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# Nitric oxide production by the alveolar compartment of the lungs in cirrhotic patients

Short title: Alveolar production of NO in liver cirrhosis

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**Abstract**

In cirrhotic patients, alveolar nitric oxide (NO) concentration is increased. This may be secondary to increased production of NO by the alveolar compartment of the lungs (V'A,NO) and/or to decreased lung transfer of NO. In advanced liver cirrhosis, NO produced by the alveoli may play a role in abnormalities of pulmonary haemodynamics and gas exchanges. In cirrhotic patients, we aimed to measure V'A,NO, and to compare V'A,NO with pulmonary haemodynamics and gas exchange parameters.

Measurements were performed in 22 healthy controls and in 29 cirrhotic patients, of whom 8 had hepatopulmonary syndrome. Exhaled NO concentrations were measured at multiple expiratory flow rates to derive alveolar NO concentration. V'A,NO was the product of alveolar NO concentration by single breath lung transfer for NO.

V'A,NO was increased in patients (median [range] 260 [177–341] $nL.min^{-1}$) compared with controls (79 [60–90], $p<0.0001$). Alveolar-arterial oxygen tension difference failed to correlate with V'A,NO. However, cardiac index correlated positively and systemic vascular resistance correlated negatively with V'A,NO ($r=0.56$, $p=0.001$, and $r=0.52$, $p=0.004$, respectively).

In cirrhotic patients, NO was produced in excess by the alveolar compartment of the lungs. Alveolar NO production was associated with hyperdynamic circulatory syndrome but not with arterial oxygenation impairment.

Keywords: alveoli - exhaled nitric oxide - liver cirrhosis - pulmonary gas exchange

## Introduction

Patients with advanced liver disease typically present with progressive systemic, splanchnic and pulmonary vasodilatation. This may lead to a hyperdynamic circulatory syndrome, which associates high cardiac index and fluid expansion in response to excessive vasodilatation.[1,2] Impaired arterial oxygenation, ranging from increased alveolar-arterial oxygen tension difference to severe hypoxaemia, is also commonly present in patients with liver cirrhosis. In the absence of overt mechanical dysfunction of the lung, these oxygenation abnormalities may be a consequence of ventilation/perfusion mismatch and/or intrapulmonary shunting and/or diffusion impairment of oxygen.[3] In addition, some patients develop hepatopulmonary syndrome (HPS), which consists of abnormal dilatation of pulmonary precapillary and capillary vessels either with or without pulmonary arteriovenous communications, whereas alveolar ventilation is preserved.[4-6] Nitric oxide (NO), a biologically active gas, is the main molecule responsible for the vasodilatation and multiple organ malfunctions that characterise hyperdynamic circulatory syndrome.[7] A loss of NO bioavailability in the endothelial cells of the hepatic microcirculation contrasts with an increase in NO production by the endothelial cells in the arteries of the systemic and pulmonary circulations. In animal models of HPS, pulmonary vascular dilatation, gas exchange abnormalities and blunted pulmonary vasopressor response were all linked to increased expression and activity of pulmonary NO-synthases.[5,8-10] In humans, the role of NO in arterial oxygenation impairment is however still debated.[11]

NO can be easily and non-invasively measured in exhaled air and is thought to reflect a balance between production and catabolism within the respiratory tract.[12] Measurement of exhaled NO at multiple controlled expiratory flow rates allows

partition into airway and alveolar NO components,[13,14] and the additional measurement of NO lung transfer allows calculation of NO production by the alveoli (V'A,NO).[15] In cirrhosis, alveolar NO concentration is increased compared with normal subjects,[16] but measurement of V'A,NO has not previously been reported. In cirrhotic patients on the liver transplantation waiting list at our institution, we aimed (1) to measure V'A,NO, and (2) to assess the relationships between V'A,NO, oxygenation parameters and pulmonary haemodynamics.

