# 1.4 Lactate, Pyruvate, Acetoacetate and 3-Hydroxybutyrate

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## 1.4.1 Introduction

Lactate, pyruvate, acetoacetate (ACAC) and 3-hydroxybutyrate (3OHB) are intermediary metabolites that normally occur in blood and play an essential role in energy production. Their accumulation in blood is a frequent cause of metabolic acidosis in children. The determination of these metabolites in biological fluids is useful in the early detection, diagnosis and treatment follow-up of abnormalities such as those of:

1. Enzymes of pyruvate "metabolism" (pyruvate dehydrogenase, PDH, or pyruvate carboxylase, PC, defects).
2. Enzymes of the Krebs cycle.
3. Enzymes of gluconeogenesis.
4. Liver glycogenolysis.
5. Oxidation of fatty acids.
6. Ketogenesis, ketolysis.
7. Mitochondrial respiratory chain.

Their relative blood concentrations are an expression of nutritional balance, providing a view of the metabolic disturbances arising in a patient. In conjunction with the measurement of unesterified fatty acids (UEFA) and glucose, they are useful tools with which to investigate intermediary metabolism in health and disease, particularly in inherited metabolic diseases.

During fasting, hormonal or metabolic modifications mobilise energy stored in adipose tissue as fat. Evaluation of different metabolite concentrations in blood provides insight into the different steps of fat metabolism.

Determinations of lactate, pyruvate, 3OHB and ACAC in biological fluids are run at different times during the day according to different periods of fasting. They are usually performed before and after meals (1 h). They are also performed after loading tests (e.g. glucose, proteins or triglycerides.)

## 1.4.2 Properties of Analytes

The plasma lactate ($CH_3$-CHOH-COOH) levels reflect the equilibrium between its production and its consumption by different tissues. Lactate is the end product of

anaerobic glycolysis, which is the main energy source for tissues such as the heart, muscles and kidney. Accumulation of lactate in blood to levels higher than 7 mmol/l leads to lactic acidosis. Hyperlactataemia can be observed either in ischaemic conditions or in many inherited metabolic diseases (e.g. PDH deficiency, PC deficiency).

During fasting, lactate is used by the liver and kidneys to provide glucose (gluconeogenesis). Blood lactate decreases during the first 15 h of fast (about 30% regardless of age). In the postprandial period, lactate is oxidised in mitochondria, producing energy for the heart, muscles and kidneys. The different metabolic pathways of pyruvate and lactate interconversion are shown in Fig. 1.4.1. The ratio of lactate to pyruvate remains unchanged independent of the fasting state.

Lactate and pyruvate are interconverted within the cytoplasm, depending upon the NAD:NADH ratio. In the cytoplasm, the ratio of lactate to pyruvate reflects the oxidoreduction state.

Pyruvate ($CH_3$-CO-COOH) is an intermediate metabolite, the product of carbohydrate, fat or protein metabolism. Pyruvate is the end point of glycolysis. In mitochondria, pyruvate may be oxidized to $CO_2$ and $H_2O$, reduced to acetyl coenzyme A (acetyl CoA) by PDH, or carboxylated by PC into oxaloacetate (Fig. 1.4.2).

