Glucagon-Like Peptide-1 (GLP-1) Reduces Mortality and Improves Lung Function in a Model of Experimental Obstructive Lung Disease in Female Mice

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The incretin hormone glucagon-like peptide-1 (GLP-1) is an important insulin secretagogue and GLP-1 analogs are used for the treatment of type 2 diabetes. GLP-1 displays antiinflammatory and surfactant-releasing effects. Thus, we hypothesize that treatment with GLP-1 analogs will improve pulmonary function in a mouse model of obstructive lung disease. Female mice were sensitized with injected ovalbumin and treated with GLP-1 receptor (GLP-1R) agonists. Exacerbation was induced with inhalations of ovalbumin and lipopolysaccharide. Lung function was evaluated with a measurement of enhanced pause in a whole-body plethysmograph. mRNA levels of *GLP-1R*, surfactants (*SFTPs*), and a number of inflammatory markers were measured. *GLP-1R* was highly expressed in lung tissue. Mice treated with GLP-1R agonists had a noticeably better clinical appearance than the control group. Enhanced pause increased dramatically at day 17 in all control mice, but the increase was significantly less in the groups of GLP-1R agonist-treated mice ($P < .001$). Survival proportions were significantly increased in GLP-1R agonist-treated mice ($P < .01$). *SFTPB* and *SFTPA* were down-regulated and the expression of inflammatory cytokines were increased in mice with obstructive lung disease, but levels were largely unaffected by GLP-1R agonist treatment. These results show that GLP-1R agonists have potential therapeutic potential in the treatment of obstructive pulmonary diseases, such as chronic obstructive pulmonary disease, by decreasing the severity of acute exacerbations. The mechanism of action does not seem to be the modulation of inflammation and *SFTP* expression. **(*Endocrinology* 154: 4503–4511, 2013)**

Glucagon-like peptide-1 (GLP-1) is a peptide secreted from the enteroendocrine L cells after oral food intake. The main function of the peptide is to stimulate insulin secretion and thereby function as an incretin hormone (1). In addition to stimulating insulin secretion in a glucose-dependent manner, GLP-1 also preserves β-cell function (2), promotes weight loss (3), and lowers circulating triglyceride levels (4). GLP-1 analogs have shown great potential in the treatment of patients with type 2 diabetes mellitus (T2DM).

In rodents, the GLP-1 receptor (GLP-1R) is expressed in pancreatic islets, lungs, brain, heart, kidney, and the gastrointestinal (GI) tract as demonstrated by Northern blotting and RT-PCR (5, 6). GLP-1R has also been reported being present in human lung (7).

GLP-1 has been extensively investigated and elicits several extrapancreatic effects. In the GI system, GLP-1 inhibits gastric emptying, gastric acid secretion, and exocrine pancreatic secretion (8–11). GLP-1 administration

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Abbreviations: COPD, chronic obstructive pulmonary disease; Ct, cycle threshold; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; OVA, ovalbumin; PAS, periodic acid-Schiff; Penh, enhanced pause; RT-qPCR, real-time-quantitative PCR; SFTP, surfactant; TBP, TATA-binding protein; T2DM, type 2 diabetes mellitus.

has been shown to exhibit cardiovascular effects, increasing arterial blood pressure and heart rate in rats (12), and interestingly, GLP-1 might be beneficial for protection against myocardial infarction (13). Reports also support a role for GLP-1 in modulation of renal function (14, 15).

Despite the presence of the receptor in lung membranes from both humans (7) and rodents (5, 6, 17–19), an exact function of the receptor in lung tissue has not been established. Vara et al (20) in 2001 have demonstrated that GLP-1 stimulates surfactant secretion in human type II pneumocytes in vitro and suggested GLP-1 as a potential treatment for respiratory distress in premature newborns. Arakawa et al (21) in 2010 investigated GLP-1 for its antiinflammatory role in atherosclerosis and described that GLP-1R is expressed on peritoneal macrophages and that GLP-1 is able to inhibit lipopolysaccharide (LPS)-induced TNF-α expression and nuclear translocation of nuclear factor-κB in these cells. Iwai et al (22) found that GLP-1 inhibited the LPS-induced increase in IL-1β in cultured cortical astrocytes and concluded that GLP-1 may be a modulator of inflammatory responses in the central nervous system, and likewise GLP-1 inhibited cytokine secretion and promoted survival after LPS-induced systemic inflammation in rats (23).

Thus, the present knowledge suggest that GLP-1 displays antiinflammatory effects, and considering the fact that GLP-1R is present in the lung, we hypothesized that systemic GLP-1 might attenuate acute lung disease in a mouse model of obstructive pulmonary disease. We developed a novel model of experimental lung disease (LD) based on two previously described mouse models of asthma and chronic obstructive pulmonary disease (COPD), models based on ovalbumin (OVA) and LPS inhalations, respectively (24, 25). With this model we were able to control the onset of exacerbation and frequently evaluate lung function during period of disease.

