Hyperinsulinemia adversely affects lung structure and function

Suchita Singh,1* Manish Badas,1* Naveen K. Bhatraju,1 Bijay Pattnaik,1 Atish Gheware,1 Praveen Kolumam Parameswaran,5 Michael Thompson,5 Michelle Freeman,5 Ulaganathan Mabalirajan,1 Reinoud Gosen,6 Balaram Ghosh,1 Christina Pabelick,5 Allan Linneberg,2,3,4 Y. S. Prakash,5 and Anurag Agrawal1

1Center of Excellence for Translational Research in Asthma and Lung Disease, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India; 2Research Centre for Prevention and Health, the Capital Region of Denmark, Copenhagen, Denmark; 3Department of Clinical Experimental Research, Glostrup University Hospital, Glostrup, Denmark; 4Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; 5Departments of Anesthesiology and Physiology and Biomedical Engineering, Mayo Clinic, Rochester, Minnesota; and 6Department of Molecular Pharmacology, Groningen Research Institute for Asthma and COPD (GRIAC), University of Groningen, Groningen, Netherlands

Submitted 27 March 2015; accepted in final form 12 February 2016

Obesity, diabetes, and asthma have attained global epidemic proportions (19, 20). Multiple studies have shown a strong epidemiological and experimental link between obesity and asthma that further relates to a diverse set of etiologic factors including altered lung mechanics, adipose hormones, and inflammatory cytokines (3, 11). Although a positive relationship between diabetes and asthma is less certain (36, 37), there is strong evidence that overt diabetes and its precursor form of insulin resistance, with consequent hyperinsulinemia, is a dependent surrogate marker or whether it independently contributes to increased risk of respiratory disease. Furthermore, although there is sufficient basis to hypothesize that hyperinsulinemia is mechanistically related to increased risk of asthma or other lung diseases, there are only limited data to support this idea, with insulin showing potentiation of airway smooth muscle (ASM) contraction in animals (15, 32), which remains to be confirmed in human airways (34). Indeed, the mechanisms by which insulin influences airway elements to produce structural and functional changes in the asthmatic airway are also not known.

Understanding the impact of hyperinsulinemia on lung health is a critical issue not just from a research perspective, but also as a global healthcare issue, given that insulin resistance is on the rise worldwide. The increasing interest in clinical use of inhaled insulin formulations that could lead to very high levels of insulin in the lung also illustrates the need for focused studies on insulin and lung health, given that there have been reports of negative effects on lung function with a previous formulation, Exubera (Pfizer) (5).

To investigate possible mechanisms of such associations and the potential causal effects of high lung levels of insulin, in vitro human lung cell models with insulin application and preclinical in vivo murine model of intranasal insulin application were tested. Overall, these studies led to a better understanding of potentially deleterious insulin-induced signaling in the lung.

MATERIALS AND METHODS

Marine Experiments

Male Balb/c mice (8–10 wk old) were obtained from National Institute of Nutrition, Hyderabad, India and were acclimatized for 1 wk. All procedures were approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), CSIR-IGIB, Delhi, India.

* S. Singh and M. Badas contributed equally to this work.

Address for reprint requests and other correspondence: A. Agrawal, Center of Excellence for Translational Research in Asthma and Lung Disease, CSIR-Institute of Genomics and Integrative Biology, New Delhi 110007, India (e-mail: a.agrawal@igib.in).
Intranasal insulin-induced hyperinsulinemia model. The model was developed in Balb/c mice: 50 μg insulin was administered intranasally, daily for 11 days. Mice were euthanized on the 12th day, by overdose of pentobarbital. The 50-μg intranasal dose was selected from pilot studies of 5–500 μg since it led to insulin levels of ~200 pmol/l, comparable to levels seen in insulin resistant humans.

Glucose tolerance test. After 11 days of insulin treatment as described above, the mice were kept fasting for 6 h and a oral glucose
load (1 g/kg body wt) was delivered into the stomach by a 20-gauge gavage needle. The blood glucose was measured via a commercially available glucometer with tail vein blood at 0 and 120 min.