## Materials and methods

*Study subjects*

From November 2006 to October 2007, 29 patients on the liver transplantation waiting list at Toulouse University Hospital were investigated. All had biopsy-proven liver cirrhosis. Physical examination findings and blood data were analysed to determine severity of liver disease according to the Child criteria.[17] None had primary lung disease. Patients were not included if they had primary cardiac disease (including systolic and diastolic left heart disease, and mitral and/or aortic stenosis and/or regurgitation) or portopulmonary hypertension. Subjects with allergy and/or asthma were also excluded. The study was approved by our institutional review board and informed consent was obtained from each patient.

*Pulmonary function tests*

Measurement of lung volumes and single-breath transfer of carbon monoxide in the lung (TL,CO) was performed according to the European Respiratory Society (ERS) guidelines.[18,19]

Arterial blood gases were analysed in patients breathing room air in a sitting position. Alveolar-arterial oxygen tension difference ($PA–a,O_2$) was calculated as follows:

$$PA–a,O_2 = PA,O_2–Pa,O_2 = FI,O_2\ (Patm–PH_2O)–Pa,CO_2/RER–Pa,O_2$$

where $PA,O_2$ is alveolar oxygen tension, $Pa,O_2$ arterial oxygen tension, $FI,O_2$ inspiratory oxygen fraction, Patm atmospheric pressure, $PH_2O$ water vapour partial pressure (47 mmHg) and RER respiratory exchange ratio (assumed to be 0.8).

The diagnosis of hepatopulmonary syndrome (HPS) was based on the ERS recommendations.[3]

*Measurement of nitric oxide concentrations in exhaled air*

Measurements were performed in the 29 patients and in 22 healthy controls. Control subjects were matched with patients for age and smoking habits. Current smokers were asked not to smoke for 24 h before NO measurement. Among subjects who were not current smokers, those who responded “no” to the question “Have you ever smoked for as long as a year?" were classified as non-smokers, and those who responded “yes” as ex-smokers.[20] Patients and controls were asked to abstain from coffee and vegetables during the 24 h prior to NO measurement.

Measurement of exhaled NO concentration (FE,NO) was performed as recommended by ATS guidelines.[21] Subjects exhaled against a positive pressure of 20 cm $H_2O$ and generated expiratory flows of 50, 100 and 200 mL/second. NO was detected with a chemiluminescent analyzer (EndoNO 8000®, SERES, Aix-en-Provence, France) with a lower limit of detection of 1 ppb and NO sampling rate of 30 L/h. Simultaneous measurements of FE,NO and expiratory flow were used to calculate NO output. Three consecutive measurements of FE,NO were performed for each expiratory flow and the calculated NO output values were represented as a function of the flow rates. Least-square linear regression over the NO output versus the flow rate data was performed and the linearity of the relationship was verified (i.e., $r^2 > 0.90$). Alveolar NO concentration (CA,NO, in ppb) and maximal airway wall flux of NO (J’aw,NO, in $nL.min^{-1}$) were estimated according to the model recently described by Condorelli *et al.*,[13] as follows:

$CA,NO = S – I [(0.001\ s/ml)/(0.53)]$

and

$J'aw,NO = (I/0.53) \times 0.06$

where S is the slope and I is the y-intercept of the linear regression over the NO output versus the flow rate.

Unlike the model of Tsoukias and George,[14] the current model takes into account axial diffusion of NO from the airways that can “contaminate” the alveolar region, and so it is more relevant when estimating alveolar NO concentration.[13]

*Measurement of NO transfer*

NO lung transfer (TL,NO) was measured during a single breath manoeuvre using an automated apparatus (Medisoft, Dinant, Belgium). Subjects were in the sitting position and wore a nose clip. They inhaled a mixture containing 14% He, 21% $O_2$ balanced with $N_2$ mixed with an $NO/N_2$ mixture (450ppm $NO/N_2$, Air Liquide Santé, France). The final concentration of NO in the mixture was 40 ppm and that of $O_2$ was 19.1%. A breathhold of 4 seconds was requested followed by a rapid expiration. The first 0.9 L of expired gas was rejected and the next 0.9 L was sampled and analysed for NO concentration.[22]

According to Perillo *et al.*,[15] V’A,NO ($nL.min^{-1}$) was calculated as the product of TL,NO ($mL.min^{-1}.mmHg^{-1}$) and CA,NO (ppb) as follows:

$V'A,NO=TL,NO \times CA,NO \times (Patm–PH_2O) \times 10^{-3}$

where Patm is atmospheric pressure and $PH_2O$ water vapour partial pressure (47 mmHg).

