Plasma oxygen during cardiopulmonary bypass: a comparison of blood oxygen levels with oxygen present in plasma lipid

I. M. PETYAEV*, A. VUYLSTEKE†, D. W. BETHUNE† and J. V. HUNT*
*Division of Chemical Pathology, Clinical Sciences Building, The Glenfield Hospital – NHS Trust, Groby Road, Leicester LE3 9QP, U.K., and †Papworth Hospital NHS Trust, Department of Anaesthesia, Papworth Everard, Cambridge CB3 8RE, U.K.

(Received 26 June/22 August 1997; accepted 1 September 1997)

1. Although not often appreciated, it is a fact that molecular oxygen is more soluble in lipids than in aqueous solution. We have recently developed a method to monitor oxygen within the lipid content of plasma. Monitoring plasma oxygen is one essential element during open heart surgery using a cardiopulmonary bypass pump and oxygenator. Currently oxygen is monitored electrochemically and is based upon monitoring the partial pressure of oxygen in a gas equilibrated with whole blood.

2. To determine the relative importance of lipid-associated oxygen in blood and assess the potential use of such a measurement we present comparisons of changes in oxygen associated with whole blood and lipid content of plasma before, during and after cardiac surgery.

3. In a limited number of patients studied (n = 28), aged between 34 and 86 years, oxygen in lipid increased with decreased extracorporeal blood temperature during cardiopulmonary bypass, increased in proportion to oxygen supplied and appeared to be a better monitor of oxygen than conventional electrochemical systems currently in use. Oxygen associated with whole blood and plasma lipid was markedly below normal on aortic declamping after cardiopulmonary bypass, suggesting an hypoxic episode at this point. Levels of oxygen in the lipid phase of plasma returned to normal presurgical values 6–8 h after surgery.

4. Calculation of the concentration of lipid-associated oxygen present in plasma suggests that plasma lipids contain up to 25% of that typically ascribed to haemoglobin. Thus, we suggest that monitoring lipid-associated oxygen may prove a better alternative to current methods of measuring oxygen status. Furthermore, we suggest that plasma lipid is a hitherto unsuspected pool of circulating oxygen which may play a significant role in tissue oxygen supply.

INTRODUCTION

The postperfusion syndrome plays an important role in the morbidity and mortality of patients who have undergone cardiac surgery. Cardiac dysfunction after cardiopulmonary bypass (CPB) has been reported by various investigators and abnormalities of post-operative ventricular function can occur even after uneventful operations [1–6]. Such surgery is accompanied by significant disturbances of oxygen and its metabolism both in the myocardium and in the blood passing through CPB [7–9]. Indeed, it is known that CPB with a pump oxygenator is associated with free-radical-mediated damage [10–12] which may result from leucocyte activation [9], arachidonic acid metabolism [13] and/or lipid peroxidation [10–12, 14]. This damage appears to continue well after surgery [9, 15, 16]. Thus, monitoring the level of oxygen present in the circulation is important during cardiac surgery using a CPB pump. Hypoxia is known to be detrimental but controversy exists with respect to the upper acceptable limits of blood oxygen. To date, no indication of upper limits exists in adult patients during extracorporeal oxygenation.

Traditionally, the oxygen content of blood is measured by means of a Van Slyke apparatus [17]. Using this method, the blood is haemolysed and the contained gas extracted by vacuum. Oxygen is then chemically absorbed and the oxygen content calculated from the resulting fall in gas pressure. Currently, clinical assessment of blood oxygen levels is based upon electrochemical detection of the partial pressure of oxygen in a gas equilibrated with blood [18]. Haemoglobin saturation can either be inferred.
from the partial pressure or measured by subsequent spectrophotometric means.

