Original Article

Postural variation of pulmonary diffusing capacity as a marker of lung microangiopathy in Indian patients with type 2 diabetes mellitus

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ABSTRACT

Introduction: Diabetes mellitus (DM) is characterized by the presence of chronic hyperglycemia and formation of advanced glycation end products (AGEs). Interaction between AGE and its receptor leads to endothelial damage and microangiopathy. This study was undertaken to investigate the possibility of using a postural variation of diffusing capacity as an early marker of lung microangiopathy and its correlation with the level of adhesion molecules, HbA1c, duration of diabetes, and insulin resistance in type 2 DM (T2DM) patients with and without microangiopathy. **Materials and Methods:** Forty patients having T2DM without any microangiopathy ($n = 20$) as well as with microangiopathy ($n = 20$), and 22 age and sex matched healthy controls were enrolled in this cross-sectional study. Measurement of lung volumes and capacities were done. DLco was measured in sitting and supine position. Levels of vascular cell adhesion molecule-1 (VCAM-1), E-selectin, fasting glucose, and insulin were estimated in plasma of the patients and compared with controls. **Results:** Restrictive type of ventilatory change was observed in DM patients. Diffusing capacity (% predicted) in the supine position ($P < 0.0001$), postural change in DLco ($P < 0.0001$), and coefficient of diffusion were significantly less in DM patients as compared to controls. Plasma levels of VCAM-1 were significantly higher in DM patients without microangiopathy and negatively correlated ($r = -0.4054$, $P = 0.0094$) with Δ DLco in all diabetic subjects. All patients had significantly higher insulin resistance. **Conclusion:** Lack of postural increase in diffusing capacity in type 2 diabetic patients along with increased VCAM-1 levels could reflect the presence of an early microangiopathy of the small pulmonary vessels.

Key words: Adhesion molecules, diabetes mellitus, diffusing capacity, microangiopathy

Introduction

Diabetes mellitus (DM) is a public health problem of global concern. It is a group of metabolic disorders which occurs due to defect in insulin secretion and/or insulin action with secondary pathophysiological changes in the structure and function of several organ systems including eyes, kidney, and nervous system.[1] Chronic hyperglycemia, a characteristic feature of DM leads to the formation of advanced glycation end products (AGEs). The receptors for AGEs (RAGEs) are present on smooth muscles, macrophages, and vascular endothelial cells. The interaction between AGE and RAGE leads to activation of pathophysiological cascades that lead to vascular damage and thus have an important role in the pathogenesis of diabetic complications.[2]

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Though a number of studies have reported significant impairment of pulmonary functions,[3,4] the lung as a possible target organ of diabetes-related injury has been highlighted recently by the approval of delivering insulin by inhalation.[5] Isotani *et al*. reported independent changes in pulmonary diffusing capacity for carbon monoxide (DLco) as a manifestation of pulmonary microangiopathy.[6] Very high vascular and ventilation reserve compensates for the partial loss of pulmonary parenchyma in the course of diabetes, due to which diabetic pulmonary microangiopathy is a clinically silent respiratory dysfunction for a long period. Hence, the commonly used single breath DLco method might not be sensitive enough to investigate the early presence of diabetic microangiopathy. Posture-related variation of diffusing capacity may thus be used to increase the sensitivity of this test; Fuso *et al*. have reported a lack of posture-related changes in DLco and capillary volume in insulin dependent DM patients as compared to healthy subjects.[7]

As vascular endothelial dysfunction precedes the development of overt diabetes pathology, assessment of vascular reactivity and endothelial function has become an important tool for screening, risk stratification, prognostic assessment, and therapeutic management of most of these patients. Endothelial cell releases adhesion molecules E-selectin, intracellular adhesion molecule-1, vascular cell adhesion molecule-1 (VCAM-1), and integrins that interact with ligands on the leukocytes and platelets. It has been demonstrated that plasma levels of adhesion molecules are increased in patients with type-2 DM (T2DM).[8,9] It may reflect endothelial damage and leukocyte activation in chronic hyperglycemic condition and thus play a role in the pathogenesis of diabetic microangiopathy.

