MEA Summary Review: Metabolites from ME/CFS implicates 'Redox' imbalance

By: Charlotte Stephens 31st January 2019

In December 2018, Prof. Maureen Hanson and colleagues from Cornell University in America, published results from a metabolomics study. They compared 823 metabolites in patients with ME/CFS and healthy controls.

Although only a few metabolites were found to be significantly different, what many of them shared was an involvement in redox reactions – which has sparked the authors theory.

The study also compared the metabolites found to be different in ME/CFS with metabolite data sets from various other diseases and found some interesting similarities, providing further support for their 'redox imbalance' theory.

They propose that a redox imbalance (explanation of this term is below) is involved in ME/CFS pathology and that this is causing increased inflammation and a lack of oxygen in cells, resulting in impaired glucose (energy) metabolism.

Although a small study, the results seem to be comparable to previous metabolomics findings from other research teams, which is promising.

Larger metabolomic studies are needed in order to define a set of metabolites that are distinct for ME/CFS – that might be used for diagnostic and monitoring purposes – as well as potentially point towards mechanisms of disease pathology and targets for treatment.

Study Highlights

- Several metabolites were identified at significantly different levels in patient's plasma compared to controls
- The findings agreed with those found in other metabolomics studies
- Some of the metabolites found to be different, as well as other disease data sets that matched the results, point towards a theory of redox imbalance
- This evidence of redox imbalance could support theories of the involvement of oxidative stress, inflammation and hypoxia (low oxygen) in ME/CFS disease pathology
- The role of possible nutritional therapy is discussed

What is metabolomics?

Metabolomics is the study of metabolites, which are small chemicals that are used in or produced by various chemical reactions that happen within cells (called metabolic reactions).

There are many different chemical reactions happening in your body all the time, which convert a substance through a series of steps into a product. These conversions are catalysed by molecules called enzymes (see fig 1).

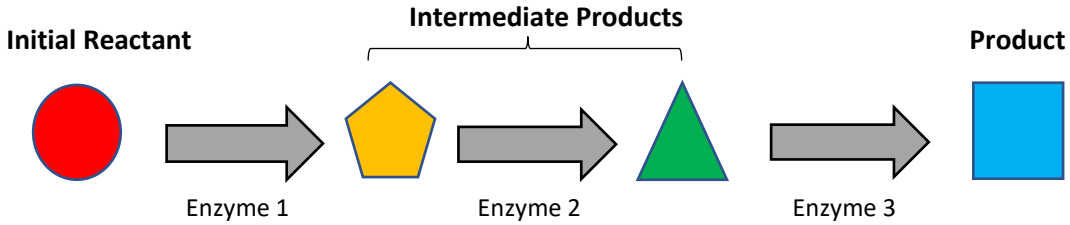


Fig 1. *Example of a metabolic pathway*

These different chemical reactions of metabolism are organised into metabolic pathways and given specific names. For example, glycolysis is the name given to the metabolic pathway that converts glucose from food into energy, which happens inside the mitochondria of cells.

Thousands of metabolic pathways exist and most involve multiple steps – some can be hundreds of steps long (see fig 2.). Therefore, there are thousands of different metabolites to study, making this a very complex area of research.

Researchers can measure the levels of particular products of metabolic pathways to see if some of the pathways are 'dysfunctional' or if something is out of balance (such as one substance being produced in too high amounts, resulting in a depletion of another substance).

Identifying metabolites that are increased or decreased compared to healthy controls can help in highlighting which pathways in the body may be affected by a particular disease.

These differing levels of metabolites can then be used as biomarkers for diagnosis, as well as being used to monitor the effectiveness of a therapeutic treatment.