**β-Catenin knockdown in mice lungs.** Mice were treated with four doses of β-catenin siRNA (Sigma), 75 μg, alongside of intranasal insulin treatment every 48 h starting from the 4th day and continued till the end of the hyperinsulinemia model. Mice were euthanized on 12th day as described above.

**Measurement of AHR.** Airway hyperresponsiveness (AHR) measurements were done after 11 days. Mice were anesthetized with intraperitoneal 16 mg/kg xylazine and 50 mg/kg pentobarbital and surgically prepared with a tracheal cannula (18 gauge). Lung function measurements were performed in anesthetized and paralyzed mice, and respiratory system parameters (airway resistance; R) were measured with a flexiVent system (SCIREQ). The methacholine nebulization was performed by using the default protocol of system software (SCIREQ) with standard particle-sized model nebulizer (4–6 μM particle size) with 10-s nebulization time, 50% duty cycle, presence of attached Drierite tube (desiccant), and regular ventilation profile. The respiratory system resistance had been shown as R or Rrs with its unit (cmH2O·s·m−3).

**Histology.** Histological staining was performed in paraffin-embedded lung sections by standard protocols (4).

**Measurement of collagen content.** Mice lungs were homogenized in RIPA buffer supplemented with DTT (1 mM) and protease inhibitor cocktail (Sigma). Collagen content in the total lung homogenate was determined by using the Sircol Collagen Assay kit (Biocolor, Carrickfergus, UK) as per manufacturer’s instructions and results were represented as relative fluorescence units per microgram lung protein.

**Immunohistochemistry.** Formalin-fixed, paraffin-embedded lung tissue sections were prepared for immunohistochemical analysis. Sections were stained with the primary antibodies for α-smooth muscle actin (rabbit polyclonal antibody; Abcam) followed by horse-radish peroxidase (HRP)-conjugated secondary antibodies (Bangs Laboratories). The signal was detected by diaminobenzidine tetrahydrochloride (DAB, Sigma), and hematoxylin was used as a counterstain.

**Cell Culture**

BEAS-2B (human bronchial epithelial cells) were directly purchased from either ECACC or ATCC and maintained under standard cell culture conditions as described by ECACC or ATCC.

**Isolation of human ASM cells.** Human ASM were isolated from surgical lung samples as previously described (31).

**Insulin treatment.** Following serum starvation, ASM cells were plated in 60-mm sterile tissue culture dishes or T75 flasks and treated with different concentrations of recombinant human insulin (Sigma) for the indicated time points. For other experiments, ASM cells were either plated in 96-well plates [extracellular matrix (ECM), proliferation, or Ca2+ assays] or on special 24-well plates provided by Seahorse Biosciences (for mitochondrial function assays).

**Cell proliferation.** ASM cells and BEAS-2B cells were plated at a concentration of 5,000 cells/well and serum starved for 16 h prior to treatment. The plates were then treated with insulin (1 μg/ml), β-catenin inhibitor (ICG-001, SelleckChem) at 1 μM concentration, and combination of insulin and β-catenin inhibitor in 1% serum-containing medium. Readouts for cell proliferation were taken after 72 h by using the MTS assay kit from Promega at 490 nm.

**Immunofluorescence protocol.** ASM and BEAS-2B cells were plated at a concentration of 10,000 cells/ml. Cells were serum starved and treated with 1 μg/ml insulin for 1 h prior to fixation with 4% paraformaldehyde. Cell were permeabilized with 0.1% Triton for 20 min and blocked with 4% BSA. β-Catenin primary antibody was used at 100 μg/ml (ab16051, Abcam) in 4% BSA and 1 mg/ml Alexa Fluor 488 secondary antibody (A21208, Life Technologies) and imaged at 490 nm in a fluorescent microscope.

