Effect of pulmonary perfusion on the slopes of single-breath test of CO$_2$

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Tusman, G., M. Areta, C. Climente, R. Plit, F. Suarez-Sipmann, M. J. Rodriguez-Nieto, G. Peces-Barba, E. Turchetto, and S. H. Böhm. Effect of pulmonary perfusion on the slopes of single-breath test of CO$_2$. J Appl Physiol 99: 650–655, 2005. First published March 31, 2005; doi:10.1152/japplphysiol.01115.2004.—The objective of this study was to evaluate the effects of lung perfusion on the slopes of phases II ($S_{II}$) and III ($S_{III}$) of a single-breath test of CO$_2$ (SBT-CO$_2$). Fourteen patients submitted to cardiac surgery were studied during weaning from cardiopulmonary bypass (CPB). Pump flow was decreased in 20% steps, from 100% (total CPB flow) to 0%. This maneuver resulted in a progressive and opposite increase in pulmonary blood flow (PBF) while maintaining ventilator settings constant. SBT-CO$_2$, respiratory, and hemodynamic variables remained unchanged before and after CPB, reflecting a constant condition at those stages. $S_{II}$ was similar before and after CPB (19.6 ± 2.8 and 18.7 ± 2.1 mmHg/l, respectively). $S_{III}$ was lowest during 20% PBF (8.6 ± 1.9 mmHg/l) and increased in proportion to PBF until exit from CPB (15.6 ± 2.2 mmHg/l; $P < 0.05$). Similarly, $S_{II}$ and the CO$_2$ area under the curve increased from 163 ± 41 mmHg/ml and 4.7 ± 0.6 ml, respectively, at 20% PBF to 313 ± 32 mmHg/ml and 7.9 ± 0.6 ml ($P < 0.05$) at CPB end. When $S_{II}$ and $S_{III}$ were normalized by the mean percent expired CO$_2$, they remained unchanged during the protocol. In summary, the changes in PBF affect the slopes of the SBT-CO$_2$. Normalizing $S_{II}$ and $S_{III}$ eliminated the effect of changes in the magnitude of PBF on the shape of the SBT-CO$_2$ curve.

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The single-breath test of CO$_2$ (SBT-CO$_2$) is a tool used for the analysis of dead space, CO$_2$ exchange, and the distribution of ventilation in anesthetized ventilated patients (8–10, 17, 27) or the perfusion in spontaneously breathing normal subjects (21). Of the several SBT-CO$_2$ indexes described, the slope of phase III ($S_{III}$) has been the most widely studied because it is closely related to the ventilation/perfusion relationship of the lungs (8, 9, 17).

The origin of this slope has been the subject of debate for many years. Convection-dependent inhomogeneities (CDI) and diffusion- and convection-dependent inhomogeneities (DCDI) within the lungs have been postulated as the main mechanisms of phase III sloping in single- and multiple-breath tests (3, 5, 28). CDI reflect large-scale inhomogeneities caused by the flow sequence between unequally ventilated units originating from the same (intraregional CDI) or different (interregional CDI) lung regions. DCDI are small-scale inhomogeneities that result from the interaction of both diffusion and convection transport observed at asymmetric branch points within lung acini.

Single- or multiple-breath tests using poorly soluble inert gases, such as N$_2$, mainly describe the effect of the distribution of ventilation on $S_{III}$ due to their minimal diffusion through the alveolar-capillary membrane. When a highly soluble gas like CO$_2$ is used instead, the effect of pulmonary blood flow (PBF) on the shape of $S_{III}$ could play an important role too. According to these concepts, the continuous transfer of CO$_2$ molecules by PBF into the alveolar space has been postulated as an additional reason for the sloping of phase III (3, 8, 24).

Schwartz et al. (24) used a mathematical model to describe the effects of PBF on $S_{III}$. They found that changes in cardiac output mostly affected the absolute value of the SBT-CO$_2$ curve with only minor effects on $S_{III}$. This theoretical finding suggests that continuous CO$_2$ elimination through the alveolar-capillary membrane does not play an important role in the sloping of phase III.