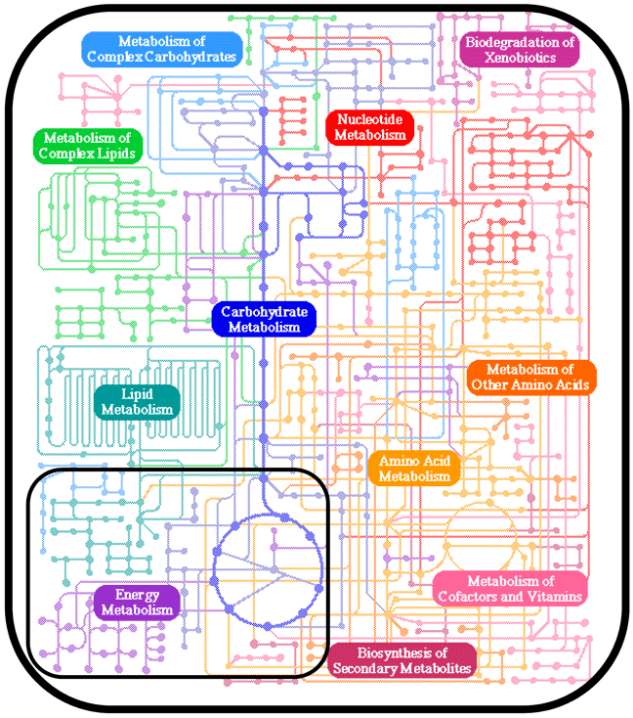


Fig 2. *A simplified map of the metabolic pathways within a cell. Source: www.urmc.rochester.edu*

What is 'Redox' and 'redox imbalance'?

'Redox' is short for 'Reduction-oxidation' reaction. These are chemical reactions that happen in various metabolic pathways. They involve the transfer of tiny, negatively charged particles called electrons (Ray *et al.,* 2012)

These reactions change the 'oxidation state' of a molecule; oxidation is a *loss* of electrons and reduction is a *gain* of electrons. Both of these things happen at the same time during the reaction – so one molecule will gain electrons from the other (see fig 3).

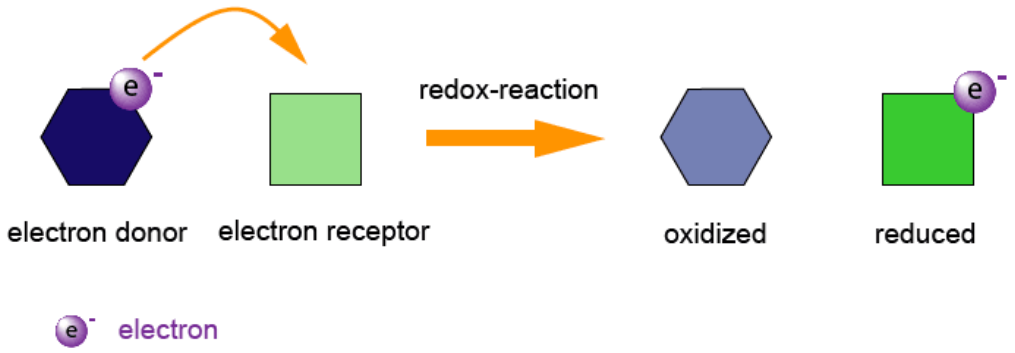


***Fig 3**. Representation of a redox reaction.*

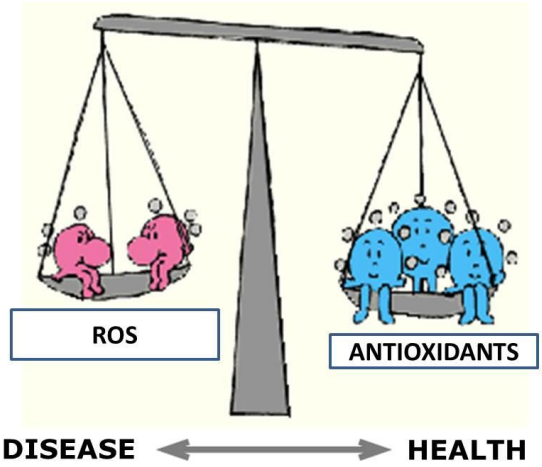
Redox reactions take place in our cells and are important for energy production. They are involved in glycolysis (the breakdown of glucose, or "food molecules", into energy).

The electrons move from glucose molecules to oxygen molecules, and it's this movement that *releases energy*. Simply put, redox reactions are how cells get energy out of glucose!

Redox imbalance

The body works to keep redox reactions in balance.

It does this through the activity of antioxidant enzymes (as well as antioxidants derived from food) in order to 'neutralize' the pro-inflammatory 'reactive oxygen species' (ROS) that are produced by redox reactions (Schieber and Chandel, 2014).



Source: antioxidants.wordpress.com

A disrupted (imbalanced) redox environment inside a cell can lead to high levels of ROS. When they are not neutralized by antioxidants, ROS can be very toxic to cells and can damage DNA and proteins.

Cells with high numbers of ROS are said to be in a state of 'oxidative stress' (Dewane and Pandit, 2012).

Redox imbalance can be caused by a number of stressors on the body. Factors that can alter the 'Redox state' include: infection, exercise, heat, dehydration, poor nutrition, psychological stress and trauma (Sies, 2015).

Redox environment is significantly altered in disease states, such as cardiovascular disease and diabetes, as well as in autoimmune inflammatory diseases (Kumar *et al*., 2010; Uttara *et al*., 2009).