*Right heart catheterisation.*

Right heart catheterisation was performed using a Swan-Ganz catheter. Cardiac output was measured by the thermodilution technique.

*Analysis*

Data were expressed as median [1st–3rd quartile]. Comparisons between more than two groups or subgroups were performed with the Kruskal-Wallis test. Comparisons between two groups or subgroups were performed with the Mann-Whitney U-test. Correlations were made by Spearman's test. A p value $< 0.05$ was considered as significant. Analysis was performed using Statview, version 5.0 (SAS Institute, Cary, NC).

## Results

*Demographic characteristics*

Demographic and clinical characteristics of patients and controls are shown in Table 1. The majority of patients were male and had cirrhosis caused by alcohol abuse. No significant difference was observed in age, smoking habits or spirometric values between patients and controls. Cirrhotic patients with HPS had lower TL,CO than patients without HPS and than healthy controls. PA–a,O2 was significantly higher in HPS patients than in non-HPS patients. Cirrhotic patients had normal mean pulmonary artery pressure (mPAP), high cardiac index (CI) and low pulmonary vascular resistance (PVR).

*NO measurements*

Table 2 summarises NO measurements in patients and controls. Median CA,NO, V'A,NO and J'aw,NO were significantly increased in cirrhotic patients compared with healthy controls. In cirrhotic patients, CA,NO, V'A,NO and J'aw,NO did not differ between patients without HPS (3.5 [2.9–6.5] ppb, 260 [178–338] $nL.min^{-1}$, and 74 [56–116] $nL.min^{-1}$, respectively) and patients with HPS (3.7 [2.5–7.0] ppb, 260 [175–359] $nL.min^{-1}$, and 74 [58–130] $nL.min^{-1}$, respectively). Patients and controls were either active smokers or non-smokers. In healthy smoking controls, J'aw,NO was significantly lower than in non-smokers but CA,NO and V'A,NO were similar. In cirrhotic patients, smoking habits had no effect on CA,NO, V'A,NO or J'aw,NO.

*Relationship between NO measurements and gas exchange parameters*

We failed to find any correlation between PA–a,O2 and CA,NO or between PA–a,O2 and V'A,NO (Figure 1). There was a significant correlation between TL,CO and

CA,NO. However, there was no correlation between TL,CO and V'A,NO (Figure 1). J'aw,NO was not correlated either with PA–a,O2 or with TL,CO (data not shown).

*Relationship between NO measurements and haemodynamics*

Cardiac index correlated positively with CA,NO (r=0.41, p=0.03) and with V'A,NO (r=0.56, p=0.001) (Figure 2). Systemic vascular resistance correlated negatively with CA,NO (r=-0.39, p=0.04) and with V'A,NO (r=-0.52, p=0.004) (Figure 2). J'aw,NO was not correlated either with cardiac index or with systemic vascular resistance (data not shown).

## Discussion

Our main findings are that in cirrhotic patients (1) production of NO by the alveolar compartment of the lungs (V'A,NO) was increased compared with healthy volunteers, and (2) V'A,NO failed to correlate with alveolar-arterial oxygen tension difference (PA–a,O2) but correlated positively with cardiac index.

An increase in exhaled NO concentrations in cirrhotic patients was first reported more than 10 years ago. Cremona *et al.* and Rolla *et al.* showed that NO concentrations measured in exhaled air from patients with advanced liver cirrhosis were increased compared with controls.[23,24] It must be stressed that the technique of collection of exhaled air in these studies markedly differed from the current recommendations.[21] Exhalations were performed without closure of the soft palate and patients wore a nose clip, two conditions which are now known to increase considerably the nasal contribution of NO measured in exhaled air.[21] Moreover, expiratory flow rates were not recorded, and it has been clearly demonstrated that NO concentration in exhaled air is inversely dependent on expiratory flow rate.[14] By measuring exhaled NO at multiple controlled expiratory flow rates and applying the two-compartment model of Tsoukias and George,[14] Delclaux *et al.* showed that alveolar NO concentration was increased in cirrhotic patients.[16] However, this two-compartment model neglected the axial diffusion of NO from the airway tree, which can contaminate the alveolar region and so lead to a falsely elevated estimate of CA,NO. We therefore used the model recently described by Condorelli *et al.* that takes axial diffusion of NO into account in the estimation of CA,NO.[13] This model was initially tested in a limited number of healthy subjects and CA,NO was found to be near zero.[13] By contrast, in our series of cirrhotic patients we found that CA,NO estimated with this model was significantly higher than in controls, indicating that

