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Immunity to influenza: Impact of obesity

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ABSTRACT

Obesity is a health concern that is recognized as a critical factor for vulnerability to influenza A/pdmH1N1 virus infection, with epidemiological and clinical impacts. In humans, obesity induces disturbances in inflammatory and immune responses to the influenza virus and in some cases, this leads to severe complications, with fatal outcomes. Obesity impairs immunity by altering the response of cytokines, resulting in a decrease in the cytotoxic cell response of immunocompetent cells which have a key anti-viral role. Additionally, obesity seems to disturb the balance of endocrine hormones, such as leptin, that affect the interplay between metabolic and immune systems. This contribution focuses on reviewing the current epidemiologic data for the immune response to immunity in obese humans and animal models. In doing so, we aim to provide potential mechanisms to enhance immunity to influenza A/pdmH1N1 virus infection and protective factors in obese people.

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Introduction

Obesity is increasingly a worldwide health concern and has been regarded as a recognized risk factor for severe complications and death caused by the influenza A/pdmH1N1 in 2009 [1]. Obesity has a deleterious effect on host immunity and increases susceptibility to infectious diseases, including the influenza A/pdmH1N1 virus [2–4]. Obesity confers a state of low-grade chronic inflammation and disturbed regulation of adipocyte-derived hormones known as adipokines, such as adiponectin and leptin. Adiponectin decreases macrophage activation and proinflammatory cytokine generation, while leptin has a pro-inflammatory effect. A higher fat load decreases levels of adiponectin while increasing leptin, suggesting a state of leptin-resistance. In obese individuals, feed-back lack of anti- and proinflammatory mediators leads to an inflammatory "storm", resulting in ineffective elimination of the influenza virus [5–7].

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The deleterious effect of obesity on immunity and resistance to influenza A/pdmH1N1 infection and bacterial-associated pneumonia in humans and animal models has been previously reviewed [1]. However, this article aimed to provide current information concerning the role of obesity, with a focus on influenza A/pdmH1N1 infection. Epidemiologic and immunologic aspects of human immunity and potential mechanisms based on murine models of obesity were also included.

Influenza epidemiology in human obesity

The epidemiologic impact of obesity on influenza A/pdmH1N1 infection has been addressed in terms of hospitalization and intensive care unit (ICU) admissions, illness severity, critical infection outcome, and mortality (Table 1).

Case-control studies have reported that obese patients visited more than two healthcare facilities before receiving a laboratory-confirmed diagnosis of influenza A/pdmH1N1 infection [8]. Data analysis from cohort studies indicated that compared with normal weight individuals (body mass index (BMI) $\leq$25 kg/m^2), obese (BMI $\geq$ 30 $\leq$ 40 kg/m^2) or morbidly obese patients (BMI $\geq$ 40 kg/m^2) were at higher risk of hospitalization as a result of seasonal (A/H3N2) or A/pdmH1N1 influenza infections [9,10]. Obese patients with confirmed influenza A/pdmH1N1 infection were more likely to be hospitalized for longer [11–13]. The association of influenza infections with hospitalization and other health-care parameters has not been confirmed by other authors. For example, in a case-cohort study, obesity was moderately or minimally associated with hospitalization from influenza-like illness (ILI) during the influenza A/pdmH1N1 infection [14]. A survey collected from households indicated no association between obesity and self-reported ILI [15]. In prospective and retrospective studies no association between obesity and medical attendance as a result of influenza A/pdmH1N1 infection was found [16]. Similar data analysis during the first influenza A/pdmH1N1 wave indicated that obese patients were more likely to develop pneumonitis, although the hospital length of stay in non-obese and obese people was similar [17].

Clinical studies of severe cases of influenza A/pdmH1N1 virus infection evidenced that obese individuals were at a high risk of admissions and a longer length of stay at intensive care units (ICU) [11,17–20]. Retrospective analysis of laboratory surveillance of patients with A/pdmH1N1 infection showed that obesity was an independent predictor for admission to ICU, but not hospitalization [21] Multivariate logistic regression models indicated no association between ICU admission and obesity in hospitalized patients with laboratory-confirmed seasonal influenza [22].

In several clinical settings obese patients with influenza A/pdmH1N1 infection were more likely to develop severe symptoms (persistent fever, severe coughing and pneumonia), as reported in adults and children [17,23–30]. Both obesity and morbid obesity were found to be independent risk factors for severe manifestations from influenza A/pdm09 virus infection [12,31,32]. Among hospitalized patients, those who were obese and morbidly obese required earlier antiviral therapy for severe influenza A/pdmH1N1 outcomes than non-obese patients [20].

In other studies, obesity comorbidities such as metabolic syndrome were an independent risk factor for severe illness as a result of influenza A/pdmH1N1 infection [33]. Paradoxically, models of multivariate logistic regression indicated that overweight and obese persons had a decreased risk of pneumonia [22]. Findings reported from a retrospective cohort study showed that influenza-associated pneumonia or pneumonia followed influenza by influenza A/pdmH1N1 were most common in underweight individuals and a decreased rate of pneumonia was associated with increased BMI [34].

Obesity plays a role in the outcome of critical complications from influenza A/pdmH1N1 infection and is associated with longer mechanical ventilation for severe acute respiratory distress syndrome and shock [11,12,19,35,36]. However, analysis of influenza A/H3N2 severity in patients admitted to ICU showed that overweight and obesity were not associated with artificial ventilation [22]. An observational study in a cohort of inpatients with critical illness by influenza A/pdmH1N1 infection showed a high rate of ICU admissions in obese patients, and patients from some communities (Hispanic or Pacific Islander) [19].

In several trials in patients with severe influenza A/pdmH1N1 infection, obesity and metabolic syndrome were risk factors for death [8,13,18,24,30,32,35,37–40]. One study reported that obesity and morbid obesity were independent factors for death by influenza A/pdmH1N1 [32]. However, other studies did not find a relationship between obesity and death as a result of influenza A/pdmH1N1 infection [10,11,16,17,36,41].

Although a complex interplay of genetic, immunological and clinical factors underlies divergent epidemiological findings, it has been suggested that metabolic syndrome, and not only BMI as a parameter of body weight, may underlie the susceptibility to influenza A/pdmH1N1 infection [33].