**Western blotting.** Total or cytosolic extracts were prepared from cells treated with insulin (1 μg/ml) alone or with wortmannin (1 μM, pretreatment for 1 h), and from lung tissue of mice administered intranasal insulin (50 μg/ml), by standard procedures. Protein estimation was done by the BCA method, and equal amounts of protein were resolved on SDS-PAGE, followed by transfer onto PVDF/nitrocellulose membrane (Millipore). The membranes were incubated with primary antibodies against pAkt, Akt, IR-1β, activated IR-1β, pGSK3β, GSK3-β, β-catenin, α-tubulin, and GAPDH (Santa Cruz Biotechnology, Abcam, or Cell Signaling). Proteins were detected using Li-Cor IR Dye secondary antibodies and imaged on the Li-Cor Odyssey. Alternatively, HRP-conjugated secondary antibody (Sigma) with DAB (3,3′-diaminobenzidine tetrahydrochloride) chromogen was used.

**ECM deposition assay.** ASM cells were serum starved and incubated with different doses of insulin for 72 h. Cells were washed in PBS and 100 μl of 0.016 N NH4OH was added to lyse the cells, thus leaving only the native deposited ECM on the well surface. Wells were washed with PBS again and incubated with 5% BSA (in PBS) or Li-Cor blocking buffer for 90 min and then incubated in 1:100 dilution of collagen-1 primary antibody (Abcam, rabbit polyclonal) for 2 h at room temperature (RT) or overnight at 4°C. Plates were washed again with 0.1% Tween 20 in PBS and then incubated with appropriate secondary antibody (Licor IRDye donkey anti-rabbit 800 CW) for 1 h at RT. After a final wash, the extent of collagen deposition was assayed by a Licor Odyssey system. Wells without any cells were used as background controls.

**Ca2+ assay.** ASM cells were serum deprived and incubated in 5 μM flou-4/AM (no. F14217, Invitrogen; excitation 495 nm; emission 510 nm) for 60 min at RT, followed by insulin (1 μg/ml) treatment for 30 min. Baseline intracellular calcium ([Ca2+]i) levels were assessed by use of a fluorescence imaging plate reader (FlexStation 3, Molecular Devices, Sunnyvale, CA). Fluo-4 levels were converted to nanomolar Ca2+ by previously described empirical calibration procedures (1).

**Seahorse experiments.** Human ASM cells were seeded into a 24-well XF24 plate and serum starved for 24 h. Cells were then treated with 1 μg/ml insulin overnight (~16 h). Prior to assay cells were equilibrated in Seahorse assay medium containing 10 mM glucose at 37°C for 1 h. Mitochondrial function was determined via a

---

Fig. 1. Intranasal insulin treatment caused hyperinsulinemia, impaired glucose tolerance, and asthma-like features in mice. Mice were intranasally administered with insulin (50 μg) or vehicle [0.01 N hydrochloric acid (HCl)] for 11 days and were euthanized on day 12 after overnight fasting. Serum samples from control and insulin-treated groups were analyzed for insulin ([I], trypglicerides ([B]), and fasting glucose levels ([C]). Glucose tolerance was measured by comparing the blood glucose levels of vehicle- and insulin-treated mice groups, after 2 h of oral glucose (25 mg) administration. The time 0 min indicates the baseline glucose levels ([D]). To assess the effect of insulin on lung structure and function a series of parameters have been measured: change in respiratory system resistance ([R]), or nonspecific bronchoconstrictor, methacholine ([E]), and collagen levels in total lung lysate, as measured by Sircol assay ([F]). Representative images of Masson’s trichrome (MT) staining ([top], periodic acid Schiff (PAS) staining ([middle], immunohistochemistry (IHC) for α-smooth muscle actin (α-SMA) ([bottom]) used to measure subepithelial fibrosis, mucus metaplasia and smooth muscle mass, respectively (G). β-Catenin levels were measured by ELISA, in total lung lysate of both insulin- and vehicle-treated mice ([H]). The result from ([H]) was corroborated by immunoblotting of β-catenin in total lung lysate of both insulin- and vehicle-treated mice ([I]). Magnification ×10 (MT and PAS staining), ×20 (IHC); scale 20 μm. Data represent means ± SE, n = 4–6, *P ≤ 0.05, compared with control. Br, Bronchi; Rrs, airway resistance.
XF Cell Mito Stress Test assay following standard protocols determined by Seahorse Biosciences. Oxygen consumption rate (OCR) and hydrogen production (ECAR) were measured in real time with fluorescent probes. OCR/ECAR measurements were taken every 5 min in the following sequence: four measurements for baseline, four measurements after addition of 9 μM oligomycin, four measurements after addition of 0.3 μM FCCP (carbonyl cyanide p-trifluoromethoxyphenylhydrazone), and four measurements following the addition of 11 μM antimycin A and 11 μM rotenone. OCR/ECAR values were normalized to well protein concentration and expressed per microgram protein. Mitochondrial ATP production, maximal respiration, and spare capacity were derived from OCR measurements by using Seahorse Biosciences suggested formulae.
Control

