



ME Association Summary Review: Changes in ‘brain chemistry’ after exercise in CFS, Gulf War Illness and sedentary controls

By Charlotte Stephens, 5th December 2017.

A [recent paper](#), from Professor James Baraniuk and Assistant Professor Narayan Shivapurkar of Georgetown University in America, reported differences in the levels of small molecules, called microRNAs, in the brains of people with chronic fatigue syndrome (CFS) and Gulf War Illness (GWI), after exercise. The accompanying [press release](#) from Georgetown University is available on the ME Association website.

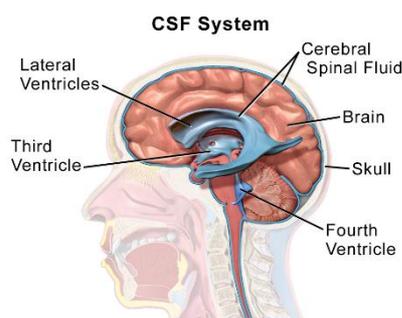
The study of miRNAs is a relatively new field, and this was the first paper to study the effects of exercise on miRNA concentrations in cerebrospinal fluid taken from sedentary controls and people with chronic fatigue syndrome.

Other studies have revealed changes in levels of circulating miRNAs in the blood, that are associated with several diseases – depression, Alzheimer’s and cancer – as well as in samples taken under physiological conditions such as pregnancy or physical exercise.

There have also been a couple of studies on miRNA levels in the blood of people with chronic fatigue syndrome. One found reduced expression of several miRNAs (Brenu *et al.* 2012), and another found increased levels of miRNAs (Petty *et al.* 2016).

However, none have examined miRNAs in cerebrospinal fluid, before and after exercise, or sought to differentiate CFS from GWI, or used a sedentary healthy control group as a comparison.

What are microRNAs (miRNAs)?



miRNAs are small molecules involved in regulating gene expression; they can repress or turn off the production of certain proteins. There may be up to 1,000 different types of miRNA’s, mainly present inside cells but also found floating in bodily fluids, such as blood and cerebrospinal fluid (the fluid that surrounds the brain and spine).

Each miRNA can influence hundreds of different genes. These genes are involved in a wide range of biological processes, such as cell growth, cell death, cardiac and skeletal muscle development, neurone growth, insulin secretion, cholesterol metabolism, aging, immune responses and viral replication.

The first disease to be associated with deregulation of miRNAs was a type of leukaemia (bone cancer). Changes in just one of these small molecules can have a big impact; a mutation in miR-96, for example, can cause progressive hearing loss, and deletion of miR-17 can cause growth defects.

miRNAs also appear to regulate the development and function of the nervous system and studies have found altered levels of miRNA in bipolar disorder and depression. Several different miRNA's have been linked to cancer and heart disease, and researchers are examining their use as biomarkers for diagnosis, as well as possible targets for treatment.

miRNAs were only discovered 15 years ago, and so research in this field is relatively new. On top of this, studying miRNA's can be very difficult as they are very small and hard to detect. They are also very similar to one another, so identifying individual mRNAs – and identifying what function they serve – can be quite challenging.

Background

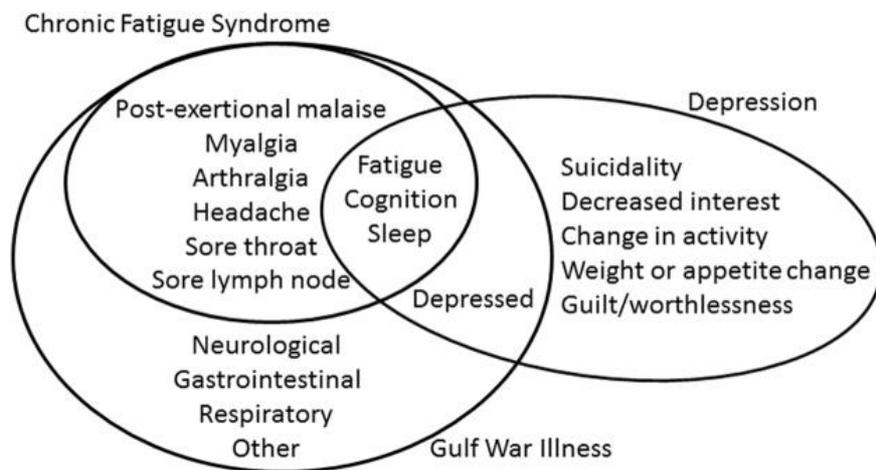
Many studies have shown the effects of exercise on miRNA concentrations, and it is generally believed that the *benefits* of exercise seen in many health conditions are because of changing levels of miRNA's.