Influenza immunity in human obesity

The impact of obesity on humoral immunity to influenza has been analyzed by assessing seroconversion (>4-fold increase in antibody titers) and seroprotection (>40-fold increase in antibody titers) after vaccination in experimental trials in human volunteers (Table 2).

A systematic review of cross-sectional and cohort studies indicated that obese adults are more likely than non-obese individuals to receive vaccination [42]. A prospective observational study documented that vaccinated obese people had double the risk of developing influenza or ILI, despite similar seroconversion or seroprotection [43]. Additional contributions support the role of obesity in the impairment of efficacy of influenza virus vaccination [44].

In contrast, clinical settings showed higher antibody titers at day 21 after a single administration of monovalent A/pdmH1N1 vaccine in adult obese patients than in lean counterparts [45]. Moreover, seroprotection and seroconversion after vaccine administration were found higher in obese than non-obese children [46]. Interplay of multifactorial events may underlie this apparent contradiction.

In order to provide insights about the impact of obesity on humoral immunity, our research group undertook, for first time, the analysis of IgA and IgG antibodies involved in virus neutralization and clearance from respiratory tract (RT) mucosa [47]. According to murine models of influenza infection, at the mucosal level, IgA is generated locally and protects the upper RT while IgG derives from serum transudation and protects lower RT [48]. Therefore, the study aimed to evaluate the influenza IgG and IgA responses in obese persons to design-based peptides derived from the surface glycoproteins hemagglutinin and neuraminidase. Hemagglutinin (HA) acts as ligand of host sialic acid and enables the fusion of the virion envelope with cell membrane whereas neuraminidase (NA) cleaves the terminal sialic acid from glycoproteins or glycolipids to facilitate the spreading of the new made infective virions [49]. This trial was conducted according to the Declaration of Helsinki and all procedures were carried out with the adequate understanding and written consent of the human volunteers. The study was approved by the Committee of Bioethics (CONBIOETICA-09-CEI-012-20160627) and Research (CI-01/25-04-2017), Escuela Superior de Medicina, Instituto Politécnico Nacional).

Table 1
Epidemiologic data for influenza and obesity.

Type of study	Finding
Australia	
Comparative analysis of prospective and retrospective data from adult obese and non-obese admitted to Intensive Care Units (ICU) with influenza A/pdmH1N1 [17]	Obese are likely to develop pneumonitis
Canada	
Cohort study of over 12 seasonal influenza in individuals aged 18–64 years [9]	Obese with or without chronic lung diseases are at risk for hospitalization during influenza seasons
China	
Meta-analysis and meta-regressions of confirmed cases by influenza A/pdmH1N1 in English and Chinese databases [35]	Obesity increases the risk of fatal and severe complications
French Territories	
Analysis of clinical and epidemiological indicators during influenza A/pdmH1N1 [24]	Severe cases and deaths were found in obese adults
Observational and descriptive study of confirmed and probable cases of influenza A/pdmH1N1 in patients [25]	Underlying conditions of severe symptoms included morbid obesity
Greece	
Retrospective and prospective data analysis of confirmed influenza A/pdmH1N1 in children [27]	Obesity aggravated the illness outcome
India	
A case-control study between deaths and survivors by influenza A/pdmH1N1 [8]	Dead patients were more likely to be obese
Iran	
Multiple logistic regression data analysis of confirmed influenza A/pdmH1N1 in adults [33]	Metabolic syndrome was an independent risk factor for severe influenza A/pdmH1N1
Italy	
Data analysis of hospitalized patients with confirmed influenza A/pdmH1N1 [28]	Obesity was found to be a risk factor for lung disorders (obstructive pulmonary disease and asthma)
Mexico	
Observational study in critically ill patients with influenza A/pdmH1N1 [36]	From 58 critical ill patients, 21 (36 %) were obese
Clinical evaluation of 100 consecutive deaths of confirmed cases of influenza A/pdmH1N1 [37]	Dead cases were found in patients with metabolic syndrome
Morocco	
Influenza surveillance for patients with influenza A/pdmH1N1 and severe acute respiratory illness (SARI) [38]	Obese patients were at increased risk of death
Serbia	
Retrospective analysis of the surveillance of laboratory-confirmed cases in 4 postpandemic seasons to evaluate predictors of hospitalization and admission to the ICU [21]	Obesity was an independent predictor for admission to the ICU but not for hospitalization
Spain	
Prospective observational and multi-center study performed in 144 ICUs of patients with confirmed influenza A/pdmH1N1 [30]	From 131 deceased patients, 83 (64 %) were obese
Turkey	
Retrospective study of hospitalized cases with confirmed influenza A/pdmH1N1 [40]	Obesity was found related to a fatal outcome
UK	
Retrospective cohort study in patients with confirmed influenza A with pneumonia [34]	Overweight was associated with decreased pneumonia rate
USA	
Retrospective cohort study to evaluate the impact of obesity on influenza disease severity [13]	Obese individuals were more likely to have lower pulmonary disease symptoms and death
Observational study from May-June 2009 in an ICU cohort of inpatients with confirmed influenza A/pdmH1N1 [19]	Obese individuals were susceptible to critical illness due to influenza A/pdmH1N1
Surveillance for hospitalizations with influenza A/pdmH1N1 to assess obesity and extreme obesity risk factor for death among case-patients older than 20 years [32]	Extreme obesity was associated with an increased odds of death
Surveillance for hospitalizations and deaths associated with influenza A/pdmH1N1 [39]	Of 47 confirmed fatal cases of influenza A/pdmH1N1, 27 (58%) were obese

Body mass index (BMI): normo-weight BMI $\geq 18 \leq 24.9\ kg/m^2$, overweight BMI = 25–29.9 kg/m^2, obese BMI $\geq 30 \leq 40\ kg/m^2$; morbid obese BMI $\geq 40\ kg/m^2$ [1].

