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Obesity Impairs the Adaptive Immune Response to Influenza Virus

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Abstract

Influenza, a highly contagious respiratory tract infection, affects millions of adults and children each year. Several high-risk populations include children, the elderly, the immunocompromised, and recently the obese. Given the dramatic rise in obesity over the past few decades, this increased risk for influenza infection poses a serious public health threat because nearly 500 million adults and children worldwide are classified as obese. Obesity impairs the immune response to influenza and influenza vaccination through alterations of the cellular immune system. Compared with vaccinated healthy-weight adults, vaccinated obese adults have twice the risk of influenza or influenza-like illness despite equal serological response to vaccination. This challenges the current standard of protection for influenza and suggests that further vaccination methods or therapeutics are required to combat this virulent respiratory virus.

Keywords: influenza; obesity; immunity; vaccination

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Influenza is a highly contagious respiratory tract infection caused by the influenza virus. On average, approximately 12,000 to 56,000 people die as a result of influenza infection in the United States each year (1). Globally, influenza has a mortality burden of approximately 250,000 to 500,000, with as many as 3 million to 5 million individuals severely affected by influenza each year (2). Influenza infection occurs primarily in cold-weather months because communal living and dry air promote the spread of the virus (3). Unlike other respiratory tract infections, influenza has a very rapid onset, characterized by a fever greater than 100°F and a cough in the absence of other causes. Other symptoms typically include headache, malaise, sore throat, congestion, body aches, and nausea or vomiting (2).

Influenza is a segmented RNA virus encapsulated by a host lipid envelope with two prominent viral peptides comprising its surface: hemagglutinin and neuraminidase. There are four strains of influenza virus—A, B, C, and D—with influenza A and B being the most common causes of infection in humans (4, 5). Antigenic drift, manifesting as small seasonal changes in hemagglutinin or neuraminidase, induced by evolutionary pressures from host immunity allows the virus to escape from antibody detection generated against the earlier influenza strain, potentially leading to reinfection of the same individual. Antigenic shift, or a combination of two or more influenza viruses to form a new influenza virus, can result in a pandemic, in which a virus spreads quickly throughout the population because of lack of previous exposure to this new influenza viral strain (4, 5).

In 1918, the "Spanish flu" pandemic impacted nearly one-third of the world's population and caused approximately 50 million deaths (6). Other influenza pandemics have occurred since 1918, often resulting from a zoonotic transfer of influenza from pigs or birds to humans. However, none has caused a similar degree of impact as the pandemic of 1918. This extreme example serves as a public health warning of the dramatic impact that a novel influenza virus can have on the overall population, highlighting the importance of prevention of this highly virulent respiratory tract infection.

Currently, vaccination remains the primary means of preventing influenza infection. Influenza vaccination typically involves an intramuscular injection of either trivalent or quadrivalent inactivated split vaccine composed of influenza A and B strains (7). Vaccination works by priming the immune system, thereby stimulating humoral and cellular immunological memory, such that upon secondary exposure, a swift and robust immune response can clear the pathogen, thus preventing infection and/or symptoms. Influenza vaccination occurs yearly to combat genetic drift in influenza, reducing the risk of potential epidemics each season. However, despite vaccination efforts, several populations of individuals, such as young children, the elderly, and the immunocompromised, have increased risk

of influenza infection (8–10). Paramount in developing new therapeutics and vaccines to combat influenza infection is developing methods for stimulating immune protection for these high-risk populations.

Obesity as a Novel Risk Factor for Influenza

Obesity has long been associated with higher risks of chronic diseases; however, recent data highlight obesity's association with a greater risk of infectious diseases (11, 12). Following the first pandemic influenza outbreak of the 21st century, the 2009 H1N1 swine flu, obesity (body mass index [BMI], $\geq$30.0 kg/m^2) was identified as an independent risk factor for increased morbidity and mortality resulting from pandemic H1N1 (pH1N1) infection (13). Louie and colleagues identified 51% of 534 adult cases of influenza in California during the 2009 H1N1 pandemic outbreak occurring in obese individuals, with 61% of influenza mortality cases happening in obese adults (13). Additionally, compared with healthy-weight adults, obese adults were found to have a higher risk of hospitalization for respiratory illness during seasonal influenza, with 1.45 times the odds of contracting seasonal influenza for adults with BMIs ranging from 30.0 to 34.9 kg/m^2 and 2.12 times the odds for adults with BMIs greater than or equal to 35.0 kg/m^2 (14).