We found that liraglutide and exenatide, stable GLP-1 analogs, dramatically improved the condition of mice induced to develop LD; however, this improvement did not appear to relate to expression of surfactants or attenuation of lung inflammation.

Materials and Methods

Animals

The animal study was approved by the Danish National Committee for Animal Studies. We used 10-week-old C57 BL/6 female mice weighing approximately 20 g (range 18.7–21.3) (Taconic). Mice were housed in air-conditioned (21°C) and humidity-controlled (55%) rooms with a 12-hour light, 12-hour dark cycle. They were given regular food (Altromin) and water ad libitum. After the last inhalation, mice were kept on heated plates to maintain sufficient body temperature. The mice were killed if they had a weight loss of 20% of body weight or had severe dyspnea and piloerection.

Mouse model of LD

We modified and combined two previously described models: a model of OVA-induced asthma (25) and a model of LPS-induced COPD (24). The mice were injected sc with 0.1 mL of homogenized heat-coagulated hen's egg white in the back of the neck. After a 14-day sensitization period, the animals were subjected to aerosolized OVA (20 mg/mL; Sigma-Aldrich) on days 14 and 16 and aerosolized LPS (2.5 mg/mL; Sigma-Aldrich) on days 15 and 17 in an exposure chamber (Buxco) with an air flow rate of 2 l/min for 30 minutes, although only 15 minutes exposure to LPS in studies 2 and 3.

Plethysmography

We used an unrestrained whole-body flow-plethysmograph (Buxco) to evaluate the pulmonary function of the mice. Our principal measure of pulmonary function was enhanced pause (Penh) but also frequency, tidal volume, minute volume, inspiratory and expiratory time, peak inspiratory and expiratory flow, and relaxation time were measured. The measurements were done in a 2-minute period, once or twice daily, at 8 AM and 8 PM.

Peptides

Mice were treated with 0.05 mL of the GLP-1R agonists liraglutide (6 mg/mL; Victoza, Novo Nordisk, Denmark) or exenatide (250 μg/mL, Byetta; Eli Lilly) twice a day given sc, starting 10 days after sensitization and continued until the animals were killed (26). This equals doses of 300 mg/kg·d of liraglutide and 1250 μg/kg·d of exenatide. The doses were chosen as the largest tolerable dose seen in pilot studies. Due to the peptide structure of liraglutide in which approximately 99% percent of the peptide is albumin bound, the applied dose of liraglutide is relatively large (27). As a control vehicle, we used the same volume of PBS.

Experimental protocol

Study 1

Sixteen mice were treated with liraglutide or vehicle (n = 8). From day 14 the mice were measured in the whole-body plethysmograph twice daily, diminished to once daily from day 20.

Study 2

Forty-eight mice were divided in three treatment groups, each composed of two × eight mice. The three treatment groups were treated with exenatide, liraglutide, or vehicle. Eight mice from each treatment group received inhalations and were measured in the whole-body plethysmograph twice daily from day 12 and euthanized at day 21 (n = 8). The remaining three groups of eight mice received inhalations but no plethysmography (n = 8). These mice were euthanized at day 17, 8 hours after the last LPS inhalation. Lung tissue was fixated in situ by the installation of ice-cold 4% paraformaldehyde into the lungs via a tracheal tube.

Study 3

Thirty-two mice were divided into four groups of eight mice. Two groups were treated with liraglutide and two groups with

vehicle. One of each treatment group was injected with an OVA pellet at day 0 and received inhalations but no plethysmography. The two remaining groups served as healthy control groups that were injected with saline at day 0, and instead of inhalations of OVA and LPS, the mice spent the same amount of time in the chamber but without receiving inhalations. All mice were killed 8 hours after the last LPS inhalation to harvest the tissue at the time at which the mice had a large increase in Penh observed in studies 1 and 2. Harvested tissue was snap frozen in liquid nitrogen and stored at −80°C for later analysis.

The outline of the three studies is depicted in Figure 1.

Histological examinations

Fixated lung tissue was embedded in paraffin and cut into 5-μm sections using a microtome. The sections representing the bronchi, trachea, and all pulmonary lobes were stained with periodic acid-Schiff (PAS)/hematoxylin. Periarterial inflammation was evaluated using a previously described method in which inflammation was graded into four levels (28): 0, no inflammation; 1, one or two concentric rows of inflammatory cells; 2, three or more concentric rows of inflammatory cells; or 4, continuous perivascular and peribronchial cell accumulation. Mucin-producing cells were evaluated by measuring the length of the bronchial circumference and counting the number of PAS-positive cells in all bronchi of a transverse section of the lung at the level of the hilus, thereby achieving a number of PAS positive cells per 100 μm of bronchial basement membrane for each lung. The area of tissue in a cross-section of the lung at the level of the hilus and the total area of the same section were measured using image processing software (Image Pro Plus; Media Cybernetics) and the air to tissue ratio was calculated.