Thus, this study was undertaken to investigate the possibility of using postural variation of diffusing capacity as an early marker of lung microangiopathy and also to investigate the endothelial activation by measuring the level of adhesion molecules E-selectin and VCAM-1 in T2DM patients with and without microangiopathy and to compare it with healthy subjects. Authors have also determined the lung volumes and capacities and correlated postural variations in DLco with levels of adhesion molecules, HbA1c, duration of diabetes, and insulin resistance in these patients.

Materials and Methods

This study was approved by the Ethics Committee of All India Institute of Medical Sciences, New Delhi, India. Prediagnosed 40 patients with T2DM were recruited from out-patient department of endocrinology and metabolism. Twenty-two age and gender-matched healthy volunteers were enrolled from institute staff after the advertisement. Prior informed written consent was obtained from all the participants

Thus, participants were classified into three Groups: Group I: T2DM without any microangiopathy (n = 20), Group II: T2DM with microangiopathy (nephropathy and/or retinopathy and/or neuropathy) (n = 20). Group III: Healthy control subjects (n = 22), Subjects with a history of macrovascular complications such as ischemic heart disease, myocardial infarction, angina, stroke, intermittent claudication were excluded from the study. Diagnosed patients having pulmonary diseases such as chronic obstructive pulmonary disease, asthma, tuberculosis, and smokers were not included in this study. Biochemical investigations and pulmonary function tests were conducted in the Department of Physiology.

Measurement of pulmonary functions

Both diabetics and healthy control subjects underwent pulmonary function testing with spirometry using a dry rolling spirometer (SPIROAIR, MEDISOFT, PK Morgan Ltd., Kent, UK). Calibration was carried out according to the manufacturer specifications each time the instrument was used. Three L syringe was used for calibration of pneumotachometer.

Measurement of lung volumes, i.e., forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and functional residual capacity (FRC) was done. Closed circuit multi-breath helium dilution technique was used for measuring FRC. Height, weight, and smoking history of each subject were taken. Pulmonary function testing was done according to the guidelines of lung function testing of the American Thoracic Society (ATS)/European Respiratory Society and the variables were recorded as a percentage predicted of the normal value.

At least three forced expiratory maneuvers were performed. The largest FVC and the largest FEV1 were selected automatically by the software for reporting even if they do not come from the same curve. For calculating FEV1/FVC, the test which gave the highest sum of FEV1 and FVC was selected. Thus, the highest value of each volume from three technically acceptable maneuvres was used for comparisons as per ATS guidelines.

DLco was measured by single breath method with correction for the subject's hemoglobin concentration. The gas mixtures for assessment of DLco consisted of 20% oxygen, 0.3% CO, 10% helium, and balance nitrogen. Before the actual recording, slow vital capacity (SVC) was recorded. If SVC was ≥1.8 L, then washout volume was

fixed to 900 ml and sample volume was also fixed to 900 ml. If SVC was ≤1.8 L, then washout and sample volume were corrected according to ATS guidelines.

Measurements of DLco were undertaken by subjects in sitting and supine positions, in accordance with the recommendations from ATS guidelines. The testing procedure began with verbal instructions given to the patient. The subjects were asked to assume the appropriate position (either sitting or supine) for 5 min before the test with an interval of 5 min between each DLco measurement. For determining a regular end expiratory baseline, four to five tidal volumes were recorded once the mouthpiece was in place. The test began with the subjects exhaling to residual volume and at this time the subject's mouthpiece was switched onto a source of test gas and then the subjects were asked to rapidly inhale the gas mixture to total lung capacity (TLC). After breath holding for 10 s at TLC, the subject exhaled again to residual volume, while the system allows the elimination of dead space clearance volume and collection of a 1 L sample volume for gas concentration analysis. Alveolar volume (VA) was determined by the single breath dilution method for each DLco measurement. The co-efficient of diffusion (Kco) was obtained by dividing the absolute values of each variable by the respective value of VA (DLco/VA). Highest DLco value was used in the statistical analysis for each subject. ΔDLco and Δ Kco were calculated for each patient as the difference between the supine and sitting DLco and Kco measurements respectively.

Calculation of body mass index

The body mass index (BMI) is a measure of obesity based on an individual's weight and height. Each BMI score was calculated by using the following formula:

BMI = Weight (kg)/[Height (m)]^2.

