

## *No Abnormal Low Molecular Weight Proteins Identified in Human Malignant Hyperthermic Muscle*

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There is no single, simple diagnostic test available to enable identification of malignant hyperthermia (MH) susceptible individuals. Recently, two novel low-molecular-weight proteins (15,000 daltons and 13,500 daltons) that were not present in normal muscle were identified in MH muscle and it was felt that this might eventually be of assistance in diagnosing MH. The authors of this report have been unable to verify these results. Polyacrylamide gel electrophoresis of the soluble proteins from muscle of four MH-susceptible and four normal individuals showed no differences in the electrophoretic fractionation patterns. Therefore, the authors conclude that the differences in protein composition previously reported in MH muscle are not characteristic of this syndrome. (Key words: Hyperthermia: malignant. Muscle, skeletal: proteins.)

MALIGNANT HYPERTHERMIA (MH), a pharmacogenetic disease of skeletal muscle, is triggered by potent inhalation anesthetics and depolarizing muscle relaxants and can be associated with a high mortality rate.<sup>1</sup> Several tests are used to screen individuals for MH susceptibility.<sup>2-6</sup> Pharmacologic testing of muscle biopsy specimens has now been firmly established as the most useful screening procedure.<sup>2</sup> However, this requires an open muscle biopsy and a laboratory equipped to cope with halothane and caffeine tension studies.

It has recently been reported that electrophoresis can be used to distinguish MH muscle from normal muscle.<sup>7</sup> Two novel low molecular weight (MW) proteins were reported to be present in the soluble fraction of vastus lateralis muscle taken from subjects who either have MH or are members of an MH family. However, we have been unable to repeat these results, and we find that there is no difference in the protein composition of normal and MH muscle.

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Received from the Clinical Neuromuscular Research Laboratory, Department of Physiology, Witwatersrand University Medical School, Johannesburg, South Africa. Accepted for publication January 16, 1986. Supported in part by a grant from the South African Medical Research Council and the Muscular Dystrophy Research Foundation of South Africa.

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### Methods

Muscle biopsies are carried out at the request of the families, the anesthetist, or the subject in order to determine the presence or absence of the carrier status. All subjects were aged between 13 and 40 yr.

Vastus medialis muscle was taken from four MH subjects. M1-M3 represents muscle taken from three subjects from different families in each of which there had been a case of MH. All were identified as carriers of MH by the caffeine contracture test.<sup>8</sup> (Subjects are classified as MH positive if a contracture response of greater than 0.7 g is obtained on exposure to 2 mM caffeine). M1-M3 each had a contracture response of greater than 1 g at 2 mM caffeine. M4 represents muscle taken from a 20-yr-old man who had had two possible MH episodes, one 13 yr before biopsy and the other 10 yr before biopsy. On both occasions he developed an elevated temperature. The caffeine contracture test proved positive for MH.

Normal muscle was obtained from individuals undergoing orthopedic surgery. None had a family history of muscle disease or an abnormal response to anesthesia and each gave a normal response to caffeine tension testing.

### PREPARATION OF MUSCLE EXTRACTS

The procedure used was that described by Blanck *et al.*<sup>7</sup> Approximately 400 mg of muscle was minced with a pair of scissors and suspended in 5 volumes of 10 mM KCl, 250 mM sucrose pH 7.3, 4° C. The minced tissue was homogenized on ice for 30 s with an ultra-turrax TP-10 tissue processor at a rheostat setting of 5. The suspension was filtered through two layers of cheesecloth and the filtrate was diluted with an equal volume of the earlier-described buffer. This was centrifuged at 49,000 × g for 35 min and the supernatant was recovered. The protein concentration of this soluble fraction was determined by the Lowry method.<sup>9</sup>

### SODIUM DODECYL SULFATE (SDS) POLYACRYLAMIDE GEL ELECTROPHORESIS

Electrophoresis was performed on the soluble fraction according to the method described by Zahler.<sup>10</sup> Samples

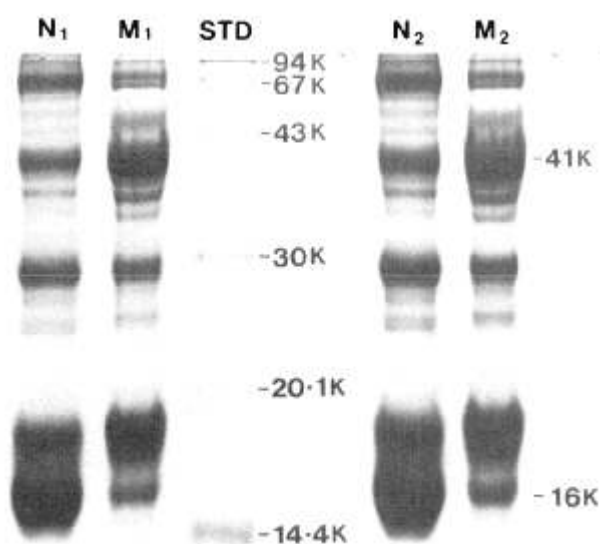


FIG. 1. 15% SDS-polyacrylamide gel electrophoresis of soluble proteins from human vastus medialis: N<sub>1</sub>, N<sub>2</sub>—normal muscle; M<sub>1</sub>, M<sub>2</sub>—MH-related patients. STD—standard MW proteins.

were made 1% with respect to SDS and 2-mercaptoethanol and after heating at 90° C for 3 min, 40 mg of protein was applied to 15% polyacrylamide gels with a 4% stacking gel. Electrophoresis was performed at pH 7.0 in 0.1 M phosphate buffer on 1.5 mm slab gels at 15 V/cm. Bromophenol blue was used as tracking dye.