It is known that oxygen has an increased affinity for hydrophobic phases. The concentration of oxygen in lipid may be an order of magnitude greater than in the aqueous phase [19–22]. Thus, a major pool of oxygen in blood may be lipids/lipoproteins which, to date, has largely defied measurement in a clinical context. We have recently developed a simple colorimetric method of monitoring oxygen quantitatively in aqueous mixtures containing lipid micelles, lipid emulsions and artificial lipoproteins. The same method can also monitor the oxygen content of lipid/lipoproteins in whole plasma and purified low-density lipoprotein. This method, described in detail elsewhere, is based upon linking a monitored oxygen-dependent reaction with oxygen in lipid components [19].

This study involved the prospective examination of 28 patients undergoing CPB and extracorporeal oxygenation during cardiac surgery. For the first time, we present a comparison of changes in blood oxygen with oxygen present within the lipid content of blood plasma, monitored by a recently developed technique based upon the contribution of lipid-derived oxygen to micellar catalysis of an oxygen-dependent reaction.

MATERIALS AND METHODS

Cardiopulmonary bypass

The patient group, containing both smokers and non-smokers lacking any obvious pulmonary disease, consisted of 6 women and 22 men aged between 34 and 86 years. Patients selected displayed no obvious abnormality in lipoprotein profile although cholesterol levels were slightly elevated; 6.3 ± 0.5 mmol/l. The majority underwent coronary artery bypass graft surgery (23 patients) and the remainder valve replacement surgery (5 patients). All patients gave informed consent for drawing blood and the trial was approved by the Local Research Ethics Committee.

A standard anaesthetic regimen was used, mainly based on a combination of infusions of methohexitol (3 mg h−1 kg−1), a loading dose of fentanyl (0.015 mg/kg) and a bolus of pancuronium (0.1 mg/kg). Atracurium (0.75 mg/kg) was added at the start of bypass. Isoflurane (0.5–1%) or sodium nitroprusside were used to control blood pressure. Morphine was given post-operatively. The CPB circuit was primed with a 1.15 litre Hartmann solution and 350 ml of mannitol (10%). A membrane oxygenator was used and extracorporeal blood was cooled to 29–31°C.

At various times during the surgical procedure blood was drawn through a catheter fixed in the left radial artery. Inspired oxygen concentration was continuously monitored using a Datex capnograph during the pre- and post-bypass phases of surgery.

Ventilation settings were adjusted as a function of the end-tidal CO2 and airway pressures. Oxygen flow was adjusted and the gas supplied was a mixture of oxygen and air.

Sampling procedure

During blood sampling, six 0.1 ml samples were added to 0.9 ml of 50 mmol/l potassium phosphate (pH 7.8) containing 1 mmol/l diethylenetriamine penta-acetic acid. Samples were mixed and centrifuged, and plasma was stored at room temperature before analysis within 2–3 h.

Oxygen measurement

Conventional electrochemical measurement of the partial pressure of whole-blood oxygen was determined immediately after sampling by means of a polarographic oxygen electrode.

The level of oxygen in lipid/lipoprotein present in plasma was determined as described elsewhere [23]. The assay is a simple chemical one and is based upon monitoring an oxygen-dependent reaction spectrophotometrically over a period of minutes. Although several similar chemical systems have been investigated, in these studies the measurement of nitroblue-tetrazolium-detectable superoxide generated by NADH and phenazine methosulphate in the presence of diethylenetriamine penta-acetic acid was used. The assays were performed in 96-well plates.

Previous studies have shown that oxygen in lipid/lipoprotein present in plasma is relatively stable with a loss of <3% over 3 h and a loss of <10% over 2 days (results not shown).

Plasma cholesterol and protein content were monitored spectrophotometrically as described previously [24, 25]. Studies of the oxygen content or human low-density lipoprotein in vitro were also conducted, as described previously [23].

Data analysis

Graphics, with lines of regression (linear or polynomial), were drawn on Cricket Graph. All statistical analyses were performed using Microsoft Excel 4.0 (Apple Macintosh).