The study population included people aged 20–50 years old, both men and women and with a BMI > 30 < 40. The vaccinated group consisted of donors who underwent submuscular injection with monovalent (A/pdmH1N1), TIV (A/pdmH1N1, A/H3N2 and B influenza strains) or both vaccines, while an unvaccinated group was included as control. Blood and saliva samples were collected within the first 6 months postvaccination. The design of peptide sequences was based upon previously described theoretical studies and experimental assays [50–52]. Peptide V (SSWSYIVETPSSDNGTCYPG) is a HA1-chain conserved sequence at vestigial (V) site nearby the binding site to sialic acid. The SM peptide (KGAINTSLPFQNIHPIPKYVKSTKLRLATC) consists in a HA2-stalk (S) sequence modified (M) with peptide amino acid residues. Q11 (IFRIEKGKIVKSVEMNAPNYHYEESSSGGGCCGGGKGAINTSLPFQNIHPITIGKSPKYVK) is a chimeric peptide that contains peptide sequences derived from HA2 and ectodomain from NA. Q14 chimeric peptide contains NA C-terminal region and HA1-derived peptide fragments (VNSDTVGWSWPDGAELPFTIDKGGGGCGGGGKTSSWPNHDSNKGVTAASPHAGAKSFYKN). The total concentration of both peptides and protein was quantified using a microtiter plate according to the Bradford method following the technical guide stated by a commercial solution (cat. no. 500-0006 BioRad Protein assay, BioRad Laboratories Hercules California, USA).

Specific serum IgG and saliva IgA levels were assessed by an indirect enzyme-linked immunosorbent assay (ELISA), according to the following successive steps using, in all cases, a volume of 100 μL of

Table 2
Influenza immunity and human obesity.

Type of study	Findings (obese BMI ≥ 30 ≤ 40 *vs* lean BMI ≥18 ≤ 24.9)
Prospective observational study of TIV in adult obese patients [43]	↔ Seroconversion and seroprotection ↑ risk of developing influenza A/pdmH1N1or influenza like illness (ILI)
Analysis of antibody titers and peripheral blood mononuclear cells (PBMC) in obese patients at 1 and 12 months after immunization with TIV [44]	↑ obesity correlated with a greater decline in circulating influenza antibody titers at 12 months postvaccination ↓ CD8+ cell activation, IFN-γ, and granzyme B + cells in PBMC cultures
Analysis of hemagglutination antibody inhibition (HAI) titer in trials of unadjuvanted influenza A/pdmH1N1 vaccine in patients stratified by body mass index (BMI) groups [45]	↑ HAI 21 days after a single vaccine dose ↔ response to influenza A/pdmH1N1 vaccine among children or adults of various BMIs following two vaccine doses
Testing of trivalent influenza vaccine (TIV) *via* intramuscular (im) in overweight (BMI ≥25 ≤ 30) and obese children [46]	↑ antibody response was slightly and persisted four months postvaccination
HAI of circulant antibodies from a clinical trial of annual influenza vaccination (A/H3N2) in obese healthcare workers [53]	↔ seroprotection from influenza challenge after immunization
Analysis of HAI titers to TIV (A/H1N1, A/H3N2 and B) in obese Human immunodeficiency virus (HIV) infected persons [54]	↔ Seroconversion and seroprotection ↓ HAI was unrelated with obesity
Analysis of circulant antibodies from older patients immunized with TIV (A/H1N1, A/H3N2 and B) [55]	no correlation between obesity and seroprotection/seroconversion to the influenza vaccine
Analysis of immune responses in young and elderly individuals vaccinated with TIV [56]	↓ circulating antibody response ↓ switched memory and transitional B cells, ↑ IL-6, ↓ IL-10
Immune response assessment in obese people with influenza [57]	↑ markers of immune activation (TNFα, TLR4 and micro-RNAs) and ↓ B cell function ↓IL-6 secretion; ↑ IgM levels in B cell cultures stimulated with anti-BCR/TLR9
Analysis of B cell response in PBMCs from non-obese and obese diabetic patients [58]	↑ B pro-inflammatory cytokine responses ↓ B ability to respond to new antigens such as seasonal influenza vaccination
Analysis of circulant mononuclear cells and cytometric bead array stimulated *ex vivo* with live influenza A/pdmH1N1 virus [60]	CD4+ and CD8+ had: ↓ CD69, CD28, and CD40 ligand and IL-12 receptor as well as ↓ levels of IFN-γ and granzyme B
Retrospective study of severe cases of influenza A/pdmH1N1 in obese patients with acute respiratory distress syndrome (ARDS) or who had died (ARDS-death) [61]	↓ slower decline in nasopharyngeal viral loads and ↑ circulating proinflammatory cytokines and chemokines in ARDS-death patients than ARDS or mild-disease
Flow cytometry analysis of PBMCs from obese adults (18-65 old years) infected *in vitro* with influenza A/PR/8/34/H1N1 [62]	↓ γδ T cells: γδ T cell displayed a blunted anti-viral IFN-γ response and reduced levels of IL-2Rα; IL-2 restored γδ T cell antiviral cytokine production
Analysis of suppressor cytokines signaling (SOCS)1 and SOCS3 in TLR-ligandactivated PBMCs from lean and obese	↓ type I interferon to TLR3-ligand priming ↑ basal expression only in SOCS3
volunteers infected during the 2009 pandemic AH1N1 influenza [63]	↑ pro-inflammatory cytokine response in response to TLR3 stimulation ↑ SOCS1 and SOCS3 in lean people

Abbreviations: increase ↑; decrease ↓; no changes ↔; Toll-like receptor (TLR).