Biochemical investigations

After 12 h of overnight fasting, blood was collected from each subject for estimation of hemoglobin, total cholesterol, serum triglycerides, soluble E-selectin, VCAM-1, insulin, and glucose.

Estimation of adhesion molecules, plasma insulin and glucose

Blood samples were collected after 12 h of overnight fast. Approximately 5 ml of blood was collected from the antecubital vein. Collected blood samples were immediately transferred to tubes with ethylenediaminetetraacetic acid. Tubes were centrifuged at 4500 rpm for 15 min at 20°C. The separated supernatant plasma was transferred to properly labeled microfuge tubes in three separate aliquots and stored at −80°C until analysis was done.

E-selectin, VCAM-1, and insulin levels were estimated by commercially available enzyme-linked immune sorbent assay kits (Diametra, Italy). Plasma glucose was estimated by a direct enzymatic colorimetric method using a commercially available estimation kit (Fortress Diagnostics, UK).

Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated according to the following formula:

$$\text{HOMA} - \text{IR} = \frac{\text{FPG} \times \text{FPI}}{22.5}$$

Where, FPG = Fasting plasma glucose (mmol/L) and FPI = Fasting plasma insulin (μIU/L).

Statistical analysis

All statistical tests were applied using GraphPad prism version 5.00 for Windows (GraphPad Software, Inc., USA). The collected data have been expressed as mean ± standard deviation or median with interquartile range (1st quartile–3rd quartile) depending on the distribution of data based on standard normality test. The normal distribution of data was checked by D'Agostino and Pearson omnibus normality testing, and Shapirov–wilk test. Data for the duration of diabetes, BMI, HbA1c, FPG, total cholesterol, triglyceride, FVC, FEV1, FEV1/FVC, DLco sitting, DLco supine, DLco/VA sitting, and DLco/VA supine were found to be normally distributed while data for Δ DLco, HOMA-IR, VCAM-1, E-selectin did not show normal distribution. To assess the significant trends in each parameter, one-way analysis of variance (ANOVA) was used to compare mean values in the three groups. In case of overall statistical significance in ANOVA, Bonferroni *post-hoc* test was used for multiple comparisons between the groups. To compare the diffusing capacity in same subjects in two postures, paired *t*-test was used. Kruskal–wallis test was used to compare median values in nonparametric data, and Dunn's Multiple Comparison test was used for comparison between the groups. Spearman's test was used to quantify the extent of correlation between Δ DLco and VCAM-1, E-selectin, HbA1c and duration of diabetes. $P < 0.05$ was considered as statistically significant.

Results

The demographic and biochemical profile of the study groups are summarized in Table 1. In spirometry, the percentage predicted value of FVC in T2DM patients with microangiopathy was found to be significantly lower than the control group ($P = 0.014$), while the percentage predicted values of FEV1 in T2DM patients with and without microangiopathy were significantly different from

the control group (P = 0.039), and FEV1/FVC ratio in T2DM patients with and without microangiopathy were comparable to the control group. The percentage predicted the value of FRC in T2DM with and without microangiopathy was significantly lower (P = 0.001) than the control group [Table 2].

Percentage predicted values of both, DLco in the supine position and ΔDLco were significantly lower in T2DM patients with and without microangiopathy as compared to controls. Contrary to the observation in healthy controls, DLco decreases in the supine position in all patients with DM as compared to sitting position. While coefficient of diffusion (Kco) was significantly less in DM patients with microangiopathy as compared to controls, ΔKco was significantly less in patients with DM irrespective of microangiopathy as compared to controls [Table 3].

