

CHRONIC FATIGUE, MITOCHONDRIAL FUNCTION & RELATED TESTS

Chronic fatigue syndrome (CFS) is a syndrome - that is a combination of symptoms and signs that form a particular clinical entity - where the cause(s) are not clearly understood or not identified.

There are a huge number of causes of long-standing fatigue, and it is essential that the possibility of these is considered and conventional tests are carried out to exclude them, when appropriate. This long list includes (this is not a complete list!!):

- Chronic infections, eg: Epstein-Barr (EBV, Glandular fever, HHV-4), Herpes simplex (HHV-1, HHV-2), Herpes zoster (HHV-3), Cytomegalovirus (CMV, HHV-5), Roseolovirus (HHV-6, HHV-7), Toxoplasma, Brucella, Borrelia burgdorferi (Lyme disease), Other tick borne co-infections (eg Babesia sp, Bartonella-like organisms, Ehrlichia, Anaplasma), HIV (HTLV-II), Syphilis, Coxsackie B, Mycoplasma, Enterovirus, Aspergillus, Legionella, Chlamydia trachomatis/psittaci, Parvovirus B19.
- Immune dysfunction.
- Hormonal and metabolic disorders, esp. hypothyroidism, adrenocortical insufficiency, diabetes.
- Autoimmune disease, esp. coeliac disease, multiple sclerosis, systemic lupus erythematosus (SLE), Hashimoto's disease.
- Cardiac, respiratory, kidney, liver, gastrointestinal, neurological, blood (esp. anaemia) diseases.
- Cancers.
- Nutritional, esp. deficiencies of B vitamins (esp. B12 and folate), vitamin D, iron, iodine.
- Depression.

Where no apparent cause is found with conventional tests, we often find one or more of the following may be contributing:

- Mitochondrial dysfunction.
- Intestinal yeast or bacterial overgrowth (dysbiosis, small intestinal bacterial overgrowth, fungal-type dysbiosis).
- Food intolerance (Type B).
- Increased small intestinal permeability ("leaky gut syndrome") - usually due to dysbiosis and/or food intolerance.
- Subclinical: hypothyroidism, adrenocortical insufficiency.
- Unrecognised, usually multiple, nutritional deficiencies - usually due to a malabsorption syndrome (milder than is usually picked up conventionally).
- Unrecognised toxicities, eg. toxic metals, pesticides and other persistent organic pollutants, volatile organic compounds, etc

MITOCHONDRIA

Mitochondria are tiny microscopic sausage shaped structures present in every cell of the body, around 2000 per cell, and they are the source of every cell's energy, whatever the type of cell. Whilst there are many different types of cell in the body (eg. those of muscle, liver, kidney, brain, lymphocyte, etc.), the way in which energy is supplied to them is exactly the same - by mitochondria. One of the main functions of mitochondria is to produce energy for the cell, and this is in the form of a molecule called ATP (adenosine triphosphate). The cell releases the energy from ATP by converting it to ADP (adenosine diphosphate). The ADP is then taken back into the mitochondria where it is reconverted to ATP - this process of recycling ADP to ATP is called oxidative phosphorylation, the Krebs' cycle or the citric acid cycle - and the ATP is exported to the cell again.

We now have a test, called "ATP Profile", which measures certain key aspects of this process in neutrophils (a particular type of white blood cell):

- 1) The total amount of ATP in the neutrophil and the amount of magnesium associated with it
- 2) How efficiently ADP is recycled to ATP
- 3) The efficiency of translocator protein - this is the mechanism by which ATP & ADP are transported across the mitochondrial membrane.

When mitochondrial function is impaired, all muscle function is impaired and this includes heart (cardiac) muscle. Indeed low cardiac output has already been demonstrated in fatigue syndromes and elegantly explains the symptoms these patients suffer from. For example, they have low blood pressure, marked postural hypotension, low blood volume and perfusion defects. Poor circulation of skin would explain cold hands, cold feet and difficulty with temperature regulation, poor circulation of the brain explains the cerebral symptoms and so on. Dr Arnold Peckerman, a cardiologist in America, believes that many of the cardiomyopathies and congestive cardiac failures are not just due to poor blood supply, but poor mitochondrial function. This cardiologist has come up with a cocktail of micronutrients which reverses the cardiac damage, namely magnesium, coenzyme Q10, acetyl-L-carnitine and D-ribose. The effect is not confined to muscles - it affects all cells and organs, including brain, liver, kidneys, etc. so may be relevant to any organ failure/inefficiency and is thought to be one of the main mechanisms underlying the ageing process.