Interest has recently shifted also to the study of the slope of phase II ($S_{II}$). It seems to be a useful index for diagnosing emphysema, evaluating the severity of bronchospasm, and detecting lung recruitment (18, 25, 27, 30). The rapid increase in the CO$_2$ concentration during expiration marks the appearance of alveolar gas at the airway opening, and its origin seems to have the same mechanism as the one described for $S_{III}$. The effect of PBF on its shape has never been studied before.

The aim of this study was to determine the role of PBF in the genesis of $S_{II}$ and $S_{III}$ of the SBT-CO$_2$. To address this question, we used an in vivo human model of patients who were submitted to cardiac surgery during the weaning from cardiopulmonary bypass (CPB). In this model, changes in PBF can be analyzed at extreme conditions while maintaining lung ventilation constant.

METHODS

After approval by the local ethics committee, we studied 14 patients under total CPB for cardiac surgery. All included patients fulfilled the following conditions: written informed consent, elective surgery, age >50 yr, ejection fraction of >30%, and absence of previous pulmonary disease or pulmonary hypertension.

On arrival at the operating room, a right radial artery indwelling catheter and a right internal jugular vein and large-bore intravenous catheters were placed under local anesthesia. Standard monitoring included ECG, rectal temperature, and invasive systemic and central venous pressure. A pulmonary artery catheter (PAC) (Baxter Health-care, Irvine, CA) was inserted through an internal jugular vein access

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in patients whose ventricular ejection fraction was lower than 40%. Thus invasive pulmonary pressures and cardiac output could be measured in those patients.

Anesthesia was induced with 1.5–2 mg/kg propofol, 10 μg/kg fentanyl, and 0.1 mg/kg vecuronium and was maintained with infusions of 80–100 μg·kg⁻¹·min⁻¹ propofol and 0.5–1 μg·kg⁻¹·min⁻¹ remifentanil. Intraoperative fluids and inotropic and vasoactive drugs were administered according to the hemodynamic status. After tracheal intubation, the lungs were mechanically ventilated with a Narkomat anesthesia machine (Heyer Medical, Germany) in a constant-flow, volume-controlled mode with the following settings: tidal volume of 8 ml/kg, respiratory rate of 15 breaths/min, inspiratory time of 33% without pause, positive end-expiratory pressure (PEEP) of 7 cmH₂O, and a fraction of inspired oxygen of 50%. Alveolar ventilation was adjusted to obtain a normal arterial PCO₂ by modifying the respiratory rate while maintaining tidal volume unchanged.

CPB was established by cannulation of the ascending aorta and the right atrium. The extracorporeal circuit was primed with 1,500 ml of a mixture of lactated Ringer solution, 4% Gelafundin (Braun Medical, Crissier, Switzerland) and mannitol. Once CPB was started, mild hypothermia (32–34°C) and hemodilution (28% hematocrit) were induced. Pump flow was maintained at 2.5 l·min⁻¹·m⁻², and mean arterial pressure was kept between 50 and 70 mmHg. During CPB, blood gases were managed according to the alpha-stat protocol (26).

Protocol. All measurements were performed during an open-chest condition with the sternum retractor in place. SBT-CO₂ was analyzed at three periods (Fig. 1): 1) 15 min before CPB outset (baseline measurement), 2) during CPB weaning, and 3) 15 min after CPB weaning.

Lung volume history was standardized before each study period to avoid the effects of FRC variations on SIII (2). For this purpose, 10 mechanical breaths in a pressure-controlled ventilation mode with a peak inspiratory pressure of 30 cmH₂O and a PEEP of 10 cmH₂O were applied. Ventilator settings were then switched back to resume baseline anesthesia ventilation as described above. After the standardization maneuver and the assurance of constant ventilation conditions, any change in SIII during CPB weaning was assumed to be the result of variations in the degree and distribution of PBF. During CPB, mechanical ventilation was stopped and the patient was disconnected from the ventilator to maintain minimal lung inflation. Ventilation was resumed 10 min before starting the CPB weaning period after lung volume was standardized once again.

During the CPB weaning protocol, pump flow was decreased in steps of 20%, from 100% CPB (2.5 l·min⁻¹·m⁻²) to 0% CPB. In this model, we assumed that PBF increased inversely but proportionally from 0 to 20, 40, 60, 80, and 100%, respectively. Every step change of 20% in the pump/pulmonary flow was maintained for at least 2 min (Fig. 1).

