Obesity, leptin and host defence of *Streptococcus pneumoniae*: the case for more human research

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Use of human challenge models could enable a better understanding of the interactions between *S. pneumoniae* exposure, colonisation and subsequent mucosal and systemic immunity in humans with obesity. https://bit.ly/3RpMIfd

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Abstract

Pneumococcal pneumonia is the leading cause of community-acquired pneumonia. Obesity is a risk factor for pneumonia. Host factors play a critical role in susceptibility to pulmonary pathogens and outcome from pulmonary infections. Obesity impairs innate and adaptive immune responses, important in the host defence against pneumococcal disease. One area of emerging interest in understanding the complex relationship between obesity and pulmonary infections is the role of the hormone leptin. There is a substantive evidence base supporting the associations between obesity, leptin, pulmonary infections and host defence mechanisms. Despite this, there is a paucity of research that specifically focuses on *Streptococcus pneumoniae* (pneumococcal) infections, which are the leading cause of community-acquired pneumonia hospitalisations and mortality worldwide. Much of the evidence examining the role of leptin in relation to *S. pneumoniae* infections has used genetically mutated mice. The purpose of this mini review is to explore the role leptin plays in the host defence of *S. pneumoniae* in subjects with obesity and posit an argument for the need for more human research.

Introduction

The global prevalence of obesity is increasing, yet little is known about the impact of obesity on bacterial pneumonia [1]. Community-acquired pneumonia (CAP) remains the leading cause of hospitalisation and mortality worldwide with *Streptococcus pneumoniae* the most prevalent pathogen [2]. Obesity is considered a risk factor for pneumonia due to impaired B- and T-cell-mediated immune responses that are important in the host defence against pneumococcal disease [3]. Additionally, the association between obesity and other major chronic conditions, such as diabetes and cardiovascular disease, increases the risk of CAP [3]. These host factors play a critical role in determining both the susceptibility to viral and bacterial pathogens and outcome from pulmonary infections.

Obesity has been frequently reported as a risk factor for adverse outcome from 2009 H1N1 influenza [4, 5] and severe acute respiratory syndrome coronavirus 2 [6] but, paradoxically, improved outcome in acute bacterial pneumonia [7–10], with studies on critically ill obese patients reporting no difference or reduced mortality compared to nonobese patients [11, 12]. Similarly, outcome data on the association between obesity and the risk and severity of pneumonia from bacterial infections is inconsistent; obesity has been linked to an increased risk of hospitalisation [13] and alternatively as acting as a protective factor during infections [2, 7]. One reason for this variability is the lack of data on the actual cause of CAP, which is not usually determined in clinical studies [2, 7, 13]. Determining the pathogen in CAP is important to advancing understanding of host defence mechanisms on patient outcomes. A bacterial infection that is highly dependent on neutrophil-mediated clearance may have a poor outcome in patients with obesity

compared to normal weight individuals. Another important consideration is whether or not bacterial pneumonia was preceded by a viral infection. Studies in human [14] and murine models [15] have shown that the precedence of pathogen exposure may determine pneumococcal infection outcomes and disease severity; pneumococcal infection after influenza may exacerbate infection, whilst pneumococcal infection prior to influenza may reduce mortality in mice [15].

One area of emerging interest in understanding the complex relationship between obesity and pulmonary infections is the role of the hormone leptin [16] – a protein produced primarily by adipocytes [17] that has cytokine-like properties with similar structure and function to cytokines and cytokine receptors of the interleukin (IL)-6 superfamily [18]. As well as regulating appetite and energy expenditure, leptin has been shown to play multiple roles in innate and adaptive immunity in mice and humans [19], and more specifically in pulmonary infections [20].

Whilst there is a substantive evidence base supporting associations between obesity, leptin, pulmonary infections and host defence mechanisms, there is a paucity of research that specifically focuses on *S. pneumoniae* infections. There is emerging evidence of the role of leptin in mice infected with *S. pneumoniae*, but there remains limited understanding of the role leptin plays in the susceptibility of obese humans to pneumococcal disease and nasopharyngeal colonisation. This mini review explores the current evidence of the role of leptin in the host defence of *S. pneumoniae* in obese subjects.

