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Fiber-type Caffeine Sensitivities in Skinned Muscle Fibers from Humans Susceptible to Malignant Hyperthermia

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Background: **The response to contracture tests may depend upon the relative proportion of muscle fiber types within the muscle specimen. To determine whether a difference in fiber-type caffeine sensitivities exists between malignant hyperthermia susceptible (MHS) and malignant hyperthermia-nonsusceptible (MHN) skeletal muscle, we compared the fiber-type caffeine sensitivities in chemically skinned muscle fibers dissected from vastus lateralis muscle from 15 MHS and 16 MHN patients.**

Methods: **Muscle fiber type was determined in each fiber by the difference in strontium-induced tension measurements and in 36 fibers, after contracture testing, by ATPase enzyme histochemistry. Caffeine sensitivity was defined as the threshold concentration inducing more than 10% of the maximal tension obtained with a calcium 1.6×10^{-2} mM solution.**

Results: **Significant difference in the mean (±SD) caffeine sensitivity was found between type I MHS fibers (2.63 ± 0.85 mM) *versus* type II MHS fibers (3.47 ± 1.2 mM) and between type I MHN fibers (5.89 ± 1.8 mM) *versus* type II MHN fibers (10.46 ± 2.6 mM). The mean (±SD) caffeine sensitivities for a given muscle fiber type (I or II) were different between groups of MHS and MHN patients. Both type I and II MHS fibers had significantly lower caffeine sensitivities, and this increase in caffeine sensitivity was significantly smaller in type I than in type II fiber.**

Conclusions: **The current study indicates that a truly MHS patient cannot have a false-negative result solely related to abnormal type II fibers contained in a given muscle strip. Although the occurrence of a very high proportion of type I fibers in MHN human muscle could result in a false-positive contracture outcome, such an occurrence is expected to be rare. (Key words: Hyperthermia, malignant. Muscle, skeletal: fiber type; skinned fiber. Pharmacology: caffeine sensitivity.)**

SEGMENTS of muscle bundles used for the *in vitro* contracture tests to diagnose malignant hyperthermia (MH) susceptibility consist of a complex admixture of type I (slow-twitch, slow oxidative) fiber and type II (fast-twitch, fast glycolytic) fiber.[1,2] The tests determine the sensitivity of cut muscle fibers to halothane or caffeine added to the bathing solution. Muscle bundles from patients susceptible to MH have lower contracture thresholds to these drugs than those from normal patients.[3,4]

In evaluation of previous studies performed on human muscle, there seems to be contradictory evidence concerning the involvement of different muscle fiber types on the *in vitro* test results. While some studies have shown that fiber-type composition does not influence contracture test results,[5,6] other authors have reported a difference in caffeine sensitivity between muscle fiber types.[7–9] These latter findings suggest that the proportion of muscle fiber types in a given muscle bundle may significantly influence the results of the caffeine contracture test leading to an inherent limitation in its sensitivity.

Recently, the skinned fiber technique has been successfully applied to separate human fibers into two classes based upon their relative sensitivities to strontium (Sr^{++}).[7,9,10] It was assumed that type I fibers had high sensitivities to Sr^{++}, and type II fibers had low

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sensitivities to Sr^{++}. Few studies validated this method by correlating it with standard histochemical staining in a large number of muscle fibers. Using skinned fiber technique, Mitsumoto *et al.*[9] showed a higher caffeine sensitivity in type I fibers from normal patients. The authors concluded that muscle fiber type influences the results of the caffeine contracture test. We therefore investigated the difference in caffeine sensitivity in Sr^{++}-sensitive and Sr^{++}-nonsensitive fibers from patients susceptible to MH. The results were compared with those obtained with muscle fibers from MH nonsusceptible (MHN) patients. In addition, the evidence of high Sr^{++} sensitivities with type I MH susceptible (MHS) fibers and low Sr^{++} sensitivities with type II MHS fibers was verified by using standard histochemical staining.