WHAT CAUSES MITOCHONDRIA TO BE LESS EFFICIENT?

At the moment our understanding is that the causes fall into two main groups, either nutritional deficiencies or toxins.

Nutritional Deficiencies

We know that mitochondria have very specific nutritional requirements, which include: Vitamins B1, B2, B3, B5, Biotin, Vitamin C, magnesium, manganese, zinc, iron, copper, carnitine - and deficiencies of one or more of these (usually several) are very common in those with poor mitochondrial function. Mitochondria also require Coenzyme Q10 and D-ribose, and the body's normal ability to make these may become much less efficient (they are not essential nutrients in our diet). If there are nutritional deficiencies one should always try to identify what is causing these, usually one or more of the following: poor diet, poor digestion and/or absorption, increased losses (in urine, diarrhoea, sweat, blood, etc) or increased requirements for some other reason (eg. stress, sleep loss, growth spurts, etc).

Toxins

A wide variety of toxins, both exogenous (coming from outside us) and endogenous (produced by our own faulty metabolism) can impair mitochondrial function. The former include: viral or bacterial DNA &/or RNA, pesticides, certain drugs, azo dyes, mercury, nickel, cadmium, copper, and many more. The latter include: lipid (fat) peroxidation products (diolien, complex aldehydes like malondialdehyde), abnormal proteins, esterases, glutathione conjugates, organic sulphate conjugates, peptide complexes (eg. from leaky gut), lactic acid & keto-acids

Antioxidant Insufficiency

Antioxidants are vital for health and even more vital when mitochondria are functioning poorly and when there is increased toxic load - both increase free radical production, which in turn amplifies any inflammatory / destructive processes taking place. We have many different antioxidants, some are vitamins and minerals that we must obtain from our diet, others are made by our body but may be compromised in ill health or by specific nutrient deficiencies. Nutritional ones include: vitamins A, C, E (tocopherols, tocotrienols), carotenoids, flavonoids. Others include the very important antioxidant enzymes: Superoxide dismutases (requires zinc, copper, manganese), Glutathione peroxidase (requires glutathione, selenium), Catalases (require iron, manganese), and still others include: glutathione, uric acid, lipoic acid, melatonin, coenzyme Q10.

TESTS RELATING TO MITOCHONDRIA

It is thanks to Dr John McLaren Howard's pioneering work, that we now have the following tests to demonstrate mitochondrial lesions - and, at present, they are not done anywhere else in the world!

ATP Profile (Acumen)

This test measures various aspects of mitochondrial function relating to ATP and ADP in neutrophils: whole cell ATP, ATP related magnesium, ADP-ATP reversion efficiency, percentage toxic blocking of active sites and translocator protein. From the results one can calculate a very approximate overall mitochondrial function score. There are a wide range of other tests relating to mitochondria, as detailed below, which can be very helpful in reaching more accurate understanding of exactly what is going on.

Mitochondrial Membrane / Translocator Protein Studies (Acumen)

This test examines mitochondria in much more detail (mitochondrial numbers, mitochondrial structure and mitochondrial DNA), can usually specifically identify toxins blocking the three main areas (oxidative phosphorylation, translocator protein and mitochondrial DNA), and the levels of calcium, magnesium, zinc, potassium (and the pH) associated with mitochondrial membranes.

Cardiolipin Profile (Acumen)

Cardiolipin is one of the three main mitochondrial membrane lipids, it is found only in mitochondrial membranes (and bacteria) and is vitally important to their function. The enzyme that makes cardiolipin (cardiolipin synthase) is manganese dependent and is impaired by manganese deficiency and may be blocked by a variety of other metals.

Mitochondrial Respiration Studies (Acumen)

This test is able to identify when oxidative phosphorylation is uncoupled from the electron transport chain. When uncoupling is present, it is due to toxins. Uncoupling may be present even when the ATP Profile and Translocator Protein Studies appear relatively normal.