At each decrement in pump flow, a partial and progressive reduction in pump venous return was performed by the perfusionist to maintain a proportional augmentation in lung blood volume and the patient’s intravascular volume status. The decrease in venous return to the patient was accomplished by partially and progressively clamping the CPB venous line in parallel with the reduction of CPB pump flow.

Criteria to move from one step of the weaning protocol to the next included a 20% decrement in blood volume inside the blood oxygenator recipient, a mean arterial pressure of ≥60 mmHg, a central venous pressure between 10 and 18 mmHg (or a wedge pressure between 10 and 18 mmHg when PAC was in place), and an increase in the end-tidal CO₂ (ETCO₂) of at least 4 Torr. We took ETCO₂ as an online marker of lung perfusion during CPB weaning (7) as ventilation and metabolism were maintained constant during the study. In preliminary patients, ETCO₂ normally increases from 0 to 5 Torr at 100% CPB to 25 to 30 Torr at 0% CPB during the CPB weaning phase. We therefore considered that ETCO₂ increments of at least 4 Torr at each step represented an increase in PBF of ~20%.

A final set of data was obtained 15 min after CPB weaning, once lung volume normalized and normal cardiovascular circulation was restored.

Expired gas measurements. SBT-CO₂ and respiratory mechanics were recorded continuously during the protocol with the COSMOpus capnograph (Novametrix, Wallingford, CT). With this device, CO₂ is measured by a main-stream sensor using the nondispersive infrared absorption technique (accuracy ±2 mmHg). Airway flow is measured by a fixed orifice differential pressure flow sensor (accuracy >3%). Before the protocol was started, a routine calibration was performed using the reference cell according to the manufacturer’s instructions.

The SBT-CO₂ was monitored and recorded online on a PC using the commercially available software Aplus for Windows (Novametrix, Wallingford, CT). CO₂ and respiratory mechanics raw data were downloaded from the Aplus and exported into Excel 98 (Windows, Microsoft) for offline analysis. To minimize error and variability of the analysis, the mean value of the last 15 breaths of every step was calculated for each variable.

Figure 2 shows a typical SBT-CO₂ curve and its derived variables as used in this study. SII and SIII were calculated automatically by a linear regression analysis between 30 and 70% of these slopes. SII and SIII were normalized by dividing them by the mean alveolar CO₂ of the corresponding expiration. Normalized SII (SII) and SIII (SIII) allowed comparison of slopes from breaths with different CO₂ excretion rates (22), as could be expected to occur during the entire CPB weaning process.
Area under the curve (VTCO₂,br) was calculated automatically by integrating the volume and CO₂ signals (11). Instrumental dead space was 20 ml, and its minimal effect on VTCO₂,br measurement was not compensated for.

Dynamic compliance of the respiratory system was calculated as expired tidal volume divided by end-inspiratory airway pressure minus total PEEP. Dynamic expiratory resistance of the respiratory system was calculated as the quotient between delta pressure and flow. These two variables related to respiratory mechanics were recorded online and used as markers for a constant ventilatory condition.

Statistical analysis. Statistical analysis was performed using the Instat software program (version 2.0, GraphPad, San Diego, CA). For comparison of variables, repeated-measurements analysis of variance (ANOVA) and Student-Newman-Keuls posttest was added for confirmation. PAC was used. If the analysis of variance (ANOVA) statistic was significant, the J Appl Physiol • VOL 99 • AUGUST 2005 • www.jap.org

II slopes are independent of PBF across subjects. As an example, Fig. 6 shows the similar effects of PBF on SIII in both groups of patients.

VTCO₂,br was lowest at 100% CPB and increased gradually during CPB weaning, reaching almost normal values at 100% of PBF (Fig. 7).

Although closing pressure of the dependent regions increased in the edematous lungs (after CPB), the standardization ventilatory maneuver used in the protocol could maintain a stable ventilatory condition in our patients. The lack of major differences in SBT-CO₂ variables in respiratory compliance and resistance before and after CPB favors the concept that the distribution of ventilation remained constant along the protocol (Fig. 8).