Patients and Methods

Diagnosis of Malignant Hyperthermia Susceptibility

Thirty-one patients presenting for a diagnostic muscle biopsy as part of investigation for MH participated in the study, which was approved by our university's Studies Ethics Committee, and informed consent was obtained from the patients for removing extra muscle. The biopsies were taken from the vastus lateralis muscle under combined block with lidocaine of the femoral nerve and lateral cutaneous nerve of the thigh. The preparation and stimulation of the muscle bundles, experimental apparatus, and methods of delivery of halothane have been described previously elsewhere.[11] Caffeine-free base was dissolved in Krebs-Ringer solution. All patients were investigated according to the protocol supported by the European MH Group.[3] The criteria of MH susceptibility (MHS) were an increase in resting tension of at least 0.2 g both with a halothane threshold concentration $\leq$2% and a caffeine threshold concentration $\leq$2 mM. A normal response (MHN) was defined as a halothane threshold >2% and a caffeine threshold >2 mM. Other results (*i.e.*, one abnormal response either with halothane or with caffeine) were classified MH-equivocal, but these patients were not included in this study since the significance of these results is not known.

Skinned Fiber Preparation

Chemically skinned single fibers were prepared as previously described by Wood *et al.*[12] Chemical skinning renders the muscle fiber sarcolemma freely permeable to external solutes.[13] Bundles containing several hundreds of fibers were attached at their extremities to a wooden stick to maintain their resting length and immediately placed in a relaxing solution at 4° C for 24 h (table 1). They were then transferred to a skinning-

Table 1. Constituents of Solution (mM)

Solution	K-Propionate	Mg-Acetate	K2-EGTA	MOPS	Ca-EGTA	Sr-EGTA
Relaxing solution	170	2.50	5.00	10	—	—
Wash solution	185	2.50	0.00	10	—	—
Ca^{++} Solution						
1.6×10^{-4}	172	2.46	3.85	10	1.152	—
1.6×10^{-2}	172	2.40	0.11	10	4.888	—
Sr^{++} Solution						
2.5×10^{-4}	172	2.48	4.96	10	—	0.025
6.3×10^{-4}	172	2.48	4.94	10	—	0.060
1.6×10^{-3}	172	2.48	4.84	10	—	0.160
4.0×10^{-3}	172	2.48	4.62	10	—	0.368
6.3×10^{-3}	172	2.46	4.42	10	—	0.568
1.0×10^{-2}	172	2.46	4.14	10	—	0.848
1.6×10^{-2}	172	2.46	3.76	10	—	1.240
3.2×10^{-2}	172	2.44	2.98	10	—	2.024
1.0×10^{-1}	172	2.42	1.3	10	—	3.688

MOPS = morpholinopropanesulfonic acid; each solution contains adenosine triphosphate (2.5 mM). The chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

storage solution that was identical to the relaxing solution except for the addition of 50% glycerol and stored at −20° C until used (1–2 weeks). This technique is identical to that employed by a number of other laboratories.[7,8,14,15] No change in skinned fiber mechanical properties could be noticed after 3–4 weeks of storage.

Single fibers were dissected from the main fascicle under a 40-power swift Model 31-400-00 binocular microscope. Each skinned fiber segment ~1 mm long was mounted horizontally between two clamps in a muscle bath (0.8 ml) filled with a relaxing solution. One clamp was attached to a Grass Model FT.03C force-displacement transducer. The muscle contracture was amplified on a Tektronix AM 502 differential amplifier and recorded on a Gould 2200 S. The length and diameter of the skinned fibers were measured under a 400-power Olympus lens. The average diameter of the fibers used in this analysis was equal to 106.4 ± 14.7 μm (n = 90) for MHS fibers and 96.7 ± 4.0 μm (n = 85) for MHN fibers. The muscle fiber was straightened by adjusting the position of the transducer, and the initial fiber length before stretching was measured. Then the resting tension was applied by stretching the fiber by 20% of its initial length.

Solutions

The concentrations of the different components in the solutions were calculated using program 3 of Fabiato and Fabiato[16] to keep the ionic strength at 200 mM. The stability constants of Orentlicher *et al.*[17] were used in the calculations: K_{CaEGTA} 1.919×10^6/M, K_{CaATP} 5.0×10^4/M, K_{MgEGTA} 40/M and K_{MgATP} 1.0×10^4/M. Composition of solutions are shown in table 1.

