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## Isolated mitochondria from frozen muscle have limited value in diagnostics

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Sir: Applegarth, Tong and Clarke [1] reported that the activities of citrate synthase, NADH-cytochrome c reductase, succinate-cytochrome c reductase, and succinate-CoQ reductase were not significantly different in mitochondria isolated from fresh and frozen muscle, while the activity of complex I was 16% higher and that of complex IV 16% lower in preparations from frozen muscle. The authors stated that investigation of mitochondria from frozen muscle is as useful as from intact muscle. This is not correct. When one is only interested in the activities measured by Applegarth et al. [1], there is no need to isolate mitochondria, because these activities can better be studied in a homogenate from (frozen) muscle.

Mitochondria from frozen muscle are different from freshly isolated mitochondria, in properties, yield and purity. They do not catalyse oxidative phosphorylation, because the protein gradient, the driving force for ATP synthesis, cannot be built up due to destruction of the inner mitochondrial membranes. Complex V catalyses ATPase instead of ATP synthetase. Mitochondrial enzymes from intermembrane and matrix space are solubilized. The fact that some mitochondrial activities were the same in the mitochondrial preparations from fresh and frozen muscle is just accidental. The activities were calculated per milligram protein, but the composition of the protein is different in the two preparations. Isolated mitochondria from frozen muscle are suitable for the determination of cytochrome redox spectra [2].

Trijbels et al. [3] advocated to study in patients suspected from having a defect in oxidative phosphorylation and/or pyruvate dehydrogenase complex, both oxidative phosphorylation in a preparation with intact mitochondria and all candidate enzymes in a homogenate from frozen muscle, to enable the detection of all possible defects present. Several groups investigate only isolated mitochondria from freshly biopsied muscle or homogenates from frozen muscle. In the view of Trijbels et al. [3], the best is to investigate both. Unfortunately this is not possible in all patients, and this will give rise to underdiag-

nosis of mitochondrial defects, and to a wrong diagnosis.

A nice example of using the combined diagnostic approach is the finding of a defect in the adenine nucleotide carrier [4], which was detected by an immunological method in frozen muscle. The suspicion of a deficiency of this translocator was raised when a phosphorylation defect was detected in oxidative phosphorylation by intact muscle mitochondria.

## References

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## Reply

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Sir: We thank Drs. Scholte and Trijbels for pointing out some important considera-

tions concerning our report on the measurement of electron transport chain activities in frozen versus fresh muscle. We agree that when possible the use of fresh muscle is preferable over that of frozen. Fresh muscle allows for the evaluation of oxidative phosphorylation in intact mitochondria and can point to a defect in complex V and the adenine nucleotide carrier, etc. Unfortunately many patients are seen and evaluated at centres that cannot provide as thorough an evaluation of muscle as could be performed by Scholte and Trijbels.

It has been our experience that many patients found to have lactic acidosis with normal activities of pyruvate dehydrogenase complex and pyruvate carboxylase in fibroblasts most commonly have defects in complexes I, II, III or IV of the electron transport chain. We have found it most convenient to be able to identify these defects by using mitochondria isolated from muscle frozen for 7 days for the reasons we gave in our original letter to the Editor. We agree with Scholte and Trijbels comments on the effect of freezing on some mitochondrial enzymes but dispute their conclusion that the similar results for complexes I, II, III and IV for mitochondria isolated from otherwise identically treated samples of fresh or frozen muscle were just accidental.

When we have isolated mitochondria from muscle samples which have been frozen for more than roughly 3 months we get low activities of the complexes we described and low activities of citrate synthase which can act as a marker enzyme against which we can assess, crudely, the reliability of the other complex activities. However, once mitochondria have been isolated under the conditions we described the mitochondrial activities of complexes I, II, III and IV are stable for at least a year if the mitochondrial aliquots are stored at  $-70^{\circ}\text{C}$ . We do caution that laboratories measuring enzyme activities on frozen muscle samples should generate their own range of normal activities for both fresh muscle and muscle which is frozen for the time period of the muscle sample they are investigating. We did not state that investigation of mitochondria from frozen muscle is as useful as from fresh, intact, muscle and the elegant data of Scholte et al. referred to by Drs. Scholte and Trijbels serve to emphasize that fresh muscle is the preferable sample for investigation of mitochondrial respiratory chain function.

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