

Calculated and measured bicarbonate: is there a difference?

Arterial blood gas analysis provides the means to assess patient oxygenation and acid-base status. Two related parameters generated during blood gas analysis reflect the carbon dioxide (CO_2) content of blood, and are partial pressure of CO_2 ($p\text{CO}_2$) and bicarbonate (HCO_3^-) concentration. Only $p\text{CO}_2$ is actually measured, bicarbonate being derived by calculation from measured pH and $p\text{CO}_2$. In many laboratories the urea and electrolyte (U&E) profile includes an assay of plasma/serum bicarbonate concentration. Although the blood gas-calculated bicarbonate concentration usually approximates fairly closely to the U&E-measured bicarbonate concentration, that is not always the case. The extent and cause of discordance between measured and calculated bicarbonate has been the subject of controversy for nearly 30 years. The many studies that have addressed this issue have provided conflicting evidence; some have demonstrated clinically acceptable agreement while others have shown quite poor agreement. The latest and probably largest study is recently reported (*Clin Chem* 2008; **54**: 1586–9). It reveals concordance between measured and calculated bicarbonate and provides the best evidence to date that the two results are, for all practical clinical purposes, interchangeable.

Bicarbonate is the principal form in which carbon dioxide is transported in blood and is a major blood buffer, helping to maintain the pH of blood within narrow physiological limits. So, an appreciation of the clinical significance of bicarbonate concentration, whether obtained by calculation or measurement, depends on a basic understanding of carbon dioxide transport and normal acid-base balance.

CARBON DIOXIDE TRANSPORT

All tissue cells depend on aerobic metabolism for generation of the energy (in the form of adenosine triphosphate [ATP]) required for survival and function. This fundamentally vital mitochondrial process is characterised by consumption of oxygen and production of carbon dioxide (CO_2). In the resting state, CO_2 is produced at the rate of 200 mL/min but can rise to five times this rate during vigorous exercise. The ultimate fate of this CO_2 is elimination from the body in expired air, and an important function of blood is transport of CO_2 from tissue cells to the lungs.

The carbon dioxide journey (Fig 1a) begins in the mitochondria of cells, where it is produced. Due to prevailing concentration gradients, CO_2 diffuses from mitochondria (where CO_2 concentration is highest) across the cytoplasm, out of the cell and into the blood flowing through the capillary network that surrounds the cell. A little of the CO_2 arriving in blood remains dissolved in the water (aqueous) phase of blood plasma and an even smaller proportion binds to the amino (NH_2) terminal group of plasma proteins forming so called carbamino compounds, but most diffuses down a concentration gradient into red cells. A little of this remains dissolved in the red cell cytoplasm and some binds

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reversibly to amino terminal groups of haemoglobin (Hb), forming carbamino-Hb, but almost all of the CO_2 arriving in red cells is rapidly hydrated to carbonic acid (H_2CO_3) by the red cell isoform of the enzyme carbonic anhydrase. At physiological pH, almost all of this carbonic acid rapidly dissociates to bicarbonate and hydrogen ions (see Equation 1). The hydrogen ions are buffered by reduced haemoglobin and most of the bicarbonate passes from red cell to plasma in exchange for chloride ions (this so-called ‘chloride shift’ preserves electrochemical neutrality).

Within the microvasculature of the lungs (Fig 1b) the CO_2 concentration gradient across the alveolar membrane determines that dissolved CO_2 passes from blood to alveoli. This loss of CO_2 from blood favours reversal of the red cell reactions described above and bicarbonate passes from plasma to red cells, buffering hydrogen ions released from haemoglobin as it becomes oxygenated. Reversal of the carbonic anhydrase reaction results in CO_2 production that diffuses down the concentration gradient from red cells to plasma and onwards to the alveoli.