Leptin signalling and immunological functions

Leptin is an adipocyte derived hormone, with cytokine like properties, whose function is mediated by binding to a leptin receptor encoded by the *LEPR* gene. Whilst there are six different isoforms of the leptin receptor, only the long isoform (LepRb) can completely tranduce leptin signalling [21]. LepRb is predominantly expressed in the hypothalamus and is present in all types of immune cells, involved in both innate and adaptive immunity [22].

Most of the biological functions of leptin occur *via* the Janus kinase 2–signal transducer and activator of transcription 3 (JAK2-STAT3) pathway (figure 1). Leptin binds to the long isoform LepRb which causes dimerisation and stimulates JAK2 autophorylation as well as phosphorylation of tyrosine residues (Tyr974, Tyr985, Tyr1077, Tyr1138) within the receptor. Leptin receptor–phosphorlyated tyrosine 1138 mediates the interaction with STAT3 which dimerise and translocate to the nucleus to activate gene transcription. Suppressor of cytokine signalling 3 (SOCS3) acts as a negative feedback signalling mechanism during prolonged stimulation of LepRb [23, 24]. Leptin induces the activation of the mitogen-activated protein kinase pathway through activation of extracellular signal-regulated kinase 1/2. This pathway is activated following JAK2 activation where Src homology-2 domain-containing protein tyrosine phosphatase-2 recruits growth factor receptor-bound protein 2 leading to activation of the signalling cascade (figure 1). Leptin further induces the phoshatidylinositol-3 kinase/protein kinase B (P13K/Akt) pathway through insulin receptor substrate 1 phosphorylation following initial JAK2 activation [21, 25] (figure 1). Both these pathways result in cell proliferation and survival.

Almost all immune cells express leptin receptors. Leptin regulates both innate and adaptive immunity through the modulation of immune cell survival and proliferation. In innate immunity, leptin increases the cytotoxicity of natural killer cells and promotes the activation of granulocytes, macrophages and dendrite cells. This activation leads to the production of pro-inflammatory cytokines (*e.g.* tumour necrosis factor-α (TNF-α), IL-6, IL-12) which facilitate the shifting of T-cells towards type 1 T-helper cell (Th1) priming [22, 25, 26]. In adaptive immunity, leptin increases the proliferation and maturation of naïve T- and B-cells whilst decreasing the inhibitory effects of regulatory T-cells on the immune response [22, 25]. Leptin promotes pro-inflammatory Th1 cytokines rather than anti-inflammatory Th2 cytokines and facilitates Th17 responses. Lastly, leptin regulates B-cell development and activates B-cells to secrete cytokines [25].

Leptin deficiency, starvation and host defence

Much of the evidence examining the role of leptin in relation to pneumococcal infections has used genetically mutated nonobese mice with leptin (ob/ob), leptin receptor (db/db) or leptin signalling (s/s, LysM-LepRb-KO and CPE$^{fat/fat}$) deficiencies. Studies using leptin-deficient (ob/ob) mice have consistently shown reduced pulmonary clearance following *S. pneumoniae* challenge when compared to wild-type mice [27, 28]. Administration of exogenous leptin has improved survival, bacterial clearance and reduced bacteraemia [27]. Following infection with lower doses of *S. pneumoniae*, ob/ob mice exhibited lower pulmonary levels of pro-inflammatory cytokines such as TNF-α and chemokines. Leptin deficiency has not been shown to affect bacterial growth in the lungs [28]. It is further recognised that leptin receptor deficiencies (db/db) and subsequent signalling (s/s) impairment affects pulmonary host defence in