This identification of obesity as a high-risk group for influenza infection presents a serious public health problem, given the exponential rise in obesity over the past few decades. Recently, for the first time in recorded human history, obese adults were found to outnumber underweight adults worldwide, suggesting that increases in the prevalence of obesity are not isolated to one particular region but reflective of an overall global trend (15). This alarming rise in the prevalence of obesity, coupled with its identification as a risk factor for influenza, increases the threat of severe infection for the nearly 500 million obese individuals worldwide (16). This observation begs several questions: How does obesity increase the risk of influenza infection? What mechanistic aspects of obesity cause greater lung pathology, complications, and/or mortality? How can data be used to better inform measures of influenza prevention, such as vaccination or therapeutic intervention?

Obesity and Influenza: Key Insights Gleaned from Animal Models

Recent findings using models of obesity have elucidated key insights into possible mechanisms of influenza infection in the obese. Murine models of obesity provide a unique translational option to study influenza's impact *in vivo* (17). Similar to epidemiological observations in obese adults, compared with lean mice, mice with diet-induced obesity infected with influenza virus have higher mortality rates (18). Obese mice developed greater lung inflammation and damage, higher numbers of cytotoxic CD8$^+$ T cells, and fewer suppressive T-regulatory cells (18, 19). Additionally, impairments in immune responses of obese mice occurred upon secondary influenza infection, whereby a primary sublethal influenza infection stimulated immunological memory much like vaccination. In these studies, compared with lean mice, obese mice infected with pH1N1 showed decreased memory CD8$^+$ T-cell production of IFN-γ, a key cytokine involved in influenza clearance (20).

Other studies using mice with diet-induced obesity infected with influenza have demonstrated decreased populations of bone marrow–resident B cells, the primary memory cells responsible for antibody production (21). This lack of memory B-cell development is further supported by the finding that influenza-specific antibodies were absent in obese mice at 35 days postinfection (19). Furthermore, Karlsson and coworkers found that obese mice had lower influenza-specific antibody production than lean mice despite increased production of neutralizing and nonneutralizing influenza-specific antibodies following adjuvant vaccination (22). Furthermore, obese mice had higher mortality even after adjuvant vaccine–stimulated production of influenza-specific antibodies, thus suggesting that obesity-impaired cellular immune responses are responsible for higher morbidity and mortality resulting from influenza (22).

These models of mice with obesity induced by a high-fat diet mimic observations seen in clinical settings. However, the question as to whether obesity or diet influences the outcome of infection remains an inherent limitation of these types of models. In other models, researchers have used global leptin receptor–knockout mice (db/db) to study the effects of obesity in response to influenza infection. Leptin, a hormone produced by adipocytes and responsible for regulating satiety, binds its receptor in the hypothalamus and thus signals a state of fullness. This type of model eliminates diet as a potential confounder because db/db mice become obese when fed a standard chow diet, due to a lack of leptin-stimulated satiety signaling. However, global depletion of leptin receptors also affects other cell types that use leptin signaling.

Compared with wild-type lean mice, leptin receptor–deficient obese mice (db/db) displayed greater mortality, higher measures of lung inflammation, and decreased viral clearance when infected with the pH1N1 influenza virus (23). Interestingly, selectively knocking out leptin receptors by crossing transgenic Cre and fully floxed (fl) mice for macrophages (LysM-Cre$^{+/+}$/LepR$^{fl/fl}$) and lung epithelial cells (SP-C-Cre$^{+/+}$/LepR$^{fl/fl}$) of influenza-infected lean mice did not alter viral clearance, lung damage, or mortality compared with wild-type mice (23). These data suggest that leptin signaling in other immune cell types may be critical in the adaptive immune response to influenza virus. Other studies support evidence suggesting that leptin plays a critical role in the immune response of T cells to pathogens through promoting the glycolytic activity of effector T cells (24).