“contamination” of the alveolar compartment by NO from the airways did not account for increased values of CA,NO in liver cirrhosis.

Increased CA,NO may suggest either an increase of the production rate of NO by the alveolar compartment of the lungs (V’A,NO) and/or a reduction of the transfer of NO from the alveolar compartment to the vascular compartment of the lungs (TL,NO).[14] In patients with systemic sclerosis, Girgis *et al.* concluded that the most likely cause of increased CA,NO was decreased transfer of alveolar NO to the lung vessels, but TL,NO was not measured.[25]

To the best of our knowledge, alveolar NO production has not previously been studied in cirrhotic patients. In our study, measurement of TL,NO in addition to estimation of CA,NO enabled us to demonstrate that the most likely cause for increased CA,NO in cirrhotic patients was an increase of alveolar NO production. However, CA,NO and V’A,NO are not strictly equivalent in these patients, as highlighted by the correlation between CA,NO and TL,CO which contrasts with the absence of correlation between V’A,NO and TL,CO.

In animal models of cirrhosis and portal hypertension, enhanced pulmonary production of NO has been clearly implicated in the development of hypoxaemia and hyperdynamic syndrome.[8,9,26,27] In cirrhotic patients, the relationships between pulmonary alveolar NO production, hyperdynamic circulation and gas exchange abnormalities remain uncertain. In our series, we found no correlation between alveolar NO (CA,NO and V’A,NO) and PA–a,O2. In contrast, Rolla *et al.* reported a strong correlation between PA–a,O2 and exhaled NO concentration,[24] but the technique of exhaled air collection differed greatly from the methods currently recommended. Delclaux *et al.* found a correlation between CA,NO and PA–a,O2.[16] The differences with our results may be due (1) to differences in study population,

because in the series of Delclaux *et al.* patients had more severe HPS, and (2) to a different mathematical model used to estimate CA,NO.[16] Interestingly, the absence of correlation between V'A,NO and PA–a,O2 may explain at least in part the results of Gomez *et al.*, who reported that acute inhibition of pulmonary NO-synthase activity (and thus acute inhibition of pulmonary NO production) by nebulised $N^G$-nitro-L-arginine methyl ester (LNAME) had no effect on PA–a,O2.[11] We also found that alveolar NO (CA,NO and V'A,NO) correlated positively with cardiac index. Increased cardiac output in response to excessive vasodilatation is a hallmark of hyperdynamic circulatory syndrome.[3,6] A possible interpretation of our finding is that NO production may be similarly increased in both the pulmonary and the systemic vessels in cirrhotic patients. In accordance with this interpretation, Gomez *et al.* showed that nebulised LNAME decreased cardiac index and increased both pulmonary and systemic vascular resistances in patients with hepatopulmonary syndrome.[11]

We aimed to minimise factors confounding the measurement of FE,NO. We verified that patients had neither allergy nor asthma because exhaled NO concentrations may be increased in these diseases.[28] Patients were also asked not to drink coffee the day of measurement because caffeine has been shown to briefly reduce FE,NO (for no more than 4 hours).[29] As nitrate-rich nutrition is presumed to increase exhaled NO concentration,[30] patients were given a vegetable-free hospital diet during the 24 h prior to the NO measurements. However, 14 of the 29 patients were regular smokers. This could be considered a bias because smoking decreases FE,NO, especially at relatively low expiratory flow rates (i.e., under 100 mL/second).[31,32] The smoking habits of the controls are not detailed because if exhaled NO concentrations are measured according to the current recommendations,