Mixed venous blood arriving at the lungs has an approximate total CO_2 content of 23.5 mmol/L, whereas (arterial) blood

SCIENCE REVIEW

leaving the lungs has a total CO_2 content of 21.5 mmol/L. This arteriovenous difference (2 mmol/L) represents the CO_2 added to blood from tissue cells and lost from blood as it passes through the pulmonary vasculature, to be eventually excreted from the body in expired air.

It should be clear from the above that most CO_2 is transported in blood plasma as bicarbonate, but there are in total four modes of transport:

- 95% is transported as bicarbonate
- 5% is transported simply dissolved in blood plasma
- <1% is transported bound to plasma proteins (carbamino compounds)
- <0.01% is transported as carbonic acid.

For practical purposes, total CO_2 in blood can be considered as the sum of bicarbonate and dissolved CO_2 because carbamino compounds and carbonic acid are present in vanishingly low concentration.

ACID-BASE BALANCE

The acidic potential of CO_2 , due to its hydration reaction to carbonic acid (see Equation 1) ensures that its concentration in blood is a major determinant of blood pH. As CO_2 concentration rises, so too does hydrogen ion (H^+) concentration. Regulation of blood CO_2 , that is matching the rate of CO_2 elimination in expired air to the rate of production in the mitochondria of tissue cells, essential for maintaining normal blood pH, is the business of the respiratory system. Respiration rate (controlled by $p\text{CO}_2$ -sensitive chemoreceptors located in the brain stem and carotid artery) is increased if $p\text{CO}_2$ rises and decreased if $p\text{CO}_2$ declines. Increased respiratory rate results in increased rate of CO_2 elimination, and decreased respiratory rate promotes CO_2 retention.

The relationship between pH and CO_2 is described by a form of the Henderson-Hasselbach equation (Equation 2). This is derived from application of the law of mass action to the hydration and dissociation reactions described in Equation 1.

As shown in Equation 3, pH of blood is dependent on the ratio of plasma bicarbonate concentration (the so-called metabolic component) to $p\text{CO}_2$ (the respiratory component). If $p\text{CO}_2$ increases without an equivalent rise in bicarbonate, pH falls. Conversely, if $p\text{CO}_2$ falls without an equivalent fall in bicarbonate, pH rises. The reverse relationship holds for bicarbonate concentration such that decreased bicarbonate without equivalent fall in $p\text{CO}_2$ results in decreased pH.

BLOOD GAS ANALYSIS AND CALCULATED BICARBONATE

The CO_2 parameters generated by the blood gas analyser are:

- partial pressure of carbon dioxide ($p\text{CO}_2$) (kPa)
- plasma bicarbonate concentration (HCO_3^-) (mmol/L).