RESULTS

Lipid-associated oxygen before CPB

Oxygen in plasma lipid increased linearly with total plasma cholesterol levels, as shown in Fig. 1.
Furthermore, plasma lipid-associated oxygen appears to be linearly related to the percentage of oxygen supplied (Fig. 2, top). On the other hand, electrochemical measurements of oxygen were linear only over a limited range of oxygen supplied (Fig. 2, bottom).

**Lipid-associated oxygen during extracorporeal blood supply**

Before narcosis an initial inhalation of 40% oxygen gas was used. This was slowly increased to 90–100% just before narcosis. During this period there is an increase in lipid-associated oxygen and also in the electrochemical detection of oxygen (Table 1). After induction of narcosis, the inspired level of oxygen was reduced from 90–100% to 60%. The level of lipid-associated oxygen also decreased (Table 1). Electrochemical measurements of oxygen failed to reflect this relatively rapid drop in oxygen supply (Table 1). During CPB, the perfusionist maintained pre-operative oxygen levels by altering the flow ratio of oxygen to air, as assessed electrochem-

![Graph](image1)

**Fig. 1.** Plasma cholesterol and plasma lipid-associated oxygen. The relationship between plasma lipid-derived oxygen and total cholesterol. Each value of lipid-derived oxygen was obtained from 3–5 patients with a cholesterol level within 7%. Values are means ± SD. Assays were performed in triplicate.

![Graph](image2)

**Fig. 2.** Comparison of electrochemical detection and measurement of lipid-associated oxygen. The measurement of plasma lipid-associated oxygen (top) and partial pressure of oxygen (bottom) as a function of administered oxygen. For each value, data are from 6–15 patients. Values are means ± SD. Data has been extrapolated through the origin to demonstrate the linear relationship between measurements of lipid-associated oxygen and inhaled oxygen. Electrochemical measurements were not linearly related to administered oxygen. Assays were performed in triplicate.
Table I. Blood oxygen in patients undergoing open heart surgery. Units of oxygen in lipid/lipoprotein present in plasma are expressed in $10^{-4}$ mol/l oxygen per mg of plasma protein. Blood oxygen was also monitored by conventional electrochemical means (units: kPa). Significant changes from pre-operative values were tested by analysis of variance.

<table>
<thead>
<tr>
<th>Stage of operation</th>
<th>Pre-operative</th>
<th>Induction of narcosis</th>
<th>Pre-bypass</th>
<th>CPB</th>
<th>End of CPB</th>
<th>End of surgery</th>
<th>6–8 h after</th>
<th>20–22 h later</th>
</tr>
</thead>
<tbody>
<tr>
<td>% oxygen gas supplied</td>
<td>60</td>
<td>90–100</td>
<td>60</td>
<td>40*</td>
<td>85–95</td>
<td>55–60</td>
<td>60</td>
<td>Ambient (20%)</td>
</tr>
<tr>
<td>Lipid-associated oxygen ($10^{-4}$ mol l$^{-1}$ mg$^{-1}$ protein)</td>
<td>2.18 ± 0.135</td>
<td>4.6 ± 0.4</td>
<td>2.0 ± 0.3</td>
<td>4.64 ± 0.35</td>
<td>0.97 ± 0.1</td>
<td>1.57 ± 0.05</td>
<td>2.0 ± 0.17</td>
<td>2.07 ± 0.13</td>
</tr>
<tr>
<td>n</td>
<td>28</td>
<td>23</td>
<td>15</td>
<td>22</td>
<td>21</td>
<td>20</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Oxygen in whole blood (kPa)

| Mean ± SD | 36.8 ± 2.9 | 49.7 ± 3.0 | 38 ± 1.0 | 36.6 ± 0.77 | 12 ± 2.0 | 27.4 ± 1.85 | 29.1 ± 1.3 | 35 ± 1.0 |
| n | 10 | 12 | 10 | 25 | 8 | 11 | 11 | 7 |
| P value | <0.005 | >0.05 | >0.05 | <0.001 | <0.02 | <0.03 | >0.05 |

*Through bypass oxygenator. Oxygen adjusted to pre-operative partial pressure, by altering oxygen/air ratio and flow.

ica. Despite this, measurements of lipid-associated oxygen suggest that the blood is fully oxygenated, resembling levels present on supplying a patient with 90–100% oxygen gas during the initial phase of narcosis (Table 1). Thus, a discrepancy exists between these two measurements.