Mouse tissues for GLP-1R determination

Tissues were obtained from five 3-month-old female C57BL/6 mice (Figure 2A) and from the control mice in study 3 (Figure 2B). Dissected tissues were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Human biopsies for GLP-1R determination

Lung biopsies where obtained from patients undergoing cardiopulmonary bypass-dependent surgery (coronary artery bypass and/or aortic valve replacement), and immediately submerged into liquid nitrogen, according to the Pulmonary Protection Trial protocol (http://www.ncbi.nlm.nih.gov/pubmed/23363494). The trial is registered at clinicaltrials.gov (NCT01614951). The muscles biopsies were obtained by the Bergström technique from the vastus lateralis muscle from middle-aged humans. The biopsies were immediately frozen in liquid nitrogen and stored at −80°C until analysis. The trials adhere to the Helsinki Declaration II and the national laws in Denmark and are approved by the Committees on Biomedical Research Ethics of the Capital Region of Denmark, the Danish Medicines Agency, and the Danish Data Protection Agency. Patients were enrolled only after informed consent. Human muscle biopsies were used in the study to serve as low expression control specimen.

RNA isolation and gene expression analysis

RNA isolation, reverse transcription, and real-time-quantitative PCR (RT-qPCR) were performed as described (29, 30). Primers used are described in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org. Expression levels were normalized to either TATA-binding protein (*TBP*) or *18S rRNA* as specified in the figure legends. When *18S rRNA* was used, the normalized expression levels were multiplied by 10^5.

Statistics

Two-way ANOVA was used to analyze the grouped data and a Bonferroni-corrected posttest was used to compare the groups on individual days. Log-rank test was used to compare survival data. Gene expression data were analyzed for statistical significance ($P < .05$) using Student's *t* test. Bonferroni correction was applied when multiple comparisons were performed.

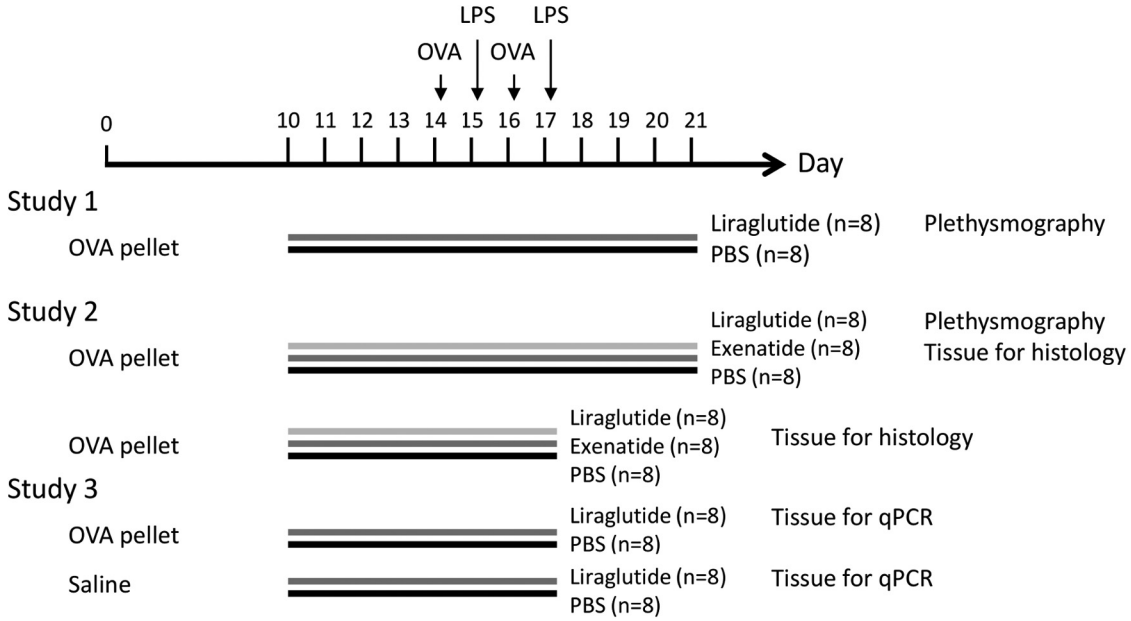

Figure 1. Schematic overview of the three experimental studies.

Results

Expression of *GLP-1R* in mouse and human lungs

We profiled the level of *GLP-1R* mRNA in nine mouse tissues (n = 5). *GLP-1R* mRNA was detectable in all tissues but was expressed at the highest level in lung, followed by intestine and brain (Figure 2A). To compare levels of *GLP-1R* in lung with that of total pancreas, we measured *GLP-1R* in another group of mice (n = 4). *GLP-1R* was expressed at the highest level in lung [average cycle threshold (Ct) value of 22.1] but with substantial expression in total pancreas (average Ct value of 25.0) (Figure 2B). Expression in skeletal muscle was low (average Ct value of 33.9). To establish the expression of *GLP-1R* in human lung tissue, we analyzed lung biopsies from patients undergoing coronary artery bypass and/or aortic valve replacement and compared it with skeletal muscle biopsies. Expression was substantially higher in lung tissue (average Ct value of 29.0) than in skeletal muscle (average Ct value of 32.1) (Figure 2C).

