Vol 2:40-46

JEMI-PEARLS

Disease Management for Adenovirus 36-Induced Obesity

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BACKGROUND INFORMATION

The World Health Organization (WHO) defines obesity as having a Body Mass Index (BMI) of 30 or greater [1]. In 1997, the WHO declared obesity as a global epidemic in developed and developing countries, with obesity rates continuing to increase exponentially [1]. In 1995, 200 million adults worldwide were obese and this number rapidly increased to 600 million in 2014 [1]. From 2011 to 2012, Statistics Canada estimated that 6.3 million adult Canadians are living with obesity, which suggest that approximately one in four Canadians are obese [1]. In addition to negatively impacting the mental and physiological health of individuals, obesity leads to large economic burdens approximating to an annual cost of 4.6 billion in Canada [2, 3]. The causes of obesity are multifactorial, with behavior factors such as overeating and low physical activity traditionally thought to be the main contributing factors [4]. Therefore, conventional treatment and prevention strategies for obesity often include restriction on diet and management of physical activity, which provide minimal effect on sustainable improvements for obesity [4]. These behavioural interventions are ineffective because such interventions are often irrespective of the cause of obesity. Therefore, cause-specific treatments and prevention strategies are urgently needed to address the economic burden associated with the exponentially increasing rates of obesity worldwide.

The Centers for Disease Control and Prevention (CDC) released a series of adult obesity prevalence maps from 1985 to 2007 showing the rapid increase in obesity prevalence in the US [5]. These map show obesity predominantly spreading from neighbouring states, suggesting that obesity may have an infectious origin. The trend from these maps also align with the term "Infectobesity", meaning obesity of infectious origin, introduced by Dr. Nikhil Dhurandhar [6, 7]. Dhurandhar *et al.* first reported the association between adenovirus and obesity, with adenovirus 36 (Ad36) being the first human adipogenic virus reported. Adenovirus serotypes 5 and 37 were also later reported to be associated with obesity in humans [4]. Previously, Other non-human viruses such as Canine distemper virus (CDV), Rous-associated virus (RAV-7), borna disease virus (BDV) and SMAM-1 were also reported to be associated with obesity [7].

Adenoviruses are members of the *Adenoviridae* family and are non-enveloped, icosahedral, doubled stranded

Received: Feb./17 **Accepted:** **Feb**./17 **Published:** July/17

Citation Zhou, B. 2017. Disease Management for Adenovirus 36-Induced Obesity. JEMI-PEARLS. 2:40-46.

DNA viruses with greater than 50 serotypes known to cause disease in humans [8]. Adenoviruses commonly infect the upper respiratory tract, gastrointestinal tract and conjunctiva and are transmitted through airborne spread, direct contact or fecal-oral route [8]. Adenovirus infections are prevalent worldwide with majority of infections occurring in young children and rarely causing serious disease in immunocompetent individuals [8].

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Ad36 is the most researched human adipogenic virus because of its low cross-reactivity with other serotypes [4]. Ad36 was first isolated in Germany from the feces of girl with enteritis and diabetes [4, 9]. Animal models of chickens, mice, rats and marmosets showed causality between Ad36 infection and adiposity, along with a nine year longitudinal study in rhesus monkeys showing that naturally Ad36-infected monkeys had a 15% increase in bodyweight [4]. Moreover, transfusion of blood from Ad36-infected animals to an uninfected recipient caused the recipient to become Ad36-infected and obese [4, 9]. Numerous studies have suggested that Ad36 promotes obesity by increasing adipogenesis and maintaining low grade chronic inflammation [9]. *In vitro* and *in vivo* studies both suggest that Ad36 promotes the proliferation and differentiation of adipose-tissue derived stem cells towards adipogenic lineage, increasing the number of adipocytes [4, 9]. The mechanisms leading to obesity from Ad36 infection is not well understood, but adiposity and low-grade chronic inflammation are speculated to have an important role in maintaining obesity. In Ad36-infected animal models, adiposity has been associated with the upregulation of PPAR-γ and C/EBP, which are key transcription factors for adipogenesis [8]. In addition, Ad36 viral E4 orf-1 gene has been shown to stimulate proadipogenic differentiation in 3T3-L1 and human adipose stem cells [8]. Adipokines, such as monocyte chemotactic protein (MCP-1), and the phagocytic role of adipose tissue contribute to maintaining low-grade chronic inflammation [4]. Therefore, literature suggest that Ad36 appear to promote chronic obesity in animal models by sustaining inflammation and expanding adipose tissue.

