TOTAL CALCIUM CONTENT OF SKELETAL MUSCLE ISOLATED FROM HUMANS AND PIGS SUSCEPTIBLE TO MALIGNANT HYPERTHERMIA

B. A. BRITT, L. ENDRENYI, R. L. BARCLAY AND D. L. CADMAN

SUMMARY

Total calcium content of both human and porcine MHS skeletal muscle is significantly less than normal. This data is consistent with the concept that some organelle (probably the sarcoplasmic reticulum, the mitochondrion or even the sarcolemma) within the MHS muscle stores less than normal amounts of calcium. The large variability between muscle specimens rules out measurement of total calcium content of skeletal muscle as a routine diagnostic test for malignant hyperthermia susceptibility.

Malignant hyperthermic susceptible (MHS) muscle has been postulated to be less capable than normal of storing calcium within some organelle or membrane. The defective organelle might be the sarcoplasmic reticulum (SR) (Britt et al., 1973; Kalow et al., 1970), the mitochondrion (Britt et al., 1974) or even the sarcolemma (Gordon, Britt and Kalow, 1973). If such an organelle did contain less than normal quantities of calcium, one would expect that the total calcium content might also be less than normal. Therefore we decided to measure the total calcium content of vastus lateralis muscle obtained from normal and MHS humans and swine.

METHODS

Anaesthetic and surgical techniques.

Pigs. The swine used were either normal York pigs of MHS York/Poland-China hogs. Susceptibility of the latter pigs was assured by: (a) a positive isometric muscle contracture test (Britt et al., 1973, 1974; Kalow et al., 1970); and (b) a subsequent halothane-induced hyperthermic rigor. The animals were premedicated with diazepam (Valium) 4 mg/kg i.m. Anaesthesia was maintained with Innovar (droperidol and fentanyl) 2 ml, nitrous oxide: oxygen (6:4 litre/min) and further diazepam as required. A field block of procaine 1% (without adrenaline) was infiltrated around, but not into, the biopsy site. One-and-a-half gram of muscle was excised from the vastus lateralis. All possible fascia and fat were cut away from the muscle before severing its blood supply. The excised muscle was immediately frozen in liquid nitrogen. The frozen muscle was then wrapped in an inner layer of parafilm and an outer layer of tinfoil to prevent moisture loss and then was kept frozen until assayed.

Humans. The normal humans were patients undergoing surgical procedures upon the hip joint or upper femur, or were relatives of malignant hyperthermia (MH) probands who were found by the isometric muscle contracture test (Britt et al., 1973; Kalow et al., 1970) to be normal. The MHS humans were patients who had recovered from MH reactions or were relatives of such individuals and who had been found by the isometric muscle contracture test (Britt et al., 1973; Kalow et al., 1970) to be MHS also. The isometric muscle contracture test was performed on each patient at the same time as the total calcium content of the muscle was measured. This tissue was not frozen.

Premedication consisted of diazepam 1.0 mg/5 kg body weight i.m. Anaesthesia consisted of Innovar (droperidol and fentanyl) 2 ml, nitrous oxide: oxygen (6:4 litre/min) and further diazepam as required. A field block of procaine 1% (without adrenaline) was infiltrated around, but not into, the surgical site. For calcium measurements, muscle was removed from the vastus lateralis and was frozen and stored in the same manner as the porcine muscle.

Biochemical techniques.

Each muscle specimen was divided into five slices each weighing approximately 0.3 g. Each piece was weighed frozen and was then rewrapped in tinfoil. After drying at 100°C for 8 hr, the muscle slices were reweighed, placed in platinum crucibles and ashed in a muffled oven at 600°C for 16 hr. The ash was then dissolved in a mixture of 0.1 N hydrochloric acid 1% LaCl₃ (LaCl₃ prevents inter-
ference by phosphate during atomic absorption reading) (Berman and Kench, 1973). The calcium concentration of the acid-ash mixture was determined by a Perkin-Elmer 303 atomic absorption photometer.

For the isometric muscle contracture study, fresh, unfrozen fascicles were prepared and measured by the method described previously by us (Britt et al., 1973). Those who developed a contracture of at least 1-g tension in the presence of less than 8.0 mM of caffeine and who had had MH, or who had had one or more relatives who had had MH, were considered to be MHS. Those who developed a contracture of less than 1-g tension in the presence of more than 9.0 mM of caffeine and who were unrelated to any individuals known to be susceptible to MH were considered to be normal.

