. Thus, it is important to identify the causes of obesity, which may lead to cause-specific treatment or prevention approaches for better management of obesity. It is known that infections can influence obesity and obesity can influence susceptibility to or severity of infections as we previously described in detail.[8] This systematic review, however, will focus on the role of infections in developing obesity. The viral etiology of obesity was first reported in 1982 when canine distemper virus was described to cause obesity in mice.[9] In 1992, an avian adenovirus, SMAM-1 was found to

increase adiposity in chickens,[10] which was reported to be associated with human obesity in 1997.[11] A substantial number of studies investigated the role of Human Adenovirus-36 (Ad36) since it was first reported to cause obesity in various animal models.[12] Ad36, which belongs to subgroup D, is one of the 50+ human adenoviruses maintained by American Type Culture Collection. This virus was first isolated in Germany from the feces of 6-year-old girl suffering from enteritis.[13] The distinctive feature of Ad36 is its paradoxical ability to decrease serum triglyceride and lipid while increasing adiposity, as well as to improve glucose disposal independent of insulin. E4orf1, an Ad36-derived 125 amino acid peptide,[14] was identified in improving glucose clearance in in-vitro and in-vivo studies. The potential of Ad36 and its E4orf1 protein in improving glycemic control is very attractive for developing an effective anti-diabetic agent. Among the adipogenic pathogens, Ad36 is the only human pathogen with the most information available that is of practical significance to human health. The objective of this systematic review was to summarize the findings of the role of Ad36 in obesity and E4orf1 protein published in the past 25 years, since its adipogenic role was first reported (Table 1).

TABLE 1 Ad36 Timeline of key findings

Year	Findings
1992	SMAM-1 adenovirus causes adiposity in chickens[10]
1997	SMAM-1 adenovirus is associated with human adiposity[11]
2000	Ad36 virus causes obesity in mice[12]
2001	Ad36 infection transmits horizontally to in-contact chicken[15]
2002	Ad36 infection is associated with body weight gain in male rhesus monkeys and causes weight gain in marmoset monkeys[16]
2004	Ad36 infection increases lipid accumulation in human pre-adipocyte cell line[17]
2005	Association of Ad36 infection with human obesity and paradoxically lower serum cholesterol[18]
2006	Ad36 decreases norepinephrine and increases C/EBPβ, C/EBPα, and GPDH[19]
2007	Ad36 suppresses leptin gene expression in adipocytes and rats. Ad36 infection of cells increases glucose uptake[20]
2008	Ad36 induces adipogenic program in human adipose derived stem cells[21]
2008	*E4orf1* gene of Ad36 induces adipogenesis[14]
2008	Ad36 infection is associated with a better glycemic control in human[22]
2008	Ad36 induced glucose uptake is via insulin independent activation of PI3K[23]
2009	Ad36 seropositivity is associated with increased risk of obesity in human[24]
2009	Ad36 infection is associated with obesity in Korean Children[25]
2010	Genomic Characterization of Ad36 reported[26]
2011	*E4orf1* gene of Ad36 increases glucose uptake in cell culture[27]
2011	Ad36 induced glucose uptake is PPARγ independent[28]
2011	Soiled bedding of Ad36 does not transmit the virus to the mice[29]
2012	Ad36 infection is associated with obesity in children and adults in Sweden[30]
2012	Ad36 improves glucose metabolism in liver cells[31]
2012	Gene expression profiling after Ad36 infection in muscle cells[32]
2013	E4orf1 protein expressing cell line developed[33]
2013	Ad36 seropositivity is associated with better glycemic control in human[34]
2014	A vaccine was developed in Korea which protects mice from Ad36 infection[35]
2015	E4orf1 promotes insulin-independent signaling in adipocytes[36]
2016	Serum neutralization assay modified for Ad36 Antibody detection[37]
2016	Hepatic Expression of E4orf1[38]

2 | METHODS

2.1 | Search strategy

The entire search process was performed using the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) shown in Figure 1.[39] Electronic databases, PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/) and the core collection, Web of Science (https://apps.webofknowledge.com/), were searched up until September 10, 2018 for published articles related to Ad36. The following set of keywords were used for search with PubMed central and using the Topic and Title with Web of Science: Adenovirus 36, Adenovirus-36, Ad-36, AD 36, Adv36, Adv-36, SMAM1, Infectobesity, and E4orf1. The search resulted in 1996 and 423 references from PubMed and the Web of Science, respectively. All references were imported to Thomas Reuters EndNote X7. After removing the duplicates, the combined search produced 2212 unique records. Upon reviewing the title, 2011 records were excluded which included studies not related to the review topic, or were patents, or non-English records. The remaining 201 records were subjected to an online full-text search using the Texas Tech Library website. The search strategies have been shown in the supporting information Table S1.