Hemodynamic data recorded before and after CPB weaning are shown in Table 2. Blood and filling pressures were stable between these moments, and only a significant increase in heart rate was observed after CPB. Only seven patients were monitored with a PAC. In these patients, cardiac index and wedge pressure showed similar values at baseline and post-CPB.
DISCUSSION

The gases used to perform breath tests come from two possible sources: 1) an external source through inhalation (SF$_6$, He, Ar, N$_2$) and 2) internal sources via blood transported by the PBF to the lung acini from body tissues (CO$_2$), from direct intravenous administration (He, SF$_6$), from gastrointestinal absorption (alcohol), or from local production by the airway mucosa (NO).

Breath tests performed with poorly soluble gases from an external source are used to describe the effects of ventilation maldistribution on the shape of $S_{III}$ (3, 5, 19, 20). On the contrary, CO$_2$ has some particular features such as its high solubility, its inverse blood-to-alveoli pathway, and its complex transport mechanism within the blood. In this regard, SBT-CO$_2$ has a greater dependence on ventilation-perfusion relationship changes, making it almost impossible to differentiate the effects of ventilation from those of perfusion on the shape of $S_{III}$.

We describe an in vivo model in which PBF is systematically controlled, allowing a close analysis of lung perfusion during the breath tests, especially those performed with highly soluble gases such as CO$_2$ that come from an internal source. Our results show that $S_{II}$ and $S_{III}$ changed as lung blood flow progressively increased during the CPB weaning phase. However, when $S_{II}$ and $S_{III}$ were normalized by dividing them by the mean expired alveolar CO$_2$, these slopes remained unchanged. Normalizing the slopes in this way cancels out the effect of the magnitude of PBF on the SBT-CO$_2$. Thus any change in $S_{II}$ and $S_{III}$ can be interpreted as being caused by alterations in the distribution of ventilation and perfusion irrespective of the magnitude of PBF.

**Phase III slope.** The present study partially reproduces the findings of the model described by Schwartd et al. (24) in real patients. They used a single-path trumpet bell model to explain a number of aspects of normal expirograms. CO$_2$ transport in the airways was simulated by a mass balance across the trumpet model to yield the airway convection-diffusion equation. The last term of this equation represents the CO$_2$ evolution from the blood into the alveoli according to the local blood flow distribution. The model controls both the amount of PBF and its distribution, predicting two facts related to $S_{III}$:

1) $S_{III}$ is minimally affected by the amount of PBF, i.e., cardiac output, when PBF is distributed to the whole lung; and 2) $S_{III}$ decreases when PBF is distributed proximally until generation 17 and then increases when PBF is distributed to the lung periphery from generation 17 to 23.

In the model, stable ventilation conditions are assumed, and changes in cardiac output from 1.8 to 13.2 l/min had minimal effect on $S_{III}$. In the present study, we found a higher increment in $S_{III}$ during extreme ranges of PBF but with a behavior similar to the one described in the model for a proximal-to-distal PBF distribution. Differences observed between these two findings could be explained by the characteristics of the trumpet model in which a single symmetric path cannot represent the true multibranched asymmetric structure of the airways and pulmonary vessels (6).
We believe that when PBF increases in our patients, it follows a proximal-to-peripheral distribution vector. This gravity-independent central-to-peripheral PBF gradient simulated in Schwardt’s model has been observed in humans (15) as well as in other mammals (1, 12, 13, 14, 29). This finding is consistent with the fractal branching pattern of pulmonary circulation where vascular resistance is increased due to branching and the existence of longer circuit pathways. Hakim et al. (16) showed in dogs that increments in cardiac output were associated with an increase in the absolute blood flow to both the central and the peripheral regions of the lung, while maintaining its central-to-peripheral gradient.