Table 1: Demographic and biochemical characteristics of participants

Parameters	Group 1 DM without microangiopathy (*n*=20)	Group 2 DM with microangiopathy (*n*=20)	Group 3 Control (*n*=22)	*P* value†
Age (years)	49.40±6.84 ns[a]	52.10±5.15 ns[b], ns[c]	47.50±6.51	ns
Body mass index (kg/m^2)	26.45±4.19 ns[a]	24.32±3.34 ns[b], ns[c]	26.57±4.97	ns
Duration of DM (years)	7.7±6.66	14.55±8.67 <0.05 c	-	0.0081
HbA1C	8.06±1.42 <0.05[a]	9.00±1.76 <0.05[b], ns[c]	5.15±0.83	<0.0001
Fasting plasma glucose (mmol/L)	10.06±3.53 <0.05[a]	11.08±3.36 <0.05[b], ns[c]	4.64±1.1	<0.0001
Total cholesterol (mg/dl)	181.10±42.62 ns[a]	203.00±48.77 <0.05[b], ns[c]	157.50±29.18	0.0026
Triglyceride (mg/dl)	166.6±44.8 <0.05[a]	158.0±51.18 <0.05[b], ns[c]	118.5±28.29	0.0009

a=Comparison between group 1 and group 3, b=Comparison between group 2 and group 3, c=Comparison between group 1 and group 2, Values are expressed as mean±SD. †One way ANOVA with Bonferroni's *post hoc* multiple comparison test. DM: Diabetes mellitus, ns: Non significant

Table 2: Lung volumes and capacities of participants

Parameter	Group 1 DM without microangiopathy (*n*=20)	Group 2 DM with microangiopathy (*n*=20)	Group 3 Control (*n*=22)	*P* value†
FEV_1 (% predicted)	69.70±14.95	67.95±15.27	78.23±10.63	0.039
FVC (% predicted)	79.70±14.34	78.00±14.43[b]	89.32±10.33	0.014
FEV_1/FVC (% predicted)	92.40±13.83	91.80±16.41	91.18±8.990	0.957
FRC (% predicted)	83.05±8.654	83.15±6.124	96.00±12.78	(<0.0001)[a,b]

a=Comparison between group 1 and 3, b=Comparison between group 2 and 3, Values are expressed as mean±SD. †One way ANOVA with Bonferroni's *post hoc* multiple comparison test. DM: Diabetes mellitus, FEV_1: Forced expiratory volume in 1 s, FVC: Forced vital capacity, FRC: Functional residual capacity

Table 3: Diffusing capacity and coefficient of diffusion in participants

parameter	Group 1 DM without microangiopathy (*n*=20)	Group 2 DM with microangiopathy (*n*=20)	Group 3 Control (*n*=22)	F value (*P* value†) (ANOVA)
DLco (% predicted sitting)	71.90±16.91	71.30±12.68	76.77±19.25	F (2,59)=0.69 (0.503)
DLco (% predicted supine)	63.70±13.88	64.20±12.29	84.59±20.25	F (2,59)=11.787 (<0.0001)[a,b]
Kco (% predicted sitting)	94.35±14.78	98.05±17.93	97.77±17.94	F (2,59)=0.29 (0.743)
Kco (% predicted supine)	97.45±16.99	95.30±18.94b	109.7±16.93	F (2,59)=4.15 (0.02)
ΔDLco	−7.50 (−11.75-5.00)	−6.50 (−10.00-4.25)	9.00 (6.25-10.00)	35.90 (<0.0001)[a,b]
ΔKco	−1.75 (3.00-7.75)	−11.50 (−4.00-2.75)	4.00 (9.00-15.75)	19.08 (<0.05)[a] (< 0.0001)[b]

a=Comparison between group 1 and 3, b=Comparison between group 2 and 3, c=Comparison between group 1 and 2, Values are expressed as mean±SD except for ΔDLCO and ΔKCOwhich are median (inter quartile range). DM: Diabetes mellitus

The values of VCAM-1, E-selectin, and HOMA-IR are presented in Table 4. The VCAM-1 levels in T2DM patients without microangiopathy was significantly higher than the control group ($P = 0.0312$), while the E-selectin levels in T2DM patients with and without microangiopathy were not significantly different than controls. The HOMA-IR values in T2DM patients with and without microangiopathy were significantly higher than the control group.

Authors have also studied the correlation between ΔDLco and adhesion molecules, duration of diabetes, HOMA-IR, HbA1c and observed that significantly negative correlation ($r = -0.4054$, $P = 0.0094$) exists between ΔDLco and VCAM-1 levels in all diabetic subjects (T2DM with and without microangiopathy). No significant correlation observed between other parameters.