Caffeine Sensitivity

The threshold concentration (caffeine sensitivity) was defined as the concentration of caffeine (caffeine anhydrate, Prolabo) that induced a tension greater than 10% of the maximal tension of the same fiber obtained with Ca^{++} 1.6×10^{-2} mM solution. Fibers were loaded with Ca^{++} 1.6×10^{-4} mM solution. After 30 s of Ca^{++} loading, fibers were rinsed twice with wash solutions to remove excess Ca^{++} and challenged with a known caffeine solution for 1 min if no increase in tension was observed. Caffeine was dissolved in wash solution at increasing concentration of 0.5, 1–15 mM. Between each successive concentration of caffeine, fibers were returned to baseline condition by rinsing with the following sequence: relaxing solution, 40 mM caffeine, and relaxing solution to load the fiber with Ca^{++} 1.6×10^{-4} mM in the same manner (fig. 1). For each muscle

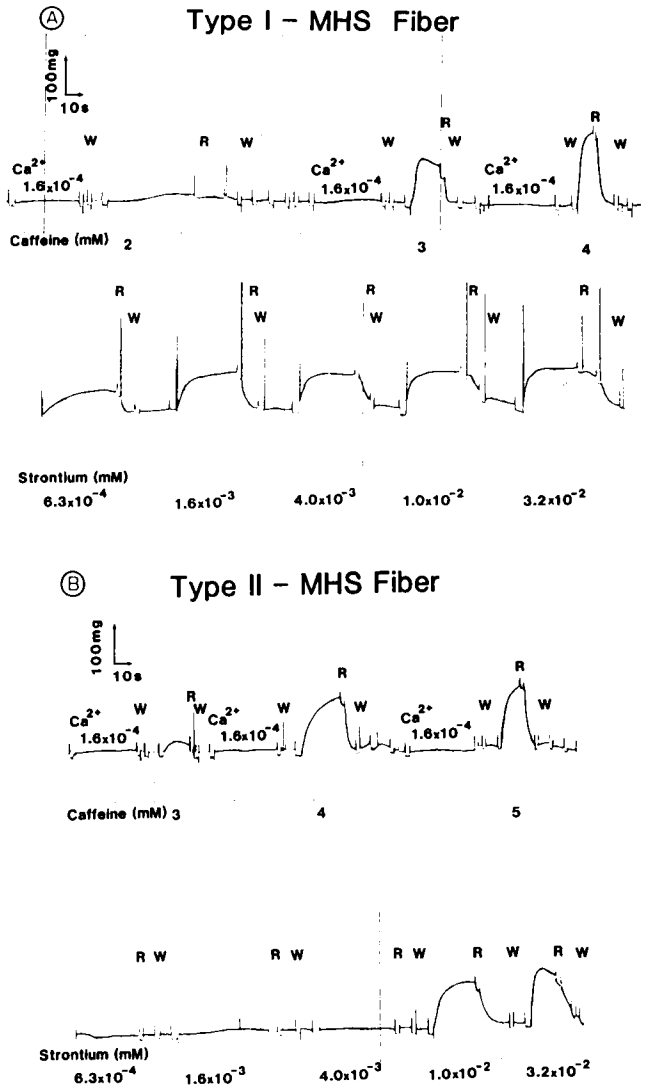

Fig. 1. Sequence for determinations of caffeine sensitivity in human skinned muscle fiber. A typical example of type I malignant hyperthermia-susceptible (MHS) fiber contracture (*A*) shows a slight contracture with 2 mM caffeine and the threshold contracture with 3 mM caffeine. Type I fiber begins to contract at low concentrations of free strontium ion (Sr^{++}) (6.3×10^{-4} mM) and the contracture was maximal at Sr^{++} 4.0×10^{-3} mM. The type II MHS fiber (*B*) begins to contract at 3 mM caffeine (caffeine threshold) and increases its tension with higher caffeine concentrations. In contrast, the type II fiber reveals no contracture at Sr^{++} 6.3×10^{-4}, 1.6×10^{-3}, and 4.0×10^{-3} mM. The fiber begins to contract at Sr^{++} 1.0×10^{-2} mM and maximal contracture is observed at 3.2×10^{-2} mM. R = relaxing solution; W = wash solution.

fiber, the contracture induced by 40 mM caffeine solution was examined as the preliminary test. Only fibers inducing tension more than 20 mg were included in the experiment.