What did the study involve?

The hypothesis for the study was that the homeostasis (keeping in balance) of metabolic networks in ME/CFS patients is disrupted.

832 different metabolites were measured from the plasma of 32 female patients meeting the Fukuda definition for ME/CFS and 19 female healthy controls.

The metabolites were grouped into 8 different biological classes of 'common pathways' they are involved in. These groups were: Amino acids, Carbohydrates, Cofactors and Vitamins, Energy, Lipids, Nucleotides, Peptides and Xenobiotics.

Explanation of terms

Amino acids are the building-blocks of proteins, Cofactors are molecules needed for certain reactions to take place, Lipids are fats, Nucleotides are the building-blocks of DNA, Peptides are small proteins and Xenobiotics are involved in the metabolism of substances such as chemicals and drugs.

Differences in metabolite levels between the patients and controls were compared.

The research team then entered their data into a computer program that compares metabolite datasets from many different diseases and finds similarities between them.

What did they find?

Initial analysis revealed 9 metabolites that were significantly different in abundance between the two groups (see fig 4). Those 9 metabolites belonged to the following categories; Cofactors and Vitamins, Energy, Nucleotides and Peptides.

One of the most significantly different metabolites identified, with *higher* abundance in patients compared to controls, was Heme – the pigment that gives blood its colour. Heme has various functions, all relating to redox reactions. Free Heme is highly toxic to cells and is pro-inflammatory (Chiabrando, 2014).

Alpha-CEHC, Gamma-CEHC and Gamma-CEHC glucuronide were all *lower* in patients and are all metabolites that belong to the vitamin E pathway. This pathway and these metabolites have antioxidant and anti-inflammatory affects. Therefore, reduced levels of these may suggest that reactive oxygen species may not be affectively neutralised- leading to increased inflammation in the body.

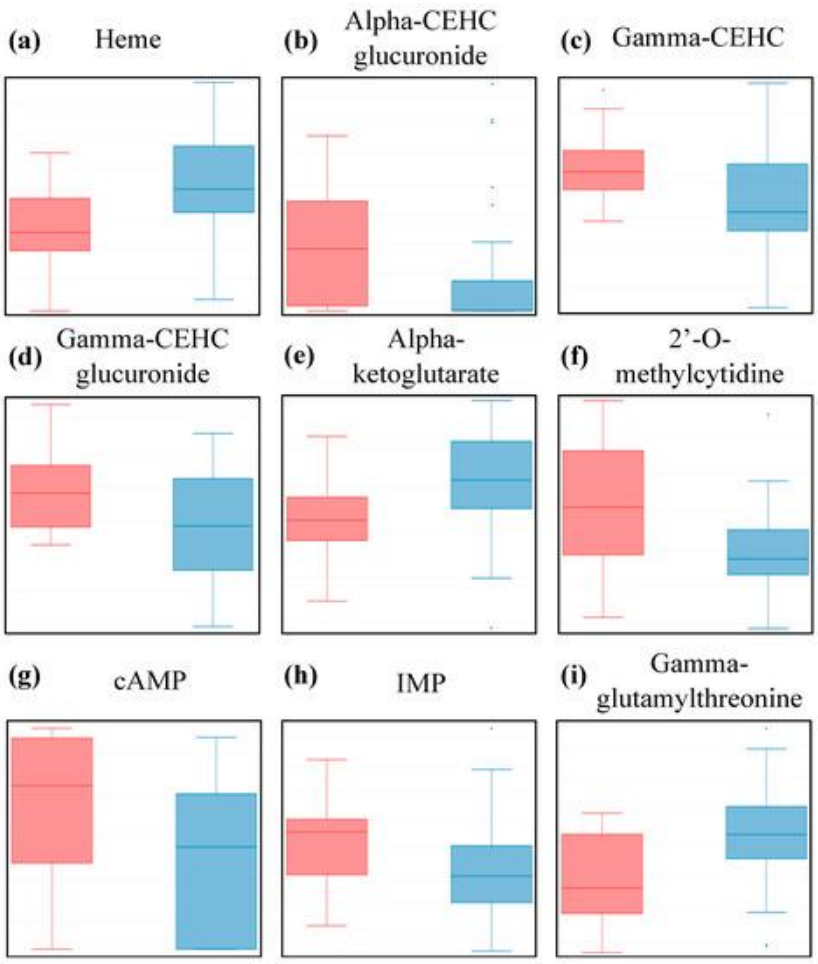


Fig 4. *A box plot of the 9 metabolites that were significantly different between the controls (red) and the patients (blue).*

Alpha-ketoglutarate is one of the 'energy' metabolites and was found to be *higher* in patients. It is found in many biological pathways and plays a role in reactions involved in energy production in the mitochondria.