each reactant: (i) for coating, 96-well microtiter plates (3590 Corning, Life Sciences New York, USA) were treated with each peptide (0.04 μg/100 μL) diluted in 0.1 M carbonate-bicarbonate buffer pH 9.6 followed by incubation at 37 °C for 1 h; (ii) four rounds of washing were carried out with 0.05% Tween-20 in phosphate-buffered saline (PBST) pH 7.2 and the washings were carried out at 37 °C and repeated before and after each step of incubation; (iii) blocking was performed with 3% fat-free milk in pH 9.6 carbonate-bicarbonate buffer and incubated at 37 ºC for 2 h; (iv) thereafter, plates were treated with samples tested in duplicate either with 1:50 serum dilution prepared in 2% fat-free milk in PBST or 1:2 saliva samples diluted in PBST and then incubated at 37 °C for 1 h; (v) for conjugation, plates were treated with either horseradish peroxidase (HRP) conjugate (goat anti-human IgG HRP conjugate (AP112 P, Millipore Temecula Calif, USA) 1:5000 in PBST) or goat anti-human IgA HRP conjugate ((A18781, Thermo Fisher Scientific, Rockford, Illinois, USA) 1:3000 diluted in 2% fat-free milk in PBST) and incubated at 37 °C for 1 h; (vi) the enzymatic reaction was developed with the substrate solution (0.4 mg/mL *o*-phenylenediamine plus 0.04% H_2O_2 in 50 mM citrate buffer, pH 5.0) and incubation at room temperature for 20 min; (vii) the enzymatic reaction was stopped with 2.5 M H_2SO_4. Absorbance was measured at 490 nm using a microplate spectrophotomer reader (BioTeck Eon, Fisher Scientific Winooski, Vermont USA). Two-fold (1:50–1:1000) serum serial dilutions or 1:2 and 1:5 dilutions of saliva samples from a patient with confirmed influenza A/pdmH1N1 infection were included as positive controls. Reciprocal sample dilution 1:50 for serum or 1.2 for saliva from the positive control showed an absorbance value ranging from 0.2 to 0.9 (0.5 mean approximately) and, therefore, these were chosen for antibody assessment. Thus, absorbance of 1:50 serum dilution or 1:2 saliva of each sample was divided between the absorbance of the corresponding dilution of the positive control and expressed as relative units. For IgA, relative units were divided between the total protein concentration and, therefore, expressed in relation to total protein. Data comparisons of median values between vaccinated (n = 15) and unvaccinated groups (n = 15) were analyzed by the Mann-Whitney U test. Differences were considered significant at $p < 0.05$. Statistical analysis was performed using the SigmaPlot statistical program for Windows version 11 (Systat Software Inc., San Jose, CA, USA). According to the results (Fig. 1) no significant differences in IgG ($p = 0.535$) and IgA ($p = 0.330$) antibody levels were found for any peptide between the two groups. These findings may be a result of the heterogenous population of study. However, they may also reflect the poor immunogenicity of virus-derived peptides or the effect of obesity on decreased antibody responses.

Previous assays reported no differences in seroprotection by influenza vaccines (A/pdmH1N1, A/H3N2 or B strains) in obese and non-obese patients [53]. In addition, a retrospective data analysis has indicated that in HIV-infected patients, obesity was not related to an impaired immune response to trivalent influenza vaccine (TIV) [54].

Additional factors may account for the divergent effects on the serologic response to vaccines. For example, age is an underly-

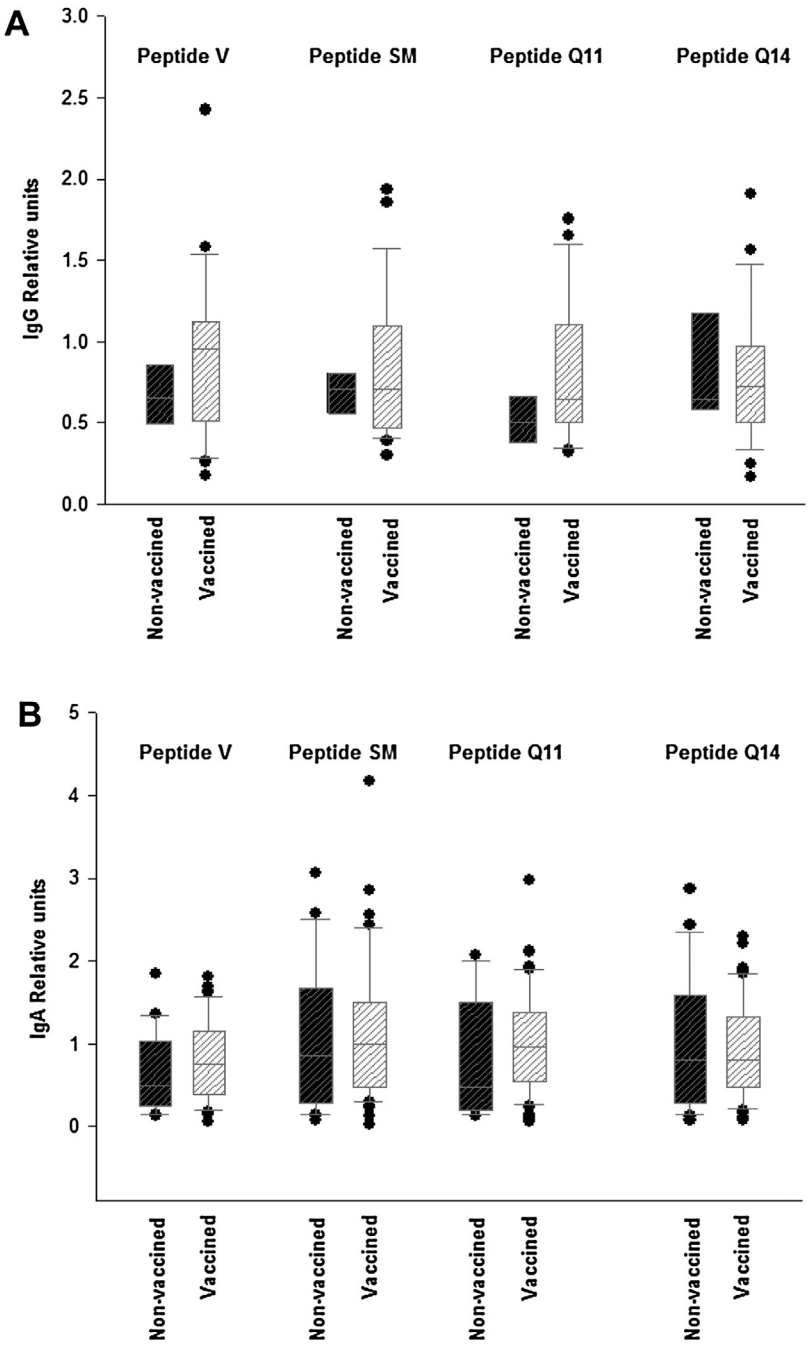

Fig. 1. Specific IgG (A) or IgA (B) antibody responses to peptides V, SM, Q11 and Q14 in vaccinated or nonvaccinated groups of obese individuals. Box plots show quartiles and median values of relative units computed as absorbance of each serum (1:50) or saliva (1:2) sample between the absorbance of the corresponding dilution of a positive control sample from a patient with confirmed influenza A/pdmH1N1 infection. For IgA, relative units were divided between the total protein concentration and expressed in regard to total protein. Significant differences were not found for IgG ($p = 0.535$) nor IgA ($p = 0.330$).

ing factor in the altered immune response to influenza vaccines in obese people [55]. Moreover, assessment of antibody titers alone may provide misleading information, therefore, additional parameters should be analyzed to determine the vaccine effectiveness [43].