2.2 | Inclusion/exclusion criteria

The first article related to adenovirus Ad36 was published in 1980, around the time when the virus was first identified from fecal sample of a girl suffering from enteritis in Germany.[13] Articles published in English after 1980 and those particularly investigated the association of Ad36 with adiposity, obesity, and diabetes were included in the review. Review articles, meta-analysis, meeting abstracts, letter to the editor, editorial focus, news items, and book chapters were excluded from the review. Finally, a total of 87 original articles investigating the in-vitro, in-vivo role, and human studies involving Ad36 were examined for qualitative synthesis, while three or more articles among these shared all three or at least two of the analysis within the same article. All the key findings for in-vitro, in-vivo, and human studies have been summarized in the supporting information Table S2, S3, and S4, respectively.

3 | FINDINGS

There were a few different ways to present the information in this systematic review. One approach would be to categorize results

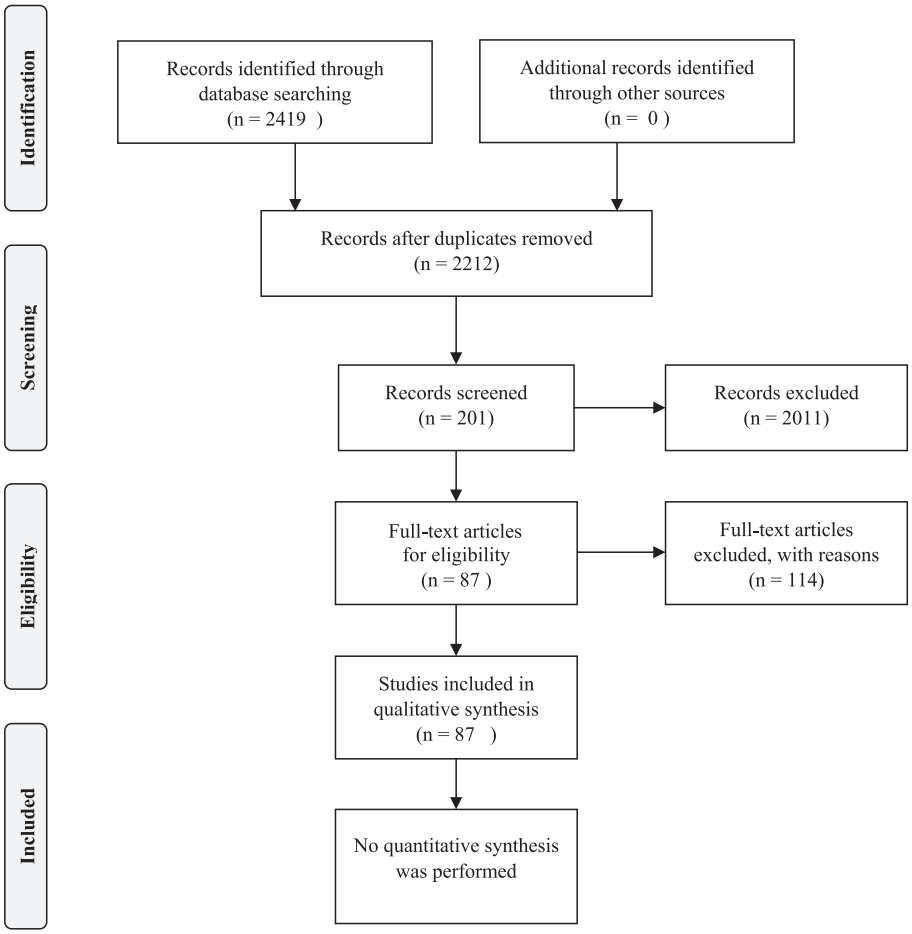

FIGURE 1 PRISMA flow diagram, summarizing the search process to identify and screen original articles examining the association of Ad36 with obesity and diabetes published through 10 September 2018

regarding Ad36 and E4orf1 individually as "in-vivo," "in-vitro," and "human data." Another approach could have been to first collectively present all information about Ad36, followed by E4orf1. However, considering its clinical relevance, we opted to categorize the data by three physiologically significant areas, namely (1) adiposity and lipid metabolism, (2) glycemic control, and (3) liver fat accumulation. Under each of these categories, we will present in-vivo, in-vitro, and human data about Ad36 and then E4orf1, as available. Considering that glycemic control is closely linked with metabolism of adipose tissue and liver, some overlap in presentation is possible. A few studies address additional areas such as the role of inflammation or skeletal muscle, which will be included under appropriate broader topics (instead of creating additional subcategories).

3.1 | Adiposity and lipid metabolism

3.1.1 | In-vivo

Ad36

In 1992, Dhurandhar et al described a chicken model that accumulates fat upon infection with an avian adenovirus SMAM-1 virus in India, with a paradoxical reduction in serum cholesterol and triglyceride.[10] However, the mechanism of this reduction in serum lipid levels was not explained at the time. No further research was performed to examine the in-vivo role in adiposity with the SMAM-1. Instead, researchers went on to investigate the adipogenic effect of another adenovirus, the human adenovirus Ad36. Experimental infection with Ad36 causes increase in visceral and total body fat accumulation in chicken and mice, with lower serum cholesterol and triglyceride levels.[12] Earlier experiments also tested additional adenoviruses for their adipogenic effect. Avian adenovirus Chick Embryo Lethal Orphan,[12] human adenovirus Ad2, Ad31, and Ad37 did not increase adiposity.[40] Whereas, human adenovirus Ad5 increases adiposity in rats.[41] These studies indicated that not all adenoviruses are adipogenic and that the adipogenic response of an animal is not simply due to any infection. Overall, Ad36 infected experimental animals displayed 60% to 100% increase in adiposity, which is defined as the top 15th percentile compared with uninfected control group.