Proteins of known MW (phosphorylase b 94,000; bo-

vine serum albumin 67,000; ovalbumin 43,000; carbonic anhydrase 30,000; soybean trypsin inhibitor 20,100;  $\alpha$ -lactalbumin 14,400§) were run on the same gel to enable the MW determination of proteins in the soluble fraction.

Gels were stained with 0.25% Coomassie Brilliant Blue R-250 in 45% methanol and 10% acetic acid and destained in 45% methanol, 7% acetic acid.

## Results

The electrophoretic separation of the soluble fraction of homogenized human vastus medialis muscle from both MH, MH-related, and normal patients are shown in figures 1 and 2.

It is evident that there is no significant difference in the protein banding patterns between normal vastus medialis and the banding pattern obtained with both muscle from the patient who has had an MH episode (M4) and the patients identified as MH positive by the caffeine contracture test (M1–M3).

With Coomassie blue staining, the color intensity of the band is proportional to the amount of protein present in that band. Even though the soluble fractions from MH and normal muscle produce identical banding patterns, it is noted that there are two proteins present in differing amounts in these samples. A protein of MW 41,000 is present in greater amounts in the MH extracts as com-

§ Low Molecular Weight Electrophoresis Calibration Kit; Pharmacia Fine Chemicals, Sweden.

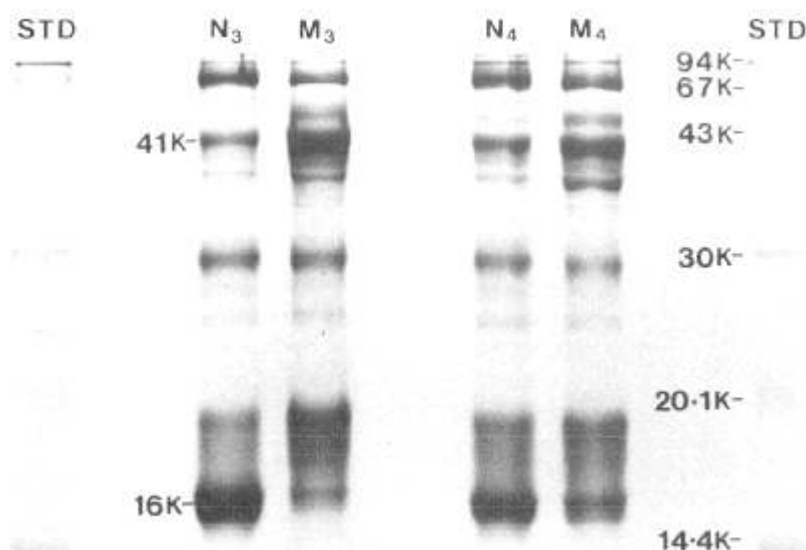


FIG. 2. 15% SDS-polyacrylamide gel electrophoresis of soluble proteins from human vastus medialis: N<sub>3</sub>, N<sub>4</sub>—normal muscle; M<sub>3</sub>—MH-related patient; M<sub>4</sub>—MH patient. STD—standard MW proteins.

pared with the normal muscle extract. The opposite applies to the protein of MW 16,000.

It must be noted that the two novel low MW proteins (MW 15,000 and 13,500) identified by Blanck *et al.*<sup>7</sup> in MH extracts are not present in any of these preparations.

### Discussion

The results of Blanck *et al.*<sup>7</sup> showed the presence of two low MW proteins in soluble extracts of vastus lateralis muscle from MH subjects, which were not present in normal muscle extracts. In direct contrast, our electrophoretic comparison of the soluble fraction of vastus medialis muscle from MH and normal subjects showed no significant differences in their protein banding patterns, and there was no evidence of the low MW proteins identified by Blanck *et al.* in the MH extracts.

Vastus medialis is used routinely in our laboratory for MH diagnosis; therefore, it was used for these electrophoretic comparisons.

As the protocol used for the fractionation of the soluble proteins was that described by Blanck *et al.*,<sup>7</sup> it is felt that the lack of the two low MW proteins in MH vastus medialis muscle was not due to procedural differences or the fact that vastus medialis muscle was used, but rather reflects the fact that the low MW proteins described by Blanck *et al.*<sup>7</sup> are not characteristic of MH.

These results support the findings of Fletcher *et al.*,<sup>11</sup> who also were unable to distinguish MH-susceptible from normal muscle using gel electrophoresis.

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