In this regard, analysis of peripheral leukocytes from patients vaccinated *via* the intramuscular route with TIV indicated that a decreased vaccine antibody response was associated with low transitional and switched memory in B cells and increases in pro-inflammatory-exhausted memory B cells [56]. In B cell cultures from lean patients, leptin increased the expression of phospho-STAT3 which was crucial for inducing pro-inflammatory cytokine tumor necrosis factor (TNF)-α production but decreased the phospho-p38 MAPK energy-sensing enzyme. These findings suggest that leptin might underlie the decreased B cell response in obese patients [56].

Other studies have provided additional evidence supporting the role of obesity in B cell dysfunction. *In vitro* cell cultures of B cells from obese patients showed decreased interleukin (IL)-6 secretion but increased IgM production after anti-B cell receptor (BCR)/toll-like receptor (TLR)-9 priming [57]. *In vitro* analysis of circulating B cells showed that, in obese diabetic patients, B cells exhibited higher production of pro-inflammatory cytokines and polyclonal stimulation, but they had a reduced ability to respond to new anti-

gens such as seasonal influenza vaccine. These findings indicate that the enhanced polyclonal B cell activation might be related to type 2 diabetes development in obese patients [58]. Furthermore, in a clinical trial of TIV in obese patients, leptin-related gene polymorphisms correlated with variability in specific antibody and B cell responses; thus, leptin polymorphisms may underlie the antibody response to influenza vaccine in obese patients [59].

Studies based on *in vitro* cultures of peripheral mononuclear cells showed that CD4+ and CD8 + T cells of obese people expressed lower levels of activation markers, including CD69, CD28 and CD40 ligands and IL-12 receptors, as well as lower levels of functional biomarkers such as interferon (IFN)-γ and granzyme B. These findings suggest a role of obesity in the impaired activation and function of CD4+ and CD8 + T cells, with a pivotal role in the regulation of antiviral interleukin production [60].

The impact of obesity on the components of innate immunity in response to influenza infection has been addressed in several studies. A clinical trial documented that obesity seemed to delay clearance of the viral load and caused a marked increase in cytokine activation in older patients with acute respiratory distress syndrome (ARDS), including those who died from ARDS (ARDS-death group) [61]. Cytofluorometric analysis of influenza A/H1N1/PuertoRico/8/34 (A/H1N1/PR/8/34) virus-infected cultures of peripheral blood from donors documented an association between obesity severity and γδ T cell reduction. Moreover, obesity damaged the γδ T cell antiviral activity, as evidenced by a decrease in IFN-γ and IL-2 receptor (IL-2) α levels [62]. These findings suggested that the anti-viral activity of γδ T cells may result from a dysregulated response to growth cell factors such as IL-2 [62]. Data from cell culture assays of peripheral blood mononuclear cells showed that obese patients infected with influenza displayed a decreased production of type I interferon in response to the TLR-3 ligand [63]. In cell cultures from obese infected patients, an increased expression of suppressor of cytokine signaling (SOCS)-3 but not SOCS-1 was found, while in non-obese people both SOCS-3 and SOCS-1 were highly expressed [63]. SOCS proteins are negative regulator of cytokines [64].

Given the multifactorial conditions underlying the influenza antibody response in obesity, analysis of the cell composition, mRNA sequence and DNA methylation using high-throughput technologies has been proposed to predict humoral immunity in response to influenza vaccination [65].

Influenza immunity in mouse obesity models

Experimental animal models of infection and vaccination-challenge provide evidence to account for the mechanisms mediated by both innate and adaptive components of immunity (Table 3).

Models of infection

Obesity is characterized by sustained, low degree inflammation. The effect of a low degree of inflammation has been studied in an experimental assay of inflammation, where lipopolysaccharide (LPS) was delivered *via* an osmotic pump implanted surgically in non-obese mice infected with influenza A/pdmH1N1 [66]. LPS-induced inflammation reduced activation markers and TNF-α and IL-6 production in *in vitro* LPS-stimulated macrophages. After influenza infection, LPS-induced inflammation increased mRNA expression of the chemokine macrophage inflammatory protein (MIP)-1α and RANTES (CCL-5), and increased infiltration of pro-inflammatory cells in the lung. These findings suggest that macrophage dysfunction favors the lung pathology caused by influenza virus [66].

Several experimental studies have addressed the impact of obesity on the dysfunctional response of inflammatory cytokines and components of adaptive immunity. The impact of obesity on phagocytic cell responses was documented in mice fed with a high-fat diet and infected with a lethal dose of the influenza A/ H1N1/PR/8/34 strain of the influenza virus. In this study, a high-fat diet promoted a higher viral titer, tissue damage, inflammation and enhanced neutrophil extracellular traps (NETs) which are associated with lung injury [67].

Experimental infection with influenza A/pdmH1N1 in diet-induced obese mice led to increased morbidity, mortality, lung pathology and alveolar virus spreading. In addition, in diet induced obese mice, IFN-β and TNF-α levels were lower in the lung, whereas IFNβ, TNF-α, and IFN-γ levels were greater in serum [68]. In obese mice infected with the influenza A/H1N1/PR/8/34 strain, an increase in lung pathology, delay in the expression of IL-6 and TNF-α and reduced mRNA expression of IFN-1α and β and natural killer cell cytotoxicity were found [69]. A model of primary influenza A/H3N2 virus infection followed by secondary influenza A/pdmH1N1 challenge in obese mice showed high mortality and reduced influenza-specific memory T CD8+ cell response with defective IFN-γ mRNA expression [70]. Moreover, obesity decreased dendritic cell presentation, attributable to alterations in IL-2, IL-6, and IL-12 cytokine generation and an altered frequency of TCD3+ and CD8+Tcells in the lung [71]. These findings support the detrimental effect of obesity on natural killer (NK) cells, dendritic cells, and TCD8+ cytotoxic lymphocytes with a prominent anti-viral role [69,71].