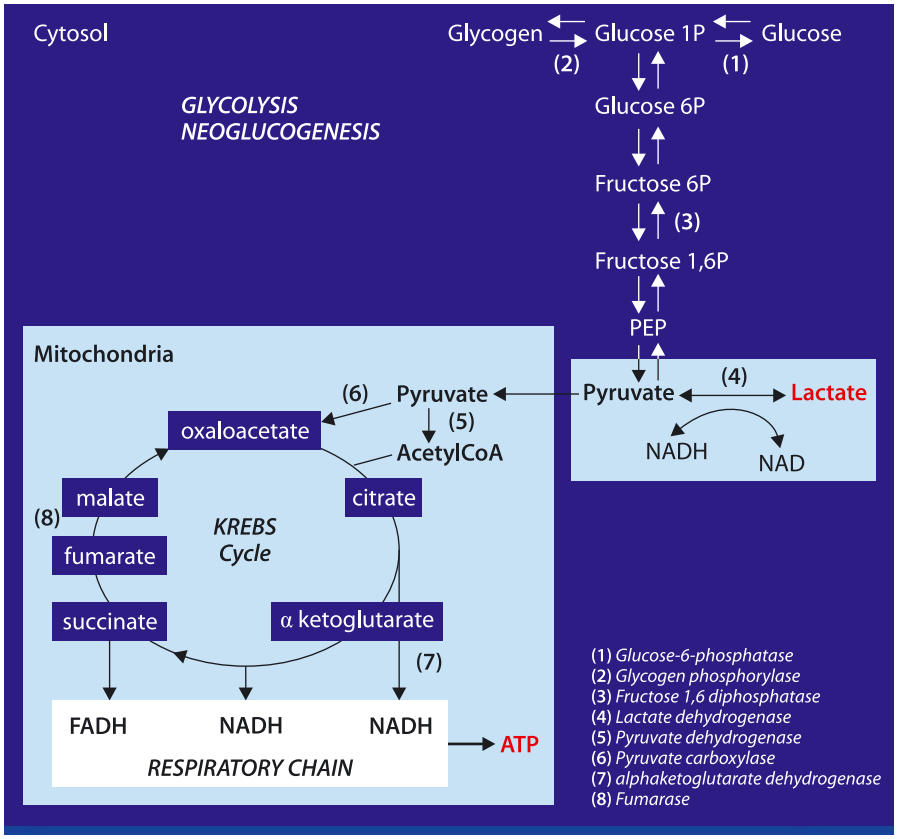


**Fig. 1.4.1** Lactate and pyruvate metabolic pathway. (*P* Phosphate, *PEP* phosphoenolpyruvate)

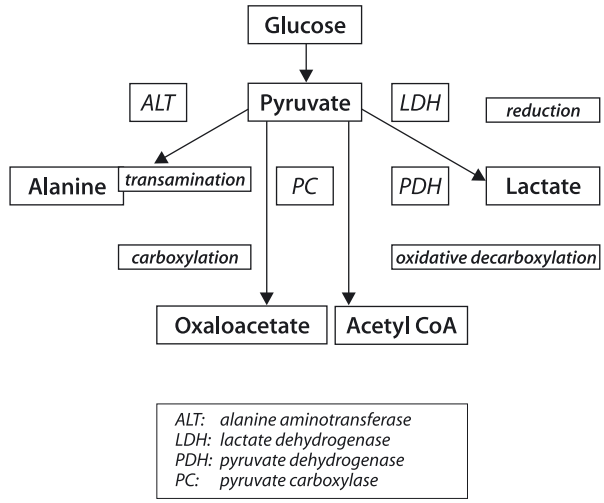


**Fig. 1.4.2** Pyruvate pathways

Pyruvate is transformed to lactate by lactate dehydrogenase (LDH) under anaerobic conditions.

Ketone bodies (KBs) circulate in the blood as 3OHB ($CH_3$-CHOH-$CH_2$-COOH) and ACAC ($CH_3$-CO-$CH_2$-COOH). The blood concentrations of these two metabolites depend upon the equilibrium between their production by the liver (ketogenesis) and consumption at the peripheral level (ketogenolysis). Abnormalities of KB metabolism manifest as ketosis, hypoketotic hypoglycaemia and inversion of the 3OHB:arachidonic acid (AA) ratio [12].

In the fed state, the KB concentrations do not usually exceed 0.2 mmol/l, except during the neonatal period, where higher concentrations are observed. The level of ACAC increases more quickly than that of 3OHB. The blood concentration of KBs increases during the fasted state, with an associated increase in the 3OHB:ACAC ratio, the result of PDH inhibition by mitochondrial acetyl CoA and NADH. KBs are primarily synthesised in the liver from acetyl CoA, the product of fatty acid oxidation.

Interconversion between ACAC and 3OHB is dependent upon the NAD:NADH ratio. Hydroxybutyrate dehydrogenase (HBDH) is localised mainly in the mitochondria. During fasting, fatty acids are transported to the liver to undergo beta oxidation.

Acyl coenzyme As are introduced into mitochondria following coenzyme A esterification in the cytoplasm. Mitochondrial entry depends upon a double membrane transport involving carnitine acyltransferases II and I. Excess acetyl CoA is used for KB synthesis. KBs are transported in the blood and ultimately metabolized via the Krebs cycle. KBs are necessary to provide energy to the brain during fasting, a true alternative substrate to glucose.

## 1.4.3 Methods

### 1.4.3.1 Principle

For simultaneous blood measurement of the four metabolites (lactate, pyruvate, ACAC and 3OHB), blood that has been deproteinised with perchloric acid is used as a sample. Spectrophotometric enzymatic methods according to reactions given in Fig. 1.4.3 were developed for automated analysers to minimise sample volume and improve precision [1, 10, 11, 17].

### 1.4.3.2 Pre-analytical

#### Specimen

Lactate and 3OHB can be measured in blood, plasma, urine and the cerebrospinal fluid (CSF). Pyruvate and ACAC are highly unstable, so deproteinisation is performed immediately after sampling. Lactate, pyruvate, 3OHB and ACAC determination can be measured in the same sample after deproteinisation using perchloric acid [17].

#### Patient Preparation

Patient preparation includes evaluation of clinical indications, and nutritional conditions (i.e. fed state, fast state, fasting duration) must be precisely defined. The patient must be fully informed of all procedures and at rest prior to blood sampling or invasive procedure. If fasting is required, the fast is scheduled for 24 h, but can be interrupted. Beginning at night, sampling is performed at 2, 15, 20 and 24 h of the fast.