Muscle Fiber Typing

After the determination of the caffeine threshold, skinned fibers were tested with increasing concentrations of Sr^{++}. The fiber was bathed once in the wash solution to eliminate traces of EGTA from the previously applied relaxing solution. The fiber was then activated with a given concentration of Sr^{++} (from 2.5×10^{-4} to 1.00×10^{-1} mM, table 1) until a plateau was observed. Intermediate tensions were expressed as a percentage of the maximal tension. Data were analyzed using a linearization of Hill's equation where relative tension = $[Sr]^{nH}/(K+[Sr]^{nH})$. The slope coefficient (nH) and the concentration of Sr^{++} for half-maximal activation (Sr_{50}) were computed. Using this type of analysis, two populations of muscle fibers were observed. Type I (slow-twitch) fibers contracted with low concentrations of Sr^{++} and were characterized by a Sr_{50} of less than 4.0×10^{-3} mM (fig. 1A). Type II (fast-twitch) fibers contracted with only high concentrations of Sr^{++} and were characterized by an Sr_{50} of more than 4.0×10^{-3} mM (fig. 1B). At the termination of the force measurements, 40 fibers classified by Sr^{++} response were carefully removed from the tissue bath and mounted with frozen media. Multiple frozen sections were cut and stained by a standard actomyosin ATPase histochemical assay at pH 9.4.[1] The staining intensity of the "test" fiber was compared with the range of staining intensities of the other fibers to determine whether the test fiber was "dark" or "light." Using basic preincubation (pH 9.4), darkly stained fibers corresponded to type II fibers and lightly stained fibers corresponded to type I fibers.[1]

Statistical Analysis

Statistical analysis was performed using a Student's paired *t* test within groups of either MHS or MHN patients and using an unpaired Student's *t* test between groups of MHS and MHN patients. Because several type I and type II muscle fibers were measured in each of the patients in the study, data were summarized and compared as follows. Means and standard deviations of caffeine concentrations by type I and type II fibers were obtained using all the measurements available. Statistical analysis was then done using Student's paired or unpaired *t* test, in which the repeat measurements of each type within a patient were first averaged so that a single type I and type II measurement for that patient could be obtained. The mean difference and standard deviation between the two fiber types in each group of MHS or MHN patients was then obtained. A P value $< .05$ was considered significant.

Results

In Vitro *Discrimination of Malignant Hyperthermia Susceptibility*

Muscle bundles from 15 patients developed a caffeine contracture of 0.73 ± 0.78 g at 2 mM caffeine and a contracture of 1.22 ± 1.12 g at 2 vol% halothane. According to the protocol supported by the European MH Group, these patients were classified as MHS (table 2). Sixteen patients did not develop any significant contracture at the above concentrations and were classified as MHN (table 3).

Caffeine Sensitivity

A total of 255 skinned fibers were tested with increasing concentrations of caffeine. Forty-seven type I (36%) and 83 type II (64%) fibers were studied from 15 MHS patients; the resulting caffeine sensitivities are plotted in figure 2A. When the difference between fiber-type caffeine sensitivity for each MHS patient was combined for analysis (n = 15), type I MHS fibers had a significantly lower caffeine threshold (2.63 ± 0.85 mM) than type II MHS fibers (3.47 ± 1.2 mM). Fifty-three type I (42%) and 72 type II (58%) muscle fibers were studied from 16 MHN patients; the resulting caffeine sensitivities are plotted in figure 2B. When the difference between fiber-type caffeine sensitivity for each MHN patient was combined for analysis (n = 15), type I MHN fibers had a significantly lower caffeine threshold (5.89 ± 1.8 mM) than did type II MHN fibers (10.46 ± 2.6 mM). The mean (±SD) caffeine sensitivities for a given muscle fiber type (type I or II) were different between groups of MHS and MHN patients (table 4). Both type I and type II MHS fibers had significantly lower caffeine sensitivities, and this increase in caffeine sensitivity was significantly smaller in type I than in type II fibers. A significant difference was found between the caffeine thresholds of type II MHS fibers and type I MHN fibers (table 4).