Ad36 infection can transmit horizontally from infected chickens to uninfected chickens sharing cages.[15] Infecting chickens with Ad36 by intra-nasal inoculation led to viral appearance in their blood, and the uninfected cage mate chickens showed Ad36 DNA in their blood in just 12 hours of sharing the cage with infected chickens,[15] indicating the high infectious potential of Ad36. Similarly, experimental infection of Ad36 in rats through intranasal and intraperitoneal routes increased epididymal fat pad weight.[42] Following infection via the intranasal route, Ad36 virus could be recovered in feces of marmosets for up

to 2 months.[16] Post infection, Ad36 spreads to various organs including heart, liver, kidney, spleen, brain, and adipose tissue, as indicated by the presence of Ad36 DNA.[16,42] Another experiment tested one of Koch's postulates of infectivity. Ad36 infected blood retrieved from infected chickens was injected intravenously in an uninfected set of animals, who also developed obesity. The viral DNA could also be recovered from the chickens receiving infected blood.[15] In addition to chickens and rodents, Ad36 is adipogenic in non-human primates as well. Experimental infection of Ad36 increased body weight 3-fold in 6-month marmosets.[16] In rhesus monkeys, natural exposure to Ad36 was associated with 15% weight gain and a 28% decrease in cholesterol over 6 months.[16] Hamsters infected with Ad36 also showed higher cholesterol LDL-fractions but no difference in total plasma cholesterol compared with controls.[43] Although, the study was the first to examine Ad36 infection in hamsters, probably, the short study period of 5 weeks was not enough to modulate lipid profile in hamsters.

The development of obesity is a complex phenomenon, which can involve both peripheral and central pathways. The pathway of increased adiposity is not clear, but several studies provided clues. Upon Ad36 infection, viral DNA was located in adipose tissue and significantly correlated with the amount of adipose tissue, suggesting a direct and local effect of Ad36 on adipose tissue growth.[16] Pasarica et al examined whether the peripheral or central mechanism is responsible for Ad36 induced obesity in Wistar rats.[19] In rats, Ad36 infection increased the expression of genes involved in adipocyte differentiation such as C/EBPβ, C/EBPα, and PPARγ, which may suggest the contribution of the peripheral pathways.[19] Whereas, Ad36 also decreased concentrations of norepinephrine (NE), dopamine, dihydroxyphenyl acetic acid, and 5-hydroxyindol acetic acid in various hypothalamic areas of the brain and also reduced serum corticosterone.[19] Authors expressed the possibility of reduced hypothalamic NE contributing to adiposity by reducing corticosterone. These experiments suggested that both, peripheral and central mechanisms may play a role in Ad36-induced adiposity.

Ad36 appears to influence state of inflammation as well. Ad36 infection of rodents showed a decrease in inflammatory cytokines such as monocyte chemoattractant protein-1 (MCP-1), IL-6, IL-18 in the short term (2-4 days) post infection,[42,44] but an increase in inflammation (TNF-α, MCP-1) about 3 month post infection.[45] Effect of Ad36 on inflammatory cytokines was studied further in-vitro, as described below.

Although cell culture studies showed that the *E4orf1* gene of Ad36 is necessary and sufficient to induce adipogenesis in cells,[14] studies that expressed E4orf1 in mice by transgenic approach or with the help of virus vectors[36,38,46,47] did not show weight gain in mice expressing E4orf1. It is possible that the expression site and intensity of E4orf1 differs when expressed as a part of Ad36 infection. It is also possible that co-expression of additional genes of Ad36 are needed to increase adiposity in-vivo.

In summary, human adenovirus Ad36 reliably causes fat accretion in various animal models. This is a property shared by some but not all viruses or all adenoviruses. It seems that Ad36 infection can be passed on readily, which may follow a feco-oral route. Ad36 may influence adipose tissue and/or the central nervous system to promote adiposity. Adipogenic effect of Ad36 even in non-human primates raises a strong possibility for similar effect of the virus in human primates.