Multiple correlational studies on different populations have been investigated in attempt to establish an association between Ad36-infection and obesity. Atkinson *et al.* released the first correlational study between Ad36 infection and obesity in humans. The study was performed in the US, showing that 30% of obese individuals compared to 11% of non-obese individuals were seropositive for Ad36 [4]. Furthermore, the study showed that Ad36 seropositive individuals, whether obese or non-obese, had a significantly higher BMI than seronegative individuals [4]. The most convincing human study showing the correlation between Ad36 and human obesity involved 26 pairs of twins, 20 monozygotic and 6 dizygotic; the study showed Ad36-seropositive twins to have a significantly higher BMI and body fat than their seronegative twin [4]. Additionally, further studies shown in Table 1 performed in the US, Italy and Turkey showed strong correlation between Ad36-seropositivity and obesity in adults and children [10, 11, 12, 13]. Although strong correlations between Ad36 infection and obesity in children and adults have been established in certain populations such as the US, Turkey and Italy, one study performed in the Netherlands showed low Ad36 prevalence and no correlation between Ad36 and obesity (Table 1) [10, 11, 12, 13, 14]. Therefore, the following article uses longitudinal studies to determine the pathological interplay between Ad36 infection and obesity in humans in order to provide cause-specific treatment and prevention strategies for improving the global obesity epidemic.

RESEARCH QUESTIONS

Discovering effective, sustainable treatment and prevention strategies for obesity is currently and will continue to be a pressing issue as obesity rates continue to increase exponentially while conventional treatments provide unsustainable benefits [4]. Studies have shown adenovirus vaccines to be safe and efficacious, suggesting that Ad36 vaccine development may be an attractive avenue against Ad36-induced obesity [15]. In addition, the gut microbiota has been shown to be a major player in obesity and should be researched

further to determine the relations between Ad36-infection, the gut microbiota and obesity [4, 8, 16, 17, 18].

This review will address three essential questions to aid the development of personalized therapies for Ad36-induced obesity with the ultimate goal of improving the global obesity epidemic. Numerous studies in humans have shown a strong correlation between Ad36-seropositivity and obesity while a few studies have shown low Ad36 prevalence with no correlation between Ad36-seropositivity and obesity [10, 11, 12, 13, 14]. This discrepancy could be an indication of susceptibility or protection against Ad36-induced obesity, which prompts further investigations into such populations to detect for biomarkers, hence leading to the first question: what biomarkers could be indicative of susceptibility or protection to adenovirus 36-induced obesity? Such biomarkers would allow monitoring of subgroups that are susceptible to Ad36-induced obesity in order to provide prevention measures using vaccines or minimize the impact of Ad36-induced obesity through modulation of the gut microbiome with antibiotics or elimination of viral replication with antivirals. This prompts the second question of determining whether Ad36 infections are age, gender or population specific; therefore, what is the reservoir for adenovirus 36-induced obesity? This second question will shed light on understanding the pathogenesis of Ad36 through longitudinal studies and help guide treatment or prevention strategies depending on how the virus maintains chronic obesity in Ad36-seropositive individuals, leading to the last essential question of this article: what treatment or prevention strategies would be most effective against adenovirus 36-induced obesity? By addressing these three important research questions, this article hopes to provide constructive solutions to address the worldwide obesity epidemic.