Recent publications have also highlighted the involvement of miRNAs in regulating the immune response induced by exercise (e.g. Makarova *et al.* 2014). Studies have also found that different types and durations of exercise, change miRNA levels in different ways (Silva *et al.* 2017).

Gulf war illness shares similar symptoms with ME/CFS (see image below), and was also believed to be a psychological illness as no biological cause had been established. This led researchers and clinicians in America to link the two diseases and wonder if there was in fact a common cause.

However, this latest study clearly shows that CFS and GWI are distinct from both the sedentary healthy control group, and from one another.

The authors also identified two distinct subgroups of GWI, separated by the presence, or absence, of exercise-induced tachycardia, and differences in brain use. These subgroups demonstrated differing results, further confirming their heterogeneity.



Symptom overlaps of CFS, GWI and depression, taken from Baraniuk *et al.* 2017

Researchers also believe that at least two different subgroups of ME/CFS exist, and that identifying these may help with more relevant investigations and therapeutic targeting. But, science has yet to determine how best to define them using biological markers.

Overview of the study

CFS and GWI patients completed questionnaires relating to the CDC case definition criteria (below) of their respective illness. All participants also completed questionnaires for quality of life, generalised anxiety disorder, depression and fibromyalgia. Participants were of similar age (an average of around 45).

‘The 1994 Center for Disease Control (CDC) criteria for CFS are: (a) moderate or severe, persistent and sustained fatigue lasting more than 6 months and causing impairment of daily activities, plus (b) moderate or severe complaints of at least 4 of 8 ancillary criteria: short term memory or problems with concentration, sore throat, sore lymph nodes, myalgia, arthralgia, sleep disturbances, new onset headaches that include migraine, and post-exertional malaise (Fig. below). Post-exertional malaise, also referred to as exertional exhaustion, is a unique characteristic of CFS that is shared by GWI subjects.’

Quality of life was significantly impaired, and fibromyalgia was more prevalent in the CFS and GWI groups compared to the sedentary controls. Depression was found in 78% of the GWI group, 64% of the CFS group, and 25% of the controls.

The higher levels of depression found in GWI and CFS were deemed most likely due to the overlapping symptoms of fatigue, sleep, and cognition, that are found in the questionnaires.

Patients with CFS, GWI, and sedentary healthy controls (SC), were split into two groups; a non-exercise group – that was tested after a night of resting – and an exercise group.

The exercise group underwent a submaximal bicycle exercise stress test on 2 consecutive days. They cycled at 70% of their age-predicted maximum heart rate for 25 minutes, followed by small increases in the bicycle's resistance, up to 85% of their maximum heart rate.

Both groups had lumbar punctures taken, to measure a variety of proteins in the extracted cerebrospinal fluid (CSF), as well as levels of miRNA's. Each group also had an MRI before and after exercise.

The authors hypothesised that there would be differences in miRNA levels between:

1. The SC, CFS and GWI groups at baseline (in the non-exercise group)
2. The SC, CFS and GWI post-exercise groups
3. Each post-exercise group and its matching non-exercise control group.

However, measures of cerebrospinal fluid total protein, albumin (a specific type of protein), and IgG (a type of antibody), and *baseline* levels of miRNA's (from the non-exercise groups) were *identical* between all three groups.

