

**Lay summary: Indistinguishable mitochondrial phenotypes after exposure of healthy myoblasts to myalgic encephalomyelitis or control serum**

**Background:** Myalgic Encephalomyelitis (ME), also known as Chronic Fatigue Syndrome, is a poorly understood disease with no diagnostic test that affects up to 400,000 individuals in the UK. Previous small studies have reported that a part of the blood, serum, from people with ME can change how healthy cells look or behave. These studies grew healthy cells in the lab and then compared them after exposure to serum either from people with ME or from healthy donors. The reported differences in cells after exposure to serum suggested that there was something in the ME serum changing the cells. Such ME blood factors could be used to develop a diagnostic test for ME.

**Our study:** In this study, we performed a large-scale replication of an experiment from Fluge et al (2016) that reported a change in the mitochondria of healthy muscle cells after they were exposed to serum from people with ME, compared to when they were exposed to serum from healthy individuals. We reperformed the original experiment with a larger number of study participants, using serum from 67 people with ME and 53 healthy donors, and generated results for over 1,700 individual mitochondrial function tests. We observed no significant differences in mitochondrial function between healthy muscle cells treated with serum from people with ME versus serum from healthy donors.

**What does this mean?** Results from our study provide strong evidence that the original finding from Fluge et al. is not true for people with ME in general. This does not mean that there are no ME blood factors, because it may be that people with ME needed to be experiencing a flare-up of symptoms or a crash when their blood was taken for us to have seen the impact of ME blood factors, or that different types of cells or tissues are affected by ME serum. Indeed, it may be that the blood is the wrong place to look for these factors. Nevertheless, our original hypothesis that serum from ME patients changes the mitochondria in healthy muscle cells appears not to be true in general, and future studies should use different methods to look for ME blood factors.