3.1.2 | In-vitro

Ad36

Many in-vitro studies examined the molecular mechanism of Ad36-induced adiposity. Adipose tissue tropism of Ad36 led researchers to selecting murine pre-adipocyte cell line (3T3-L1) or human primary stromal vascular cells (hASC) for cell signaling studies. Overall, Ad36 accelerates replication and differentiation of human or murine preadipocytes into adipocytes and increases lipid accumulation.[14,17,20,48,49] In the presence of adipogenic media, Ad36 accelerates adipogenic differentiation.[14] Even in the absence of adipogenic media, Ad36 can induce differentiation and significant lipid accumulation.[50] In fact, the adipogenic influence of Ad36 is so robust, that it induces adipogenic differentiation in hASC that are exposed to osteogenic media and as such are expected to have osteogenic differentiation.[21] Ad36 suppresses expression and release of leptin in 3 T3-L1 cells, which may act as an autocrine/paracrine influence in promoting adipogenic differentiation and adipose tissue growth.[20] Overall, studies showed that in adipocyte progenitors, Ad36 downregulates genes of preadipocyte status such as Pref1 and Wnt10b, and upregulates cAMP and PI3K pathways, leading to upregulation of the genes involved in the adipogenic cascade, including C/EBPβ, C/EBPα, PPARγ, lipoprotein lipase, aP2, fatty acid synthase, glycerol phosphate dehydrogenase, and adiponectin.[14,17,19-22,28,44,50-52] This adipogenic effect is not limited to adipocyte progenitors. Even human adipose tissue pieces (explants) exposed to Ad36 infection in-vitro show very similar upregulation in genes of adipogenic cascade.[22] Many research groups extended the investigation of adipogenic effect of Ad36 in additional cell types. Cell death inducing DNA fragmentation factor alpha-like effector A (cidea) and fat-specific protein 27 (FSP27/cidec) are known to promote accumulation of triglycerides in adipocytes in-vivo and in-vitro,[53,54] whereas perilipin protects the lipid droplets in adipocytes from oxidation. In humans skeletal muscle cells (HSKM), Ad36 upregulated Cidec/FSP27, perilipin, and decreased AMP-activated protein kinase (AMPK) activities but not PPARγ signaling, which seems to be a way to reduce fatty acid oxidation and induce adipogenesis.[55] Ad36 infection of human bone marrow mesenchymal stem cells showed upregulation of 35 adiposity-related genes including LPL, ATF3, FABP4, C/EBPα, and C/EBPγ along with PPAR-γ and inflammation pathways.[51]

A potential consequence of this adipogenic effect of Ad36 was apparent in an experiment that studied differentiation potential of human adipocyte progenitors obtained from Pima Indian subjects who have very high prevalence of obesity.[21] In a blinded study, adipose tissue samples of the subjects were screened for the presence of Ad36 DNA as evidence for Ad36 infection and separately screened for the adipogenic differentiation of their adipocyte progenitors. Subjects positive for Ad36 infection had 8-fold greater ability for their adipocyte progenitors to differentiate into fat cells, compared with the individuals not exposed to Ad36. This suggested that natural Ad36

infection is associated with greater potential for adipogenic differentiation.

E4orf1

In 2008, Rogers et al reported that Ad36 induces adipogenesis in hASC and 3T3-L1 cells via its gene E4 open reading frame (orf)-1.[14] This gene was necessary and sufficient to induce adipogenesis in-vitro and consequential lipid accumulation, even in absence of adipogenesis inducing media and any other inducers (MDI) to induce adipogenesis in hASC and 3T3-L1 cells.[14] Although the *E4orf1* gene of another human adenovirus, Ad9, was reported previously to upregulate the phosphatidylinositol-3-kinase (PI3K) pathway by its PDZ-protein binding domain,[56] Ad36 derived E4orf1 was reported for the first time to activate cAMP and PI3K pathways evidenced by increased cAMP levels, PKB activity, cell proliferation, and lipid accumulation.[14] In addition to the improvement in systemic glucose level, E4orf1 induction was also reported to promote whitening in the brown adipose tissue evidenced by the reduction in gene markers of browning (UCP1, PGC1α, Cidea, Otop1, and Prdm16).[36]

3.1.3 | Human studies

Association of adenovirus with human obesity was first revealed in 1997, where an avian adenovirus SMAM-1 was reported to be linked to obesity in humans.[11] Subsequently, Ad36 was reported to be associated with human obesity in several epidemiological studies from China,[52,57] Czech Republic,[58,59] India,[11] Italy,[24,60] Mexico,[61] South Korea,[25,62-64] Poland-Sweden,[30,65] Turkey,[66-69] and United States.[18,34,44,70-74] Most of the studies were observational which examined the presence of antibody against the Ad36 virus in human serum by serum neutralization assay or by an Enzyme-Immuno Absorbant method. These assays were only able to detect Ad36 antibody which indicate past Ad36 infection but not necessarily the time of infection. The prevalence of Ad36 infection among people with obesity varies from 7.1% to 64.7%.[24,44] Regardless of the association of the Ad36 and SMAM-1 virus with obesity, interestingly some studies reported paradoxically lower triglycerides and serum cholesterol level and better glycemic control. Atkinson et al examined the presence of Ad36, Ad-2, Ad-31, and Ad-37 antibodies status in human serum samples collected from three states of United States. They found 27%, 58%, and 20% of the obese individuals were significantly positive only for Ad36 antibody presence in Florida, New York, and Wisconsin, respectively, and the serum cholesterol was also lower than the Ad36 antibody negative patients.[18] They also studied 26 pairs of adult human twins who were discordant for the presence of Ad36 antibodies. Adult human twins usually have similar body weights, probably due to the shared genetic basis. However, those who were positive for Ad36 antibodies were heavier and fatter than their co-twins who were Ad36 antibody negative. Although this is an association, it strongly suggests that the twins exposed to Ad36 infection may have gained weight and fat in response to Ad36 infection, compared with their respective co-twins who were uninfected. A study from San Diego, California, reported that 22% children with obesity were exposed to Ad36, as determined by Ad36 antibody presence. Median BMI of Ad36 antibody positive children was also higher compared with Ad36 antibody negative children.[25,62,70] In Italy, the odds ratio for the risk of obesity due to Ad36 antibody seropositivity was 6.879.[24] The association of human obesity with Ad36 antibody presence could not be confirmed in several studies including a US military personnel study,[75] in individuals of Netherlands and Belgium,[76] in Korean adults,[63] in US children from Georgia and Indiana,[77] Chinese Han population,[78] and in Young Finns study.[79] Nonetheless, a meta-analysis of studies about the association of Ad36 antibody presence with human obesity shows that the odds ratio of having obesity is 1.60 for those exposed to Ad36 infection.[80] Compared with adults, the association of Ad36 infection with obesity has been more consistently reported for children. In Sweden, prevalence of Ad36 seropositivity in lean children increased from ~7% in 1992-1998 to 15%-20% in 2002-2009, which corroborated with a parallel increase in obesity in the country. Association of Ad36 seropositivity with obesity is also reported in South Korean children.[25,62]