PROJECT NARRATIVE

What biomarkers could be indicative of susceptibility or protection to adenovirus 36-induced obesity?

As shown in Table 1, the percentage of Ad36-seropositivity is significantly higher in obese than non-obese individuals in the US, Italy and Turkey [10, 11, 12, 13]. On the contrary, the Netherland population shows no significant correlation between Ad36-seropositivity and obesity [14]. Obesity is a metabolic disease and substantial numbers of studies have reported the contribution of the gut microbiota in obesity [4, 8, 16, 17, 18, 19, 20]. Particularly, a higher Firmicutes to Bacteriodetes ratio has been detected in obese humans and mice models [8, 16, 17]. In addition, the gut microbiota is associated with the breakdown of non-digestable plant-derived polysacchides into short chain fatty acids (SCFAs) that may contribute to the integrity of the intestinal epithelial and prevent endotoxin translocation into the systemic circulation [8, 18]. Tambo *et al.* describes the "gut-to-adipocyte axis" signaling as the communication between the gut microbiome and adipocytes through the release of endotoxins [8]. This signaling involves three systems, the endocannabinoid, apelinergic and enterondocrine, which are shown to influence obesity through promoting adipogenesis, maintaining chronic low grade inflammation or disrupting intestinal integrity [8]. Therefore the composition of symbiotic bacteria in the gut microbiota is implicated in the maintenance of the gut immune system and many metabolic diseases such as obesity.

TABLE 1 Correlation between adenovirus 36-seropositivity and obesity in the US, Italy, Turkey and the Netherlands.

Country	Number of Subject	Ad36 Prevalence (%)		Ad36 Prevalence (%)	Mean Age (years)	Source
		Obese	Non-obese			
US (adult)	502	30	11	24.7	42	[10]
US (children)*	124	22	7	15	14	[12]
Italy	203	65	33	43.3	46	[13]
Turkey (adult)*	130	18	4	21.5	40	[11]
Turkey (children)*	146	27	6	33.1	11	[11]
Netherlands	509	6	4	5.5	≥19	[14]

*Obesity as ≥95th percentile of 2000 Center for Disease Control and Prevention growth charts

The importance of the gut microbiota in influencing the development of obesity is evident from the immense amount of literature supporting such a hypothesis, but no research has investigated the role of Ad36 on the gut microbiome composition and whether such alterations in the gut microbiome may lead to obesity in certain groups of individuals. Therefore, this article seeks to investigate the gut microbiome and metabolite composition of populations with strong and no correlation between Ad36-seropositivity and obesity in search of biomarkers that could indicate susceptibility or protection to adenovirus 36-induced obesity. Figure 1A illustrates the experimental design in biomarker discovery for Ad36-induced obesity. To address this research question, two major groups will be investigated: the first group will include populations showing strong correlation between Ad36 and obesity such as the US, Italy and Turkey; and the second group will include populations showing no correlation between Ad36 and obesity such as the Netherlands. The two groups will be further divided into subgroups of non-obese Ad36-seropositive, obese Ad36-seropostive, obese Ad36-seronegative and non-obese Ad36-seronegative. The obese Ad36-seropostive subgroup may suggest susceptibility factors, while the non-obese Ad36-seropositive subgroup may suggest protective factors against Ad36-induced obesity. The non-obese Ad36-seropositve subgroup may suggest factors that contribute to obesity but are irreverent to Ad36 infection and the non-obese Ad36-seronegative subgroup will act as the control group. Comparison between populations showing strong correlation and no correlation between Ad36-seropositivity and obesity may also indicate addition protective or susceptibility factors to Ad36-induced obesity. The gut microbiome composition and metabolite profile of each subgroup will be determined through microbiome sequencing coupled to metabolic profiling using mass spectrometry, resulting in the discovery of viral or host cell metabolites that could be indicative of susceptibility or protection to Ad36-induced obesity. Furthermore, research to assess the accuracy of the determined biomarkers and the specificity of Ad36 infections should be investigated using longitudinal studies.