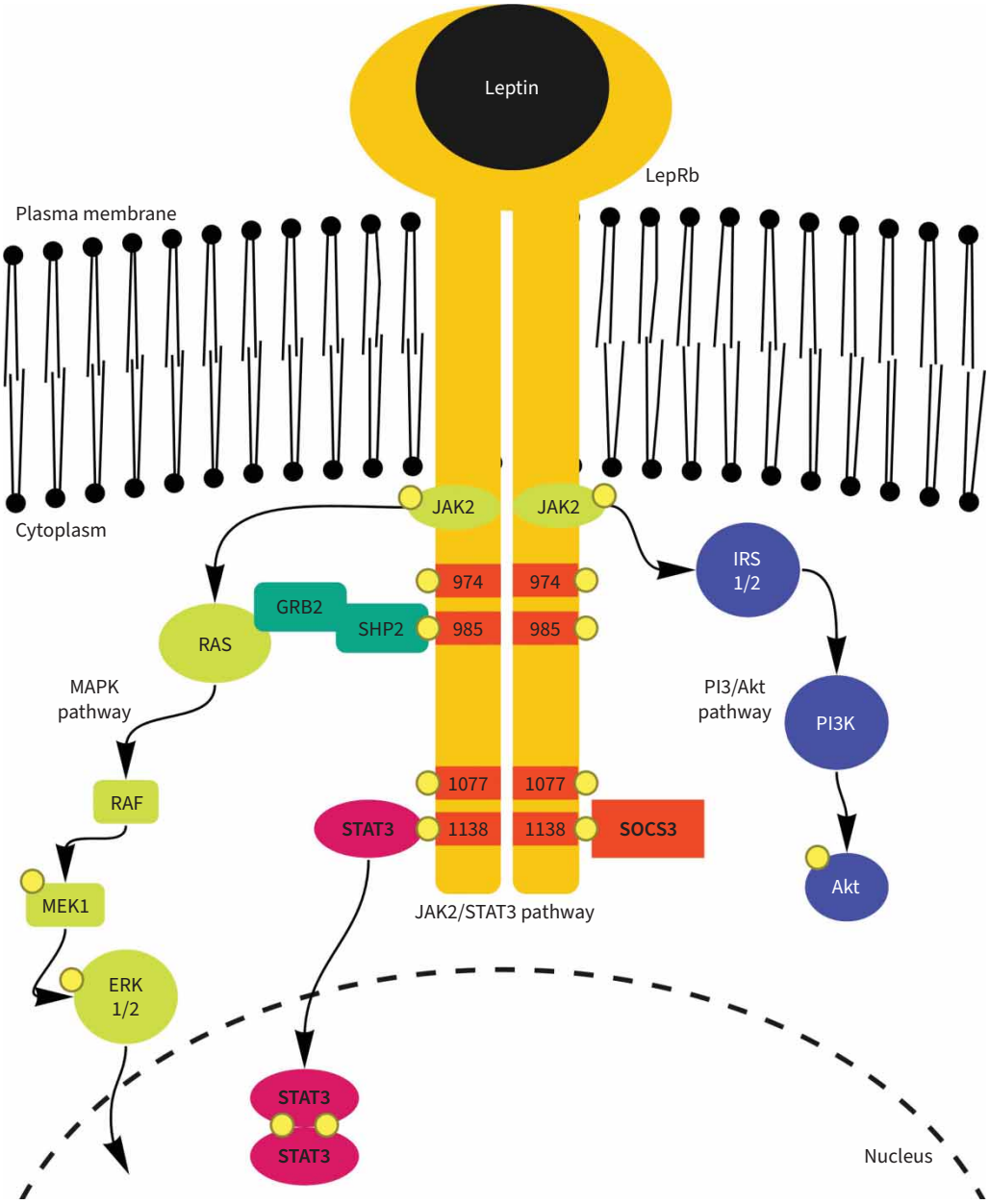

FIGURE 1 Leptin receptor and intracellular leptin signalling pathways. Leptin binds to LepRb causing dimerisation and phosphorylation of Janus kinase 2 (JAK2) and tyrosine residues within LepRb. Activation of JAK2 initiates three signalling pathways. 1) JAK2/signal transducer and activator of transcription 3 (STAT3) pathway: LepRb phosphorylated Tyr1138 mediates the dimerisation and translocation of STAT3 into the nucleus to activate gene transcription. Suppressor of cytokine signalling 3 (SOCS3) acts as a negative feedback mechanism during prolonged stimulation of LepRb. 2) Mitogen-activated protein kinase (MAPK) pathway: leptin induces the activation of Src homology-2 domain-containing protein tyrosine phosphatase-2 (SHP2), which recruits the adaptor protein growth factor receptor-bound protein 2 (GRB2) to initiate the RAS/RAF/MAPK kinase 1 (MEK1)/extracellular signal-regulated kinase 1/2 (ERK1/2) signalling cascade. 3) Phoshatidylinositol-3 kinase/protein kinase B (P13K/Akt) pathway: leptin mediates P13K/Akt activation *via* the insulin receptor substrate 1/2 (IRS1/2). These pathways cause cell proliferation and survival.