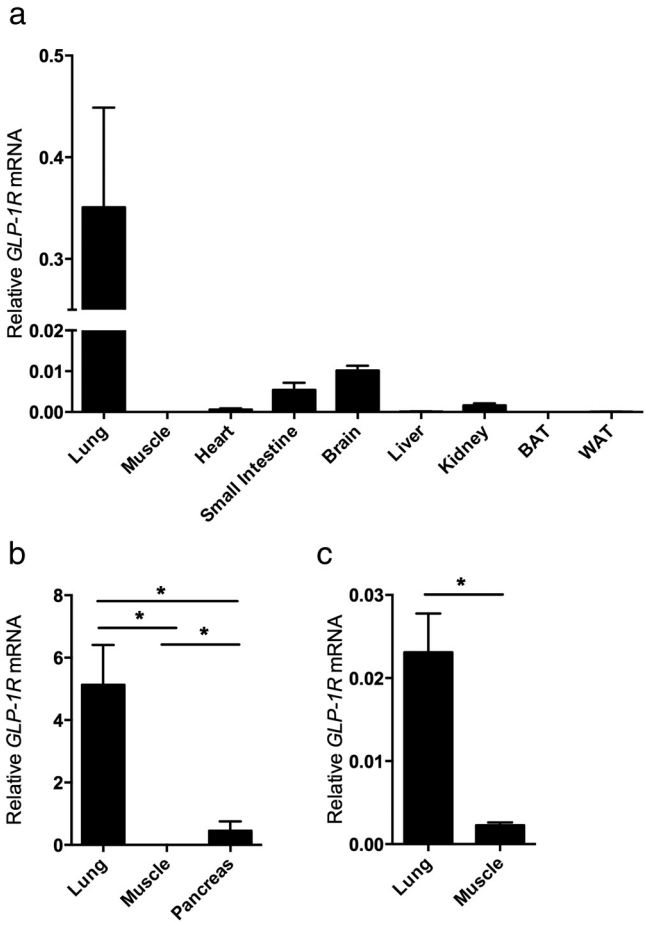

Figure 2. RT-qPCR profiling of *GLP-1R* gene expression in mice and humans. A, Profile of *GLP-1R* expression measured in nine different mouse tissues (n = 5). B, Expression of *GLP-1R* in lung, muscle, and total pancreas from mice (n = 4). C, *GLP-1R* gene expression measured in human lung tissue (n = 9) and skeletal muscle (n = 4). Relative mRNA expression levels were determined by normalization to expression levels of *18S rRNA*. Data represents mean ± SEM. *, $P < .05$, *t* test.

Behavior and clinical observations

In the mouse model of LD, the vehicle-treated mice differed noticeably from both exenatide- and liraglutide-treated animals in behavior and clinical appearance, visible already from the first inhalation with LPS. The exenatide- and liraglutide-treated animals retained normal grooming and inquisitive behavior and showed no signs of illness in terms of piloerection, dyspnea, or periorbital darkening, whereas the vehicle-treated animals tended to seclude themselves from the group, be inactive, and show some degree of piloerection and dyspnea.

Survival

The mice suffer from the exacerbation from day 17, and if death occurs, it happens within 36 hours after the last inhalation. When inhalations are stopped, the airway obstruction diminishes and the mice who survives the exacerbation will not die unless they are killed. In study 1, the liraglutide-treated group had a significant improved survival (100% vs 37.5%, $P < .01$); in the vehicle group, five of eight mice died 24 hours after the second LPS inhalation and no mice died in the liraglutide-treated group (Figure 3A). In study 2, the survival was increased to 62.5% in the vehicle group due to shortening of the LPS inhalation to 15 minutes, but this was still significantly less than the 100% survival in both the exenatide- and liraglutide-treated groups (Figure 3B).

Plethysmography

The course of the inflammatory reaction was as expected, with negligible response to the first three inhalations at days 14, 15, and 16 and a pronounced response to the last LPS inhalation on day 17.

In study 1, there was a highly significant difference between the liraglutide-treated animals and the vehicle-treated control group 12 and 24 hours after the last LPS inhalation ($P < .001$) (Figure 4A). In study 2, there were highly significant differences ($P < .001$) between the vehicle-treated control group and both the exenatide- and liraglutide-treated animals 12, 24, and 36 hours after the last LPS inhalation (Figure 4B).There were significant differences between the control groups and the treatment groups in all parameters measured in the plethysmograph in both studies (Supplemental Figures 1 and 2).