In addition to the association of Ad36 seropositivity with adiposity, studies also reported serum lipid profile in individuals, which is summarized in Table 2. In 2015, Dusatkova et al reported significantly higher prevalence of obesity risk carrier alleles *PCSK1*rs6232, *PCSK1* rs6235, and *BDNF* rs923461 in Ad36 positive Czech adolescents.[59] *APMI* and *visfatin* are known to modulate metabolic processes including glucose metabolism and fatty acid oxidation, both genes were upregulated in naturally Ad36 infected individuals and also in 3T3-L1 cells upon Ad36 infection.[52] Collectively, the data show unequivocally that exposure of animals to Ad36 infection increases adiposity. On the other hand, in humans, ethical reasons preclude such experimental infection. The observational studies do not establish causative role of Ad36 in increasing adiposity in humans. Nonetheless, collective data from animal and human studies and mechanistic explanations from in-vitro studies seem to build a strong case for the adipogenic role for Ad36 in humans.

3.2 | Glycemic control

3.2.1 | In-vivo

When rats were experimentally infected with Ad36, they improved systemic glycemic control as determined by lowering of fasting serum insulin levels compared with control, and better insulin sensitivity as determined by lower HOMA-index. Ad36 infection produced a significant decrease in NE concentrations in the paraventricular nucleus of the infected rats.[19] However, depletion of NE causes suppression of corticosterone[81] which may increase insulin sensitivity by promoting glucose transport in adipocytes and a parallel reduction in lipolysis.[82] Krishnapuram et al reported that Ad36 improved glycemic control in chow-fed and high-fat fed mice model and outlined a working model for the mechanism of improving glycemic control.[44] To understand the molecular basis for lower fasting serum insulin level, they examined insulin receptor signaling. It was revealed that Ad36 in fact inhibited the proximal insulin signaling and yet increased the distal insulin signaling leading to increased cellular glucose uptake. In cells of adipose tissue, skeletal muscle, and liver, Ad36 increased the distal insulin signaling via the activation of Ras-PI3K pathway, which in turn increased the glucose transporters (GLUT 4 and GLUT 1) in skeletal muscle and adipose tissue and lowered GLUT 2 and Glucose-6-

TABLE 2 Ad36 and E4orf1 related signaling and functions

Increased					
Cell	Adipocytes[17] aP2[21,22,50] ATF3[51] ACC[55] Adiponectin[22,28] APMI[52] cAMP[14] CREB[14]	C/EBP-α[51] C/EBP-β[14,21,50] C/EBPΔ[50] C/EBP-γ[51] GAPDH[17,22,50] Cidec[55] FSP27[55]	FABP4[51] FAS[22] GLUT1[23] GLUT4[23,27,33] HMGR[55] IL-6[84] LPL[21,22,51] Lactate production[20]	MCP-1[83] PPARγ[14,21,22,50,51] PKB[14,22] p38[14] pAKT[83] PI3K[22,23,27,50]	PECAM-1[22] Perilipin[55] Ras[22,23,27] SREBP-1c[55] SERBP2[55] TNF-α[84] Visfatin[52]
Animal	AdipoR1[44] AdipoR2[44] Adiponectin[44] AMPK[44,85] ACC1[20] ApoB[44] CD68[44]	C/EBP-α[19] C/EBP-β[19] CPT I[44] Cholesterol in LDL fraction[19,43] ENPP1[86] FoxO1[44]	FAS[20] GAPDH[19] GLUT4[36,44] GLUT1[44] 5-Hydroxytryptamine[19] IRS-1[44]	IRS-2[44] Leptin[19] MTP[44] MCP-1[44,45] M1/M2[86] NRF-1[85] NF-κB[45] PGC-1α[85]	p-AKT[36] PPARγ[44] Total Body Fat[12,15] TNF-α[44,45] Triglyceride[19] UCP-1[85] Visceral Fat[12,16]
Human	Adiponectin[52] *BDNF*rs923461[59] Fasting Insulin[60]	IL-6[77] LDL Cholesterol[58,62] MCP-1[63,77]	Macrophage infiltration[52] TNF-α[77]	*PCSK1*rs6232[59] *PCSK1*rs6235[59] Serum TG[24,62]	Serum Cholesterol[58,62] VEGF[77] Visfatin[52]
Decreased					
Cell	Adiponectin[84] AMPK[55]	Leptin[20] MCP-1[22]	RunX2[21] UCP3[55]	Wnt10b[14,50]	
Animal	Cholesterol[10,12,15,16] Serum TG[10,12,15]	Norepinephrine[19] GLUT2[44] G-6-pase[44]	IL-6[44] IL-18[42]	MCP-1[42] SREBP-1c[44]	Corticosterone[19]
Human	Fasting glucose[44,58] Fasting Insulin[44]	HDL Cholesterol[24,60]	LDL Cholesterol[62,75]	Serum Cholesterol[18,62,73]	Serum TG[18,52,63,73,87]