mice [29, 30]. Disruption to different signalling pathways produces opposing effects on the host defence. Disruption to leptin receptor (LepRb) JAK2/STAT3 signalling enhanced leukotriene production and pulmonary bacterial clearance [29]. Whereas ablation of the leptin receptor (LepRb) in myeloid cells impaired *S. pneumoniae* pulmonary clearance and alveolar macrophage bactericidal function [30].

How this murine work applies to the regulation of bacterial pneumonia inflammation in the human lung needs greater consideration.

A further area of study has been the application of acute starvation models to understand how the metabolic regulatory role of leptin influences the host defence during pneumococcal infection [31]. In studies of nonobese mice, acute starvation during *S. pneumoniae* infection rapidly decreased leptin levels impairing host defence with associated reduced bronchoalveolar lavage fluid (BALF) neutrophil counts and levels of IL-6 and macrophage inflammatory protein 2. Leptin administration restored bacterial clearance in the lungs and increased levels of BALF neutrophils, cytokines, alveolar macrophages and leukotriene B4 synthesis.

The studies described above focus on leptin deficiency, receptor and signalling defects or conditions of starvation and not obesity *per se*. These distinctions are important because the pathogenesis of obesity directly influences our understanding of the role of leptin in pneumococcal disease: genetic mutations that cause obesity [27–30] and diet-induced obesity [1, 32], which is the main cause of obesity in humans. New evidence suggests that prolonged overnutrition leading to diet-induced obesity, hyperleptinaemia and leptin resistance, a hallmark of obesity, play a critical role in the immune response to bacterial pathogens [1, 20, 33].

Overnutrition, hyperleptinaemia and host defence

In diet-induced obesity, subjects have sustained higher serum leptin levels (hyperleptinaemia) and leptin resistance from prolonged high fat diet overnutrition than lean humans [34]. One study [1] examining the effect of obesity on the host defence against *S. pneumoniae* noted that $CPE^{fat/fat}$ mice (carboxypeptidase E enzyme deficient mice; enzyme critical to processing prohormones and proneuropeptide regulation of appetite and energy expenditure) developed hyperglycaemia, raised serum leptin and triglyceride levels, and increased blood neutrophil count prior to *S. pneumoniae* infection. These findings, in conjunction with emerging evidence of hospitalised patients with severe pneumonia, suggest that it is not body weight *per se* but chronic hyperleptinaemia that is the link between obesity and increased risk of pulmonary infection [20].

Therefore, leptin resistance and the impact of altered inflammatory signalling in diet-induced obesity [35] need further consideration. Mutation in the leptin gene or the leptin receptor as described in the murine studies above are not the main causes of leptin resistance [35]. Instead, leptin resistance is induced by altered leptin transportation across the blood–brain barrier, deterioration in function of the leptin receptors accompanied by hypothalamic inflammation, endoplasmic reticulum stress and defective autophagy [34, 36, 37]. In high fat diet fed mice, leptin transport across the blood–brain barrier is substantially decreased [38]. In obese humans with severe hyperleptinaemia, leptin levels in the cerebrospinal fluid are only slightly increased [36]. Therefore, it is possible that pulmonary host defence may be impaired differently, in various models of obesity, such as diet-induced obesity with or without leptin resistance.