To confirm whether obesity independent of diet accounts for the observed impairments in response to influenza, our laboratory used a hypothalamic leptin receptor–knockout (LepR$^{H-/-}$) model in mice. After crossing a fully floxed leptin receptor mouse with a Cre transgenic mouse under control of the Nkx2.1 promoter [C57BL/6J-Tg(Nkx2-1-cre)2S], mice lacking hypothalamic leptin receptors became obese as a result of hyperphagia while consuming the same chow diet as their lean controls (LepR$^{HFlox/Flox}$) (18). In this model, hypothalamic leptin–deficient obese mice (LepR$^{H-/-}$), compared with lean mice (LepR$^{HFlox/Flox}$) fed identical diets, were found to have increased mortality following infection with pH1N1 influenza. Additionally, obese

$LepR^{H-/-}$ mice had fewer $CD4^+$ and $CD8^+$ T cells as well as reduced numbers and activity of bronchoalveolar lavage T-regulatory cells (18). Interestingly, compared with lean controls, both mice with diet-induced obesity and obese $LepR^{H-/-}$ mice had altered metabolic profiles in lung tissue following pH1N1 infection (18).

Further investigations revealed that, following influenza infection, obese mice displayed altered metabolic profiles in lung, liver, mesenteric white adipose tissue, serum, urine, and feces (25). These studies demonstrate impairments in the metabolic profile as well as the immune response of obese mice compared with wild-type lean mice in response to influenza infection. Furthermore, leptin may play a critical role in the immune response of specific cell types in response to influenza virus. Obesity is inherently a metabolic condition in which prolonged caloric intake results in hormone and nutrient dysregulation and a proinflammatory environment. This altered metabolic milieu suggests that metabolic factors may account for higher rates of influenza infection and mortality.

Obesity and Influenza: Translational Insights into Possible Mechanisms

In addition to model studies using mice, clinical research studies with human subjects have also yielded great insight into how obesity alters the immune response to influenza. In 2011, our group established an observational clinical study in which participants received the influenza vaccine and underwent pre- and postvaccination venous blood draws to isolate serum and peripheral blood mononuclear cells. We found that at 30 days postvaccination, there were no significant differences in influenza-specific serological responses to the vaccine between healthy-weight, overweight, and obese individuals. Interestingly, however, overweight and obese adults had a greater decline in influenza-specific antibody titers at 1 year postvaccination (26).

In addition, peripheral blood mononuclear cells from influenza-vaccinated healthy-weight, overweight, and obese adults were stimulated with H1N1 influenza virus *in vitro*. We found that $CD4^+$ and $CD8^+$ T cells from obese and overweight adults expressed fewer activation and functional markers than those from healthy-weight adults (27). These impairments included less expression of activation markers CD28, CD40 ligand, CD69, and IL-12R as well as reduced expression of IFN-γ and granzyme B, critical cytokines produced by effector T cells involved in influenza clearance (25). Additionally, there were no differences in dendritic cell activation or function, suggesting that obesity affects T-cell activation and function independently of antigen presentation (25).

In the 2013–2014 and 2014–2015 influenza seasons, our group examined the influenza-specific antibody responses of 1,022 study participants monitored weekly for laboratory confirmed influenza or influenza-like illness. We found that, compared with vaccinated healthy-weight adults, vaccinated obese adults had twice the risk of influenza or influenza-like illness despite equivalent influenza-specific antibody responses to the vaccine (28). This finding suggests that impairments in the cellular immune response, namely $CD4^+$ and CD^+ effector T cells, are responsible for the higher observed risk of obese adults for influenza. This finding challenges the current standard of protection for influenza vaccination. This suggests that obese adults may need alternative approaches to achieve protection from influenza, much like the elderly and immunocompromised.

Obesity, as a metabolic condition, often leads to hyperinsulinemia, hyperleptinemia, and nutrient dysregulation. Furthermore, how T-cell metabolism drives cell homeostasis, proliferation, and function is well established (29, 30). Given impairments of T cells in murine models of influenza infection coinciding with alterations in the metabolic milieu of obese mice, it is plausible that alterations in the metabolic profile of T cells stemming from obesity may drive functional impairments. Recent studies have demonstrated how insulin and leptin signaling in T cells can drive glycolytic profiles of effector T cells, supporting cell proliferation and function (24, 31). Other factors, such as inflammatory cytokines and adipocyte activation of T cells, have been implicated in the skewing of T-cell populations, thus promoting a proinflammatory microenvironment and impairing the immune response to influenza (32–34). However, how excess insulin or leptin signaling, along with other obesogenic factors, alters the metabolic profile and thus the function of these critical immune cells remains unknown.