Significant differences were only observed *post-exercise* in the CFS and GWI groups and were not seen in the sedentary healthy controls (SC). The results suggest, therefore, that miRNA's may not be useful as a diagnostic tool for CFS, or GWI, but could instead be used to demonstrate the effects of post-exertional malaise (PEM).

Results and implications

There were no differences seen in miRNA levels between the *non-exercise* SC, GWI and CFS groups. Therefore, baseline levels of miRNA in cerebrospinal fluid cannot be used as a diagnostic marker for CFS or GWI.

Levels of miRNAs were also the same between the *post-exercise groups*, with only a slight difference seen between the two separate phenotypes of GWI in one miRNA. The differences in miRNAs were only seen when comparing each post-exercise group with its respective non-exercise comparison group;

- **Elevations:**

- Exercise elevated the levels of several miRNA's in the *sedentary control* exercise group, compared with the control non-exercise group.
- The post-exercise *GWI* group also showed elevated levels of some miRNAs compared with the *GWI* non-exercise group.
- The post-exercise *CFS* group did not have any elevations of miRNA levels compared to the *CFS* non-exercise group.

- **Reductions:**

- All the *post-exercise* groups (SC, GWI and CFS) had significantly reduced levels of 2 miRNA's (miR-328 and miR-608) compared to their respective non-exercise groups. These may be *general markers of exercise effects* on the brain that affect everyone, regardless of their disease status.
- The *GWI* and *CFS* post-exercise groups had reduced levels of 3 different miRNAs (miR-let-7i-5p, miR-200a-5p, and miR-93-3p) compared to their non-exercise groups. These miRNAs were unchanged in the sedentary control groups, meaning these miRNAs could be demonstrating some model of PEM.
- *CFS* was distinguished from the other groups by having notable reductions in 12 miRNAs (significantly; miR-126-5p, miR-186-3p, miR-19b-3p, miR-92a-3p and miR-505-3p) compared to the non-exercise *CFS* group. "The large number of exercise-induced reductions in miRNAs differentiated *CFS* from *SC* and the *GWI* phenotypes."

So, what do each of these miRNA's do?

Although we do not yet know the exact physiological functions of each individual miRNA, we have some information from existing studies:

miR-let-7i, which was reduced in both GWI and CFS post-exercise, has been found to be reduced in the blood of athletes after exercise but has also been found to be decreased in rat models of depression. miR-let-7i is thought to target IL6 (a type of inflammatory cytokine), and so decreased levels of this miRNA may lead to *increased* levels of IL6 (possibly leading to increased inflammation). It has also been shown to represses T-cell activation and production of IL-8 (a molecule that attracts immune cells) and HMGB1 (a pro-inflammatory cytokine), and so, again, decreased levels of miR-let-7i could lead to *increased inflammation* (Jickling *et al.* 2016).

miR-126-5p, which was reduced in CFS, may be anti-apoptotic (prevents cell death), and promote the formation of blood vessels (Schober *et al.* 2014). Therefore, since it's reduced in CFS, this *could* mean there is more cell death and less blood vessel formation taking place. It has also been shown to repress the migration of leukocytes (immune cells) across the blood-brain barrier (layer of cells that controls what moves in and out of the brain) (Poissonier *et al.* 2014). So, if levels of miR-126-5p are reduced, this could mean that leukocytes (immune cells) may be allowed to cross the blood brain barrier into the brain more freely. This *might* then support theories of *neuroinflammation* (inflammation of the nervous tissue) in the development of CFS.

miR-186-3p, which was reduced in CFS, is also decreased in aging mice, and has been linked to the breakdown of certain proteins (Amyloid peptides) that increase the risk of brain disease, such as Alzheimer's.

miR-196-3p, which was reduced in CFS, is also reduced in serum from Alzheimer's patients.

miR-92a-3p, also reduced in CFS, was increased in a type of cancer and was shown to reduce tumour apoptosis (cell death) (Songh *et al.* 2016). Therefore, its reduction in CFS after exercise *may* promote apoptosis (cell death).

miR-505-3p, also reduced in CFS, has been associated with regulating cytokines and its reduction has been linked with *increased inflammation* (Escate *et al.* 2017).

miRNAs appear to play a large role in protecting the blood-brain barrier (which controls what is allowed in and out of the brain). Exercise could decrease levels of miRNAs that usually protect this barrier, allowing things to move into the brain.