#### Blood collection

Blood is collected as follows:

1. Blood sampling (venipuncture) must be performed without tourniquet, venestasis or muscular hand activity. Use of a catheter is recommended. Once the catheter (Cathelon; Critikon, Tampa, Florida, USA) is installed, the first milliliter (1 to 2 volumes of the catheter) should be discarded before a blood sample is taken.
2. Blood volume: 0.6–1 ml is collected into a heparinised tube.
3. The period of the blood collection is determined according to the diagnostic and therapeutic processes chosen, in accord with the suspected disease (i.e. before meals, after meals, fasting state, fasting test, loading test). For example, when screening for mitochondrial cytopathy, redox has to be evaluated in the fed state or after a glucose loading test, and after 10 h of fast.
4. Document in the patient's charts and requests the exact time of sampling and delays after last meals.
5. Avoid capillary blood.
6. Choose venous or arterial blood and document which has been taken.

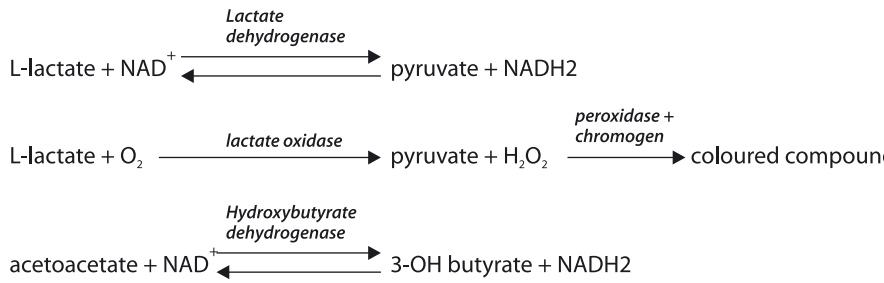


**Fig. 1.4.3** Reactions involved in the enzymatic measurement of 3-hydroxybutyrate, acetoacetate, lactate and pyruvate

## Sampling volume

Blood lactate, pyruvate, ACAC, 3OHB, glucose and redox ratio should be measured using the same sample for a reliable calculation of redox status.

## Blood Pre-treatment

Deproteinisation using acids is required to avoid glycolysis, but has to be performed as soon as possible after sampling (within 5 min). Simultaneous determination of lactate, pyruvate, ACAC and 3OHB is possible using this sample.

Different procedures are described for pre-treatment of blood, but perchloric acid is the most widely used agent: blood collected in a tube containing heparin is immediately deproteinised with a perchloric acid solution (1 mol/l) that has been refrigerated at + 4°C (two volumes for one volume of blood). Deproteinised samples may be frozen for up to 5 days.

### Plasma Lactate and 3OHB

Lactate and 3OHB can be measured in plasma. Blood is collected in a tube with an anticoagulant (heparin, EDTA, oxalate) and antiglycolytic agent (fluoride – without which there is an artefactual increase in lactate levels). The tube is transported in ice water and plasma is separated from the blood within 15 min following sampling.

Lactate levels in plasma sampled according to the aforementioned conditions are stable for up to 1 week at + 4°C and for up to 1 month at –20°C.

### Lactate in CSF

CSF sampling is performed by lumbar puncture, avoiding haemolysis. No pre-treatment of the sample thus obtained is necessary.

## Specimen Preparation Procedure

### Deproteinisation

Deproteinisation is carried out immediately after collection (< 5 min) as follows:

1. Measure accurately 1 ml (0.5 ml) blood collected in a tube containing heparin; transfer this to a tube containing 2 ml (1 ml) perchloric acid 1 M (which was measured accurately and refrigerated at 4°C previously).
2. Agitate the blood/perchloric acid mixture thoroughly to obtain a brown coagulum.
3. Keep the tube frozen (–20°C) for a period less than 5 days.

### Neutralisation

1. After thawing, deproteinised samples are centrifuged (+ 4°C) for 10 min at $3000 \times g$.
2. Mix 1 ml of the supernatant with 0.5 ml of phosphate buffer. If the volume of the supernatant available is < 1 ml, keep the ratio at one volume of supernatant to half a volume of buffer.
3. Mix. Place the tubes for 10 min at –20°C.
4. Check that the pH is 7 with pH paper (range 1–14.).
5. If needed, adjust pH as follows, noting the added volume for future calculation:
   a. if pH< 7 add phosphate buffer M (50 µl to 100 µl) (see reagent preparation)
   b. if pH> 7add $HCl0_4$ M (50 µl to100 µl).
6. Centrifuge for 5 min at $3500 \times g$ at + 4°C.
7. The deproteinised sample is thawed and centrifuged and the supernatant has to be neutralised using tripotassium phosphate buffer.

The stability in the filtrate of lactate, pyruvate, 3OHB and ACAC is 5 days at –20°C.

## Reagents and Chemicals

Lists of reagents, and recipes for working solutions and reagents are provided in Tables 1.4.1 and 1.4.2, respectively. The lactate reagent is prepared as per the manufacturer's instructions. The reagent required for the deproteinisation step is a solution of perchloric acid 1 mol/l (dilute 8.6 ml of $HCLO_4$ 70% in 100 ml distilled water or 11 ml of $HCLO_4$ 60% in 100 ml distilled water). That required for the neutralisation is phosphate tripotassic buffer 1 mol/l: add 5.3 g of phosphate tripotassic in 25 ml distilled water. This solution will be stable for 1 year at 25°C.