RESULTS

(Table I.) In both humans and swine, the total calcium content was significantly lower in the MHS muscle compared with normal muscle. No significant differences in calcium content were observed between normal human and normal porcine muscle, nor between MHS human and MHS porcine muscle. Standard errors for the human muscle were greater than for the porcine muscle.

| Table I. Calcium content of human and pig skeletal muscle |
|-----------------|-----------------|-----------------|-----------------|
|                 | Wet weight†     | Dry weight§     |                 |
|                 | Human           | Pig             | Human           | Pig             |
| Normal          | 57.7 (±9.0)     | 51.9 (±4.0)     | 246 (±36)       | 263 (±16)       |
| MHS             | 31.6 (±4.8)     | 28.3 (±2.4)     | 156 (±24)       | 133 (±11)       |
| t               | 2.75*           | 4.86†           | 2.16*           | 7.44†           |

* P < 0.05; † P < 0.001; ‡ mg calcium/g wet muscle; § mg calcium/g dry muscle.

DISCUSSION

These values for skeletal muscle calcium content are in agreement with those reported by Berman and Kench (1973), who measured total calcium of skeletal muscle excised from several pigs during an MH hyperthermic reaction. They are not, however, in agreement with measurements made by Bianchi and reported by Auerbach and colleagues (1973). Bianchi measured the calcium content of skeletal muscle obtained from one human patient shortly after recovery from MH crisis. It may be that calcium distribution in muscle convalescing from an MH reaction is different from that which exists before or during the reaction.

The decreased calcium content observed in both human and porcine MHS muscle supports the premise that some organelle or membrane within such muscle stores less than its normal quota of calcium. Myoplasmic calcium would have to be within the normal range, otherwise normal muscle contraction could not occur, and we know that muscle flaccidity is not a feature in MHS persons (Gordon, Britt and Kalow, 1973; Britt and Kalow, 1970). In MHS humans, calcium uptake into the SR is less than normal (Britt et al., 1973; Kalow et al., 1970), while in MHS swine, calcium uptake into the mitochondria is impaired (Britt et al., 1975). In normal muscle each of these organelles stores large quantities of calcium, the concentration in the SR being 1,000-2,000 times greater than in the myoplasm (Porter and Franzini-Armstrong, 1965; Ebashi, 1965; Siekevitz, 1965; Martonosi and Feretos, 1964; Carvalho, 1966), and in the mitochondria several hundred times greater than in the myoplasm (Lehninger, 1956, 1970). Thus, a reduction in the amount of calcium in either of these organelles would be expected to have a significant effect in lowering total muscle calcium content. The ability of MHS sarcolemma to store and transport calcium has not been studied. It is possible that a defect in this membrane may also contribute to a low total muscle calcium content.

The greater standard errors for the human than for the porcine muscle were probably a reflection of the greater genetic variability of the human subjects but may have been a result of the greater variability in surgical technique for taking the human muscle. This was especially a problem for the normal humans as in all of these our biopsies were, for ethical reasons, part of another surgical procedure.

We had originally hoped that muscle calcium content determination might prove to be a useful diagnostic test for the MH trait, because of the small amount of muscle required and the ease of measurement. The large standard errors between subjects and the overlap between the lower end of the normal range and the upper end of the MHS range (table I), however, have ruled out this possibility.

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REFERENCES

TENEUR TOTALE EN CALCIUM DU MUSCLE SQUELETTIQUE ISOLE DES HUMAINS ET DES PORCS ET SUSCEPTIBLE D’HYPERTERMIE MALIGNE
RESUME
La teneur totale en calcium squelettique susceptible d’hyperthermie maligne des humains et des porcs est tres inferieure a la normale. Cette donnee correspond au specchio que certains organites (probablement le reticulum sarcoplasmique, le mitochondrion ou meme le sarcolemma) se trouvant a l’interieur mme du muscle susceptible d’hyperthermie maligne contiennent moins de calcium que la normale. La grande diversite des spemens de muscles interdit la mesure de la teneur totale en calcium du muscle squelettique afin d’établir un test de diagnostic routinier pour la susceptibilite à l’hyperthermie maligne.

DER TOTALE KALZIUMGEHALT VON SKELETTMUSKELN, ISOLIERT BEI MENSCHEN UND SCHWEINEN MIT ANFALLIGKEIT AUF MALIGNE HYPERTHERMIE
ZUSAMMENFASSUNG

CONTENIDO TOTAL EN CALCIO DEL MUSCULO ESQUELETICO AISLADO DE HUMANOS Y CERDOS SUSCEPTIBLE A HIPERTERMIA MALIGNA
SUMARIO
El contenido en total en calcio del músculo esquelético MHS tanto de humanos como de cerdos es significativamente menor que el normal. Estos datos son consistentes con el concepto de que algunos órganos (probablemente el retículo sarcoplásmico, el mitocondrio o incluso el sarcolema) dentro del músculo MHS guardan menos cantidad de calcio que la normal. La gran variedad entre los especímenes musculares establece la regla de medición del contenido total de calcio del músculo esquelético como una prueba rutinaria de diagnóstico para la susceptibilidad a la hipertermia maligna.