In some cases, this may be a good thing and could explain why exercise has a positive impact on depression for example. However, in the case of CFS, it *could* be allowing immune cells and pro-inflammatory cells into the brain, as well as *possibly* causing increased cell death.

Differing levels of miRNAs have been reported in depression, fibromyalgia, and Alzheimer's, however these miRNA changes were not found in any of the groups in this study. This could provide further evidence that these are all separate conditions, or it could mean those results were not reproducible due to small sample sizes and the different methods employed.

Comments

The use of sedentary healthy controls was good as it rules out the differences in results being due to deconditioning/low fitness levels. It was also good that the authors made everyone exercise to the same amount, so differences in results can't be contributed to people trying harder or not trying hard enough.

There were some issues with sample size numbers, for example, there were 43 CFS patients in the non-exercise group but only 16 in the post-exercise group. This gives a very small sample size in the post-exercise group, which could have affected the results. This could have been because there were less willing or able volunteers to do the exercise stress test, due to the significant and often long-lasting negative affects it can have on people with CFS.

This study was very intensive for CFS patients, and those able to cycle at 70% of their maximum heart rate for 25 minutes were likely to be of mild-moderate severity. However, if studying this severity group produced these results, it would be very interesting to see the results in a severe cohort – although studying this group would be challenging, given the relative inability to carry out an exercise stress test. So, perhaps we need to develop a more suitable exercise-test that would allow everyone with ME/CFS to take part, regardless of severity, and which would elicit the kind of miRNA expressions we have seen above.

It is a shame that the non-exercise and post-exercise results were from completely different people; it would be better to observe the before and after effects of exercise on the same people. However,

this may not have been possible as it may be unsafe to carry out two lumbar punctures in close succession. It would therefore be good to see a repeat of this study using blood samples, instead of cerebrospinal fluid, as they would be easier to extract and monitor before and after exercise.

Measuring miRNA in cerebrospinal fluid isn't ideal because it is an invasive procedure. It would be better to instead focus on miRNAs circulating in the blood. In fact, it is quite surprising that the effect of exercise on blood levels of miRNA in CFS has not yet been studied.

Although MRIs were taken before and after exercise, the results of these were not presented in this paper and so perhaps there will be another paper yet to come from the researchers.

Conclusion

This study is yet another to add to the many great pieces of biomedical research to have emerged this year, further supporting what we already know; chronic fatigue syndrome is very real and not 'in the mind' but – in the brain.

Significant pathophysiological differences were demonstrated between CFS and GWI, distinguishing them as two separate conditions. The paper concluded:

'Despite the symptom overlap of CFS, GWI and other illnesses in the differential diagnosis, the distinct exercise-induced miRNA patterns in cerebrospinal fluid imply separate mechanisms for post-exertional malaise in these diseases.'

Although these results do not point towards a diagnostic marker or treatment yet, it certainly adds to the knowledge of potential brain inflammation and demonstrates the very real effects of PEM. As the field of miRNA research expands, so will our knowledge of what these results mean for people with ME/CFS.

The ME Association has provided a research grant to Professor Elisa Oltra at the [University of Valencia](#) in Spain, to study miRNA in the blood of severely affected patients, using samples from the UK ME/CFS Biobank. Although this research will not be looking at miRNA expression before and after exercise, it is hoped that it will reveal biomarkers similar in scope to previous research from this team that examined miRNA expression in fibromyalgia.

Professor Maureen Hanson's team from [Cornell University](#) in America, is also examining miRNAs in the blood of people with ME/CFS as part of the NIH collaborative research centre project. They will be using exercise stress-tests to help determine if resultant changes in miRNA expression cause a dysregulation of the immune system. This aspect of the project is led by Dr Andrew Grimson whose lab specialises in the genomics of gene regulation.

CSF image credit: Medical gallery of [Blausen Medical](#) 2014

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