Insulin 1μg for 1hr

Insulin (24 hours)

Statistical analysis. Data are expressed as means ± SE. Significant differences between two groups were estimated by unpaired Student t-test. For experiments with more than two groups, data were analyzed by one-way ANOVA followed by Tukey post hoc testing. Statistical significance was set at *P ≤ 0.05.

RESULTS

Intranasal Insulin Administration to Mice Induces Insulin Resistance, Increased Lung β-Catenin Levels, Collagen Deposition, and AHR

Repeated intranasal insulin administration, at a dose sufficient to increase circulating insulin to physiological hyperinsulinemic levels, was associated with insulin resistance as indicated by increased serum triglyceride levels and glucose intolerance (Fig. 1, A–D). This was further associated with increased AHR to methacholine challenge (Fig. 1E). Increased insulin levels also led to a significant increase in lung collagen levels (Fig. 1F). Histological analysis of lung sections of these mice shows increased peribronchial collagen deposition (Fig. 1G, top), without a significant increase in mucin content (Fig. 1G, middle). Furthermore, immunohistochemistry for α-smooth muscle actin (α-SMA) in the insulin-treated mice showed a significant increase in its levels suggesting an increase in proliferation of smooth muscle cells (Fig. 1G, bottom). Taken together, the data indicate that hyperinsulinemia induces asthma-like pathophysiological features. β-Catenin, a known mediator of smooth muscle cell proliferation, has been reported to be induced by insulin. To
investigate whether β-catenin is involved in the aforementioned insulin-mediated effects, we measured β-catenin level in total lung protein. A significant increase in lung β-catenin level was observed in hyperinsulinemic mice (Fig. 1, ELISA and Western blot).

β-Catenin Is Causally Associated with the Insulin-Induced Altered Lung Function in Mice

To establish the causal role of β-catenin in insulin-induced asthma-like features, we investigated the effects of β-catenin knockdown on the lung structure and function of hyperinsulinemic mice. We found a significant decrease in the airway responsiveness to methacholine challenge, in insulin + β-catenin siRNA-treated mice, compared with mice treated with insulin + scrambled siRNA (Fig. 2A). This observation was further corroborated by less peribronchial collagen deposition (Fig. 2B, top) and decreased smooth muscle actin (Fig. 2B, bottom) in lung sections of insulin and β-catenin siRNA-treated mice, compared with mice treated with similar doses of insulin and scrambled siRNA. To gain a better mechanistic understanding of how intranasal insulin administration induced asthma-like features, we also investigated the effects of insulin on cultured cells. Insulin treatment induced a proliferative phenotype in human ASM cells in vitro in a β-catenin-dependent manner (Fig. 2C, right). Treatment with a β-catenin inhibitor, alone or in combination with insulin, resulted in a significant reduction of ASM cell proliferation, compared with vehicle control and cells treated with insulin, respectively (Fig. 2C, right). Figure 2C, left, shows the proliferative effects of insulin and the antiproliferative effects of β-catenin inhibition in BEAS-2B cells in vitro. Freshly isolated primary epithelial cells from human lung tissue also showed increased proliferation with identical insulin treatment, with proliferating cell nuclear antigen being increased by ~20%. In view of identical effects between the primary cells and BEAS-2B cell line, BEAS-2B cells were used for further studies of mechanisms by which β-catenin was upregulated.