Cooling of extracorporeal blood during CPB had a significant influence on the content of lipid-associated oxygen (Fig. 3A). Oxygen content appears to be greater at low temperature (28–30°C). Above 31°C lipid-associated oxygen, obtained from patients before CPB, decreased significantly. Studies of puri-
Lipid-associated oxygen after CPB

In the first 3–9 min after CPB plasma lipid-associated oxygen and electrochemical measurements of oxygen dropped to less than 60% of plasma levels before surgery (Table 1), despite a supply of 100% oxygen gas. Figure 4 shows the CPB levels of plasma lipid-associated oxygen as a function of bypass duration both immediately after CPB (A) and 6–8 h later (B). Table 1 shows that there is a return to presurgical levels of blood lipid oxygen content after surgery within 6–8 h after surgery.

DISCUSSION

Oxygen in plasma lipid appears to be linearly related to total plasma cholesterol (Fig. 1) and increases in oxygen supply lead to an increase in lipid-associated oxygen (Fig. 2, top). The true significance of oxygen within the lipid components present in blood has yet to be examined. In our opinion, it would not be at all surprising that lipids constitute a major route of oxygen supply to tissue. After all, even haemoglobin releases oxygen into the aqueous phase of blood. Once in the blood the oxygen must gain access to tissues and eventually to the intracellular environment; it can do this relatively slowly by diffusion alone. Alternatively, oxygen can enter blood lipids/lipoproteins where it is far more soluble. Blood lipid/lipoprotein components might serve as 'packages of oxygen' which may interact with membranes better than water itself. The aqueous phase of plasma may even be considered an insulator exemplified by the stability of oxygen in the lipid fraction of plasma (results not shown). A question of access might also apply. Blood plasma and its contents may gain access to places and spaces inaccessible to the haemoglobin-containing erythrocytes. These observations raise an interesting question of whether or not the control of plasma lipid content, in common practice for patients with cardiovascular disease, is wise in prospective CPB patients. Low lipid levels may reduce tissue oxygen supply whereas high levels might accelerate free radical production. Given the detrimental effects of increased plasma lipid in cardiovascular disease and the reported benefits of plasma lipid reduction, a more detailed investigation of plasma lipid content, changes in lipid-associated oxygen during surgery and the recovery rate of the patients after surgery is essential before any valid assessment can be made.

Assuming equilibration between 20% oxygen gas and an aqueous system, water-soluble oxygen amounts to $2.4 \times 10^{-4}$ mol/l. Oxygen dissolved in
blood water is assumed to account for approximately 1.5% of total blood oxygen; the rest is assumed to be in haemoglobin. On the assumption that the aqueous content of blood reflects that of water equilibrated with ambient oxygen tension, haemoglobin contains 0.016 mol/l oxygen. Our measurements of lipid-associated oxygen, when patients inhale 20% oxygen, amount to $1.2 \times 10^{-4}$ mol/l oxygen per mg of plasma (see Table 1 or Fig. 2) which is equal to 0.0042 mol/l oxygen per litre of plasma (plasma typically contains 25 mg/ml protein). Thus, up to 25% of total oxygen present in whole blood is within the lipid fraction of plasma.

A comparison of the measurement of lipid-associated oxygen with the conventional electrochemical system for measuring oxygen levels suggests that the measurement of lipid-associated oxygen is a better reflection of the oxygen supplied. In other words, lipid-associated oxygen is linearly related to inhaled oxygen supply (Fig. 2, top), whereas this linearity only occurs over a very limited range in the case of electrochemical detection (Fig. 2, bottom) [18].