What is the reservoir for adenovirus 36-induced obesity?

Publications on the epidemiology, peak age and clinical features of Ad36 infections are limited [21]. The only longitudinal study investigating the relation between Ad36-seropositivity and obesity in humans was performed in Finland [21]. This study followed young Finns from childhood to adulthood with a baseline to follow-up time ranging from 21 to 31 years [21]. The data from the Finn study demonstrated no causative role for Ad36 in human obesity, but major limitations of the study prevented the authors from reaching any definitive conclusions. The limitations included having only single sampling time points at childhood and adulthood and performing serological test with an ELISA test rather than the gold standard Ad36 serum neutralization assay [21].

To address the lack of longitudinal studies in the literature on the causative role of Ad36 in human obesity, this study attempts to monitor Ad36 replication throughout the life course from childhood to adulthood in order to establish the reservoir for adenovirus 36-induced obesity. As demonstrated in Figure 1B, the experimental design would require regular physician monitoring of Ad36 replication in serum samples using polymerase chain reaction (PCR) in high Ad36 prevalent populations throughout childhood to adulthood. Regular monitoring of the biomarkers discovered in the previous research question will allow accuracy assessment of the biomarker in predicting the progression of Ad36-induced obesity. The longitudinal study involving regular physician monitoring of Ad36 replication and associated biomarkers will help determine whether Ad36 infection is age, gender or population specific. Therefore, the subgroups at risk for Ad36-induced obesity and the window for intervention can be identified to guide treatment and prevention strategies. Furthermore, the most effective treatment or prevention strategies would depend on the mechanism of action that Ad36 employs to induce chronic obesity and therefore, elucidating such mechanisms would be necessary.

What treatment or prevention strategies would be most effective against adenovirus 36-induced obesity?

Multiple studies have shown that the knockdown of genes involved in inflammation and adiposity, the administration of anti-inflammatory agents or Ad36-inactivated vaccines are effective in reducing Ad36-induced obesity in animal models [15, 22, 23, 24]. Na *et al.* showed that Ad36-infected mice pre-inoculated with UV-inactivated Ad36 had similar body weight and epididymal fat to controls, whereas, Ad36-infected mice that was not pre-inoculated with UV-inactivated Ad36 showed enlarged epididymal fat pads and increase levels of inflammatory cytokines [22]. Therefore, UV-inactivated Ad36 inoculation prevented the increase in inflammation and body fat, suggesting that inactivated Ad36 vaccine may provide promising prevention

strategies for Ad36-induced obesity [22]. In addition, live oral vaccines against adenovirus serotypes 4 and 7 developed for US military recruits show high efficacy and safety profiles, therefore, further supporting that vaccines against Ad36 may be a promising prevention option [15]. In addition, Na *et al.* showed that Ad36 infection activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF_KB) which upregulates MCP-1 and macrophage infiltration into adipocytes, resulting in chronic inflammation and lipid metabolism