Expression of surfactants

To probe whether the beneficial effect of liraglutide was due to increased expression of surfactants (SFTPs), we

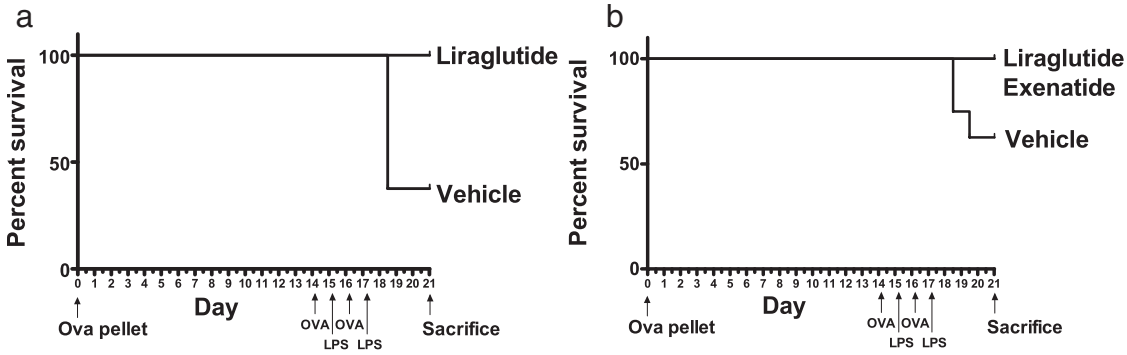

Figure 3. Survival proportions. A, Study 1. Five of eight control mice died after the last LPS inhalation, whereas none of the liraglutide-treated mice died. B, Study 2. Three of eight control mice died after the last LPS inhalation, whereas none of the GLP-1R agonist-treated mice died. In both studies there were a significant difference in mortality between the treatment groups (study 1, $P < .01$; study 2, $P < .05$, log-rank test).

measured expression of mRNAs encoding the four *SFTPs*. *SFTPB* and *SFTPC* were down-regulated 3- to 4-fold in LD mice compared with healthy control mice, irrespectively of liraglutide treatment, whereas expression of *SFTPA* was unaffected (Figure 5, A–C). *SFTPD* expression was increased in the liraglutide-treated LD mice compared with the corresponding control mice (Figure 5D).

Histological examinations and expression of inflammatory markers

In the LD mice, there was no difference in grade of inflammation, air to tissue ratio, or mucin content in goblet cells between groups treated with GLP-1R agonists or PBS, neither in the acute phase (8 hours after the last LPS inhalation) nor at day 21 when Penh was normalized (data not shown). Both groups appeared severely inflamed, with accumulation of neutrophils in the alveoli, periarteriolar lymphocyte infiltration, and presence of mucus-producing cells in the epithelium of the bronchi and larger bronchioles (Figure 6, A and B). In accordance with this severe inflammation, mRNA levels of the macrophage marker *CD68* were increased 3-fold in LD mice compared with healthy control mice, irrespectively of liraglutide treatment (Figure 6C). Another macrophage marker, *F4/80*, appeared to increase in LD mice, however, reaching significance only in the liraglutide group (Figure 6D). Expression of the proinflammatory cytokines monocyte chemotactic protein-1 (*MCP-1*) and IL-6 (*IL-6*) as well as of the antiinflammatory cytokine *IL-10* was strongly induced in LD mice (Figure 6, E–G). Expression of another proinflammatory cytokine, *IL-8*, was significantly increased in liraglutide-treated LD mice but tended to increase only in vehicle-treated LD mice (Figure 6H). Expression of macrophage markers and cytokines was not affected by liraglutide treatment in either the healthy control group or in the LD group.

Discussion

In this report we provide evidence, supporting that GLP-1R agonists might display beneficial effects in the treatment of lung diseases such as COPD. Our main finding was a highly significant and remarkable improvement of survival and lung function in GLP-1R agonist-treated mice compared with controls in a new mouse model of obstructive LD.

In addition to the improved survival, we judged all GLP-1R agonist-treated mice to be of better health, including retainment of normal grooming and inquisitive behavior.

Traditionally COPD is induced in experimental animals by cigarette smoke; still these models do not resembles COPD in human subjects, and even if they exhibit the hallmarks features of COPD, they may not respond to traditional treatments such as glucocorticoids (31). Short-term models of cigarette smoke induced COPD can be used

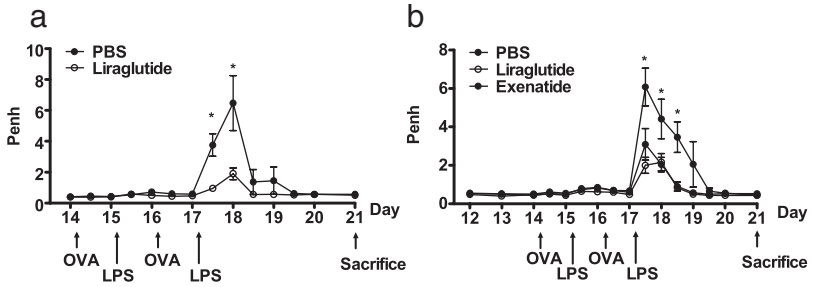

Figure 4. Lung function measurements using Penh as a variable to estimate airway resistance. A, Study 1. The increase in Penh in control mice after the last LPS inhalation was significantly attenuated in the liraglutide-treated group (12 h after last LPS inhalation, $P < .001$; 24 h after last LPS inhalation, $P < .0001$, two way ANOVA). B, Study 2. The increased Penh in the control group after the last LPS inhalation was significantly attenuated in the liraglutide- and exenatide-treated groups (12, 24, and 36 h after the last LPS inhalation, $P < .0001$, two way ANOVA).