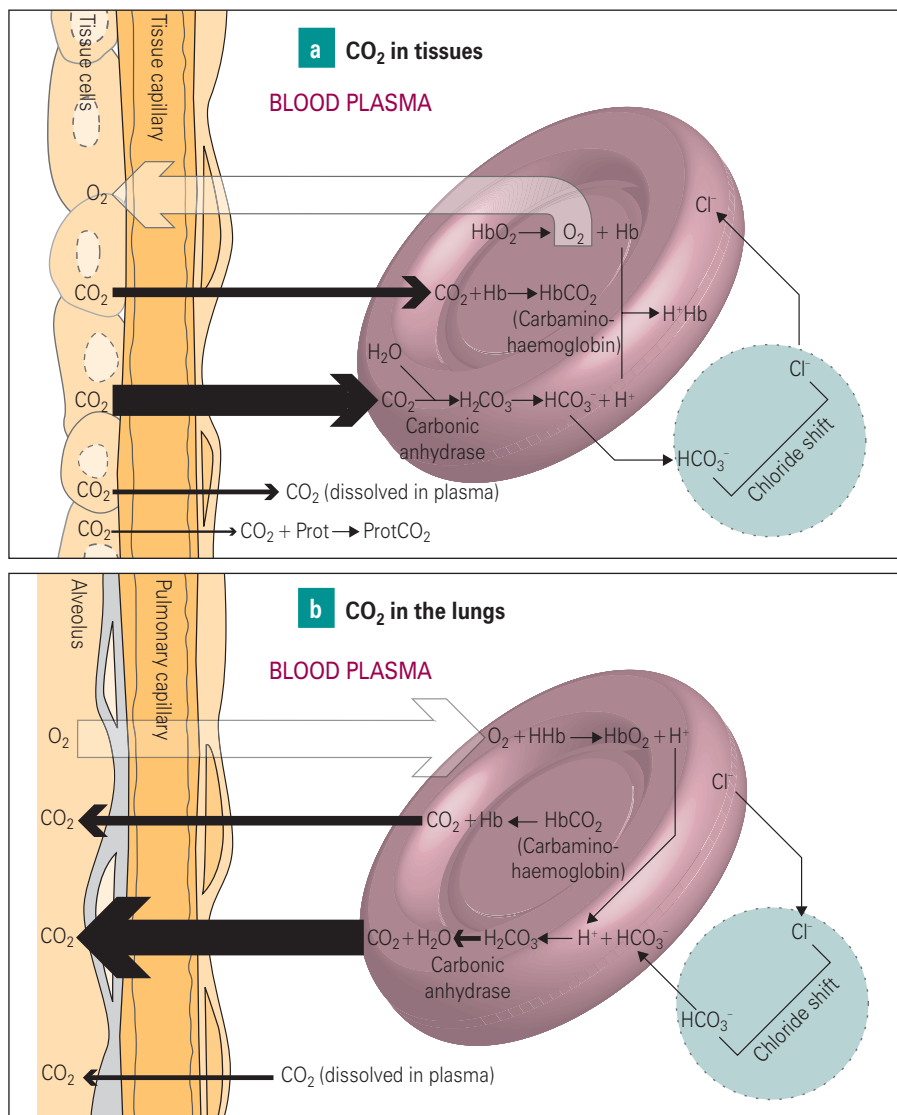


Fig 1. Carbon dioxide a) in tissues, and b) in the lungs.

Partial pressure of $p\text{CO}_2$ is a measure of the pressure exerted by that small portion ($\approx 5\%$) of total CO_2 in blood that remains in the gaseous state, 'dissolved in' the aqueous phase of plasma. The measurement is made using a CO_2 -specific pH electrode incorporated in blood gas analysers. In health, $p\text{CO}_2$ of arterial blood is maintained within the range 4.7–6.0 kPa, while $p\text{CO}_2$ of venous blood is a little higher at 5.6–6.8 kPa.

Plasma bicarbonate concentration (HCO_3^-) is the quantity of bicarbonate in unit volume of plasma. As previously noted, this is the form in which most carbon dioxide (95%) is transported in plasma. This

parameter cannot be measured but is generated by calculation during blood gas analysis. The calculation used (Equation 4) is a rearrangement of Equation 2 and depends on having values for pH and $p\text{CO}_2$, both of which are measured during blood gas analysis. In health, arterial plasma bicarbonate is maintained within the approximate reference range 22–28 mmol/L. Venous bicarbonate is a little higher at 24–30 mmol/L.

In some laboratories a distinction is made between 'actual' bicarbonate (the parameter described above) and 'standard' bicarbonate. Standard bicarbonate is a second calculated parameter and is the plasma bicarbonate concentration of blood that has been equilibrated with gas of normal $p\text{CO}_2$ (5.3 kPa) tension. In health, actual bicarbonate concentration is the same as standard bicarbonate concentration because $p\text{CO}_2$ is normal in both instances. By normalising $p\text{CO}_2$, the respiratory component of acid-base balance, standard bicarbonate is a measure solely of the metabolic component and is therefore considered helpful for elucidating the cause of disturbance in acid-base balance.