Discussion

We found clearly an association between lung function of deterioration and T2DM. Mean percentage predicted values FEV1 and FVC were significantly decreased, but the ratio of FEV1/FVC did not significantly decrease in diabetic subjects as compared to controls. This implies a restrictive type of ventilatory change.

The pathophysiological mechanism of the restrictive pattern is still not clear. It has been suggested that nonenzymatic glycosylation of connective tissue, especially collagen may be responsible for both lung and joint abnormality.[10,11] Collagen is most abundant connective tissue protein in the human lung and is predominantly found in the large bronchi, major pulmonary vessels and the interstitium. Any qualitative or quantitative abnormality in collagen can cause restrictive pulmonary disease.[12] AGE product formed due to nonenzymatic glycosylation of collagen has low physiological turnover rates,[13] more stiffness[14] and more resistance to digestion by pepsin and collagenase than nondiabetic collagen.[15] Thus, a likely explanation of our results is that chronic hyperglycemia caused an increase in the glycosylation of collagen in lungs resulting in less compliant pulmonary parenchyma and the observed restrictive changes in pulmonary function. Hence, a reduction in the lung volume demonstrated in DM subjects may, therefore, be regarded as a manifestation of the widespread abnormalities of the connective tissue that is known to occur in diabetes.

In this study, there was a significant decrease in FRC in both T2DM without microangiopathy and with microangiopathy patients compared to healthy control subjects. Schnapf in 1984 found similar results in insulin dependent DM patients with limited joint mobility which was explained by the reduced pulmonary elastic recoil of lung parenchyma probably due to the stiffness of collagen that leads to decreased FRC.[16]

In this study, the diffusing capacity was measured in sitting and supine posture. In sitting posture, diffusing capacity was statistically comparable among the three groups but it decreased from sitting to supine position in T2DM patients with and without microangiopathy, compared to healthy subjects. This postural variation of diffusing capacity has been proposed to increase the sensitivity and the diagnostic usefulness of DLco in the assessment of pulmonary microangiopathy in type 2 diabetes patients. Regarding DLco and type 2 diabetes, two reports have been published by Isotani *et al.* and Marvisi *et al.*, who found a significant reduction in DLco/VA and in DLco, respectively, in diabetic patients, as well as a correlation between DLco and diabetic complications.[6,17] Normally, the upper regions of the lungs are relatively poorly perfused in sitting posture because of gravity dependent gradient of perfusion so, carbon monoxide absorption is greater in well perfused, lower portions of the lung than the upper portions.[18] The diffusing capacity in a healthy subject is increased in the supine position because the related gravity effect is abolished and more uniform perfusion of lungs takes place.[19] According to Fick's law, diffusion of gases is directly proportional to surface area and inversely proportional to the thickness of blood gas barrier. Two mechanisms that are responsible for increasing the surface area for diffusion in the supine position are recruitment, that is, opening up of previously closed blood vessels[20] and distension, that is, increase in caliber of pulmonary capillaries.[21] Several mechanisms that have been hypothesized for development

Table 4: Circulating levels of VCAM-1, E-selectin and HOMA-IR

Parameter	Group 1 DM without microangiopathy (*n*=20)	Group 2 DM with microangiopathy (*n*=20)	Group 3 Control (*n*=22)	Kruskal Wallis statistic (*P* value†)
VCAM-1 (ng/ml)	191.3[a] (241.1-334.4)	186.29 (186.2-305.1)	138.1 (138.1-247.8)	6.94 (0.0312)
E-selectin (ng/ml)	43.22 (25.18-58.70)	44.97 (33.85-53.78)	37.15 (23.63-49.30)	2.42 (0.298)
HOMA-IR	12.25[a] (8.796-15.60)	14.59[b] (12.56-19.48)	0.5772 (0.4340-1.345)	44.51 (<0.0001)

a=Comparison between group 1 and 3, b=Comparison between group 2 and 3, c=Comparison between group 1 and 2, Values are expressed as median (inter quartile range). †Kruskal Wallis test with Dunn's multiple comparison tests was applied to compare the three groups in pairs. DM: Diabetes mellitus, VCAM: Vascular cell adhesion molecule-1, HOMA-IR: Homeostatic model assessment-insulin resistance