Conclusions

Influenza is a highly contagious respiratory tract infection that causes seasonal impacts each year. Furthermore, influenza poses a significant public health threat because the introduction of a novel virus has the potential to create a highly pathogenic pandemic. Recent identification of obesity as an independent risk factor for influenza morbidity and mortality increases the risk of influenza infection for nearly 500 million obese individuals worldwide. Understanding the mechanistic role obesity plays in increasing the risk of influenza morbidity and mortality remains fundamental to combating this virulent pathogen.

Recent findings that vaccinated obese adults have twice the risk of influenza or influenza-like illness compared with vaccinated healthy-weight adults despite equal serological response to vaccine challenge the current standard of protection for influenza. Translational data suggest that an alteration in the metabolic profile of T cells in obese individuals impairs the activation and function of these critical adaptive immune cells. How do systemic metabolic alterations of obesity directly affect immune responses to influenza? We propose that hyperinsulinemia or hyperleptinemia may lead to the metabolic dysregulation of T cells, thus impairing their function in response to influenza. Hence, metabolic restoration of cellular immune cells may be critical to restoring their function and reduce the risk of influenza morbidity and mortality in obese individuals. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1 Rolfes MA, Foppa IM, Garg S, Flannery B, Brammer L, Singleton JA, Burns E, Jernigan D, Reed C, Olsen SJ, *et al*. Estimated influenza illnesses, medical visits, hospitalizations, and deaths averted by vaccination in the United States. Atlanta, GA: Centers for Disease Control and Prevention [updated 2017 Apr 19; accessed 2017 Jul 18]. Available from: https://www.cdc.gov/flu/about/disease/2015-16.htm

2 World Health Organization (WHO). Influenza (seasonal): fact sheet. Geneva, Switzerland: WHO [updated 2016 Nov; accessed 2017 Jul 18]. Available from: http://www.who.int/mediacentre/factsheets/fs211/en/

3 Lowen AC, Mubareka S, Steel J, Palese P. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* 2007;3:1470–1476.

4 Chiu C, Ellebedy AH, Wrammert J, Ahmed R. B cell responses to influenza infection and vaccination. In: Oldstone MBA, Compans RW, editors. Influenza pathogenesis and control. Vol. II. New York: Springer; 2015. pp. 381–398.

5 Kreijtz JH, Fouchier RA, Rimmelzwaan GF. Immune responses to influenza virus infection. *Virus Res* 2011;162:19–30.

6 Watanabe T, Kawaoka Y. Pathogenesis of the 1918 pandemic influenza virus. *PLoS Pathog* 2011;7:e1001218.

7 Lambert LC, Fauci AS. Influenza vaccines for the future. *N Engl J Med* 2010;363:2036–2044.

8 Whitley RJ, Monto AS. Prevention and treatment of influenza in high-risk groups: children, pregnant women, immunocompromised hosts, and nursing home residents. *J Infect Dis* 2006;194(Suppl 2): S133–S138.

9 Madan RP, Tan M, Fernandez-Sesma A, Moran TM, Emre S, Campbell A, Herold BC. A prospective, comparative study of the immune response to inactivated influenza vaccine in pediatric liver transplant recipients and their healthy siblings. *Clin Infect Dis* 2008;46:712–718.

10 He XS, Holmes TH, Zhang C, Mahmood K, Kemble GW, Lewis DB, Dekker CL, Greenberg HB, Arvin AM. Cellular immune responses in children and adults receiving inactivated or live attenuated influenza vaccines. *J Virol* 2006;80:11756–11766.

11 Huttunen R, Syrjänen J. Obesity and the outcome of infection. *Lancet Infect Dis* 2010;10:442–443.

12 Milner JJ, Beck MA. The impact of obesity on the immune response to infection. *Proc Nutr Soc* 2012;71:298–306.

13 Louie JK, Acosta M, Samuel MC, Schechter R, Vugia DJ, Harriman K, Matyas BT; California Pandemic (H1N1) Working Group. A novel risk factor for a novel virus: obesity and 2009 pandemic influenza A (H1N1). *Clin Infect Dis* 2011;52:301–312.

14 Kwong JC, Campitelli MA, Rosella LC. Obesity and respiratory hospitalizations during influenza seasons in Ontario, Canada: a cohort study. *Clin Infect Dis* 2011;53:413–421.

15 NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet* 2016;387:1377–1396.

16 Huttunen R, Syrjänen J. Obesity and the risk and outcome of infection. *Int J Obes* 2013;37:333–340.

17 Nilsson C, Raun K, Yan FF, Larsen MO, Tang-Christensen M. Laboratory animals as surrogate models of human obesity. *Acta Pharmacol Sin* 2012;33:173–181.