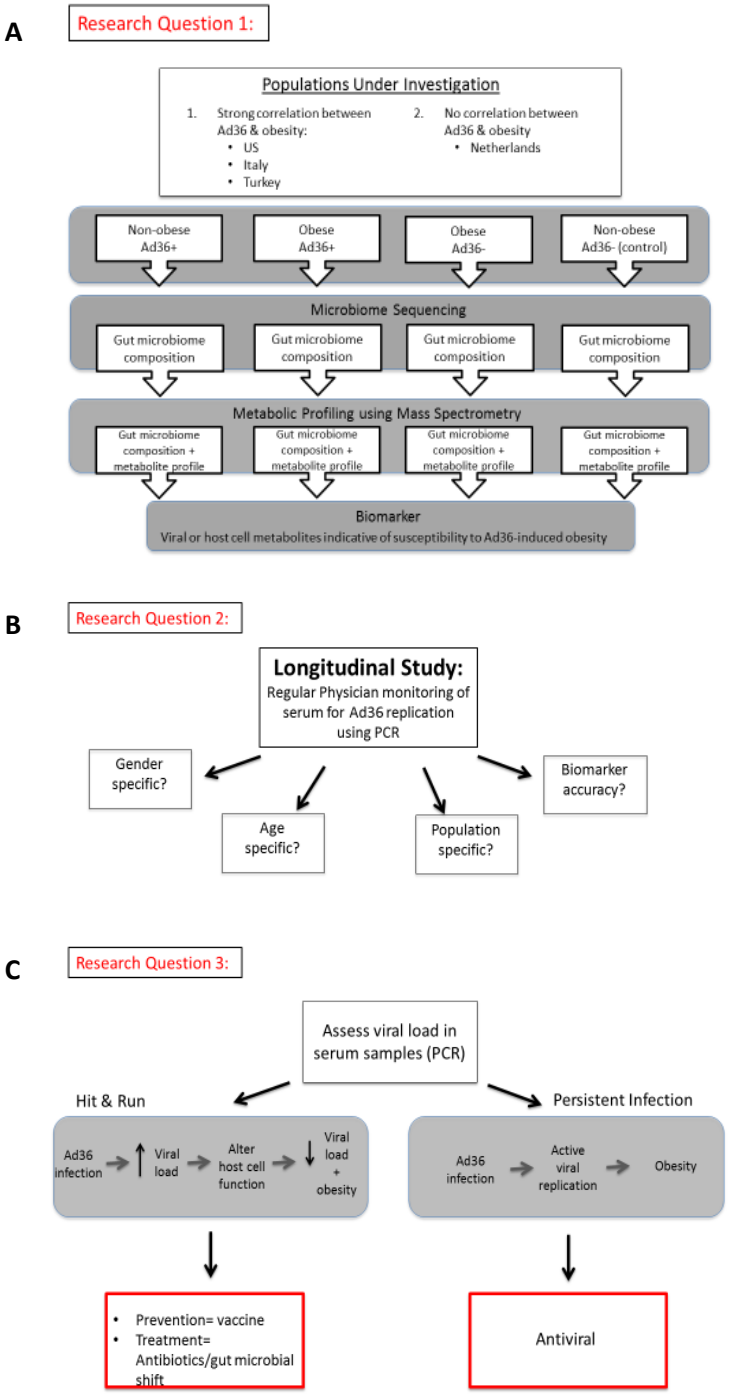

FIG. 1 Diagram integrating the three essential research questions of this study. **A)** Couple microbiome sequencing with metabolite profiling to discover biomarkers indicative of susceptibility or protection against Ad36-induced obesity. **B)** To determine the reservoir of Ad36-induced obesity using a longitudinal study involving regular physician monitoring of Ad36 infection in serum with PCR. **C.** Use PCR monitoring of Ad36 viral load in serum of Ad36-infected individuals to determine the mechanism of action of Ad36, either hit-and-run process or persistent infection, to develop cause-specific treatment and prevention strategy for Ad36-induced obesity.