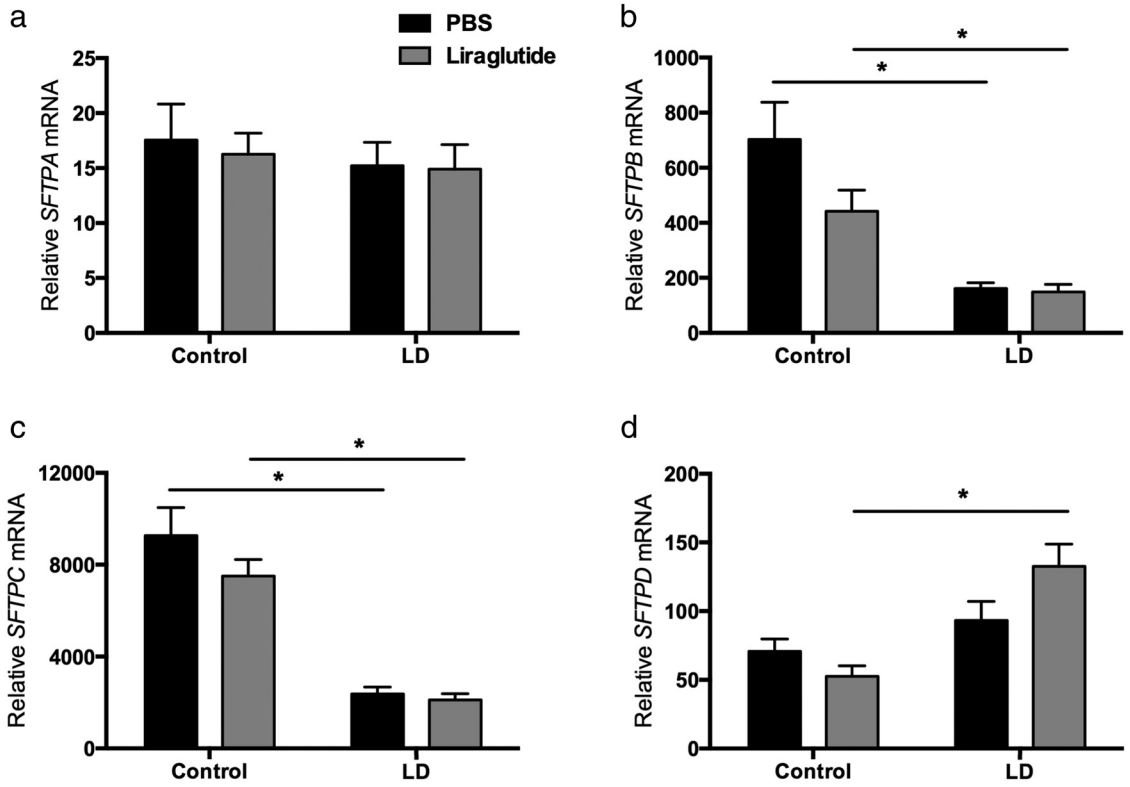

Figure 5. Surfactants in LD mice. Lung samples from study 3 were analyzed by RT-qPCR. A, *SFTPA*. B, *SFTPB*. C, *SFTPC*. D, *SFTPD*. Relative mRNA expression levels were determined by normalization to levels of *TBP*. Data represent mean ± SEM [control (n = 4); LD (n = 6–8)]. *, $P < .05$, *t* test.

for the evaluation of the early smoke induced inflammatory response, but mice in these models do not have diminished lung function (31). Chronic models (up to 9 months of exposure) of cigarette smoke induced COPD result in airway remodeling but only mild alterations in pulmonary function, and the airways needs to be challenged with methacholine to obtain measurable parameters for pulmonary function, which actually is a measure of airway responsiveness and not airway obstruction (32). The long duration of the experiment and the short life span of a mouse limit the use for these chronic models. Our model is based on two previously described models of asthma and COPD (24, 25), based on OVA and LPS inhalations, respectively. Our pilot studies had established that combining these two models resulted in severe pulmonary inflammation with neutrophil granulocyte and macrophages infiltration as well as goblet cell hyperplasia. Furthermore, it was possible to measure a decrease in pulmonary function in a whole-body plethysmograph without challenging the animals with methacholine and thereby be a model of obstructive pulmonary diseases such as COPD. We show here that the increase in Penh in this model is accompanied by a large increase in expression of several markers of inflammation and an overall decrease in expression of surfactants. We also showed that there is a coincidence in the large increase in Penh and death of the animals.