Assays revealed that TCD8+ and Treg cell responses were increased in the lung in mice fed with a high-fat diet and infected with the influenza A/ H1N1/PR/8/34 virus strain followed by a lethal dose of influenza A/pdmH1N1 virus. However, the suppressive activity of Tregs on inflammation was blunted. Data indicated that excessive inflammation and impaired Treg may account for the greater severity of influenza A/pdmH1N1 mediated illness [72].

The role of obesity in bacterial infections that arise as a complication of primary influenza infection has been documented in mice non-lethally infected with influenza virus strains and followed by infection with *Streptococcus pneumoniae* [73]. In this experimental setting, obesity increased mortality regardless of the type of bacteria, influenza virus strain (A/H3N2 or A/pdmH1N1) or timing of co-infection. In obese mice, higher bacteria and virus titers correlated with greater lung tissue damage and increased platelet-activating factor receptor expression, a host receptor with a critical role in pneumococcal invasion [73].

Some experimental settings have analyzed the presumed role of endocrine hormones, such as leptin, in the immune response to influenza in obesity. Long-term follow up of influenza infection with influenza X-31 virus showed that obesity reduced the expression of IL-2 receptor β (IL-2Rβ, CD122) but did not alter the expression of IL-17 receptor α (CD127), both of which are required for CD8+ memory T cell maintenance. These alterations were related to peripheral leptin resistance caused by reduced mRNA expression of leptin receptors in lungs along with increased mRNA expression of SOCS-1 and SOCS-3 [74]. Both SOCS-1 and SOCS-3 are negative feedback regulators of leptin signaling in association with leptin resistance [74].

Other findings showed that in obese mice, circulating leptin and testosterone levels were reduced or increased, respectively, during infection with the A/pdmH1N1 strain of influenza virus [68]. The role of leptin and testosterone was not fully addressed but may control the energetic balance for the control of influenza infection in obese mice [68].

According to other studies, higher levels of leptin and lower adiponectin levels in obese mice infected with a lethal dose of influenza A/pdmH1N1 virus strain were related to mortality,

Table 3
Influenza immunity and mouse obesity.

Experimental setting	Findings (obese vs lean or control)
Murine pdmAH1N1 infection in non-obese and obese-mice fed high-fat diet (HFD) with or without docosahexanoic acid (DHA) [57]	↓ Antibody titer that was blunted with DHA ↑ Pro-resolving mediators (SPMs) 14-, 17-hydroxydocohexaenoic acids, and protectine DX were induced by DHA
Mice treated by osmotic pump with tween saline (control) or LPS (model of chronic inflammation in obesity) for 3 weeks and then infected with influenza A/pdmH1N1 virus [66]	The LPS-treated group had ↓ Φ activation markers in the steady state; ↓ TNF-α, IL-1β, IL-6 inflammatory cytokines in re-stimulated peritoneal Φ
Non-obese and HFD obese mice with pneumonia by influenza A/PR/8/34H1N1 virus [67]	↑ neutrophil extracellular traps (NETs) despite the absence of a significant difference in disease progression
Normo weight and obese mice infected with influenza A/pdmH1N1 virus [68]	↑ higher mortality; ↑ alveolar virus spreading; ↓ IFN-1β and inflammatory cytokines in lung; ↑ serum cytokine levels
Lean and obese mice infected with influenza A/PR/8/34H1N1 [69]	↑ mortality rate, lung pathology ↓ IFN-α and IFN-β expression in infected lungs and delayed expression of IL-6 and TNF-α and ↓ natural killer (NK) cell toxicity
Obese mice with primary infection with influenza A/H3N2 virus and a secondary influenza A/pdmH1N1 virus challenge [70]	↑ mortality, lung pathology, lung viral titers ↓ mRNA IFN-γ, influenza-specific TCD8+ cells producing IFN-γ ↓ memory TCD8+ cells with a lower capacity to secrete IFN-γ
Lean and obese mice infected with influenza A/PR8/34 virus [71]	↓ delayed mononuclear cell entry ↓ dendritic cells (DC) throughout infection; ↓ DC antigen presentation; altered cytokine profiling IL-2, -6 and -12
Obese mice infected with influenza A/PR/8/34H1N1 and five weeks later challenged with a lethal dose of heterologous influenza A/pdmH1N1 virus [72]	↑ viral titers, lung inflammation, damage ↑ cytotoxic memory TCD8+ cells in lung ↑ Treg cells but were less suppressive
Lean and obese mice infected with influenza X-31 virus (findings at 84 days post-infection) [74]	↓ memory T cells, IL-2 receptor β expression (IL-2Rβ, CD122) ↓ mRNA leptin-receptor in the lungs ↑ mRNA SOCS1 and SOCS3 in the lungs
Obese mice infected with influenza A/pdmH1N1 virus to analyze lung pathogenesis and immune response [75]	↑ mortality and initial lung viral titer, severe inflammation; ↑ inflammatory cytokine and chemokine levels; ↑ preexistent leptin but ↓ pre-existent adiponectin level. anti-leptin antibody treatment ↑ survival of infected mice associated with ↓ IL-6 and IL-1β
Influenza A/WSN/33H1N1 infection in wildtype mice, obese mice globally deficient in leptin receptor (db/db), and non-obese mice with leptin-receptor deletion in lung epithelium Φ and type-II cells [76]	Compared with wild-type mice, the bronchoalveolar fluid of db/db mice had: ↓ virus clearance, ↓NK+ cells ↑ monocytes and neutrophils
Lean or obese mice infected with influenza A/PR/8/34H1N1, and metabolic profiling was assessed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and multivariate statistical data analysis [77]	Metabolic profiling of obese mice showed perturbations in nucleotide, vitamin, ketone body, amino acid, carbohydrate, choline and lipid metabolic pathways (immunemetabolic interactions)
Lean or obese mice were infected with influenza A/pdmH1N1 [78]	↑ pH1N1 mortality, lung inflammation and excess lung damage ↓ broncho-alveolar Φ and Treg cells
Lean and obese mice exercised for eight weeks by treadmill running and infected with influenza A/PR8/34H1N1 virus. The immune response was analysed in serum and bronchial alveolar lavage (BAL) [79]	↑ Serum anti-influenza IgG2c abs; ↑ % specific TCD8+ cells in BAL and ↓ TNF-α by specific TCD8+ cells.
Regulate fat diet (RFD) or high fat diet (HFD) mice groups underwent influenza cell-culture base vaccine [80]	↑ Th1 and ↓Treg; ↑ phagocytic Φ activity ↓ CD86 expression *in vitro* Φ cultures; ↓ IL-6 and TNF-α in LPS-primed Φ ↓ influenza-vaccine antibodies
Lean and obese mice vaccinated adjuvanted influenza A/H7N9 virus vaccine followed by a homologous or heterologous virus challenge [81]	↓ Antibody titer Adjuvant improved seroconversion but not protection
Lean and obese mice vaccinated with influenza A/pdmH1N1 vaccine and then challenged with homologous influenza A/pdmH1N1 virus [82]	↑ pdmAH1N1 specific ab response and neutralizing activity; ↑ lung virus titers after challenge; ↑ inflammatory cytokine and chemokine expression in lung; ↑ severe lung inflammation; ↑ mortality rate
Non-obese and HFD obese mice immunized with influenza A/pdmH1N1 cell culturebased vaccine and egg-based vaccine and challenged with influenza A/pdmH1N1 virus [83]	Obese mice prior challenge: ↑ mRNA cytokines monocytechemoattract protein-1 (MCP-1) in serum and adipocytes. ↓ neutralizing antibody titers and virus-specific effector memory TCD8+ cells ↑ mRNA cytokines monocytechemoattract protein-1 (MCP-1) in serum and adipocytes.