In the pre-bypass period, inhaled oxygen was reduced from 90–100% to 50% inhaled oxygen (Table 1 and Fig. 3). Measurements of lipid-associated oxygen reflected this reduction better than the electrochemical measurements. Again there would appear to be a differing sensitivity of the two measurements. It is possible, but unlikely, that the period before CPB is relatively short (up to 45 min) and the discrepancy between levels of lipid-associated oxygen and electrochemical detection may occur as a result of differences in diffusion rates of oxygen in aqueous (electrochemical detection) and hydrophobic lipid environments (lipid-associated oxygen detection). In other words, diffusion of oxygen into lipids is faster and thus levels of lipid-associated oxygen respond more rapidly to changes in inhaled oxygen supply.

Although the solubility of oxygen in aqueous systems, and in organic solutions too, is decreased with a drop in temperature, the effects of temperature shown in Fig. 3 may be due to changes in lipid fluidity. Noteworthy is the 31°C phase transition temperature of lipids which also appears to be the transition between differences in plasma lipid-associated oxygen with temperature (Fig. 3A). Studies of lipid-associated oxygen in purified low-density lipoprotein in vitro suggest that the temperature effect is indeed due to changes in lipid fluidity rather than any other effect of cooling blood (Fig. 3B). Perhaps by reducing blood temperature there is then an increase in tissue oxygen supply via lipid-associated oxygen, thus explaining some of the observed benefits of lowering blood temperature during CPB.

During CPB plasma lipid-associated oxygen increased, an unexpected observation given that blood during this time is deliberately oxygenated to a level resembling normal presurgical values, as assessed electrochemically. Reasons for this discrepancy between the two forms of oxygen measurement remain uncertain. They may reflect the effect of haemodilution during bypass. Whereas the level of lipid-associated oxygen is corrected for any dilution effect by expressing units per mg of plasma protein, conventional electrochemical measurements are not. Alternatively, the higher level of lipid-associated oxygen during this period may be due to a drop in temperature which increases lipid-associated oxygen (Fig. 3), as mentioned above. The discrepancy is likely to be due to a combination of both haemodilution and temperature. More significant is the observation itself. This period may be associated with an increase in cellular debris, which may also include an increase in transition metals. An increase in transition metals at the same time as an increase in lipid-associated oxygen might be expected to enhance the deleterious processes of lipid peroxidation.

Plasma oxygen decreased by about 60% once CPB had terminated as detected electrochemically or by assessment of lipid-associated oxygen (Table 1), suggesting an hypoxic episode. This may have been due to a disturbance in oxygen provision by the respiratory system; this occurs as a result of microatelectasis due to accumulation of extravascular fluid in the mediastinum and reversible alveolar collapse [26–30]. The degree to which this occurs is likely to extend with duration of bypass. Such a possibility is suggested by the observation that post-CBP lipid-associated oxygen was inversely related to duration of bypass (Fig. 4A), a relationship which diminishes with time of analysis after surgery (Fig. 4B). Conventional electrochemical and lipid-associated oxygen analysis are able to detect this short period of anoxia (Table 1). The novel assay of lipid-associated oxygen may well serve in the evaluation of this potentially damaging phase of CPB. Oxygen levels appear to have normalized 6–8 h after the end of the operation.

To summarize, measuring lipid-associated oxygen appears to reflect changes in plasma oxygen better than the conventional electrochemical method. The measurement of the partial pressure of oxygen by conventional electrochemical means is limited at concentration extremes. These studies have shown that lipid-associated oxygen increased during narcosis and stages before and after CPB. Once CPB was terminated the plasma levels of lipid-associated oxygen were significantly lower than normal for a period of some minutes. This suggests a period of potentially damaging hypoxia. Lipid-associated oxygen then normalized within 6–8 h of surgery.

Measurement of lipid-associated oxygen seems to reflect subtle changes in the oxygen content of the blood and appears to be at least as good as the conventional electrochemical measurements. The relevance of this measurement has yet to be fully
ACKNOWLEDGMENTS

J.V.H. would like to thank the British Heart Foundation for his personal support.

REFERENCES