Clinical studies

There are very few clinical studies examining the relationship between leptin levels in obesity and *S. pneumoniae* infections in humans. Díez *et al*. [32] specifically examined the relationship between leptin and the outcomes of hospitalised patients with confirmed diagnoses of CAP. This study showed no difference in the serum leptin levels between hospitalised CAP patients and the healthy control group, when adjusting for body mass index. At the time of admission, approximately 5 days from symptom onset, a linear relationship between leptin levels and body mass index, body fat and muscle mass was observed suggesting that leptin acts as a nutritional marker, rather than an inflammatory reactant. However, the role of leptin in the host defence of pneumococcal disease and pneumococcal nasopharyngeal colonisation, in the absence of infection-induced starvation, remains unknown.

Determining the susceptibility or risk of infection in relation to obesity requires carefully controlled human infection studies that investigate pre and post exposure to *S. pneumoniae*. Similarly, the timing of infection in regards to leptin levels is critical to understand the severity of illness. In mice, leptin levels rise immediately after infection and decline after 24 h [31]. In humans, the time between infection and hospital admission is likely to be greater than 24 h and vary considerably between patients.

Often clinical studies involve obese patients who have multiple comorbidities, known to increase susceptibility to pneumococcal disease, thus making direct links with obesity difficult. Clinical data supports the notation that type 2 diabetes is a risk factor for pneumococcal and other types of CAP [39, 40]. Since approximately 90% of patients with type 2 diabetes are overweight or obese [41], it is difficult to determine if obesity or diabetes is responsible for impaired host defence in humans. Research

that includes normal weight patients with diabetes is needed to address this issue. Likewise, the question of greater rates of colonisation in humans with obesity requires carefully controlled human infection studies. Patients with and without type 2 diabetes would need to be included in these studies since this is an important distinction that will affect understandings of host defence.

Body mass index measurements are frequently used in clinical studies as a proxy for adiposity despite not directly measuring fat mass. Given that leptin is primarily produced in adipocyte cells, collecting data on waist circumference, bioelectric impedance and dual energy X-ray absorptiometry scans will provide more accurate data. Furthermore, 10–30% of people with obesity have normal metabolic parameters (metabolically healthy obesity) [42]. Recording metabolic syndrome or measures of metabolic status will provide a more accurate assessment of metabolic health and assist in the distinction between the host defence mechanisms resulting from obesity and diabetes.

Conclusion

Collectively, the evidence suggests that there are both metabolic and immune responses that influence the role of leptin in the host defence of *S. pneumoniae* in obese subjects; the effects of these responses being intricately related to the pathogenesis of obesity (genes or diet), the acute phase of illness, nutritional status, and the severity of the disease. Furthermore, the evidence suggests that not only leptin deficiency but prolonged hyperleptinaemia and leptin resistance may impair the pulmonary host defence as observed in diet-induced obesity. As most of our understanding about the impact of obesity on the host defence to pneumococcal disease comes from research in genetically mutated mice models, direct comparisons of murine models with diet-induced obesity in humans may not be feasible. There is a paucity of clinical research specifically examining pneumococcal nasopharyngeal colonisation, the prerequisite for pneumococcal infection, in obesity. Further research is required to understand how *S. pneumoniae* colonisation is regulated in obese populations and informs our understanding of susceptibility to subsequent pneumococcal disease; and how hyperleptinaemia in diet-induced obesity influences nasopharyngeal colonisation and lung bacterial burden. The distinction between the influence of obesity and type 2 diabetes on host defence to *S. pneumoniae* exposure, colonisation and pneumococcal disease needs further exploration. The use of human challenge models [43–45] in people with an extreme body mass index could enable a better understanding of the interactions between *S. pneumoniae* exposure, colonisation and subsequent mucosal and systemic immunity in humans with obesity.