RESTORING OPTIMAL MITOCHONDRIAL FUNCTION

Mitochondrial function can usually be restored and symptoms improved by identifying, where possible, the causes and predisposing factors, which may include:

- Identifying and supplementing for deficiencies of nutrients / substrates that the mitochondria require to function (magnesium, d-ribose, vitamins B3, B12, coenzyme Q10, L-carnitine are probably the most important, but deficiencies of vitamins biotin, vitamins B2, B3, B5, C, zinc, copper, potassium, manganese, iron) may also be relevant. One should also, of course, aim to identify and correct the causes of multiple deficiencies - usually dietary and gastrointestinal dysfunction.
- Identifying any toxins that may be significantly impairing mitochondrial function, and then avoiding further exposure and aiding their detoxification / excretion.
- Identifying and supplementing for deficiencies of antioxidants, antioxidant enzymes and their cofactors (esp. superoxide dismutase, glutathione peroxidase, zinc, copper, manganese, selenium, vitamin B12).
- Identifying and addressing stress factors, coping strategies, predisposing factors; improving stress management by careful pacing regimes, relaxation exercises, graded exercise regime, improving sleep patterns, etc.
- Optimising diet.

INVESTIGATIONS AT ACUMEN, BIOLAB AND ELSEWHERE

It must be noted that because Acumen laboratory's work is so pioneering, there is unfortunately nowhere else in the world duplicating and validating their work, as yet. All Acumen's work is based on very sound science - there is plenty of published research concerning the basic principles on which each of the tests is based. Acumen has a document detailing the science and scientific references for each of the tests, which is available to any doctors, on request. If there are any biochemists interested in replicating his work, Dr McLaren Howard would be delighted to help out - we desperately need this invaluable work externally validated.

As a clinician who has been using Acumen's tests for many years on a day to day basis, I can say that, even though there is no external validation, there is a huge degree of internal validation - the tests are highly repeatable, there is frequent confirmation of many of the findings when tested in a totally different ways (this is particularly easy regarding toxic compounds), the results correspond very well with the clinical history and picture of the patients I

see and test, both in the first instance and on follow up testing. I am extremely eager to carry out and publish audit studies - I have such a wealth of important findings that pass through my clinic, but unfortunately at present I am so limited by lack of time, energy and funding - hopefully this will change in the not too distant future.

The Basic Mitochondria Related Tests I like to do, if possible, are:

- **CFS Profile** (Acumen, £125) - comprises the following, at a reduced price, when all done together:
 - **ATP Profile** (Acumen, £85)
 - **Cell Free DNA** (Acumen, £30): indicates whether there is increased cell destruction, whatever the cause - and is consistently elevated in CFS / ME, especially if the sufferer is not giving into the fatigue.
 - **Superoxide Dismutase Studies** (Acumen, £20): an absolutely vital antioxidant enzyme, with three main forms: cytoplasmic zinc/copper-SOD (SOD1), mitochondrial manganese-SOD (SOD2), extracellular zinc/copper-SOD (SOD3). These SODase studies to measure all three forms independently and assess whether the genes coding for them are normal, blocked, deleted or polymorphic. I much prefer this test to the Biolab version.
 - **Vitamin B3 functional test** (Acumen, £20): measures NAD Activation - vitamin B3 is vital for mitochondrial function.
- **Serum L-Carnitine** (Acumen £40): L-carnitine is essential for the transport of fatty acids across the inner mitochondrial membranes. The oxidation of fatty acids inside the mitochondria is a basic and essential source of cellular energy.
- **Coenzyme Q10** (Biolab, £32): an absolutely vital antioxidant enzyme protecting the mitochondria from damage and is intimately involved in oxidative phosphorylation. It's actually made by the mitochondria, so the less efficient they are, the less Coenzyme Q10 they can make - thus a vicious circle!
- **Red Cell Glutathione Peroxidase (GSH-PX)** (Acumen £18): another absolutely vital antioxidant enzyme whose functioning is dependent on adequate levels of selenium - another very common deficiency. At present I prefer the Acumen test, the Biolab one gives very different results.
- **Red Cell Glutathione** (Acumen £18; Biolab, £23): glutathione is essential for detoxification pathways, the antioxidant enzyme glutathione peroxidase, and functions itself as an antioxidant.

