

Evaluation of Mitochondrial Function in Disorders of Iron

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Synopsis:

The occurrence or consequence of mitochondrial dysfunction in human diseases has not been studied comprehensively owing to a lack of suitable assays. Recent technological improvements, particularly the development of antibodies directed against mitochondrial protein subunits and native gel electrophoresis, have led to several-fold improvement in the sensitivity of the assays. These advances now make it feasible to use a small amount of peripheral blood to study the mitochondrial respiratory complexes.

Iron is an essential metal required for oxygen transport and diverse metabolic processes in the cells. Both iron deficiency and excess are known to increase oxidative stress and mitochondrial dysfunction in animal models. Alteration in body iron would have a profound effect on the synthesis and activity of iron-containing enzymes involved in cellular energy metabolism. Our hypothesis is that iron disorders (deficiency or excess) affect activity and relative abundance of the respiratory complexes in platelet-derived mitochondria, which can be used to develop and validate assays measuring mitochondrial health.

Specifically, we propose to use peripheral blood samples from individuals with iron deficiency (either nutritional or blood loss) and overload (thalassemia and sickle cell anemia). We will compare the relative abundance and activity of respiratory complexes with normal controls. Since the assays require only a small amount of blood, we will use the leftover EDTA blood samples obtained from the clinical laboratory after the complete blood count has been performed.

We will separate platelets derived from peripheral blood and measure the following:

1. Relative abundance of mitochondrial respiratory complexes with blue-native polyacrylamide gel electrophoresis and a new dipstick assay that uses gold-labeled sandwich antibody technique.
2. Specific activities of mitochondrial respiratory complexes after separation by native electrophoresis or immobilization on the dipsticks.
3. Oxidative damage to the mitochondrial respiratory complexes with 4-hydroxynonenol antibody or protein carbonyl formation.