Table 2. Halothane and Caffeine Threshold for Tests Recommended by the European Malignant Hyperthermia Group and Corresponding Caffeine Concentrations of Type I and Type II Skinned Muscle Fibers from 15 Malignant Hyperthermia Susceptible Patients

Patient No.	Age (yr)	Sex	Halothane Threshold (%)	Caffeine Threshold (mM)	Caffeine Sensitivity (mM) (Mean ± SD)	
					Fiber Type I	Fiber Type II
1	22	M	0.5	0.5	2.71 ± 0.48	3.00 ± 1.51
2	26	F	0.5	0.5	1.87 ± 1.31	2.00 ± 0.00
3	30	M	0.5	1.0	1.90 ± 1.81	3.25 ± 2.87
4	48	M	2.0	0.5	1.00 ± 0.00	1.66 ± 0.51
5	42	F	2.0	2.0	3.33 ± 0.57	2.00 ± 0.00
6	56	M	2.0	2.0	2.50 ± 0.70	4.00 ± 0.81
7	57	M	0.5	1.0	1.33 ± 0.57	3.11 ± 1.76
8	14	M	0.5	0.5	2.00 ± 0.00	3.25 ± 0.88
9	45	M	1.0	1.5	3.00 ± 0.00	6.16 ± 1.32
10	16	M	0.5	0.5	3.33 ± 1.52	3.28 ± 0.75
11	43	F	1.0	2.0	3.20 ± 0.44	3.00 ± 0.00
12	24	F	0.5	0.5	2.75 ± 0.50	3.88 ± 1.16
13	25	F	1.0	2.0	3.00 ± 0.00	4.50 ± 1.29
14	45	M	1.0	1.5	4.00 ± 0.00	3.75 ± 0.95
15	23	M	2.0	2.0	3.50 ± 0.70	5.25 ± 1.75

Muscle Fiber Typing

Of the 40 fibers investigated by histochemical staining, 36 fibers could be rigorously typed. Six MHN fibers and six MHS fibers with high Sr^{++} sensitivity were found on ATPase histochemistry frozen sections to be lightly stained indicating that they were effectively type I fibers. Nine MHN fibers and 15 MHS fibers with low Sr^{++} sensitivity were darkly stained by the same technique, which was consistent with histochemical type II fibers. Figure 3 illustrates the experimental relative tensions

Table 3. Halothane and Caffeine Threshold for Tests Recommended by the European Malignant Hyperthermia Group and Corresponding Caffeine Concentrations of Type I and Type II Skinned Muscle Fibers from 16 Malignant Hyperthermia Nonsusceptible Patients

Patient No.	Age (yr)	Sex	Halothane Threshold (%)	Caffeine Threshold (mM)	Caffeine Sensitivity (mM) (mean ± SD)	
					Fiber Type I	Fiber Type II
1	18	F	N	8	5.33 ± 1.03	6.00 ± 0.00
2	19	M	N	4	5.00 ± 0.00	8.33 ± 0.57
3	43	M	N	4	4.50 ± 2.12	7.33 ± 1.50
4	15	M	N	8	6.40 ± 2.51	12.20 ± 3.83
5	37	M	N	4	7.50 ± 0.70	12.83 ± 2.48
6	43	M	N	8	7.00 ± 1.73	12.00 ± 3.00
7	30	F	N	8	5.50 ± 2.12	9.33 ± 1.15
8	46	F	N	8	10.20 ± 2.04	14.20 ± 1.50
9	31	M	N	8	8.00 ± 1.63	12.40 ± 2.51
10	15	M	N	8	4.00 ± 0.00	12.50 ± 2.73
11	47	F	N	4	6.66 ± 1.15	13.50 ± 3.00
12	44	F	N	8	6.87 ± 1.88	—
13	47	F	N	8	4.75 ± 1.70	11.25 ± 2.98
14	31	M	N	3	5.50 ± 0.70	10.33 ± 4.04
15	35	F	N	8	4.00 ± 0.00	7.25 ± 2.06
16	39	M	N	4	3.00 ± 1.41	7.50 ± 4.96

N: no contracture response to increasing concentrations of halothane.