More Specialised Mitochondria Related Tests

I usually ask Dr McLaren Howard to do any of these if they are well indicated from the initial ATP profile - as long as the patient is happy for this to be done:

- **Mitochondrial Translocator Protein Studies** (Acumen, £90) (to identify the specific toxins blocking the mitochondrial translocator protein). I request this to be done *only if the ATP profile indicates a toxic block*.
- **Mitochondrial (Leucocyte) Respiration Studies** (Acumen, £120). I usually request this to be done *only if there are indications from the other tests that it might be very helpful*.
- **Cardiolipin Profile** (Acumen, £60). I usually request this to be done *if there are indications from the other tests that it might be very helpful*.
- **Full Intracellular Calcium Studies** (Acumen £80), **Intracellular Calcium Screening Test** only (Acumen £30): elevated intracellular calcium is a frequent finding where there is poor mitochondrial function and can severely impair mitochondrial & cellular function. I usually request this to be done *only if there are indications from the other tests that it might be very helpful*.
- **Actin & Cytoskeleton Investigations** (Acumen £120): disruption of calcium binding to actin can produce an abnormal cytoskeleton which in turn reduces cell motility. I usually request this to be done *only if there are indications from the other tests that it might be very helpful*.

Other Investigations that may be important or /and useful:

NUTRITIONAL STATUS:

- **Vitamins B1, B2, B6 functional tests** (Biolab, about £24 each or £64 for all three): important to check where there are neuromuscular and cognitive symptoms and fatigue; B1 & B2 are vital for mitochondrial function.
- **Vitamin Profile** (Biolab: A, C, E, Carotenes, B1, B2, B6; £108).
- **Vitamin D Profile** (Biolab £46).
- **Biotin** (Biolab, £72): biotin is a less well known B vitamin that is essential for oxidative phosphorylation. The test is a functional one measuring pyruvate carboxylase activation in white blood cells.

- **Serum Vitamin B12 and Red Cell Folic Acid** (can be done by your GP on the NHS, or at Biolab).
- **Active Vitamin B12** (holotranscobalamin - the metabolically active form of serum B12) + **Serum Methylmalonic Acid** (increases with increasing B12 deficiency) - Biolab £140.
- **Serum Ferritin** (can be done by your GP on the NHS at any laboratory, approx. £32): a sensitive test for iron deficiency (iron stores) - which can be missed if anaemia is not present.
- **Plasma Mineral Profile with RBC Magnesium** (Biolab: Ca, Cr, Cu, Fe, Mg, Mn, Se, Zn + RBC Mg, £60)
- **Red Cell Magnesium** (Biolab, £22)
- **Plasma Zinc** (Biolab £22)
- **Plasma Copper** (Biolab, £22).
- **Red Cell Selenium** (Biolab, £22), **Plasma Selenium** (Biolab £22)
- **Plasma Manganese** (Biolab, £22).
- **Red Cell Essential Fatty Acids** (Biolab £60).
- **Urinary Iodine** (Biolab, about £32), **Iodine : creatinine ratio** (Biolab, about £44).
- **Urinary Sulphite** (Biolab £23): the presence of urinary sulphites indicates molybdenum deficiency.