Higher levels of this could suggest one of two things; that either the reactions involved in energy production are happening a lot more than usual, in order to produce more energy, or that the compound is not being utilized in these reactions and so there is excess.

2'-O-methylcitidine, cAMP and IMP belong to the 'Nucleotides' class and are *lower* in patients. Not much is known about 2'-O-methylcitidine. cAMP and IMP are both linked to energy metabolism. cAMP is also known to be a regulator of hormonal pathways.

Finally, Gamma-glutamyl-threonine was significantly *higher* in patients than controls. Little is known about its affects. However, interestingly, it is cited in a patent as a potential biomarker for liver toxicity (Milburn *et al.,* 2014)

Further exploration of the data

Further analysis revealed some other significantly different metabolites, which were all in *higher* abundance in patients compared to controls. These included TUDCA, a cell-protective agent, BHBA, involved in ketosis (a form of energy metabolism where fat is the main energy source instead of glucose), Piperine, found in herbs and spices, and Histamine, a compound involved in many aspects of the body, including immune responses.

Continuity with previous studies

The researchers used a programme to compare their results with other similar metabolic studies and found very comparable results, with not many metabolites differing from one study to the next.

> The authors comment "The patient cohorts that have been used in our studies and others differ in geographical location, diet, treatment regimes, yet there are remarkable similarities among the findings".

This level of continuity is rare in ME/CFS research, which is promising and offers some real hope from the field of metabolomics.

Comparing to other diseases

The researchers used an analytical tool to compare their metabolite data set with other known disease-associated metabolite sets.

> The authors note, "While this tool identifies particular metabolites that vary similarly in other diseases to alterations detected in ME/CFS, it is not intended to be used for implying that all ME/CFS metabolite alterations are identical to those that occur in particular other diseases."

10 metabolite sets were identified as being similar to the ME/CFS dataset (listed in the table below). Interestingly, 4 out of the 10 diseases identified involved deficiencies in enzymes (dehydrogenase, oxidase, transferase) that may be linked to redox imbalance.

Deficiencies in these areas had also been identified by previous metabolic studies in ME/CFS (Armstrong *et al.*, 2015; Fluge *et al*., 2016).

Disease-associated metabolite sets with similarities to ME/CFS dataset (number of similar metabolites out of total metabolites in disease set)	
Anoxia (4/8)	CPT II transferase deficiency (4/8)
Heart failure (3/10)	LCHAD dehydrogenase deficiency (2/10)
P-5'-P oxidase deficiency (2/3)	CoA transferase deficiency (1/3)
Primary systemic Carnitine deficiency (1/4)	Persistent hyperinsulinemic hypoglycaemia of infancy (1/3)
Diabetic Ketoacidosis (1/2)	Obesity (1/2)

Anoxia is an extreme form of hypoxia – a state in which a region of the body has extremely low levels of oxygen – which has been previously highlighted in regard to ME/CFS pathology, and was touched on in our review of the recent study on red blood cell deformability.

It was suggested that this low oxygen status of tissue could be due to the presence of too much Heme, as found to be the case in ME/CFS.

Symptoms of hypoxia include fatigue, confusion, headaches and numbness of extremities, which are also symptoms of ME/CFS. Reduced cerebral blood flow, which could result in inadequate brain oxygenation, as well as reduced blood volume, have both been found in ME/CFS (Streeton and Bell, 2011)

Ketosis and diseases relating to ketosis was a recurring find, also supported by the earlier findings of elevated levels of BHBA – involved in the process of ketosis. This finding suggests a disturbance in the utilisation of carbohydrates for energy in ME/CFS.

Correlations with metabolites of other diseases included seizures and epilepsy, which may suggest a possible link to altered brain function, as well as several syndromes involving eye health, with eye symptoms and sensitivity to light being common in ME/CFS.

Comments and criticisms

It must be noted that significant differences in metabolites were not found by just comparing the two groups.

The researchers had to group the metabolites into the 'super pathways' first before any differences were found and they also carried out multiple analysis techniques on the data set.

This was most likely because the sample size was too small for the number of metabolites they were looking at.

However, despite the fact that multiple analysis had to be carried out in order to identify differences, the main findings of this study did appear to agree with findings from other studies, which is promising.

And yet the ranges in the patient data overlap with the control data in all of the identified metabolites, potentially making it difficult for any of them to be used as reliable biomarkers.