β-Catenin Activation and Nuclear Translocation Is Via the PI3-Akt-GSK3β Pathway

Proliferative effects of β-catenin are known to be dependent on its nuclear translocation. Therefore, we next investigated for nuclear translocation of β-catenin in insulin-treated ASM and BEAS-2B cells. Increased nuclear localization of β-catenin

![Fig. 4. Insulin enhances β-catenin levels in human lung epithelial cells. The key molecular intermediates of PI3K/Akt signaling have been assessed for their role in insulin-induced increase in β-catenin levels. Human transformed bronchial epithelial (BEAS2B) cells were treated with insulin (1 μg/ml) with/without wortmannin [Wort (1 μM)] pretreatment in vitro. After 24 h the total cell protein was analyzed, by immunoblotting, for activated insulin receptor 1β (IR1β) (A), phosphorylated Akt (pAkt; B), phosphorylated GSK3β (p-GSK3β; C), and β-catenin (D). Data represent means ± SE. n = 6, *P ≤ 0.05; **P < 0.01 (significant insulin effect); ***P < 0.001 (significant wortmannin effect), compared with wortmannin alone or untreated control. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.](http://ajplung.physiology.org/).
was observed in insulin-treated ASM cells (Fig. 3A). Figure 3B shows a time-dependent increase in nuclear localization of β-catenin upon insulin treatment in ASM cells. Immunofluorescence imaging for β-catenin in insulin-treated BEAS-2B cells showed an increased colocalization with nucleus compared with untreated controls (Fig. 3, C and D). The BEAS-2B imaging studies were performed at 24 h and earlier signaling events were dissected through Western blots.

Insulin is known to induce β-catenin through either Wnt signaling or the PI3/Akt pathway, of which the second is part of classical insulin signaling. To determine the role of PI3-Akt-GSK3β activation in insulin-induced β-catenin activation and nuclear translocation, wortmannin, a PI3K inhibitor, was used. Insulin treatment unexpectedly led to increase in the activated insulin receptor-1β (IR1β), which is known to mediate PI3K activation via insulin receptor substrate (Fig. 4A). PI3K inhibition by wortmannin pretreatment reduced phosphorylation of Akt and GSK3β (Fig. 4, B and C, left and right). This was associated with significant reduction of the β-catenin levels in both untreated and insulin-treated cells (Fig. 4D).

Taken together, these results suggest that insulin induces β-catenin expression in a PI3/Akt pathway-dependent manner.

Insulin Induces a Procontractile and Profibrotic Phenotype in ASM Cells

To understand the importance of the identified insulin-PI3/Akt-β-catenin axis in clinical context, we further investigated the consequences of insulin-treatment on ASM phenotype. First, we confirmed that primary human ASM cells express IR1β (Fig. 5A), and insulin treatment to these cells induces phosphorylation of IR1β (Fig. 5A), and insulin treatment to these cells induces phosphorylation of Akt (Fig. 5B). Previous studies have shown that insulin induces proliferation of human lung fibroblasts and bovine tracheal smooth muscle cells (15, 40). We found that recombinant human insulin exposure leads to about eightfold increase in human ASM cell number by 72 h (Fig. 5C). We further demonstrate that insulin increases [Ca2+]i levels in human ASM cells (Fig. 5D). Both proliferative and contractile ASM phenotypes place metabolic demands on the cells, usually met by mitochondrial oxidative phosphorylation (7, 35).