18 Milner JJ, Rebeles J, Dhungana S, Stewart DA, Sumner SC, Meyers MH, Mancuso P, Beck MA. Obesity increases mortality and modulates the lung metabolome during pandemic H1N1 influenza virus infection in mice. *J Immunol* 2015;194:4846–4859.

19 Milner JJ, Sheridan PA, Karlsson EA, Schultz-Cherry S, Shi Q, Beck MA. Diet-induced obese mice exhibit altered heterologous immunity during a secondary 2009 pandemic H1N1 infection. *J Immunol* 2013; 191:2474–2485.

20 Karlsson EA, Sheridan PA, Beck MA. Diet-induced obesity in mice reduces the maintenance of influenza-specific $CD8^+$ memory T cells. *J Nutr* 2010;140:1691–1697.

21 Kosaraju R, Guesdon W, Crouch MJ, Teague HL, Sullivan EM, Karlsson EA, Schultz-Cherry S, Gowdy K, Bridges LC, Reese LR, *et al*. B cell activity is impaired in human and mouse obesity and is responsive to an essential fatty acid upon murine influenza infection. *J Immunol* 2017;198: 4738–4752.

22 Karlsson EA, Hertz T, Johnson C, Mehle A, Krammer F, Schultz-Cherry S. Obesity outweighs protection conferred by adjuvanted influenza vaccination. *MBio* 2016;7:e01144-16.

23 Radigan KA, Morales-Nebreda L, Soberanes S, Nicholson T, Nigdelioglu R, Cho T, Chi M, Hamanaka RB, Misharin AV, Perlman H, *et al*. Impaired clearance of influenza A virus in obese, leptin receptor deficient mice is independent of leptin signaling in the lung epithelium and macrophages. *PLoS One* 2014;9:e108138.

24 Saucillo DC, Gerriets VA, Sheng J, Rathmell JC, MacIver NJ. Leptin metabolically licenses T cells for activation to link nutrition and immunity. *J Immunol* 2014;192:136–144.

25 Milner JJ, Wang J, Sheridan PA, Ebbels T, Beck MA, Saric J. ^{1}H NMR-based profiling reveals differential immune-metabolic networks during influenza virus infection in obese mice. *PLoS One* 2014;9: e97238.

26 Sheridan PA, Paich HA, Handy J, Karlsson EA, Hudgens MG, Sammon AB, Holland LA, Weir S, Noah TL, Beck MA. Obesity is associated with impaired immune response to influenza vaccination in humans. *Int J Obes* 2012;36:1072–1077.

27 Paich HA, Sheridan PA, Handy J, Karlsson EA, Schultz-Cherry S, Hudgens MG, Noah TL, Weir SS, Beck MA. Overweight and obese adult humans have a defective cellular immune response to pandemic H1N1 influenza A virus. *Obesity (Silver Spring)* 2013;21: 2377–2386.

28 Neidich SD, Green WD, Rebeles J, Karlsson EA, Schultz-Cherry S, Noah TL, Chakladar S, Hudgens MG, Weir SS, Beck MA. Increased risk of influenza among vaccinated adults who are obese. *Int J Obes (Lond)* 2017;41:1324–1330.

29 MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* 2013;31:259–283.

30 Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. *J Exp Med* 2015;212:1345–1360.

31 Fischer HJ, Sie C, Schumann E, Witte AK, Dressel R, van den Brandt J, Reichardt HM. The insulin receptor plays a critical role in T cell function and adaptive immunity. *J Immunol* 2017;198:1910–1920.

32 Green WD, Beck MA. Obesity altered T cell metabolism and the response to infection. *Curr Opin Immunol* 2017;46:1–7.

33 Xiao L, Yang X, Lin Y, Li S, Jiang J, Qian S, Tang Q, He R, Li X. Large adipocytes function as antigen-presenting cells to activate $CD4^+$ T cells via upregulating MHCII in obesity. *Int J Obes* 2016;40:112–120.

34 Park HL, Shim SH, Lee EY, Cho W, Park S, Jeon HJ, Ahn SY, Kim H, Nam JH. Obesity-induced chronic inflammation is associated with the reduced efficacy of influenza vaccine. *Hum Vaccin Immunother* 2014;10:1181–1186.