phosphatase in the liver. Reduced hepatic GLUT2 suggested reduced hepatic glucose release in the presence of Ad36. Thus, Ad36 appears to improve systemic glycemic control by increasing skeletal muscle and adipose tissue glucose uptake, and by decreasing hepatic glucose output, thereby reducing circulating glucose levels. Similar signaling mechanism was observed when HSKM and adipose tissue explants were experimentally infected in-vitro.[22,23] In mice adipose tissue, Ad36 infection increased the expression of inflammatory cytokines such as TNF-α, MCP1, and CD68 mRNA, which are known down regulators of proximal insulin signaling.[88,89] Moreover, Adiponectin is a key adipokine, which exists in higher-, medium-, and lower-molecular-weight forms, among which higher molecular weight is linked to insulin sensitivity.[90] Ad36 significantly increases the levels of total adiponectin in high fat fed mice, probably via PI3K upregulation.[91,92] Adiponectin can also activate AMPK and promote glucose uptake in skeletal muscle, which may contribute to the total glucose disposal in Ad36 infected mice.[93]

A search for identifying the gene of Ad36 that is responsible for its effect on glycemic control revealed that *E4orf1* gene of Ad36 is necessary and sufficient to increase cellular glucose uptake.[14] Next, E4orf1 was tested extensively in-vivo. E4orf1 was expressed in mice in different ways. In one model, E4orf1 was expressed in the liver and muscle of the C57Bl/6J mice using a retrovirus vector.[46,86] In other experiments, E4orf1 was expressed in the livers of diet induced obese mice (DIO) and db/db mice by using the recombinant adeno-associated viral (rAAV) serotype vector Rec 2[38] and in wild type mice via adipocyte targeting sequence (ATS).[47] Apart from the delivery of E4orf1 in mice through vectors, a novel transgenic mice model (E4orf1-Tg) was generated where the mice express E4orf1 in adipose tissue upon doxycycline induction.[36] These different models developed by different research groups yielded very similar findings.

When wild-type mice were challenged with high-fat diet for 2 weeks followed by intra-peritoneal, intramuscular, and subcutaneous injections with pBabe-E4orf1 or pBabe-puro retrovirus,[46] the E4orf1 group showed improvement in blood glucose clearance in 1 week. The improvement in glycemic control faded within the next week, only to be recaptured when the mice were re-infected again 7 days later. Along with the improvement in glycemic control, pBabe-E4orf1 receiving mice had impaired proximal insulin signaling in epidydimal fat as determined by downregulation of phosphor-tyrosine (p-Tyr) expression of insulin receptor.[86]

rAAV induced hepatic expression of E4orf1 in *db/db*, DIO, and WT mice models was described by McMurphy et al.[38] Lower fasting blood glucose and serum insulin levels were consistent among all three mice models; they also showed improved glycemic control during glucose tolerance tests (GTTs). The level of insulin was measured only in the *db/db* mice during GTT and was significantly lower compared with the control group.

ATS mediated delivery of *E4orf1* gene in HFD fed mice improved glucose disposal during GTT and ITT.[47] ATS-E4orf1 lowered the blood glucose level in streptozotocin-treated mice within 4 to 8 hours of injection. The effect was not significant after 12 hours but was lower than the control mice. The GTT performed in the streptozotocin-treated mice also showed faster reduction in glucose level despite the lack of insulin.

In E4orf1-Tg mice, in response to E4orf1 induction by doxycycline, glucose disposal as determined by GTT was minimally better compared with WT mice. However, insulin release in response to glucose load was markedly and significantly lower in the E4orf1-Tg

mice, compared with WT mice. This suggested that E4orf1 expressing transgenic mice may have lower requirement for insulin.[36] In the subcutaneous white adipose tissue of E4orf1-Tg mice, insulin signaling cascade showed marked reduction in p-Tyr of insulin receptor, and significant upregulation of pAKT and GLUT4. Overall, it appears that the *E4orf1* gene is responsible for the glycemic effect of Ad36, and it increases glucose disposal, which, in turn, may reduce the need for insulin, an effect termed as Insulin Sparing Effect.[94]