We speculate that, under constant ventilation conditions, as PBF increases and reaches more peripheral regions during CPB weaning, the asymmetric geometry of the vascular bed creates lung areas with different alveolar CO2 concentrations. Similar to the CDI mechanism, inter- and intraregional gradients of CO2 are produced in the alveoli by the regional pulmonary to the CDI mechanism, inter- and intraregional gradients of CO2 concentration mainly depends on the rate at which CO2 molecules were transported into the lungs to be eliminated by ventilation. Our findings with respect to VTCO2,br behavior is consistent with the fractal branching pattern of pulmonary circulation where vascular resistance is increased due to branching and the existence of longer circuit pathways. Hakim et al. (16) showed in dogs that increments in cardiac output were associated with an increase in the absolute blood flow to both the central and the peripheral regions of the lung, while maintaining its central-to-peripheral gradient.

We assume that the amount and distribution of PBF are interrelated phenomena, whether normalization has any effect on the distribution of PBF will have to be confirmed in further studies in which PBF magnitude and distribution can be manipulated separately.

**Phase II slope.** The model described by Schwartz et al. (24) did not study the effects of PBF on SII, and to our knowledge the hemodynamic-related effect on SII has not been documented yet. On the contrary, the effect of ventilation distribution on SII is now a better understood phenomenon. Asthmatic and emphysematous patients (18, 25, 30) show a decrease in SII when compared with normal subjects. In mechanically ventilated patients with anesthesia-induced atelectasis, we have shown that, after a lung-recruitment maneuver in which the lung is reexpanded, SII increases (27). The fact that lung recruitment increases SII indicates that the reopening of collapsed small airways results in a more homogeneous distribution of ventilation. Therefore, an inhomogeneous distribution of ventilation seems to influence SII.

In a recent study, Schulz et al. (23) describe the ventilation-induced changes on phase II independent from the influences of phase III by using labeled CO2 (C18O2). This technique might be very helpful in studying the determinants of PBF in more detail.

Despite the known influence of the distribution of ventilation on SII, our results have shown that PBF has an additional effect on this slope. SII showed its highest values before and after CPB. The lowest values were found during the highest pump flow (lowest PBF), observing a progressive increment as PBF was increased.

**VTCO2,br.** VTCO2,br depends directly on PBF. VTCO2,br progressively increased during CPB weaning because more CO2 molecules were transported into the lungs to be eliminated by ventilation. Our findings with respect to VTCO2,br behavior agree with those observed in the theoretical simulation performed by Schwartz et al. (24). These authors demonstrated that cardiac output and venous CO2 tension have a direct relationship with VTCO2,br values because average alveolar CO2 concentration mainly depends on the rate at which CO2 molecules are delivered by PBF.

**Pitfalls.** We assumed that PBF increases proportionally and inversely to the step decreases in CPB pump flow. However, this proportionality does not necessarily exist in the beating heart because the cardiac output is dependent on many factors such as contractility, cardiac rate, preload, and afterload. These factors undergo important changes in response to the ischemia-reperfusion phenomena responsible for the post-CPB stunning heart and the effects of the proinflammatory state that is associated with CPB circulation.

In addition, during CPB weaning, preload depends mainly on the control exerted by the perfusionist over the venous return from the pump. In this subjective maneuver, the perfu-
sionist clamps the venous line returning to the patient to achieve a gradual and proportional increase in preload. It is common to reach the normovolemic state during CPB weaning within several minutes after all residual volume of blood inside the pump is reinfused to the patient through the arterial cannula. Thus, strictly speaking, the step changes in PBF could only yield a rough approximation of the protocol-targeted 20%. In our view, however, the progressive change in PBF and not the exact rate of change was important to study the effects of PBF on SBT-CO₂.

The SBT-CO₂ variables immediately after CPB weaning did not reach baseline and post-CPB values. The “stunning” heart (i.e., a myocardium failure observed immediately after CPB weaning caused mainly by ischemia reperfusion, electrolyte dysbalance, and the inflammatory response) and the transient hypovolemic state observed at this moment of the protocol were responsible for this limitation, a situation that cannot be overcome in clinical cardiac surgery under CPB.

In conclusion, this in vivo human model showed that PBF plays an important role in the genesis and morphology of the SBT-CO₂ slopes. Nonnormalized S₁, and S₃ were affected by the different degrees of PBF, whereas normalized slopes were not. Normalized slopes describe ventilatory and perfusion inhomogeneities in mechanically ventilated patients independent of changes in the magnitude of PBF. The role of slope normalization on PBF distribution remains to be determined.

REFERENCES