GLP-1R agonists have the advantage of being successfully marketed to treat T2DM and of having a satisfying safety profile and few side effects (33). Because of the insulin-stimulating effect of GLP-1, one could be concerned about hypoglycemia as a side effect, but GLP-1-stimulated insulin secretion is glucose-dependent, and liraglutide has in large clinical trials showed great potential as an antiobesity drug, without any risk of inducing hypoglycemia in healthy obese persons (34). The side effects of GLP-1R agonist treatment is most often GI related, with nausea being the most frequent. This is, however, diminished after a few weeks of treatment (34). The weight-reducing effect of GLP-1R agonists could be a disadvantage in the treatment of patients with lung diseases such as COPD. However, epidemiological findings suggest that COPD is associated with a high prevalence of abdominal obesity (35) and T2DM (36). To our knowledge, no reports have elucidated whether patients with T2DM and COPD experience better lung function and fewer or less severe exacerbations if treated with GLP-1R agonists for their T2DM.

MKC253 is a GLP-1/Tecnosphere formulation used for inhalations that will carry GLP-1 deep into the lung. This

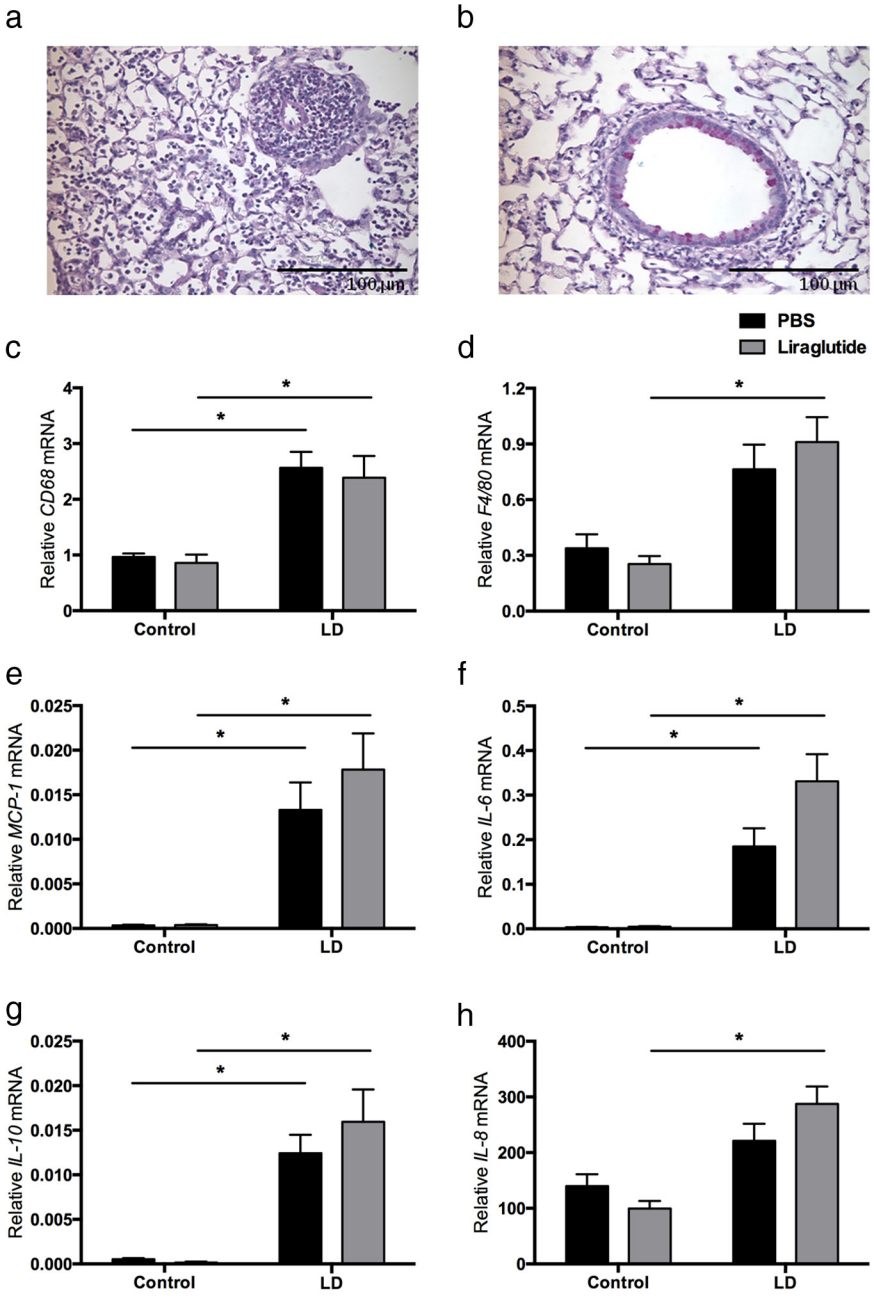

Figure 6. Histology and expression of inflammatory markers in LD mice. A and B, Two examples of inflammatory changes as seen in a lung from a liraglutide-treated mice. A, Inflammatory cells in the alveoli and a small arteriole surrounded by inflammatory cells, mainly monocytes, lymphocytes, and macrophages. B, Bronchiole with mucus-producing cells in the epithelium. The normal mouse lung has no mucus-producing cells in the bronchi, but in this diseased lung, there are only ciliated cells and mucus-producing, goblet cell-like cells. C–H, Lung samples from study 3 were analyzed by RT-qPCR. C, *CD68*. D, *F4/80*. E, *MCP-1*. F, *IL-6*. G, *IL-10*. H, *IL-8*. Relative mRNA expression levels were determined by normalization to levels of *TBP*. Data represent mean ± SEM [control (n = 4); LD (n = 6–8)]. *, $P < .05$, *t* test.