3.2.2 | In-vitro

Ad36 infection increases glucose uptake in rat adipocytes in the absence or presence of insulin.[20] Ad36 increased glucose uptake in HSKM cells and adipose tissue explants of human diabetic and non-diabetic subjects via insulin independent PI3K pathway, and increased Ras, and phosphor-PI3K protein abundance.[23] Considering that Ad36 increases adiposity, improves glycemic control and strongly upregulates PPARγ, an unanswered question was if the Ad36 action was similar to that of thiazolidinediones, which are PPARγ agonist anti-diabetic agents that also increase adiposity.[95] However, several experiments showed that Ad36 enhances glucose uptake irrespective of chemical inhibition, downregulation, or absence of PPARγ.[28]

As infection with Ad36 is not a viable treatment option for improving glycemic control, researchers searched for and identified the Ad36 derived E4orf1 protein described earlier[14] that improves glucose disposal. E4orf1 mediates anti-hyperglycemic action of Ad36. E4orf1 promotes glucose uptake in pre-adipocytes, adipocytes, and myoblasts, and reduces glucose release from hepatocytes.[27]

Many studies investigated the molecular mechanism by using cell lines that inducibly express E4orf1 in response to doxycycline treatment. The E4orf1 expressing 3T3-L1 cells also showed upregulation of the Ras/PI3K/GLUT4 pathway.[27,33,86] Furthermore, based on the studies, it was proposed that E4orf1 interacts with the PDZ domain binding motif of Drosophila discs-large (Dlg1) protein in cells, and the complex activates Ras, which in turn upregulates PI3K/GLUT4 pathway, leading to greater glucose uptake in cells.[46]

3.2.3 | Human studies

Ad36 enhances glucose disposal in cell and animal models, which led to the expectation that those humans who are naturally exposed to Ad36 would have better glycemic control. A study of over 1400 Caucasian, African American, and Hispanic men, women, and children from four cohorts showed that past Ad36 infection is associated with better glycemic control, independent of age, sex, race, or adiposity.[44] Ad36 seropositive subjects have greater adiponectin levels[28] which may contribute to the better glycemic control. Even in insulin resistant states, Ad36 infection shows a relatively protective association with glycemic profile. Past Ad36 infection was also associated with significantly lower HbA1c levels, better glycemic control, and greater systemic glucose disposal rate even in the presence of greater adiposity.[22,28,44,96] Most interestingly, a longitudinal study of 1400 Hispanic individuals with a mean age of about 40 years showed that individuals who were exposed to Ad36 at baseline gained significant amount of weight in the following 10 years but had less age-related deterioration in glycemic control, compared with those who were unexposed to Ad36.[34]

Overall, Ad36 or E4orf1 induce greater glucose uptake in cells, enhance glycemic control in rodent models, and Ad36 shows cross-sectional and longitudinal associations with better glycemic control in humans. There were no studies reporting the role of E4orf1 in humans. Collectively, the findings strongly suggest a causative role of Ad36 and E4orf1 in enhancing glycemic control in humans.

3.3 | Liver fat accumulation

3.3.1 | In-vivo

Generally, increase in adiposity is linked with hepatic steatosis. However, despite Ad36's role in increasing adiposity in animals, it shows protective effects in the liver. Ad36 infection protects the liver from hepatic steatosis and inflammation, thus may hinder the progression to nonalcoholic steatohepatitis. Ad36-infected high fat fed mice significantly increased glycogen and lowered lipid content in the liver compared with control.[44] In addition, Ad36 also reduced hepatic glucose release, lipogenesis, inflammation (downregulated inflammatory markers IL-6, INFγ, TNF-α), insulin resistance, and fibrosis and upregulated lipid oxidation and export in the liver.[44] Lipid accumulation and hepatic triglyceride level were less in a mice model upon high fat feeding where Ad36-E4orf1 was induced transgenically.[36] Also, a study that introduced Ad36-E4orf1 in wild type mice with ATS found fewer and smaller fat droplets in the liver along with lowered mRNA expression of pro-inflammatory cytokines TNF-α and MCP-1.[47] Ad36 upregulates adiponectin which may activate AMPK[44] and, in turn, may protect the liver against steatosis.[97] Reduced lipogenesis by Ad36 is inferred based on the downregulation observed for FAS (fatty acid synthase), SREBP-1c (sterol response element-binding protein-1c), FOXO1 (forkhead box O1), and increased lipid oxidation as suggested by the upregulation of AdipoR1 and AdipoR2 (adiponectin receptors), CPT I (carnitine palmitoyltransferase I), LXR (liver X receptor), and PPARγ.[44] It appears that Ad36 may increase lipid export by upregulating MTP (microsomal triglyceride transfer protein) and apoB (apolipoprotein B). Hepatic E4orf1 transduction upregulated genes involved in glycolysis Hk2, Pgam2 (encoding 6-phosphofructo-2-kinase) in DIO, db/db, and WT mice and downregulated gluconeogenesis (glucose-6-phosphatase catalase subunit) in DIO and WT mice.[38]

3.3.2 | In-vitro

In addition to clinical and animal studies, Ad36 or its E4orf1 protein downregulates GLUT2 (the key hepatic glucose transporter) and suppresses glucose output in hepatocytes.[31] The same in-vitro study also reported suppressed de novo-lipogenesis, increased lipid export, and decreased ratio of incomplete to complete fat oxidation in hepatocytes.