of microangiopathy are the formation of AGE, hyperosmolarity, aldolase reductase pathway, AGE product pathway, reactive oxygen intermediate theory and protein Kinase C pathway. Hyperglycemia induces many changes in the vascular cell, which include hemodynamic alteration, endothelial dysfunction, activation of inflammatory cells and changes in the expression of vascular and neurotrophic factors.[22,23] AGE can alter cellular function by binding to its receptor RAGE[24] found on the mononuclear and endothelial cell. This binding produces a cascade of cellular signaling events. It stimulates macrophage production of interleukin-1, insulin-like growth factors-1, tumor necrosis factor-alpha (TNF-α) and granulocyte/macrophage colony-stimulating factor.[25] Thus, the levels of these cytokines are increased. At these increased levels, the synthesis of type IV collagen is increased in the basement membrane of alveoli and pulmonary capillaries,[26] so that there is an increase in the thickness of the diffusing barrier, leading to the decreased saturation of the erythrocytes. Autopsy or animal studies have shown an increase in the thickness of basal lamina of both the alveoli and pulmonary capillary.[27] TNF-α is also responsible for increasing the smooth muscle mass in the arterial wall by blocking the anti-proliferative effect of NO.[28] This ultimately produces a narrowing of the lumen of the vessel.[29] AGE binds with RAGE at the endothelial cell and produces reactive oxygen species,[30] which activates the free radical sensitive transcription factor nuclear factor kB (NF-κB), a coordinator of numerous "response to injury genes."[23] This NF-κB causes activation of endothelial cells. Dysfunctional endothelium is associated with, and likely precedes clinical complications of DM by promoting increased vascular permeability. Increased vascular matrix formation that produced narrowing of vessel lumen increased basement membrane deposition and induction of smooth muscle cell proliferation all together produced decrease pulmonary blood volume in supine position that leads to decrease diffusing capacity in the supine posture. Microangiopathy results in low compliance of the capillary bed and could detain the redistribution of blood, especially in the apex of the lung in the supine position.

Conclusion

Lack of postural increase in diffusing capacity in type 2 diabetic patients along with VCAM-1 levels increase could reflect the presence of an early microangiopathy involvement of the small pulmonary vessels. Hence, postural variation of diffusing capacity may be used as a diagnostic procedure for early assessment of pulmonary impairment in diabetic patients.

The present study also shows that lung volume decreases in diabetic subjects due to the widespread abnormalities of connective tissue, so that regular measurement of FEV1 and FVC may provide a simple method for monitoring progressive defect in collagen with regular DLco measurements for early detection of lung microangiopathy.

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Conflicts of interest

There are no conflicts of interest.

References

1. DeFronzo RA, Eldor R, Abdul-Ghani M. Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. Diabetes Care 2013;36 Suppl 2:S127-38.
2. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: A "glycoxidation-centric" point of view. Nutr Metab Cardiovasc Dis 2013;23:913-9.
3. Davis TM, Knuiman M, Kendall P, Vu H, Davis WA. Reduced pulmonary function and its associations in type 2 diabetes: The Fremantle Diabetes Study. Diabetes Res Clin Pract 2000;50:153-9.
4. Schuyler MR, Niewoehner DE, Inkley SR, Kohn R. Abnormal lung elasticity in juvenile diabetes mellitus. Am Rev Respir Dis 1976;113:37-41.
5. Cefalu WT. Concept, strategies, and feasibility of noninvasive insulin delivery. Diabetes Care 2004;27:239-46.
6. Isotani H, Nakamura Y, Kameoka K, Tanaka K, Furukawa K, Kitaoka H, *et al.* Pulmonary diffusing capacity, serum angiotensin-converting enzyme activity and the angiotensin-converting enzyme gene in Japanese non-insulin-dependent diabetes mellitus patients. Diabetes Res Clin Pract 1999;43:173-7.
7. Fuso L, Cotroneo P, Basso S, De Rosa M, Manto A, Ghirlanda G, *et al.* Postural variations of pulmonary diffusing capacity in insulin-dependent diabetes mellitus. Chest 1996;110:1009-13.
8. Matsumoto K, Sera Y, Abe Y, Ueki Y, Miyake S. Serum concentrations of soluble vascular cell adhesion molecule-1 and E-selectin are elevated in insulin-resistant patients with type 2 diabetes. Diabetes Care 2001;24:1697-8.
9. Fasching P, Waldhäusl W, Wagner OF. Elevated circulating adhesion molecules in NIDDM – potential mediators in diabetic macroangiopathy. Diabetologia 1996;39:1242-4.
10. Kohn RR, Schnider SL. Glycosylation of human collagen. Diabetes 1982;31:47-51.
11. Lange P, Groth S, Kastrup J, Mortensen J, Appleyard M, Nyboe J, *et al.* Diabetes mellitus, plasma glucose and lung function in a cross-sectional population study. Eur Respir J 1989;2:14-9.
12. Laurent GJ. Lung collagen: More than scaffolding. Thorax 1986;41:418-28.
13. Monnier VM, Kohn RR, Cerami A. Accelerated age-related browning of human collagen in diabetes mellitus. Proc Natl Acad Sci U S A 1984;81:583-7.