deregulation to promote obesity [22]. The study showed that MCP-1 knockdown mice infected with Ad36 did not show increase in body weight and levels of MCP-1 and TNF-α, suggesting that MCP-1 knockdown mice were protected from inflammation and obesity [22]. As a result, MCP-1 appears to be a key regulator in maintaining inflammation and promoting adipogenesis in Ad36-induced obesity and may be a potential therapeutic target for the development of indirect-acting antivirals (IAA) against Ad36-induced obesity [22]. Furthermore, Na *et al.* demonstrated that Ad36-infected mice fed with mulberry extract had reduced total body weight and epidermal fat pads, along with 70% reduction in viral replication compared to untreated Ad36-infected mice [24]. In addition, Roger *et al.* demonstrated that the Ad36 E4-orf1 gene increased transcriptional factors such as CCAAT-enhancer binding proteins and peroxisome proliferator-activated receptor, which increases adiposity and promotes obesity [25]. Therefore, the E4 orf-1 gene may serve as a potential therapeutic target for developing a direct-acting antiviral (DAA) against Ad36-induced obesity. Dhurandhar proposed that Ad36 may infect through two mechanisms, either through the hit-and-run process or persistent infection [6]. To implement effective treatment or vaccines for Ad36-induced obesity, PCR will be used to monitor serum samples for Ad36 viral load to determine the mechanism of action that Ad36 employs to induce chronic obesity. As shown in Figure 1C, the hit-and-run process maintains chronic obesity by increasing viral load after Ad36 infection causing alterations in host cell function, which maintains obesity even after elimination of viral load. If Ad36 employs the hit-and-run mechanism, the most effective prevention strategy would be to implement a vaccine for Ad36-seronegative individuals and antibiotic treatment to shift the gut microbiota back to healthy, non-obese promoting conditions for Ad36-seropositive individuals. However, if Ad36 employs persistent infection to maintain chronic obesity through active viral replication, then antivirals would be required to reduce viral replication and eliminate obesity. The most effective antiviral treatment would involve the combination of IAA and DAA, which would reduce resistance to treatment and increase efficacy. As proposed above, the combination of an IAA against MCP-1 and a DAA against *E4 orf-1* may serve as an effective treatment regimen. One major downfall with antivirals is that Ad36 infection may cause permanent damage to host functions that cannot be reverted even after the elimination of viral load. Therefore, elucidating the mechanism of action of Ad36 by assessing the viral load in the serum of Ad36-infected individuals will help guide effective strategies to treat or prevent Ad36-induced obesity in at risk individuals.

SUMMARY AND CONCLUSION

The declaration of the worldwide obesity epidemic by WHO was over two decades ago, but an effective treatment or prevention regimen has still not been developed to address the increasing obesity rates [1, 4]. Conventional treatments for obesity are often unsustainable because these regimens fail to address the cause of obesity; therefore, cause-specific regimens will need to be developed through a personalized medicine approach. Dhurandhar introduced the concept of obesity of infectious origin termed "Infectobesity" [6, 7]. Research on Ad36 has shown the causality between Ad36-infection and obesity in animals, but such causality still needs to be established in humans [4]. Many studies have shown the strong correlation between Ad36-seropositivity and obesity in humans, indicating a promising area for further investigation of cause-specific therapies to decrease the unresolved global obesity epidemic. Microbiome sequencing coupled with metabolite profiling will allow the development of biomarkers and enable screening for individuals at risk for Ad36-induced obesity. Longitudinal studies on Ad36 infection in humans will allow the determination of whether Ad36 infection is gender, age or population specific in order to guide intervention strategies against Ad36-induced obesity. Lastly, investigating the viral load in serum samples of Ad36-seropositive individuals will reveal how Ad36 maintains chronic obesity in infected individuals and help determine whether vaccine implementation or antiviral development would be the most appropriate regimen in reducing Ad36-induced obesity.

As with any personalized medicine studies involving the collection and analysis of personalized data, bioethical considerations are necessary. This study will require strict regulations to ensure proper storage of personal information of all participants. Microbiome sequencing and metabolite profiling is an emerging field with rapid improvements in sequencing techniques and bioinformatics tools, but the analysis and interpretation of unknown metabolites in complex biological samples still remain ambiguous [26]. Therefore, another major challenge of this study involves the requirement for robust computational software for the analysis and biological interpretation of sequencing data.

As a result, this article propose to couple microbiome sequencing with metabolic profiling and use

longitudinal studies to guide treatment and prevention strategies for groups susceptible to Ad36-induced obesity where behavioural interventions have minimal effect on improving obesity. The development of cause-specific treatment and prevention strategies will hopefully provide insight and solutions to address the worldwide obesity epidemic.

ACKNOWLEDGEMENTS

I would like to thank Dr. François Jean for his guidance and important feedback throughout the development of this research project and my peers for all the helpful feedback.

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