## Instrumentation and Calibration

An automated method can be run after validation using multiparametric open analysers [1, 11, 17], For example, KONELAB 30 (Thermo Fisher Scientific, Waltham, USA). There is no standard for pyruvate, ACAC or 3OHB. The results are calculated taking into account the molecular extinction coefficient of NADH ($6.3\ mmol^{-1} \cdot cm^{-1}$). A lactate standard is provided in the kit.

■ ***Table 1.4.1*** *List of reagents.* 3OHB *3-Hydroxybutyrate,* ACAC *acetoacetate,* HBDH *hydroxybutyrate dehydrogenase,* LDH *lactate dehydrogenase*

| Name | Provider | Reference | Storage temperature |
|---|---|---|---|
| Perchloric acid 70–72% | Merck | 100591000 | 20–25°C |
| Lactic acid | Sigma | L2250 | +4°C |
| ß NAD grade I | Sigma | N 1511 | –20°C |
| ß NADH grade II | Sigma | N 8129 | +4°C |
| Lactate | Randox | LC 2389 | +4°C |
| Phosphate tripotassic buffer | Sigma | 04347 | 20–25°C |
| Tris buffer | Merck | 1083820500 | 20–25°C |
| Paper pH 1–14 | MN | 902 04 | 20–25°C |
| ACAC powder | Sigma | A8509 | –20°C |
| D,L-3OHB powder | Sigma | H6501 | +4°C |
| 3-HBDH | Roche | 127841 | +4°C |
| LDH | Roche | 107069 | +4°C |
| $NaHCO_3$ | Merck | 1063290500 | +4°C |
| Pyruvate powder | Sigma | P8574 | +4°C |

■ ***Table 1.4.2*** *Reagent preparation and working solutions. Lactate reagent was prepared according to the manufacturer's instructions*

| | ACAC | 3OHB | Pyruvate |
|---|---|---|---|
| Tris 0.1 mol/l buffer | pH 6.98: dissolve 3.0285 g of Tris buffer into 200 ml distilled water. Adjust pH to exactly 6.98 with 1.2 M HCl, then adjust the volume to 250 ml. | pH 9.5. dissolve 3.0285 g of Tris buffer into 200 ml distilled water. Adjust the pH to exactly 9.5 with 1.2 M HCl, then adjust the volume to 250 ml. | pH 7.4: dissolve 3.082 g of Tris buffer into 200 ml distilled water. Adjust the pH to exactly 7.4 with 1.2 M HCl, then adjust the volume to 250 ml. |
| Stability at +4°C | 1 month | 1 month | 1 month |
| *β* NADH reduced, stock solution, 0.6 mmol/l | | | Dissolve 0.85 mg of NADH into 2 ml $NaHCO_3$ 5% |
| Stability | | | 7 days at +4°C |
| Working solutions: R1 | *β* NADH reduced solution at 0.4 mmol/l: dissolve 2.8 mg of NADH in 10 ml of Tris buffer (pH 6.98) | Solution of NAD 12 mmol/l: dissolve 80 mg of NAD in 10 ml of Tris buffer (pH 9.5) | Solution of NAD 0.12 mmol/l: dilute 1.2 ml stock solution NADH in 4.8 ml Tris buffer pH 7.4 |
| R2 | Mix: 750 µl Tris pH 6.98 + 250 µl HBDH (> 15 U/ml) | Mix: 750 µl Tris buffer pH 9.5 + 250 µl HBDH (> 15 U/ml) | Mix: 80 µl of LDH solution (7500 U/ml) + 4720 µl of Tris buffer pH 7.4 |

#### Quality Control

The quality control samples are prepared as described in Table 1.4.3. Target values are calculated according to the amount of weighed product, and values obtained must be within ± 15% of the target value.

### 1.4.4 Analytical

#### 1.4.4.1 Methods

The same deproteinised blood as used for lactate, pyruvate, 3OHB, and ACAC assays described above is used for enzymatic methods employing spectrophotometric measurement (Fig. 1.4.3) [10, 17]. The enzymes involved are LDH for pyruvate, lactate oxidase for lactate and HBDH for ACAC and 3OHB.

#### Procedure

The operating procedure for this method is given in Table 1.4.4. Note that lactate is quantified according to the manufacturer's instructions.

#### Calculation

For the filtrate samples, results have to be recalculated according to the dilution ratio (e.g. × 4.5). If acid base or buffer was added to correct the pH, an additional correction must be made according to the following equation:

$$\frac{\text{observed result} \times \text{final volume}}{\text{initial volume}}$$

#### Validation of the Methods

The results of method validation are given in Table 1.4.5.

#### 1.4.4.2 Post-analytical

#### Interpretation – Biological Variation

Blood lactate values are usually lower than plasma levels (–15 %) depending upon haematocrit values. KB concentration depends on whether the patient is in a fed or fasted state and age; the concentration is low in the fed state, increasing as the fasting period increases, with some variability according to age in children (e.g. KB elevations are higher in younger, more precocious children; see Fig. 1.4.4) [7]. In the fed state, KB levels are less than 0.2 mmol/l and the ratio 3OHB:ACAC is less than 1. The blood concentration of KBs increases during fasting. The 3OHB:ACAC ratio increases with the concentration of KBs during fasting (2.5–3.5). Plasma values of

■ ***Table 1.4.3*** *Preparation of quality control samples*

| | Lactate | Pyruvate | ACAC | 3OHB |
|---|---|---|---|---|
| Stock solution | 50 mmol/l | 10 mmol/l | 20 mmol/l | 10 mmol/l |
| Dissolve into 10 ml albumin 50 g/l solution | 0.048 g of lactate (sodium salt) | 11 mg of pyruvate (sodium salt) | 25.5 mg ACAC | 25.7 mg of D-l, 3OHB |
| Stability | 6 months at – 20°C | 6 months at – 20°C | 6 months at – 20°C | 6 months at – 20°C |
| Prepare three different level solutions | | | | |
| Level 1 | 10 µmol/l | 100 µmol/l | 10 µmol/l | 0.25 mmoll |
| Level 2 | 50 µmol/l | 200 µmol/l | 50 µmol/l | 0.5 mmol/l |
| Level 3 | 100 µmol/l | 400 µmol/l | 100 µmol/l | 1 mmol/l |
| Stability | Do not store | Do not store | Do not store | Do not store |
| Target values and acceptable limits | 10 µmol/l ± 1.5<br>50 µmol/l ± 7.5<br>100 µmol/l ± 15 | 100 µmol/l ± 15<br>200 µmol/l ± 30<br>400 µmol/l ± 60 | 10 µmol/l ± 1.5<br>50 µmol/l ± 7.5<br>100 µmol/l ± 15 | 0.25 mmol/l ± 0.03<br>0.5 mmol/l ± 0.075<br>1 mmol/l ± 0.15 |

■ ***Table 1.4.4*** *Operating procedure*

| | Pyruvate | ACAC | 3OHB |
|---|---|---|---|
| Sample (neutralised supernatant) | 60 µl | 60 µl | 40 µl |
| R1 (buffer + coenzyme) | 100 µl | 105 µl | 120 µl |
| Mix thoroughly, incubate at 37°C, measure absorbance at 340 nm A1 | | | |
| R2 (enzyme) | 20 µl | 12 µl | 10 µl |
| Wait time at 37°C | 300 s | 450 s | 600 s |
| Measure absorbance at 340 nm A2 | | | |
| Calculation factor (µmol/l) taking into account the sample dilution (×4.5) | 475<br>2142 | 466<br>2686 | 671<br>3019 |

■ ***Table 1.4.5*** *Analytical performances of the methods [17].* CV *Coefficient of variation*

| | Lactate | Pyruvate | ACAC | 3OHB |
|---|---|---|---|---|
| Reproducibility within run (CV%) | < 3 | < 3 | < 3 | < 3 |
| range (mmol/l) | 0.8–8 | 0.1–0.5 | 01–2 | 0.4–5 |
| Reproducibility run to run (CV%) | < 5 | < 5 | < 5 | < 5 |
| range (mmol/l) | 0.8–8 | 0.1–0.5 | 0.1–2 | 0.4–5 |
| Linearity limits (mmol/l) | 0–15 | 0–15 | 0–15 | 0–9 |
| Detection limit (mmol/l) | 0.1 | 0.01 | 0.02 | 0.02 |

KBs are 10–20% higher than blood values. No difference has been observed between venous and arterial blood in this regard.

## Interpretation – Reference Values

Reference values are given according to age, fed state, fasting state, diet and nutritional status (Table 1.4.6). Figure 1.4.4 shows the evolution of KB level as a function of fasting time and age [4, 17]. The reference values for lactate/creatinine/pyruvate are as follows:

1. Urine: lactate: creatinine ratio < 0.2 mmol/mmol creatinine.
2. CSF [2, 9]:
   a. Lactate: 1.1–2.2 mmol/l
   b. Pyruvate: 0.05–0.15 mmol/l
   c. Lactate:pyruvate ratio: 15–20

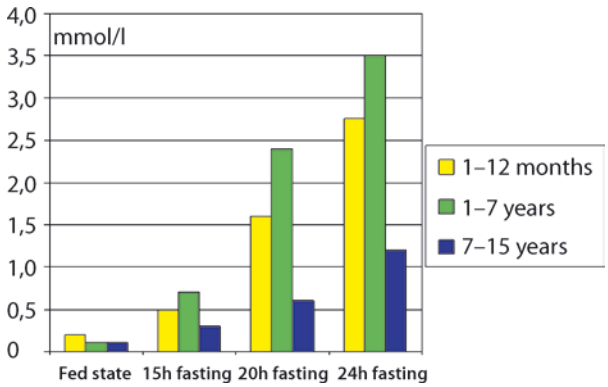


**Fig. 1.4.4** Ketone body levels according to fasting time and age

***Table 1.4.6*** *Reference values for blood lactate, pyruvate, ACAC and 3OHB according to age, fed state and fasting state.* L:P *lactate:pyruvate ratio*

| | Lactate (L) (mmol/l) | Pyruvate (P) (mmol/l) | L:P | 3OHB (mmol/l) | ACAC (mmol/l) | Ketone bodies (mmol/l) | Ratio 3OHB: ACAC |
|---|---|---|---|---|---|---|---|
| **Children (0–1 year)** | | | | | | | |
| Fed state, 1 h after meals | 0.6–2.2 | 0.04–0.14 | 6–14 | 0.10–0.2 | 0.10–0.25 | 0.10–0.30 | < 1 |
| **Children (1–7 years)** | | | | | | | |
| Fasting 10 h | 0.7–1.8 | 0.09–0.17 | 6–14 | 0.02–0.3 | 0.04–0.20 | 0.02–0.6 | < 2.5 |
| Fed state | 0.9–1.8 | 0.08–0.17 | 6–14 | 0.02–0.1 | 0.04–0.13 | 0.02–0.2 | < 1 |
| **Children (7–15 years) and adults** | | | | | | | |
| Fasting 10 h | 0.7–0.9 | 0.04–0.12 | 6–14 | 0.02–0.3 | < 0.2 | 0.1–0.4 | 0.4–2.3 |
| Fed state | 1.0–1.55 | 0.08–0.16 | 6–14 | 0.02–0.1 | 0.04–0.13 | < 0.20 | < 1 |

### 1.4.4.3 Main Causes of Hyperlactataemia, Hyperketonaemia and Hypoketonaemia

The main causes of acquired hyperlactataemia and hereditary hyperlactataemia are given in Tables 1.4.7 and 1.4.8, respectively. The main causes of hyperketonaemia and hypoketonaemia are given in Tables 1.4.9 and 1.4.10, respectively.

***Table 1.4.7*** *Main aetiologies for hereditary hyperlactataemias [3]*

| Primary hyperlactataemias | Secondary hyperlactataemias |
|---|---|
| Glycogen metabolism disorders<br>Amylo-1,6-glucosidase defect<br>Liver phosphorylase defect<br>Glycogen synthetase defect | Organic acidaemias<br>Methylmalonic aciduria<br>Propionic aciduria<br>Isovaleric aciduria |
| Gluconeogenesis defects<br>Glucose-6-phosphatase defect<br>Fructose-1,6-biphosphatase defect<br>Phosphoenol pyruvate carboxykinase defect | Congenital hyperammonaemia: citrullinaemia<br>Fat oxidation disorders |
| Pyruvate disorders<br>Pyruvate carboxylase defect<br>Pyruvate dehydrogenase defect | |
| Krebs cycle abnormalities<br>Ketoglutarate dehydrogenase defect<br>Fumarase defect | |
| Respiratory chain abnormalities<br>NADH coenzyme Q reductase defect (complex I)<br>Succinate coenzyme Q reductase defect (complex II)<br>Coenzyme Q cytochrome C reductase defect (complex III)<br>Cytochrome oxidase C defect (complex IV)<br>ATPase defect (complex V) | |

***Table 1.4.8*** *Main causes for acquired hyperlactataemia [3, 6]*

| **With anoxia, resulting in tissue hypoperfusion** | **Without anoxia** |
|---|---|
| Heart stroke, endotoxins or haemorrhagic | Diabetes |
| Serious anaemia | Renal insufficiency |
| Intensive muscular disease | Intoxications: salicylate, cyanide, alcohol, antiretroviral drugs, biguanides |
| Left ventricular insufficiency | |
| Convulsions | |
| Hypocapnia | |

***Table 1.4.9*** *Causes for hyperketonaemias*

| Over-production | Decreased ketone body utilisation at the peripheral level |
|---|---|
| Diabetes – insulin dependent | Ketolysis defects:<br>Succinyl coenzyme A:3-keto acid transferase<br>ACAC coenzyme A thiolase |
| Methyl malonic, propionic or isovaleric acidaemias | |
| Pyruvate carboxylase and multiple carboxylase deficiency | |
| Gluconeogenesis enzyme deficiency: glucose-6-phosphatase, fructose-1,6-diphosphatase or abnormality of glycogen synthesis (glycogen synthase) | |

***Table 1.4.10*** *Hypoketonaemic states*

| |
|---|
| Hyperinsulinism |
| Multiple acyl coenzyme A dehydrogenase |
| Long chain fatty acid oxidation defect |
| Carnitine palmitoyl transferase I and II |
| Systemic carnitine deficiency |
| Long-chain acyl coenzyme A dehydrogenase defect |
| Long-chain 3-hydroxy-acyl coenzyme A dehydrogenase defect |
| Medium chain acyl coenzyme A dehydrogenase deficiency |
| Hydroxymethyl glutaryl coenzyme A lyase defect |

### 1.4.4.4 Typical Pathological Values

#### Respiratory Chain Abnormalities

Data from a patient affected with mitochondrial respiratory chain abnormalities (complex I, III and IV deficiencies) are given in Table 1.4.11. Permanent hyperlactataemia is associated with an increased lactate:pyruvate ratio and postprandial ketosis with an increased 3OHB:AA ratio. Pyruvate, the product of glycolysis, is metabolised in the mitochondria to acetyl CoA by PDH. Pyruvate can be reduced to lactate by LDH or may be utilized for gluconeogenesis. Mitochondrial respiratory chain dysfunction results in decreased acetyl CoA metabolism. Pyruvate metabolism shifts towards other metabolic routes, including reduction to lactate and gluconeogenesis. Lactate cannot be cleared as rapidly as it is being produced, resulting in acidosis. Increased gluconeogenesis results in hyperglycaemia.

A fully functional PDH complex leads to acetyl CoA accumulation. Overproduction of acetyl CoA, without utilisation in the respiratory chain complex, results in accumulation of acetyl CoA in the cytoplasm, where it serves as a substrate for fat production. An inability to metabolise acetyl CoA also leads to increased circulating levels of ACAC and 3OHB [8, 13].

***Table 1.4.11*** *Data from a patient affected with a complex I, III and IV deficiency*

| | Lunch | | Dinner | |
|---|---|---|---|---|
| | Before meals | After meals | Before meals | After meals |
| Lactate (mmol/l) | 6.00 | 8.50 | 5.00 | 8.90 |
| Pyruvate (mmol/l) | 0.24 | 0.31 | 0.18 | 0.28 |
| L:P | 24.00 | 27.50 | 28.00 | 32.00 |
| ACAC (mmol/l) | 0.22 | 0.14 | 0.16 | 0.20 |
| 3OHB (mmol/l) | 1.53 | 0.90 | 1.10 | 1.27 |
| Ketone bodies (mmol/l) | 1.75 | 1.04 | 1.26 | 1.45 |
| 3OHB:AA | 7.00 | 6.40 | 6.90 | 6.40 |
| Glycaemia (mmol/l) | 5.00 | 5.80 | 3.30 | 5.20 |

## PC Defect

In the initial step of gluconeogenesis, pyruvate is carboxylated to oxaloacetate by PC (Fig. 1.4.2). Two forms of PC deficiency are observed. Patients with type B disease develop symptoms in the neonatal period, including severe lactic acidaemia, hypotonia, seizures, failure to thrive, psychomotor retardation and hepatomegaly. The lactate:pyruvate ratio is increased due to a deficiency in aspartate, which is involved in maintaining the mitochondrial redox status. Hypoglycaemia is mild and helps to distinguish this condition from type I glycogen storage disease (glucose-6-phosphatase deficiency), which also produces lactic acidosis. Most patients with type B disease do not synthesize any PC protein. Death usually occurs within the first 6 months of life. Type A disease manifests as less severe lactic acidaemia (3–6 mmol/l) with acute metabolic decompensation usually observed in association with an illness or fasting. These patients demonstrate low muscle tone and developmental delays, mental retardation with cerebral atrophy, and abnormalities of brain myelinisation.

Metabolically, acetyl CoA that is generated is diverted to ketogenesis, and urea cycle activity is decreased, leading to hyperammonaemia associated with fasting hypoglycaemia, increased lactataemia associated with an increased lactate:pyruvate ratio, and increased ketonaemia with a 3OHB:AA ratio < 1. Data from a patient affected with a PC defect are presented in Table 1.4.12.

## PDH Defect

Data from a patient affected with a PDH defect are presented in Table 1.4.13. In general, hyperlactataemia (increasing with meals) associated with a normal lactate: pyruvate ratio and normal ketonaemia is observed. The ketogenic diet is a rational treatment for PDH complex deficiency.

■ ***Table 1.4.12*** *Fed and fasting state for a patient affected with a pyruvate carboxylase defect (type B)*

| | Before meals | After meals |
|---|---|---|
| Lactate (mmol/l) | 22.00 | 11.00 |
| Pyruvate (mmol/l) | 0.55 | 0.22 |
| L:P | 40.00 | 50.00 |
| ACAC (mmol/l) | 0.80 | 0.50 |
| 3OHB (mmol/l) | 0.35 | 0.25 |
| Ketone bodies (mmol/l) | 1.15 | 0.80 |
| 3OHB:AA | 0.40 | 0.50 |
| Glucose (mmol/l) | 2.40 | 5.60 |

■ **Table 1.4.13** *Data from a patient affected with a pyruvate dehydrogenase defect*

| | Breakfast | | Lunch | |
|---|---|---|---|---|
| | Before meals | After meals | Before meals | After meals |
| Lactate (mmol/l) | 9.30 | 13 | 8.80 | 10.00 |
| Pyruvate (mmol/l) | 1.07 | 1.50 | 0.66 | 0.90 |
| L:P | 8.70 | 8.70 | 13 | 11.00 |
| ACAC (mmol/l) | 0.07 | 0.03 | 0.04 | 0.06 |
| 3OHB (mmol/l) | < 0.02 | < 0.02 | < 0.02 | < 0.02 |
| Ketone bodies (mmol/l) | < 0.20 | < 0.20 | < 0.20 | < 0.20 |
| 3OHB:AA | < 1 | < 1 | < 1 | < 1 |

## Beta Oxidation Defect

Hypoketotic hypoglycaemia was observed in a patient with consanguinity and a history of hypotrophy and neonatal hypoglycaemia. She was hospitalised at 6 months of age for anorexia, hypotonia, progressive asthenia and failure to thrive [15, 16]. Associated findings included liver insufficiency, icterus and hepatomegaly. Metabolic evaluation of lactate, pyruvate, ACAC and 3OHB (associated with increased UEFA; Table 1.4.14) resulted in an evaluation of a fatty acid oxidation defect. Characterization of plasma acylcarnitines and oxidation of long-chain fatty acids (myristic and palmitic acid) in cultured fibroblasts demonstrated a long-chain 3-hydroxyacyl-coenzyme A dehydrogenase defect.

**Table 1.4.14** *Data from a patient affected with a long-chain 3-hydroxyacyl coenzyme A dehydrogenase defect.* UEFA *Unesterified fatty acids*

| | Before meals | 1 h after | 3 h after | 4 h after |
|---|---|---|---|---|
| Lactate (mmol/l) | 1.50 | 2.20 | 1.50 | 2.30 |
| Pyruvate (mmol/l) | 0.15 | 0.18 | 0.15 | 0.18 |
| L:P | 10.00 | 12.00 | 10.00 | 13.00 |
| ACAC (mmol/l) | 0.06 | 0.07 | 0.07 | 0.05 |
| 3OHB (mmol/l) | 0.05 | 0.02 | 0.04 | 0.04 |
| Ketone bodies (mmol/l) | < 0.20 | < 0.20 | < 0.20 | < 0.20 |
| 3OHB:AA | < 1 | < 1 | < 1 | < 1 |
| UEFA (mmol/l) | 1.00 | 0.07 | 1.20 | 3.50 |
| Glucose (mmol/l) | 4.00 | 5.80 | 2.50 | 2.40 |

***Table 1.4.15*** *Data from a patient affected with succinyl coenzyme A:3-oxo acid transferase deficiency*

| | Fed state | After 20 h fasting |
|---|---|---|
| Lactate (mmol/l) | 1.50 | 1.30 |
| Pyruvate (mmol/l) | 0.15 | 0.11 |
| L:P | 10.00 | 12.00 |
| ACAC (mmol/l) | 0.25 | 4.20 |
| 3OHB (mmol/l) | 0.20 | 6.00 |
| Ketone bodies (mmol/l) | 0.45 | 10.00 |
| 3OHB:AA | < 1 | 1.40 |
| UEFA (mmol/l) | 0.10 | 3.00 |
| Glucose (mmol/l) | 4.50 | 3.00 |

## Ketolysis Disorder

### Succinyl Coenzyme A:3-Oxo Acid Transferase Defect

Succinyl coenzyme A:3-oxo acid transferase catalyses the transformation of ACAC into acetoacetyl coenzyme A in the mitochondria of extra-hepatic tissues. This enzyme defect may be suggested in cases of severe ketoacidosis often associated with neurologic dysfunction [16].

The data presented in Table 1.4.15 are derived from a 2-year-old child in the fed and fasting states and document the results of a metabolic evaluation of this patient. Permanent hyperketonaemia increased with fasting, in comparison to UEFA, which are lower in comparison to the KB concentration, suggests a defect in utilizing KBs in the periphery, not overproduction of KBs from UEFA.

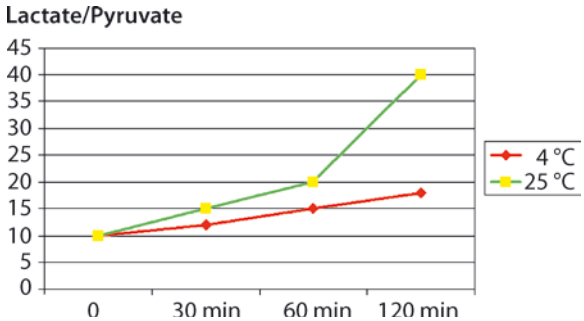


**Fig. 1.4.5** Effects of blood storage temperature and delay between sample preparation and measurement on the lactate:pyruvate ratio

## 1.4.5 Pitfalls

### 1.4.5.1 Pre-analytical Pitfalls

The main sources of variation are linked to the time of collection, conditions of treatment and the transport and storage of the sample [5, 14].

#### Deproteinisation

1. An increased lactate:pyruvate ratio is observed when the delay between the moment of blood collection and deproteinisation exceeds 5 min (Fig. 1.4.5).
2. Inappropriate volume of the deproteinised sample will lead to errors in measurement.
3. Inappropriate pH of the filtrate may reflect a non-uniform dilution ratio between the blood sample volume and $HClO_4$ volume, or can be due to errors in $HClO_4$ molarity or instability due to inappropriate storage of the acid.
4. Lactate, pyruvate, 3OHB and ACAC must be measured using the same deproteinised sample; if not, the lactate:pyruvate ratio may be incorrect.

#### Sample Stability

Pyruvate and ACAC are not stable at 20–25°C.

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