Abbreviations: macrophage (Φ); increase (↑), decrease (↓), no changes (↔). In experimental studies using as standard strain C57BL6 mice at 14 week-age old a body mass index >0.40 (g/cm^2) or Lee index ≥ 344.32 (g/cm) indicate obese [84].

severe lung injury and inflammation, as well enhanced proinflammatory cytokine levels [75]. Administration of anti-leptin antibodies decreased mortality in infected obese mice in association with decreased IL-6 and IL-1β levels in lung tissue but not with pulmonary viral titers [75]. Leptin antibody neutralization was suggested as a potential therapeutic strategy against influenza infection in obesity [75].

The role of leptin was analyzed in a model of infection by the influenza A/pdmH1N1 virus in wild-type non-obese mice, obese mice deficient in leptin receptor (db/db) and non-obese mice with a specific deletion of leptin receptor in lung epithelium (SPC-Cre/ LepR fl/fl). Compared with wild-type mice, viral clearance was impaired in obese db/db mice; in wild-type and SPC-Cre/LepR fl/fl mice, viral clearance was enhanced after virus infection [76]. In comparison to wild-type mice, obese mice showed higher mortality, whereas the survival of SPC-Cre/ LepR fl/fl mice was improved. Thus, a global loss of leptin receptor instead of a specific loss of leptin receptor in lung epithelial cells increased mortality and reduced viral clearance. These data suggest that impaired leptin-receptor signaling in dendritic cells and/or T cells may underlie susceptibility to influenza infection in obese people [76].

The complexity of the interplay between immunity and endocrine pathways in obesity when there is influenza seems to underlie the impact of immune impairment. The metabolome profile of lung tissue from high-fat diet fed and genetically obese mice infected with the influenza A/pdmH1N1 strain suggested that obesity induced alterations in fatty acids, phospholipids