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EQUATIONS

EQUATION 1



EQUATION 2

$$\text{pH} = \text{pK}'_1 + \log \frac{[\text{HCO}_3^-]}{S \times \text{pCO}_2}$$

where: pK'_1 = 'apparent' dissociation constant of carbonic acid = 6.1

$[\text{HCO}_3^-]$ = concentration of plasma bicarbonate (mmol/L)

S = solubility coefficient for CO_2 at 37°C (0.23)

pCO_2 = partial pressure of pCO_2 (kPa)

The denominator in this equation ($S \times \text{pCO}_2$) is the concentration of carbon dioxide (mmol/L) dissolved in blood plasma (ie around 5% of total CO_2).

By removing all constants, Equation 2 can be simplified to:

EQUATION 3

$$\text{pH} \propto [\text{HCO}_3^-] / \text{pCO}_2$$

EQUATION 4

$$\log [\text{HCO}_3^-] = \text{pH} + \log (S \times \text{pCO}_2) - \text{pK}'_1$$

U&E PROFILE AND MEASURED BICARBONATE

The traditional urea and electrolyte (U&E) profile of blood plasma includes bicarbonate measurement. As U&E is ordered much more frequently than arterial blood gases, measured bicarbonate can provide the first indication of disturbance in CO_2 (and therefore acid-base) homeostasis. The use of the term bicarbonate to describe this measured parameter is not strictly accurate because the chemical methods measure all CO_2 liberated from plasma (or serum) by the addition of strong acid, or alternatively all bicarbonate produced as a result of adding a strong alkali to plasma. Thus, it includes not only bicarbonate but also dissolved CO_2 , carbamino compounds and carbonic acid. Some laboratories use the more correct term 'total carbon dioxide' to describe measured bicarbonate.

DISCORDANCE BETWEEN MEASURED AND CALCULATED PLASMA BICARBONATE

There are two sound theoretical reasons for measured bicarbonate to be slightly higher than calculated bicarbonate. First, as outlined above, measured bicarbonate includes an additional component due to non-bicarbonate CO_2 , which amounts to around 1.2 mmol/L.

Second, the plasma or serum sample used to measure bicarbonate is derived from venous blood, which has a slightly higher bicarbonate concentration than arterial blood, the sample used to generate calculated bicarbonate.

Notwithstanding these theoretical differences, which amount to no more than 2.0 mmol/L, in general, calculated and measured bicarbonate do agree. However, reports in the literature in the early 1980s suggested that it is by no means rare for results to be wholly discordant, with difference between measured and total bicarbonate in individual patients as high as 15 mmol/L.

Despite numerous studies over the intervening years, there remains no consensus on the cause or extent of this non-systematic discordance. Several studies conducted in recent years have identified a particular

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patient group, the critically ill, as more likely to show discordance, and this has led to advice from some quarters that for critically ill patients at least it might be prudent to abandon calculated bicarbonate in favour of measured bicarbonate.

The most recently published study (*Clin Chem* 2008; 54: 1586-7) to compare measured and calculated bicarbonate comes from the Mayo Clinic in Rochester, Minnesota. From laboratory computer records covering a 10-month period spanning 2006/07, investigators retrieved 17,621 records of both measured and calculated bicarbonate concentration for samples drawn simultaneously from the same patients. The calculated values were obtained using a Radiometer 725 blood gas analyser, and the assay for measured values was an enzymatic (phosphoenolpyruvate) method performed on a Roche discrete analyser. Bicarbonate concentration range was 5-49 mmol/L.

Statistical analysis revealed the following regression equation for measured (y) and calculated (x) bicarbonate concentrations: $y = 0.96x + 0.68$ mmol/L. The mean (standard deviation [SD]) difference between measured and calculated values was just -0.36 (1.23) mmol/L. Values agreed to within 2.0 mmol/L of each other for 16,800 (95.3%) of the 17,621 pairs and within 3.0 mmol/L for 17,538 (98.5%) of the pairs. With just 0.65% of paired results demonstrating what might be considered clinically significant discordance (difference >4 mmol/L), this very large database, generated in conditions of normal laboratory practice, has provided reliable evidence that, for the vast majority of patients, it is immaterial whether bicarbonate is determined by calculation or chemical assay. ■