no dose–response relationship between the number of cigarettes smoked and the levels of exhaled NO has been found in healthy subjects.[20] In accordance with previous results on the effects of tobacco smoking on exhaled NO, we found that smokers without cirrhosis (controls) had lower NO airway production than non-smokers. Smoking habits had no significant influence on CA,NO and V'A,NO in control subjects. Moreover, our results in cirrhotic patients are in accordance with the findings of Delclaux *et al.*,[16] who found no significant difference in alveolar NO concentration or bronchial and alveolar NO outputs between smoking and non-smoking cirrhotic patients.

We acknowledge some limitations in this study. First, we studied a small series of patients. Second, we included a large majority of patients without HPS or with mild to moderate HPS. Therefore, caution is required in extending our results to patients with severe or very severe HPS.

In summary, our results indicate that NO is produced in excess by the alveolar compartment of the lungs in advanced liver cirrhosis. In our series, alveolar NO production was associated with hyperdynamic circulatory syndrome but not with arterial oxygenation, suggesting that acute inhibition of alveolar NO production may decrease cardiac output without altering gas exchanges. Further measurements of alveolar NO output, pulmonary haemodynamics and gas exchanges following long-term NO-synthase inhibition or liver transplantation may help to understand the role of alveolar NO synthesis in advanced liver diseases.

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**Figure legends**

**Figure 1:** Relationship between alveolar NO and oxygenation parameters in cirrhotic patients (n=29). **(A,B)** There was no correlation between alveolar NO concentration (CA,NO) and alveolar-arterial oxygen tension difference (PA-a,O2), or between alveolar NO output (V'A,NO) and PA-a,O2. **(C,D)** There was a weak but significant correlation between CA,NO and lung transfer for carbon monoxide (TL,CO), but no correlation between V'A,NO and TL,CO.