In order to determine their reliability as biomarkers, they would need to be measured again in a much larger cohort, as well as be measured against people with other chronic or fatiguing illnesses.

The sample size of the study is relatively small, making it hard to draw accurate conclusions. Also, as always, it would have been better to have used a 'sedentary' control group for better comparison with ME/CFS.

Finally, to be sure that the changes in metabolites correlate with symptoms experienced by patients and are a cause, and not an effect, of having ME/CFS, you would need to observe them over time to see if the metabolite levels change when symptoms become worse or are alleviated.

It would be interesting to see if the levels of these metabolites become more distinct from controls during an episode of PEM for example.

What could these findings mean?

As most of the metabolites found to be different either have multiple known actions within the body or are relatively unknown, it is very difficult to pinpoint a singular pathway involved in ME/CFS pathology.

Additionally, jumping to conclusions about what a particular metabolite's presence might mean can be a bad idea.

The main theory the authors have is that the differences in metabolites point towards a redox imbalance, leading to an abundance of ROS, resulting in oxidative stress, which may contribute to a state of hypoxia (shortage of oxygen).

Hypoxia results in further generation of ROS by mitochondria, resulting in the activation of protective systems (which are often inflammatory) (Smith *et al.,* 2017). Oxidative stress has been previously associated with ME/CFS in a number of studies (Morris *et al*., 2014)

Many studies in ME/CFS point towards a state of hypoxia (lack of oxygen). Patient response to exercise demonstrates an inability to affectively deliver oxygen to muscles. Reduced blood volume has been exhibited in ME/CFS patients, affecting the oxygenation of many tissues (Biswall *et al.,* 2011).

> The authors comment: "Disturbances in circulation and provision of oxygen to tissues could underlie many symptoms of ME/CFS."

This lack of oxygen in cells in turn impacts glucose (energy) metabolism, as the chemical reactions involved in the process only happen in the presence of oxygen.

A role for nutrition?

Many ME/CFS patients self-report finding symptom-relief through specialized diets and supplements.

The adoption of restrictive diets, such as gluten/dairy free, or ketogenic diets and fasting have been reported as helpful by some patients (Craig, 2015). Supplements such as NADH, coQ10 have also been found to be helpful.

However, systematic reviews of these methods has not led to clear recommendations to the patient community (Jones et al., 2017).

The authors pointed out that a commonality between all of the nutritional management strategies in ME/CFS is their effects on redox metabolism.

> The authors comment: "Clearly, our current work as well as other reports suggests that nutritional alterations might be of assistance to patients, though further research is necessary before any recommendations can be made."

Some of the results suggest a problem with carbohydrate metabolism and instead a utilisation of the state of ketosis (using fats as a primary energy source, instead of carbohydrates). Many patients find ketogenic diets helpful and they are widely used in other chronic conditions (Craig, 2015).

However, as ketogenic diets are very restrictive and are often high in saturated fats, they may not be very healthy long-term and are often not sustainable. Additionally, they are primarily used for weight loss and so this would not be suitable for those who struggle to keep weight on (Campos, 2017).

Source: 123rf.com

Antioxidants are known to reduce inflammation and oxidative stress through helping to balance redox states. Therefore, dietary inclusion of these may help alleviate symptoms. (Lobo *et al.,* 2010; Arulselvan *et al*., 2016).

Polyphenols, carotenoids and vitamins C and E are all powerful antioxidants.

Foods high in these include: Flaxseeds, sunflower seeds, almonds, dark chocolate, celery, carrots, sweet potatoes, winter squash, dark leafy greens, tomatoes, bell peppers, brussel sprouts, broccoli, cauliflower, oranges, mangoes, kiwis, berries, avocadoes and olive oil (Carlsen *et al.,* 2010; Hussain *et al.,* 2016).

Conclusion

This study adds to the rapidly growing metabolomics data in ME/CFS and may help bring us one step closer to unravelling the dysfunctional pathways behind the pathology of ME/CFS.

The findings add further weight to the hypotheses of redox imbalance, oxidative stress and hypoxia. But larger studies are needed in order to identify a reliable biomarker for the diagnosis, monitoring and treatment of ME/CFS.

> The authors concluded: "ME/CFS biomarkers, as a mean of unambiguous diagnosis and monitoring of efficacy of therapies, are one of the urgently needed developments in this field."
>
> "Future work in which a larger and independent cohort is analyzed and compared to other fatiguing illnesses… will reveal whether plasma metabolomics may serve as a reliable tool for objective identification and monitoring of ME/CFS patients."

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