OTHER TESTS

- **Plasma Homocysteine** (Biolab £55)
- **Plasma Histamine** (Biolab £35)
- **Urinary Kryptopyrroles** (Biolab £28)
- **Urinary Organic Acids** - a variety of laboratories offer measurements of a variety of organic acids. These tests give a metabolic “snapshot” based on the products the body discards through the urine. These small organic acid molecules are by-products of human cellular activity, the digestion of foods, and the metabolism of gastrointestinal flora. Certain organic acids can be indicators of toxicities and “markers” of metabolic pathways and nutritional deficiencies. Metabolites of yeast or gastrointestinal bacteria appear against the background of normal human metabolites and provide an assessment of yeast and bacterial activity.
 - **Organic Acid Test** (OAT, 73 compounds, by Great Plains Laboratory, USA, www.greatplainslaboratory.com) via Biolab £160.
 - **Genova Diagnostics**: Metabolic Analysis Profile, about 40 tests, www.gdx.uk.net/index.php?page=shop_product_details&flypage=flypage.tpl&category_id=1&option=com_virtuemart&Itemid=116&product_id=53&vmcchk=1&Itemid=116, about £208
 - **European Laboratory of Nutrients (ELN)**: Organic Acids in Urine, about 74 tests, www.europeanlaboratory.nl/pages/body.php?menu=371&PHPSESSID=bb24f471dcd70154a2daf5d53c1cf182, about €259
 - **Metamatrix**: Organix Comprehensive Profile, about 46 tests, www.metamatrix.com/test-menu/profiles/organic-acids/organix-comprehensive, about £297
- **Carbonic Anhydrase Studies** (Acumen £60) - this test is useful in the diagnosis of hyperventilation - see separate information sheet.
- **Adrenal Stress Profile** - cortisol and DHEA measured in saliva samples taken at four times in the day.
 - Doctor’s Data (via Biolab) £67; or with sIgA £91.
 - Genova £75; or with sIgA £97

For tests relating to gastrointestinal dysfunction and thyroid, please see my separate information sheets: Gut Dysfunction, Hypothyroidism.

TESTS RELATING TO TOXINS AND DETOXIFICATION PATHWAYS:

- **Glutathione S-Transferase Profile** (Acumen, £50): GST is a vital detoxification enzyme family, one of the eight main phase II liver detoxification pathways. Elevated levels indicate increased exposure to toxins that are detoxified by glutathione conjugation. The test measures both total serum and red cell GST levels and affinity chromatography of both can help identify whether toxic exposures are single or multiple; red cell glutathione is also measured. It is a very useful screening tool and often enables specific identification of toxic metals. This is one of my favourite screening tests for toxic metals, along with metallothionein (below).
- **Metallothionein Studies** (Acumen £50): Metallothionein is a family of cysteine-rich enzymes that bind metals. Blood metallothionein is the main transport protein for zinc, copper and toxic metals and appears to be essential

for zinc and copper homeostasis and effective metal detoxification. The test measures the amount of the enzyme and the identity and ratio of the metals bound to it. It can give a useful indication of zinc and copper status and is very useful for identifying the presence of many toxic metals, especially ones one might not have suspected. This is one of my favourite screening tests for toxic metals, along with red cell GST (above).