Fig. 5. Insulin induces human airway smooth muscle (ASM) cell proliferation. Insulin-induced phenotypic changes in ASM cells were quantified in primary airway smooth muscle cells isolated from lungs of human donors. Enzymatically dissociated primary human ASM cells, from lung samples of 3 different patients, maintained in culture dishes in vitro were first investigated for the expression of insulin receptor 1β (IR1β) by immunoblotting (A). The effect of insulin (1 μg/ml) on human ASM cells was quantified by measuring the following parameters: pAkt levels via immunoblot (B), cell proliferation by CellTiter 96 AQueous One Solution Cell Proliferation Assay (C), fluorescence-based intracellular basal calcium concentration ([Ca2+]i; D), adenosine triphosphate (ATP) (E), basal mitochondrial respiration (F) by Seahorse Mitochondrial Stress Test, and extracellular collagen-I deposition by a modified in-cell Western blot technique (G). The data in D–G are represented as mean ± SE of technical replicates for individual patients (Pt.). *P ≤ 0.05, compared with respective controls. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Veh, vehicle; Ins, insulin; OCR, oxygen consumption rate.
Induction of ASM cells with insulin led to increases in ATP production and basal mitochondrial respiration in human ASM cells (Fig. 5, E and F). Interestingly, ASM cells treated with increasing concentrations of insulin demonstrate deposition of collagen-I, a major ECM protein involved in airway remodeling (Fig. 5G, left and right). Overall, these data suggest that insulin can alter human ASM mass and functional phenotype.

**DISCUSSION**

Previous epidemiological findings have reported associations between low lung function, insulin resistance, and diabetes (6, 14, 38, 41). Inhaled insulin (Exubera, Pfizer) was associated with a decline in forced expiratory volumes in more than half of a small group of patients, before it was withdrawn (5). However, the knowledge of mechanistic underpinnings of these associations is limited and whether insulin may have direct deleterious effects on lung structure or function is not known. The present study importantly fills this gap and finds potential deleterious effects of high levels of insulin on the lung that could help understand the aforementioned epidemiological and clinical observations. Our data provide a partial mechanistic understanding of such effects and implicate activation of the PI3/Akt-β-catenin axis in hyperinsulinemic effects on the lung. β-Catenin activation in primary human airway smooth muscle cells, in an insulin-dependent manner, leads to a proconstrictive and profibrotic phenotype that fits the mixed obstructive as well as restrictive lung function changes in clinical studies of diabetes and inhaled insulin, in relation to respiratory health.

In our experimental studies, mice treated with intranasal insulin showed peribronchial thickening, collagen deposition, and increased AHR. Furthermore, we found that insulin can directly induce molecular signaling events associated with airway smooth muscle proliferation, collagen deposition, and epithelial mesenchymal transition (EMT). A link between insulin resistance, consequent hyperinsulinemia, and asthma is well supported by previous evidence (2, 34, 38). Increased fasting insulin levels are previously reported in humans with insulin resistance, and increased levels of insulin are associated with a decline in forced expiratory volumes in more than half of a small group of patients, before it was withdrawn (5). However, the knowledge of mechanistic underpinnings of these associations is limited and whether insulin may have direct deleterious effects on lung structure or function is not known. The present study importantly fills this gap and finds potential deleterious effects of high levels of insulin on the lung that could help understand the aforementioned epidemiological and clinical observations. Our data provide a partial mechanistic understanding of such effects and implicate activation of the PI3/Akt-β-catenin axis in hyperinsulinemic effects on the lung. β-Catenin activation in primary human airway smooth muscle cells, in an insulin-dependent manner, leads to a proconstrictive and profibrotic phenotype that fits the mixed obstructive as well as restrictive lung function changes in clinical studies of diabetes and inhaled insulin, in relation to respiratory health.