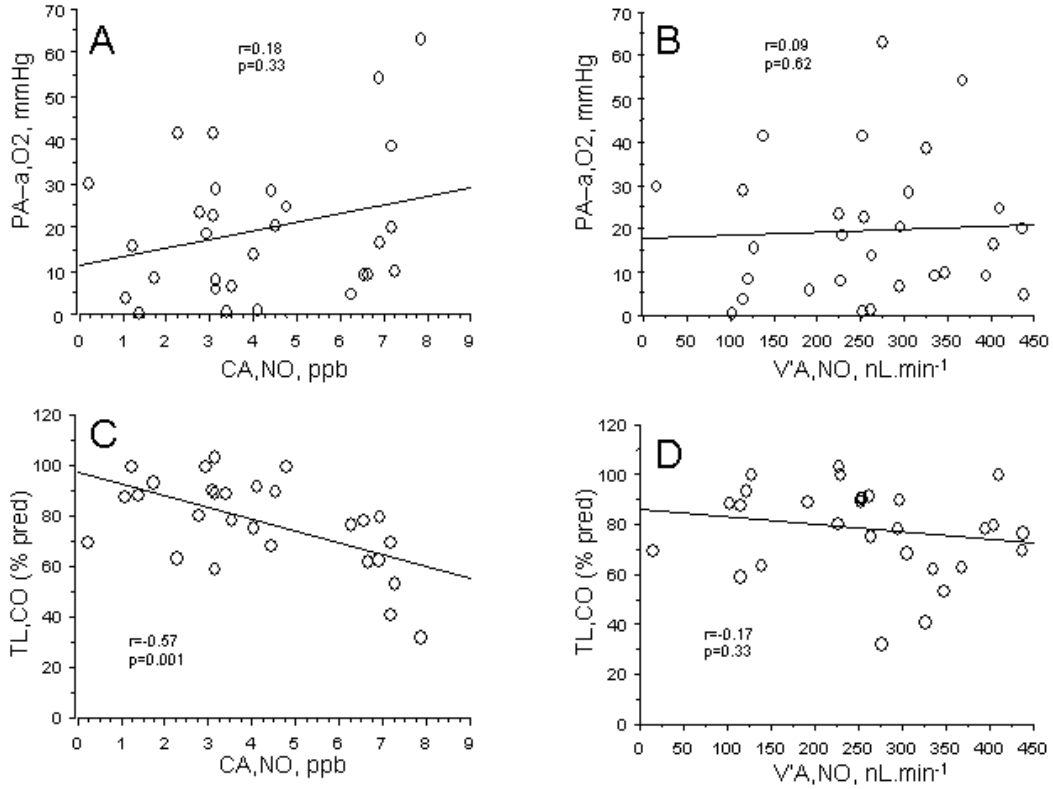


**Figure 1**

**Figure 2:** Relationship between alveolar NO and haemodynamic parameters in cirrhotic patients (n=29). (**A,B**) Cardiac index (CI) correlated positively with alveolar NO concentration (CA,NO) and with alveolar NO output (V'A,NO) (r=0.41, p=0.03 and r=0.56, p=0.001, respectively). **(C,D)** Systemic vascular resistance (SVR)

correlated negatively with CA,NO and with V'A,NO (r=-0.39, p=0.04 and r=-0.52, p=0.004, respectively)