3.3.3 | Human studies

In a series of studies conducted in Italy, Trovato et al reported an association of Ad36 exposure with lower occurrence of non-alcoholic fatty liver diseases and significant reduction in the bright liver disappearance in Ad36 seropositive non-alcoholic fatty liver disease patients.[98,99] Additional studies from the USA also reported that exposure to Ad36 was associated with lower intrahepatic lipid.[44]

Overall, Ad36 and E4orf1 reduce hepatic accumulation in animals even in the presence of high fat diet. Cell signaling studies suggest that the reduced lipid in liver may be due to reduced lipid uptake, greater oxidation, and export of lipid from liver. It is interesting that human observational studies reflect findings similar to those in in-vivo and in-vitro studies. Considering that fatty liver is associated with impaired insulin action in liver, skeletal muscle, and adipose tissue the phenotype of lower hepatic lipid in Ad36 infected individuals may be particularly beneficial.

3.4 | Ad36 and inflammation

A chronic state of low-grade inflammation contributes to the maintenance of obese state.[100,101] The MCP-1 stimulates macrophage infiltration into adipocytes.[102] The MCP-1 signaling induces adipogenesis in preadipocytes independent of PPARγ activation,[103] and it has an angiogenic effect on endothelial cells.[104] Ad36 infection stimulates MCP-1, probably through the activation of nuclear factor κB.[45] In fact, experiments with MCP1 knockout mice show that MCP-1 is necessary for Ad36-induced adiposity.[45] In addition, Ad36 infection of adipocytes produces greater amount of inflammatory cytokines such as interleukine-6 and TNF-α.[84]

Interestingly, after experimental Ad36 infection of rats (2-4 days), animals show an acute reduction in MCP-1 and IL-18.[42] Whereas, following chronic Ad36 infection for several months, mRNA levels of TNF-α and pro-inflammatory M1 macrophages (Cd64) were upregulated in the fat pad of Ad36 infected mice. Ad36-induced increase in MCP-1 can be attenuated by exercise intervention, independent of weight loss.[85] Nam et al reported that Mulberry extract reduces inflammatory cytokines MCP-1, and TNF-α and M1 macrophage induced by Ad36.[105]

Serum MCP-1 levels are higher in humans exposed to Ad36.[45] Although Ad36 increases inflammatory cytokines in various studies, its *E4orf1* gene does not seem to share this pro-inflammatory property. Instead, E4orf1 expression in 3T3-L1 cells decreased MCP-1 expression and improved glucose uptake.[83]

Overall, it appears that right after infection, Ad36 reduces inflammatory response, which is a typical self-serving feature of a viral infection for its survival and spread. Chronically, however, Ad36 infection appears to increase inflammatory cytokines. Considering that greater adiposity is associated with inflammatory response, Ad36 increasing adiposity may be the driver for the observed inflammatory response after Ad36 infection. On the other hand, Ad36 appears to require MCP1 for inducing adiposity in mice.[45] Additional information is needed to clarify the relationship of Ad36 with inflammation and glucose disposal.

4 | SUMMARY AND CONCLUSIONS

Adenoviruses SMAM-1 and Ad36 increase adiposity in various animal models including chickens, mice, rats, and marmosets. Substantial number of in-vitro studies have delineated the molecular mechanism underlying this adipogenic effect. Additional adipogenic adenoviruses such as Ad5 or Ad9 were later discovered, but not all adenoviruses are adipogenic. Some adenoviruses such as Chick Embryo Lethal Orphan, and Ad2 are not adipogenic. Considering that humans cannot be infected experimentally to determine a direct causal effect, the key question, whether adipogenic adenoviruses cause obesity in humans, is not yet resolved conclusively. Cross-sectional and longitudinal studies show strong association of past natural exposure to Ad36 with risk of obesity in humans and rhesus monkeys. A small percent of studies did not report this association. A human virus that causes obesity in animal models using identifiable molecular pathways and shows association with adiposity or fat gain upon natural exposure in humans collectively in about 10,000 subjects[106] is likely to impact human adiposity. However, more conclusive evidence will have to come from creatively designed additional observational studies.

Meanwhile, there are several implications based on the information generated thus far.[107] The reports lend strong support to the concept of Infectobesity and add infections as one of the multiple contributors of obesity. Recently, infections, in general, were reportedly linked with greater weight gain.[108-110] The ability of a few microbes to cause obesity should prompt search for identifying additional and specific adipogenic microbes. Possibility of an infection leading to obesity should take away the guilt and stigma felt by many sufferings from the disease obesity. Importantly, this research has led to efforts to develop vaccines to prevent Ad36 induced obesity.[35]

Another important implication is about the development of antidiabetic agents based on the action of the E4orf1 protein. In particular, its insulin sparing effect[94] is highly desirable. In-vivo and in-vitro reports thus far show a strong effect of E4orf1 on glucose disposal. Its benefit or adverse effects in higher animals or humans are not yet known. Overall, the field seems to have made substantial progress in the past 25 years, with contributions from several research groups. Several gaps in knowledge still exist, which may be addressed by future research.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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