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References

1 Mancuso P, O'Brien E, Prano J, *et al.* No impairment in host defense against *Streptococcus pneumoniae* in obese CPE$^{fat/fat}$ mice. *PLoS One* 2014; 9: e106420.
2 Singanayagam A, Singanayagam A, Chalmers JD. Obesity is associated with improved survival in community-acquired pneumonia. *Eur Respir J* 2013; 42: 180–187.
3 Kopelman PG. Obesity as a medical problem. *Nature* 2000; 404: 635.
4 Fezeu L, Julia C, Henegar A, *et al.* Obesity is associated with higher risk of intensive care unit admission and death in influenza A (H1N1) patients: a systematic review and meta-analysis. *Obes Rev* 2011; 12: 653–659.
5 Riquelme R, Jiménez P, Videla AJ, *et al.* Predicting mortality in hospitalized patients with 2009 H1N1 influenza pneumonia. *Int J Tuberc Lung Dis* 2011; 15: 542–546.
6 Chu Y, Yang J, Shi J, *et al.* Obesity is associated with increased severity of disease in COVID-19 pneumonia: a systematic review and meta-analysis. *Eur J Med Res* 2020; 25: 64.

7 Corrales-Medina VF, Valayam J, Serpa JA, *et al.* The obesity paradox in community-acquired bacterial pneumonia. *Int J Infect Dis* 2011; 15: e54–e57.
8 Inoue Y, Koizumi A, Wada Y, *et al.* Risk and protective factors related to mortality from pneumonia among middleaged and elderly community residents: the JACC Study. *J Epidemiol* 2007; 17: 194–202.
9 LaCroix AZ, Lipson S, Miles TP, *et al.* Prospective study of pneumonia hospitalizations and mortality of U.S. older people: the role of chronic conditions, health behaviors, and nutritional status. *Public Health Rep* 1989; 104: 350–360.
10 Nie W, Zhang Y, Jee SH, *et al.* Obesity survival paradox in pneumonia: a meta-analysis. *BMC Med* 2014; 12: 61.
11 Decruyenaere A, Steen J, Colpaert K, *et al.* The obesity paradox in critically ill patients: a causal learning approach to a casual finding. *Crit Care* 2020; 24: 485.
12 Oliveros H, Villamor E. Obesity and mortality in critically ill adults: a systematic review and meta-analysis. *Obesity* 2008; 16: 515–521.
13 Kornum JB, Nørgaard M, Dethlefsen C, *et al.* Obesity and risk of subsequent hospitalisation with pneumonia. *Eur Respir J* 2010; 36: 1330–1336.
14 Rylance J, de Steenhuijsen Piters WAA, Mina MJ, *et al.* Two randomized trials of the effect of live attenuated influenza vaccine on pneumococcal colonization. *Am J Respir Crit Care Med* 2019; 199: 1160–1163.
15 McCullers JA, Rehg JE. Lethal synergism between influenza virus and *Streptococcus pneumoniae*: characterization of a mouse model and the role of platelet-activating factor receptor. *J Infect Dis* 2002; 186: 341–350.
16 Vernooy JHJ, Ubags NDJ, Brusselle GG, *et al.* Leptin as regulator of pulmonary immune responses: involvement in respiratory diseases. *Pulm Pharmacol Ther* 2013; 26: 464–472.
17 White SJ, Taylor MJ, Hurt RT, *et al.* Leptin-based adjuvants: an innovative approach to improve vaccine response. *Vaccine* 2013; 31: 1666–1672.
18 Tartaglia LA. The leptin receptor. *J Biol Chem* 1997; 272: 6093–6096.
19 Martí A, Marcos A, Martínez JA. Obesity and immune function relationships. *Obes Rev* 2001; 2: 131–140.
20 Ubags NDJ, Stapleton RD, Vernooy JHJ, *et al.* Hyperleptinemia is associated with impaired pulmonary host defense. *JCI Insight* 2016; 1: e82101.
21 Cordero-Barreal A, González-Rodríguez M, Ruiz-Fernández C, *et al.* An update on the role of leptin in the immuno-metabolism of cartilage. *Int J Mol Sci* 2021; 22: 2411.
22 Procaccini C, La Rocca C, Carbone F, *et al.* Leptin as immune mediator: interaction between neuroendocrine and immune system. *Dev Comp Immunol* 2017; 66: 120–129.
23 Park H-K, Ahima RS. Leptin signaling. *F1000Prime Rep* 2014; 6: 73.
24 Bates SH, Stearns WH, Dundon TA, *et al.* STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* 2003; 421: 856–859.
25 Francisco V, Pino J, Campos-Cabaleiro V, *et al.* Obesity, fat mass and immune system: role for leptin. *Front Physiol* 2018; 9: 640.
26 Maurya R, Bhattacharya P, Dey R, *et al.* Leptin functions in infectious diseases. *Front Immunol* 2018; 9: 2741.
27 Hsu A, Aronoff DM, Phipps J, *et al.* Leptin improves pulmonary bacterial clearance and survival in ob/ob mice during pneumococcal pneumonia: leptin restores pulmonary host defence. *Clin Exp Immunol* 2007; 150: 332–339.
28 Wieland CW, Stegenga ME, Florquin S, *et al.* Leptin and host defense against Gram-positive and Gram negative pneumonia in mice. *Shock* 2006; 25: 414–419.
29 Mancuso P, Peters-Golden M, Goel D, *et al.* Disruption of leptin receptor–STAT3 signaling enhances leukotriene production and pulmonary host defense against pneumococcal pneumonia. *J Immunol* 2011; 186: 1081–1090.
30 Mancuso P, Curtis JL, Freeman CM, *et al.* Ablation of the leptin receptor in myeloid cells impairs pulmonary clearance of *Streptococcus pneumoniae* and alveolar macrophage bactericidal function. *Am J Physiol Lung Cell Mol Physiol* 2018; 315: L78–L86.
31 Mancuso P, Huffnagle GB, Olszewski MA, *et al.* Leptin corrects host defense defects after acute starvation in murine pneumococcal pneumonia. *Am J Respir Crit Care Med* 2006; 173: 212–218.
32 Díez M-L, Santolaria F, Tejera A, *et al.* Serum leptin levels in community acquired pneumonia (CAP) are related to nutritional status and not to acute phase reaction. *Cytokine* 2008; 42: 156–160.
33 Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, *et al.* Role of leptin in inflammation and *vice versa*. *Int J Mol Sci* 2020; 21: 5887.
34 de Git KCG, Adan RAH. Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation. *Obes Rev* 2015; 16: 207–224.
35 Gruzdeva O, Borodkina D, Uchasova E, *et al.* Leptin resistance: underlying mechanisms and diagnosis. *Diabetes Metab Syndr Obes* 2019; 12: 191–198.
36 Morris DL, Rui L. Recent advances in understanding leptin signaling and leptin resistance. *Am J Physiol Endocrinol Metab* 2009; 297: E1247–E1259.
37 Zhou Y, Rui L. Leptin signaling and leptin resistance. *Front Med* 2013; 7: 207–222.