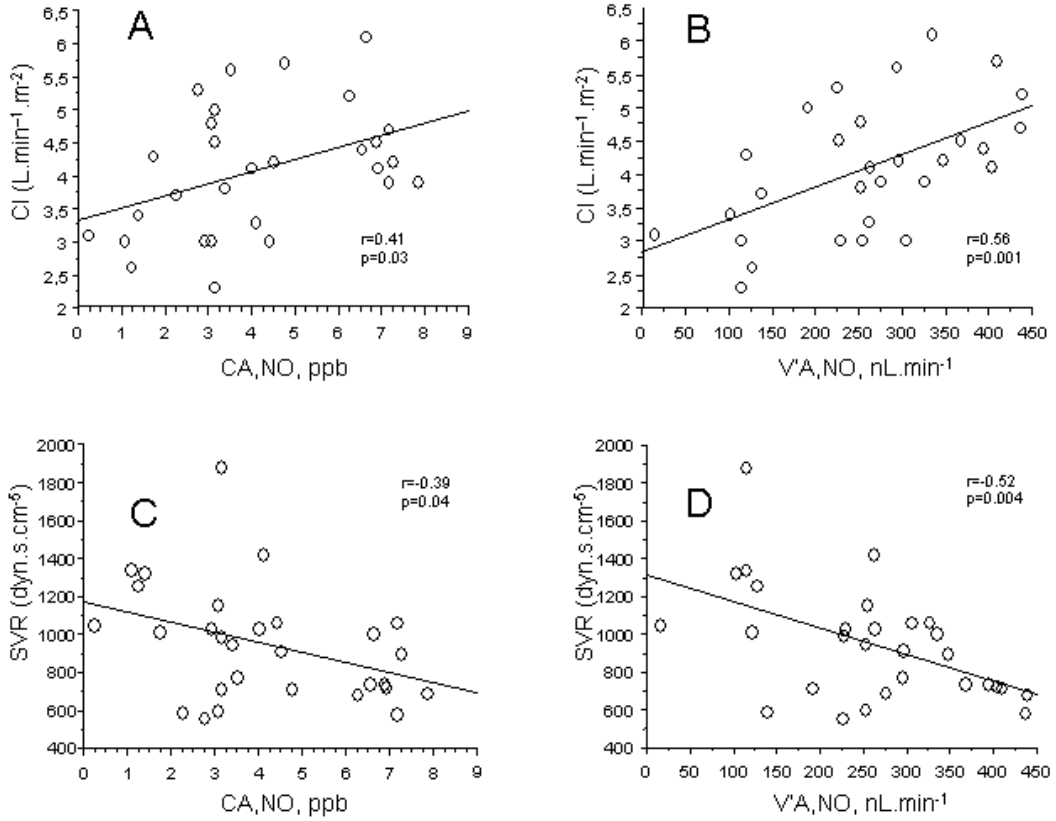


**Figure 2**

**Table 1**: Demographic, functional and haemodynamic characteristics of cirrhotic patients and healthy volunteers

| | Cirrhotic patients | | Controls (n=22) |
|---|---|---|---|
| | non-HPS (n=21) | HPS (n=8) | |
| Age (years) | 52.9 [40.9–58.4] | 56.1 [48.8–60.6] | 48.3 [36.9–55.4] |
| Gender (M : F) | 13 : 8 | 7 : 1 | 16 : 6 |
| Tobacco smoking (yes : no) | 9 : 12 | 5 : 3 | 10 : 12 |
| Alcohol : viral hepatitis : others | 7 : 6 : 8 | 7 : 1 : 0 | - |
| Child grade (A : B : C) | 3 : 3 : 15 | 0 : 3 : 5 | - |
| TLC (% predicted value) | 99 [90–110] | 93 [90–96] | 99 [94–110] |
| FEV1/VC (%) | 82 [79–86] | 80 [74–87] | 80 [77–85] |
| TL,CO (% predicted value) | 88 [76–92] | 66 [52–75]*# | 96 [85–101] |
| Pa,O2 (mmHg) | 96 [87–107] | 64 [58–79] # | |
| Pa,CO2 (mmHg) | 33 [31–36] | 33 [31–37] | |
| PA–a,O2 (mmHg) | 9 [6–19] | 40 [29–48]# | - |
| Mean PAP (mmHg) | 14 [12–16] | 15 [12–19] | - |
| Cardiac index ($L.min^{-1}.m^{-2}$) | 4.2 [3.2–4.8] | 3.9 [3.4–4.7] | - |
| PVR ($dyn.s.cm^{-5}$) | 56 [30–89] | 57 [44–96] | - |
| SVR ($dyn.s.cm^{-5}$) | 988 [737–1186] | 720 [594–1060] | |

HPS: hepatopulmonary syndrome; M: male; F: female; TLC: total lung capacity; FEV1: forced expiratory volume in 1 second; VC: vital capacity; TL,CO: lung transfer for carbon monoxide; PA–a,O2: alveolar-arterial differential pressure for oxygen; PAP: pulmonary artery pressure; PVR: pulmonary vascular resistance; SVR: systemic vascular resistance

*: $p<0.05$ compared with controls

#: $p<0.05$ compared with non-HPS cirrhotic patients.

**Table 2**: NO measurements in control subjects and in patients with liver cirrhosis

| | n | FE50,NO (ppb) | CA,NO (ppb) | TL,NO, (mL/min/mmHg) | V'A,NO (nL/min) | J'aw,NO (nL/min) |
|---|---|---|---|---|---|---|
| Healthy controls | 22 | 14.4 [11.9–21.0] | 1.4 [0.6–2.3] | 93 [84–112] | 79 [60–89] | 37 [23–51] |
| Non-smokers | 12 | 17.8 [14.1–20.3] | 1.3 [0.6–1.9] | 96 [85–110] | 78 [58–85] | 44 [31–52] |
| Smokers | 10 | 11.7 [8.1–14.5]‡ | 1.6 [0.8–2.3] | 93 [80–112] | 80 [61–89] | 33 [22–45]† |
| Cirrhotic patients | 29 | 20.7 [16.5–26.5] | 3.5 [2.9–6.5]# | 92 [84–111] | 260 [178–338]# | 74 [56–116] |
| Non-smokers | 15 | 20.0 [16.7–23.7] | 3.9 [3.1–5.2]# | 92 [85–106] | 289 [172–350]# | 74 [56–98] |
| Smokers | 14 | 20.7 [16.5–35.0] | 3.3 [2.5–6.9]# | 95 [79–113] | 253 [181–336]# | 79 [56–155] |

Definition of abbreviations: FE50,NO: exhaled NO concentration at expiratory flow of 50 mL/second; CA,NO: alveolar concentration of nitric oxide; TL,NO: lung transfer factor for NO; V'A,NO: output of nitric oxide produced by the alveoli; J'aw,NO: maximal bronchial output of nitric oxide

† $p<0.01$ compared with healthy nonsmoking subjects.

‡ $p<0.001$ compared with healthy nonsmoking subjects.

# $p<0.0001$ compared with healthy subjects whatever their smoking history