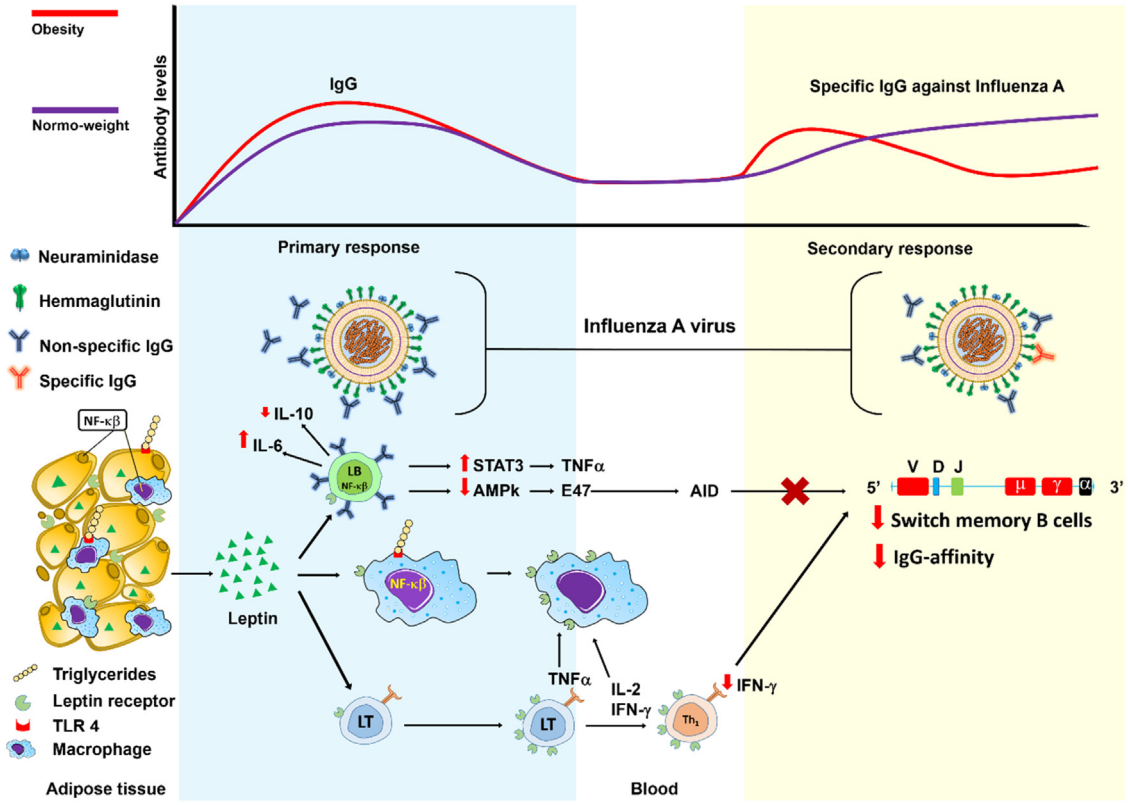

Fig. 2. Hypothetical mechanisms of the impact of obesity on the primary and secondary responses to influenza virus infection. Influenza virus expresses surface antigens like hemagglutinin and neuraminidase that enable binding to host cells, resulting in the elicitation of the host immune response. The primary response against influenza virus generates high levels of circulating IgG antibodies, due in part, to the excess of leptin produced by adipose tissue. Leptin favors the expression of proinflammatory signal pathways that stimulate the response of tumor necrosis factor (TNF)α and phagocytic mononuclear cells *via* leptin-receptor surface. Leptin also drives the response of inflammatory lymphoid cells and blunts the activation of T cell populations that favor antibody generation. Under conditions of chronic proinflammation caused by fat over-load, the specificity of IgG antibodies is low due to a decreased activity of adenosine monophosphate-activated protein kinase (AMPk) causing the downmodulation of E47 protein, essential for activation of cytidine deaminase (AID). The latter determines the immunoglobulin class-switch isotype, isotype-affinity increase and the generation of B memory cells. In comparison with non-obese persons, obese individuals vaccinated against influenza virus have higher IgG titers in the early months post-infection; as the time passes by, the IgG response decreases due to a defective memory B cell response.

and nucleotide metabolism that seemed to underlie an aberrant influenza A/pdmH1N1 immune response [77,78].

Protocols of exercise and dietary components seem to improve immunity to influenza in obesity. An experimental assay in lean and obese mice infected with influenza A virus analyzed the role of exercise in the anti-viral immune response in the respiratory tract [79]. In obese mice, exercise delayed cell infiltration and restored cytokine and chemokine generation and IFN-α gene expression; these findings support the beneficial effects of exercise in obese mice by improving the delay in immune activation, as found under healthy weight conditions [79].

Experimental infection with the influenza A/ H1N1/PR/8/34 virus strain was assessed in obese mice that received a Western diet with or without docosahexaenoic acid (DHA) [57]. Compared with obese mice without DHA, DHA-fed obese mice had higher levels of circulating antibodies. DHA did not have a direct impact on B cells but elicited downstream specialized pro-resolving lipid mediators (SPMs), which in turn elicited elevated murine Ab levels after influenza infection. These findings indicate that DHA counteracts the impact of obesity on the down-modulation of humoral immunity *via* SPM mechanisms [57].

Models of vaccination and virus challenge

Experimental studies have addressed the impact of obesity on the response to influenza virus vaccines. In high-fat diet fed mice vaccinated with the influenza A/pdmH1N1 virus, obesity increased the basal phagocytic activity of peritoneal macrophages, but decreased CD86+ expression in peritoneal un-primed macrophages as well as IL-6 and TNF-α secretion in LPS-primed macrophages. In splenocytes, obesity increased the Th1 subpopulation or decreased Treg cells; moreover, the antibody titer induced by influenza vaccine rapidly decreased under obesity conditions. These findings indicated that the reduced vaccine efficacy in obesity may result from impaired macrophage function [80].

In a model of diet-induced obesity in mice non-lethally infected with influenza virus strains followed by a S. *pneumoniae*, obesity impaired the protection conferred by the bacterial or influenza vaccines, but antibiotic therapy for secondary bacterial infection could effectively reduce the mortality [73]. Experimental settings of vaccination in mice using a squalene-based adjuvant have been designed to evaluate the role of obesity in the protection conferred by the influenza vaccine [81]. In comparison to lean mice, obese mice had a lower antibody neutralizing response to hemagglutinin (HA) and neuraminidase antigens. Moreover, even the increase in the neutralizing antibody titer after vaccination and protection conferred by the adjuvant vaccine in lean mice was significantly reduced in obese mice, as evidenced by a higher viral load in the respiratory tract after viral challenge [81].

The role of obesity in the impairment of vaccination has also been confirmed in high fat diet fed mice vaccinated with the monovalent influenza A/pdmH1N1 vaccine followed by challenge with the homologous influenza A/pdmH1N1 virus [82]. In that study, obesity impaired the protection conferred by the vaccine and was

associated with increased mortality and decreased neutralizing antibody titers. At a local level, higher viral titers were consistent with greater tissue damage and inflammation, as well increased expression of pro-inflammatory cytokines and chemokines in the lung [82].

The impact of obesity in the protective response conferred by influenza vaccines has been addressed in murine models. Obese vaccinated mice had higher monocytechemoattract protein-1 (MCP-1) levels and inflammatory markers in serum and adipose tissue; moreover, obese mice had lower circulating neutralizing antibody titers and virus-specific effector memory TCD8+ cells. After challenge with influenza virus, obese mice showed a severe inflammatory response in the lung. These findings indicate that the inflammatory condition in obesity suppresses the efficacy of influenza vaccination [83].

Taken together, the mechanisms underlying the effect of obesity on influenza infection are not fully known but entail a complex relationship between metabolic and immune pathways. Some approaches such as exercise and the modification of dietary components such as essential fatty acids seem to restore the immune response to viral infection. Based on all the information above, we have derived a model of the likely mechanism which describes the impact of obesity on primary and secondary antibody responses, and this is depicted in Fig. 2.

Concluding remarks

Clinical trials and experimental models have demonstrated potential mechanisms that provide valuable insights for pharmacological interventions to enhance immunity to influenza infection and the protective effect of influenza vaccines in obese people. Although the development of pharmacological drugs and immunomodulators to control and/or prevent influenza infection is in progress, the control of obesity by exercise, a healthy diet and good nutritional habits encompass easy and cheap ways to prevent the detrimental effect of obesity on influenza virus infection.

Disclosure and conflicts of interest

Authors have no conflicts of interest to declare.

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Ethics statement

This trial was conducted according to the Declaration of Helsinki and all procedures were carried out with the adequate understanding and written consent of the human volunteers. The study was approved by the Committee of Bioethics (CONBIOETICA-09-CEI-012-20160627) and Research (CI-01/25-04-2017), Escuela Superior de Medicina, Instituto Politécnico Nacional).

CRediT authorship contribution statement

Sandra Angélica Rojas-Osornio: Conceptualization, Data curation, Investigation, Formal analysis, Writing - original draft. **Teresita Rocío Cruz-Hernández:** Methodology, Project administration, Software. **Maria Elisa Drago-Serrano:** Writing - original draft, Writing - review & editing. **Rafael Campos-Rodríguez:** Funding acquisition, Resources, Supervision, Validation, Visualization.

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