form of delivery produced peak concentrations in plasma within 5 minutes (37) and could therefore be relevant as first-line treatment in acute respiratory impairment. It is not known whether this compound acts on GLP-1R in lung tissue because the method is developed for the treatment of T2DM.

The mechanisms behind the beneficial effects observed in the animals treated with the GLP-1R agonists are unknown. We show here that the *GLP-1R* mRNA is expressed in mouse and human lungs. We have not demonstrated that the GLP-1R protein is also present. GLP-1R activation could have an antiinflammatory effect, as suggested for other organ systems, ie, in the vascular bed and central nervous system (21, 22). Our histological analysis showed severe inflammation of the lung tissue in LD mice, but the grade was unaffected by liraglutide and exenatide. Expression of several inflammation markers (eg, *CD68, MCP-1, IL-6,* and *IL-10*) was highly increased in the inflamed lung tissue, but liraglutide treatment did not attenuate this expression.

As mentioned, Vara et al (20) in 2001 found that GLP-1R activation stimulated SFTP secretion from human type II pneumocytes. Pulmonary SFTPs have important properties in maintaining alveolar and airway stability, by reducing surface tension and preventing alveolar and airway collapse (39). Our *SFTP* mRNA measurements indicated that *SFTPC* was most highly expressed, followed by *SFTPB*, *SFTPD*, and *SFTPA*. Expression of *SFTPB* and *SFTPC* was strongly decreased in LD mice, independent of liraglutide treatment. However, a limitation of our study is that we have not determined surfactant protein levels. Therefore, we cannot rule out the possibility that liraglutide influences protein levels of surfactants.

We found a clear coincidence in Penh and death and because both the GLP-1-treated mice with a low Penh and the vehicle-treated mice with a high Penh showed severe histological inflammation and an increase in expression of inflammatory markers, we suggest the airway obstruction to be the cause of death. A small vasorelaxant effect of GLP-1 directly administered to isolated preconstricted truncus pulmonalis rings from rats have been dem-

onstrated by Golpon et al (40) in 2001. In the isolated perfused rat lung, a high dose of GLP-1 administered by the artery reduced the perfusion pressure; this effect was lost when inhibiting nitric oxide with N-ω-nitro-L-arginine methyl ester. GLP-1 had no effect on isolated tracheal rings (40). Our experiments suggest GLP-1 to have an effect on airway obstruction. Airway obstruction is a consequence of hyperplasia/metaplasia of the airway epithelia, hypersecretion of mucus, emphysema, increased perfusion pressure, and a constriction of smooth muscle cells surrounding the bronchi and the bronchioli (41). We did not find any change in mucus-producing cells or other histological features. Therefore, we suggest GLP-1 to mediate relaxation of smooth muscle cells in the conductive airways and the vascular bed.

The vasodilatory effect of GLP-1 is also shown by Nyström et al (16) to be independent of endothelial substances; however, the dilatory effect was also modest in this study. Kim et al (38) recently showed that GLP-1 had no direct vasodilatory effect in aortic rings but elegantly demonstrated that the perfusate from isolated hearts infused with GLP-1 had a remarkable vasodilatory effect, which was dependent on the GLP-1R-mediated secretion of atrial natriuretic peptide from heart muscle cells. The possible reduction in perfusion pressure mediated by GLP-1 treatment could contribute to the decrease in Penh; however, in our study the reduction in airway obstruction was substantial, and therefore, an effect on the bronchi/bronchioli smooth muscle cells probably also plays a role either by a direct stimulation of GLP-1R-positive smooth muscle cells or by a second factor secreted in response to GLP-1. This hypothesis needs further investigations.

We suggest GLP-1R agonists as a possible new treatment for lung diseases such as COPD and asthma. We found a highly significant reduction in mortality and morbidity in a mouse model of obstructive lung disease upon treatment with the two marketed GLP-1 analogs, liraglutide and exenatide. Further experiments are needed to elucidate whether these results can be extrapolated to humans and also to determine the mechanism of action of this remarkable effect.

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Author contributions include the following: N.V. and H.K. planned and performed the animal experiments. M.S.I. and J.B.H. performed the gene expression analyses. S.S.P. performed the histological analysis. K.B.B. collected the human lung biopsies. N.V., M.S.I., J.B.H., and H.K. wrote the manuscript. All authors commented and approved the final version of the manuscript.

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