- **Lymphocyte Sensitivity Test** (Acumen, about £60 for up to 12 substances and £5 extra for each additional substance tested): measures influx of calcium into lymphocytes on exposure to a test substance. Excellent test for assessing sensitivities to metals, pesticides, VOC's, PBBs, phthalates, salicylates, sulphates, benzoates, formaldehyde and other common compounds. Foods and biological inhalant allergens not tested.
- **Toxic Effects Test** (Acumen, about £80 for up to 5 test substances): I consider this test to be a major breakthrough as it is the first clinically available test that actually measures to what extent a potentially toxic substance is currently inhibiting cellular function in a given patient. The test first measures the metabolic activity of mixed white blood cells and then the percentage inhibition of this by the substance(s) being tested. This test was introduced in early 2011 and is proving to be of considerable benefit for: 1) measuring this important marker of how toxic a substance is in the individual patient, 2) prioritising treatment for those substances that have the greatest toxicity where multiple toxins are involved, and 3) monitoring the effectiveness of detoxification.
- **DNA Adducts** (Acumen £95): if toxins are found to be impairing mitochondrial function it is common to find the same toxins on genomic DNA, where they may be blocking important genes. This test identifies whether there are toxins on genomic DNA and whether they are on or close to specific known genes. See separate information sheet.
- **Fat Cell Pesticide & Related Substances Assay** (Acumen, £75): many pesticides are fat soluble and are therefore retained in fat stores. Pesticides are cleared from the blood fairly quickly, so blood levels mainly reflect recent exposure, while pesticide levels in fat cells give a better view of what is stored in tissues).
- **Volatile Organic Substances (VOC's)** (Blood: Biolab £74, Acumen £75; Fat: Acumen £75): quantitative assessment of 20 common VOC's (eg. Benzene, Toluene, Xylene, Styrene, Trimethylbenzene, Dichloromethane, Chloroform, Trichloroethane, Tetrachloroethylene, Dichlorobenzene, Carbon tetrachloride, Ethylbenzene, Trichloroethylene, Bromodichloromethane, N-Pentane, Cyclopentane, 2-Methylpentane, 3-Methylpentane, N-Hexane, N-Heptane)
- **Toxic metal levels:** I choose the laboratory and specific tests on the basis of the range of metals we need to test for - one or more of the following:
 - Hair
 - Urine - preferably before and after a chelating agent, eg. DMSA, N-acetyl cysteine
 - Stool
- **Urinary Porphyrins:** This is a useful initial screening test when xenobiotic toxicity is suspected, particularly for mercury. Specific urinary porphyrin patterns are associated with mercury, arsenic, lead and some toxic chemicals, eg. hexachlorobenzene (HCB), dioxins, polyvinylchloride (PVC), polybrominated biphenyls (PBBs). Urinary porphyrins are oxidized intermediate metabolites of haem synthesis and have been associated with genetic disorders, metabolic disturbances/diseases, poor nutritional status, oxidative stress, and high level exposure to toxic chemicals or metals. Several laboratories offer urinary porphyrin profiling:
 - **Metametrix** www.metametrix.com/content/DirectoryOfServices/0060PorphyrinsProfile, about £133
 - **Doctor's Data** www.doctorsdata.com/test_info.asp?id=125
 - **Laboratoire Philippe Auguste** www.labbio.net/index.php?page=porphyrines_en
- **Melisa Test** (memory lymphocyte immuno stimulation assay, see: www.melisa.org) (through Biolab): this is a sensitivity test that is able to measure immune response to a range of metals and drugs, and also thimerosal, gluten, Candida and Borrelia (Lyme). Considerably more expensive than Acumen's Lymphocyte sensitivity test - but is measuring immune response rather than a cell membrane effect - and it is now widely accepted in conventional circles (Acumen's is not yet).

- **Detoxification Profile** (Genova, about £145): assesses functional efficiency of some of phase 1 detoxification (cytochrome P450 system) and some of phase 2 detoxification (glucuronidation, sulphation, and glutathione and glycine conjugation)
- **DetoxiGenomics** (Genova, about £327): assesses the genes responsible for certain detoxification enzymes: Cytochrome P450 enzyme (8 genes), Glutathione S-transferase (3 GST genes - glutathione conjugation), N-acetyl transferase (7 NAT genes - acetylation), Catechol-O-methyltransferase (COMT gene - methylation) and also Superoxide dismutase (2 SOD1 & 1 SOD2 genes).

RESOURCES

For more detailed information on some of these tests, please see my website.

Information sheets available from my office (full list on my website):

- DNA Adducts
- Toxic metals: Mercury, Nickel, Arsenic, Cadmium, Lead, Aluminium, Chromium VI, Titanium, Tin, Bismuth.
- Other toxins: Volatile organic compounds, Pesticides, Organochlorines, Bromine compounds, Organofluorines, Perfluorinated compounds, Silicones, Para-dichlorobenzene, CTAB (Cetrimide), Formaldehyde, Nitrosamines.
- Detoxification Program

Some Published Research Papers:

- Sarah Myhill, Norman E. Booth, John McLaren-Howard. **Chronic fatigue syndrome and mitochondrial dysfunction.** Int J Clin Exp Med (2009) 2, 1-16. <http://www.ijcem.com/files/IJCEM812001.pdf>
- Norman E Booth, Sarah Myhill, John McLaren-Howard. **Mitochondrial dysfunction and the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).** Int J Clin Exp Med 2012;5(3):208-220. <http://www.ijcem.com/files/IJCEM1204005.pdf>
- Sarah Myhill, Norman E Booth, John McLaren-Howard. **Targeting mitochondrial dysfunction in the treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) – a clinical audit.** Int J Clin Exp Med 2013;6(1):1-15. <http://www.ijcem.com/files/IJCEM1207003.pdf>
- **Acumen Tests are Anchored in the Medical and Scientific Literature** - Dr John McLaren-Howard