38 Myers MG, Cowley MA, Münzberg H. Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol* 2008; 70: 537–556.

39 Campling J, Jones D, Chalmers JD, *et al.* The impact of certain underlying comorbidities on the risk of developing hospitalised pneumonia in England. *Pneumonia* 2019; 11: 4.

40 Lopez-de-Andres A, Albaladejo-Vicente R, de Miguel-Diez J, *et al.* Incidence and outcomes of hospitalization for community-acquired, ventilator-associated and non-ventilator hospital-acquired pneumonias in patients with type 2 diabetes mellitus in Spain. *BMJ Open Diabetes Res Care* 2020; 8: e001447.

41 Centers for Disease Control and Prevention. National Diabetes Statistics Report. www.cdc.gov/diabetes/data/statistics-report/index.html Date last updated: 18 January 2022. Date last accessed: 03 June 2022.

42 Blüher M. Metabolically healthy obesity. *Endocr Rev* 2020; 41: bnaa004.

43 Ferreira DM, Neill DR, Bangert M, *et al.* Controlled human infection and rechallenge with *Streptococcus pneumoniae* reveals the protective efficacy of carriage in healthy adults. *Am J Respir Crit Care Med* 2013; 187: 855–864.

44 Trimble A, Connor V, Robinson R, *et al.* Pneumococcal colonisation is an asymptomatic event in healthy adults using an experimental human colonisation model. *PLoS One* 2020; 15: e0229558.

45 Wright AKA, Ferreira DM, Gritzfeld JF, *et al.* Human nasal challenge with *Streptococcus pneumoniae* is immunising in the absence of carriage. *PLoS Pathogens* 2012; 8: e1002622.