14. Andreassen TT, Seyer-Hansen K, Bailey AJ. Thermal stability, mechanical properties and reducible cross-links of rat tail tendon in experimental diabetes. Biochim Biophys Acta 1981;677:313-7.
15. Sternberg M, Cohen-Forterre L, Peyroux J. Connective tissue in diabetes mellitus: Biochemical alterations of the intercellular matrix with special reference to proteoglycans, collagens and basement membranes. Diabete Metab 1985;11:27-50.
16. Schnapf BM, Banks RA, Silverstein JH, Rosenbloom AL, Chesrown SE, Loughlin GM. Pulmonary function in insulin-dependent diabetes mellitus with limited joint mobility. Am Rev Respir Dis 1984;130:930-2.
17. Marvisi M, Bartolini L, del Borrello P, Brianti M, Marani G, Guariglia A, *et al*. Pulmonary function in non-insulin-dependent diabetes mellitus. Respiration 2001;68:268-72.
18. West JB, Dollery CT. Distribution of blood flow and ventilation-perfusion ratio in the lung, measured with radioactive carbon dioxide. J Appl Physiol 1960;15:405-10.
19. Daly WJ, Giammona ST, Ross JC, Feigenbaum H. Effects of pulmonary vascular congestion on postural changes in the perfusion and filling of the pulmonary vascular bed. J Clin Invest 1964;43:68-76.
20. Warrell DA, Evans JW, Clarke RO, Kingaby GP, West JB. Pattern of filling in the pulmonary capillary bed. J Appl Physiol 1972;32:346-56.
21. Glazier JB, Hughes JM, Maloney JE, West JB. Measurements of capillary dimensions and blood volume in rapidly frozen lungs. J Appl Physiol 1969;26:65-76.
22. Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. JAMA 2002;288:2579-88.
23. Potenza MA, Gagliardi S, Nacci C, Carratu' MR, Montagnani M. Endothelial dysfunction in diabetes: From mechanisms to therapeutic targets. Curr Med Chem 2009;16:94-112.
24. Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb 1994;14:1521-8.
25. Gugliucci A. Glycation as the glucose link to diabetic complications. J Am Osteopath Assoc 2000;100:621-34.
26. Brownlee M, Vlassara H, Cerami A. Nonenzymatic glycosylation and the pathogenesis of diabetic complications. Ann Intern Med 1984;101:527-37.
27. Vracko R, Thorning D, Huang TW. Basal lamina of alveolar epithelium and capillaries: Quantitative changes with aging and in diabetes mellitus. Am Rev Respir Dis 1979;120:973-83.
28. Hogman M, Cerami A, Bucala R. Advanced glycosylation endproducts block the antiproliferative effect of nitric oxide. J Clin Invest 1992;90:1110-3.
29. Rumble JR, Cooper ME, Soulis T, Cox A, Wu L, Youssef S, *et al*. Vascular hypertrophy in experimental diabetes – Role of advanced glycation end products. J Clin Invest 1997;99:1016-27.
30. Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, *et al*. Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors binding proteins. J Biol Chem 1994;269:9889-97.