In our experimental studies, mice treated with intranasal insulin showed peribronchial thickening, collagen deposition, and increased AHR. Furthermore, we found that insulin can directly induce molecular signaling events associated with airway smooth muscle proliferation, collagen deposition, and epithelial mesenchymal transition (EMT). A link between insulin resistance, consequent hyperinsulinemia, and asthma is well supported by previous evidence (2, 34, 38). Increased fasting insulin levels are previously reported in humans with features of insulin resistance (12, 27). We have also confirmed that our in vivo insulin model develops signs of early insulin resistance (Fig. 1D). Recent work shows a vagally mediated bronchoconstrictor effect of hyperinsulinemia in rats (28), whereas previous work has shown a hypercontractile effect in bovine ASM (32). We report here that insulin induces proliferation and increased respiration in human ASM cells and speculate that insulin-induced increase in ASM mass, with a procontractile and profibrotic phenotype (see Fig. 5), causes airway contraction and thickening (10), culminating in development of asthma-like features. Toward developing a mechanistic understanding of the effects of insulin on the lung, we show that β-catenin signaling is enhanced by insulin in vitro and in vivo. β-Catenin has been previously implicated in cell proliferation and EMT (22, 30). Previous studies have also confirmed the role of β-catenin in ASM cell contraction (18). Interestingly, β-catenin is also known to translocate into the mitochondria and modulate its functions (26), which is interesting in the context of recent work showing the importance of mitochondria in lung health (7). Activation of β-catenin appears to be mediated largely by PI3K/Akt activation and subsequent inhibition of GSK-3β by phosphorylation. This pathway, which is distinct from Wnt-dependent β-catenin activation (13), is also used by TGF-β1 to induce β-catenin activation in ASM and lung fibroblasts (9). It remains to be investigated whether modulation of β-catenin pathway could be a relevant therapeutic strategy to revert insulin-mediated deleterious changes in the human lung. Additional pathways of insulin action also merit future consideration.

Our work has important general implications that should hopefully lead to questioning of many current trends or practices including use of inhaled insulin formulations in diabetes. We have shown that high insulin levels within the physiological range adversely affect lung structure and function. This should discourage blind use of insulin secretagogues and external insulin. Furthermore, it seems likely that restrictive low lung function in apparently healthy populations may relate to hyperinsulinemia. This has never been explicitly tested, and further studies in cross-sectional and birth cohorts are warranted.

**GRANTS**

A. Agrawal was supported by the Lady Tata Memorial Trust. This work was supported by MLP5502 grant from Council of Scientific and Industrial Research (CSIR); CSIR-Mayo Clinic Collaboration Project (MLP-1201); Indian Council of Medical Research (ICMR) for fellowship to S. Singh, and National Heart, Lung, and Blood Institute Grants R01 HL088029 and R01 HL056470 (Y. S. Prakash).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


Hypertension, obesity, and hyperinsulinemia: links to mechanistic alterations in lung homeostasis and inflammatory responses.

Hypertension and obesity are main risk factors for chronic obstructive pulmonary disease (COPD). The development of COPD is a result of chronic inflammatory processes in the lung. This process is fostered by environmental factors such as tobacco smoke and simultaneously by systemic factors. Hyperinsulinemia is one of these systemic risk factors. To test whether hyperinsulinemia can be recognized as a risk factor for COPD, we evaluated the effects of different treatments on lung function and metabolic factors in hyperinsulinemic rats, including 1) smoking and 2) expression of the wild-type form of a gene encoding a receptor for the IGF-1 ligand, a receptor that has been shown to be involved in the pathogenesis of COPD.

In this study, we investigated the effects of hyperinsulinemia on lung function and metabolic factors in hyperinsulinemic rats. Smoking and treatment with a wild-type form of a gene encoding a receptor for the IGF-1 ligand were evaluated. Smoking significantly decreased lung function and increased metabolic factors in hyperinsulinemic rats. Treatment with the wild-type form of the gene encoding the IGF-1 receptor significantly decreased lung function and metabolic factors in hyperinsulinemic rats. These findings support the hypothesis that hyperinsulinemia is a risk factor for COPD and that the IGF-1 receptor plays a role in the pathogenesis of COPD.

References: