Free fatty acids (plasma, serum)

1 Name and description of analyte

1.1 Name of analyte
Free fatty acids

1.2 Alternative names
NEFA, non-esterified fatty acids

1.3 NMLC code
To follow

1.4 Description of analyte
Free fatty acids (FFAs) are chains of hydrocarbon groups attached to a carboxyl group. They vary in chain length and saturation. Saturated FFAs are the predominant type.

1.5 Function of analyte
FFAs are intermediary metabolites that are formed from the hydrolysis of triacylgycerols (triglycerides) by lipoprotein lipase and hormone-sensitive lipase. FFAs are hydrophobic and are transported in the blood bound to albumin to tissues where they are metabolised in the mitchondria through cycles of β-oxidation to generate energy. They are the preferred fuel type in cardiac and skeletal muscle. In hepatocytes their fate differs depending upon energy needs, hormone balance and substrate availability: they can be used for energy production; re-packaged into triglycerides and exported as very low density lipoproteins or stored within the liver. They are also the substrate for ketone formation.
Although they are an important energy source, saturated fatty acids are not essential to life, whereas certain unsaturated fatty acids are. Unsaturated fatty acids are components of structural (e.g. cell membrane) phospholipids and are precursors of prostaglandins, thromboxanes and leukotrienes.

2 Sample requirements and precautions

2.1 Medium in which measured
Plasma (not lithium heparin) or serum

2.2 Precautions re sampling, handling etc.
- Samples must be taken at the time of hypoglycaemia (see 3.1) to allow accurate interpretation. However, correcting hypoglycaemia should not be delayed if the blood is difficult to obtain.
- Upon receipt, specimens should be centrifuged as soon as possible and frozen if not assayed immediately.
- Heparinised plasma (i.e. from blood taken from patients on heparin therapy or collected into lithium heparin sample tubes) is not acceptable as heparin activates LPL, which increases *in vitro* [FFA].

3 Summary of clinical uses and limitations of measurements

3.1 Uses

Measurement of free fatty acids is part of the National Metabolic Biochemistry Network (metbio.net) algorithm for the investigation of hypoglycaemia in infants and children. Free fatty acids are measured and interpreted in conjunction with β-hydroxybutyrate concentrations during hypoglycaemia.

3.2 Limitations

Measurement of β-hydroxybutyrate is necessary to fully interpret the metabolic response to hypoglycaemia and to determine whether any metabolic defects are present.

4 Analytical considerations

4.1 Analytical methods

Enzymatic colorimetric methods measure total NEFA. The method involves the acylation of coenzyme A (CoA) by the fatty acids in the presence of added fatty acyl-CoA synthetase (ACS, EC 2.3.1 86)). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD, acyl CoA:oxygen 2-oxidoreductase, EC 1.3.3.6)) with generation of hydrogen peroxide. In the presence of peroxidase (POD, EC 1.11.1.7)), oxidative condensation of 3-methy-N-ethyl-N(β-hydroxyethyl)-aniline (MEFA) with 4-aminoantipyrine occurs, to form a purple adduct that absorbs light at 550nm. This allows the concentration of NEFA to be determined from the optical density measured at 540–550 nm.

Methods such as GC, HPLC, and MS can be used to separate and identify specific fatty acid species, although this is rarely necessary.

4.2 Reference method

Gas-liquid chromatography can be used to measure specific fatty acids, although this is not required in routine clinical practice.

4.3 Reference materials

An international reference preparation is not available.

4.4 Interfering substances

Minimal interference from other serum components such as uric acid, ascorbic acid, bilirubin, and haemoglobin (Wako method insert).

4.5 Sources of error

Delay in separation of red cells and improper storage of specimens can falsely increase [FFA]. Long term frozen storage can decrease measured [FFA].

5 Reference intervals and variance

5.1.1 Reference interval

Wako method insert: 0.1–0.6 mmol/L (fasting)

5.1.2 Extent of variation

5.1.2.1 Interindividual CV: 32%

5.1.2.2 Intraindividual CV: 45%

5.1.2.3 Index of individuality: 1.41

5.1.2.4 CV of method: 4.1%

5.1.3 Critical difference: 125%

5.1.4 Sources of variation

Plasma concentrations of FFAs are strongly influenced by nutrition and the effects of several hormones such as insulin, glucagon and adrenaline.

6 Clinical uses of measurement and interpretation of results

6.1 Uses

- The major use is in the investigation of the cause of hypoglycaemia in infants and children.
- High concentrations of FFAs are associated with the clustering of risk factors that comprise the metabolic syndrome, and are associated with increased risk of cardiovascular disease (CVD). However, they are not routinely measured for this purpose as other well-established lipid markers of CVD risk are available, such as HDL:LDL cholesterol ratio and ApoB.

6.2 Confounding factors

Neonates may have a poor physiological response to hypoglycaemia; patients on chemotherapy or parenteral nutrition, and patients with liver disease may produce an unusual pattern of intermediary metabolites in response to hypoglycaemia.

7 Causes of abnormal results

High or low [NEFA] results must be interpreted in conjunction with the results of measurement of glucose and ketones, if investigating for a metabolic defect.

7.1 High values

7.1.1 Causes

- A raised [FFA] indicates a lipolytic response, which is appropriate if the patient is hypoglycaemic.
- If the molar ratio of [FFA]/[βOHB] is >2.0 in the presence of hypoglycaemia, this indicates a fatty acid oxidation defect.

7.1.1 Investigation

Specific acylcarnitine species will be raised if there is a fatty acid oxidation defect (FAOD), although most of these individuals will now be identified through the National Neonatal Screening Programme for medium chain acylCoA dehydrogenase disorders (MCADD).

7.2 Low values

7.2.1 Causes

An absence of lipogenesis or ketogenesis during hypoglycaemia is inappropriate and could indicate hyperinsulinism or possibly panhypopituitarism. [S why 'possibly'? just because v uncommon in infants? W]

7.2.1 Investigation

Detectable insulin in a hypoglycaemic sample indicates hyperinsulinism. Low [cortisol] and [growth hormone] during hypoglycaemia may suggest hypopituitarism. Dynamic function testing is required for confirmation.

7.3 Notes

None

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions
No data available

9 Systematic reviews and guidelines

9.1 Systematic reviews
None identified

9.2 Guidelines
National Metabolic Biochemistry Network Guidelines for the investigation of hypoglycaemia in infants and children.
http://www.metbio.net/docs/MetBio-Guideline-REBA404702-25-05-2009.pdf (Last accessed 15.iii.2012)

9.3 Recommendations
None identified

10. Links

10.1 Related analytes
None

10.2 Related tests
Glucose, ketones, β-hydroxybutyrate

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