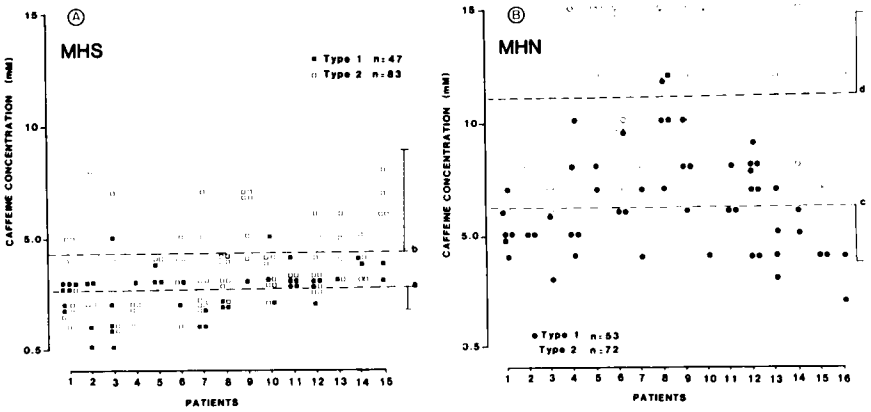

Fig. 2. Caffeine sensitivities in human type I and II muscle fibers by skinned fiber technique. The caffeine concentration that induced 10% of the maximal contracture with calcium 1.6×10^{-2} mM is shown in *A* for each MHS muscle fiber (square) and in *B* for each MHN muscle fiber (circle). In MHS patients, a significant difference was found between type I fibers (filled square; 2.63 ± 0.85 mM, broken line a) and type II fibers (open square; 3.47 ± 1.2 mM, broken line b). In MHN patients, type I fibers showed a significant increased caffeine sensitivity (filled circle; 5.89 ± 1.8 mM, broken line c) compared with type II fibers (open circle; 10.46 ± 2.6 mM broken line d). MHS = malignant hyperthermia-susceptible; MHN = malignant hyperthermia-nonsusceptible.

and the computed curves. No significant difference was found for Sr_{50} and Hill coefficient in a given muscle fiber type between MHS and MHN group (table 5). Significant differences in Sr_{50} values with concomitant changes in the Hill coefficient attested the difference in Sr^{++} sensitivity between muscle fiber type (table 5).

Strip Muscle Contracture Test Versus *Fiber-type Caffeine Sensitivity*

In vitro contracture responses for halothane-caffeine thresholds and corresponding caffeine concentrations (mean ± SD) of type I and type II skinned muscle fibers are given in tables 2 and 3. Caffeine sensitivity of type I MHS fibers from patient 14 (table 2) overlapped that found for type I fibers from three MHN patients (10, 15, and 16; table 3). One MHN patient (1, table 3) developed a type II caffeine sensitivity similar to that of MHS patient 9 (table 2). Correlation coefficients for fiber-type caffeine sensitivities *versus* standard halothane-caffeine thresholds are given in table 6. No correlation was found in the MHN group, whereas there were positive correlations in the MHS group for caffeine thresholds and type I alone and type I plus II caffeine sensitivities. No correlation was found in this group for halothane thresholds.

Table 4. Comparison of Fiber Type Caffeine Sensitivity between Groups and within Group of Malignant Hyperthermia Susceptible (MHS) and Malignant Hyperthermia Nonsusceptible (MHN) Patients

	Caffeine Sensitivity (mM)	
Fiber Type	MHS	MHN
I	2.63 ± 0.85* (15)	5.89 ± 1.8†‡ (16)
II	3.47 ± 1.2§ (15)	10.46 ± 2.6† (15)
II–I	0.84 ± 1.1	4.67 ± 1.9†

Each value is the mean ± SD with number of patients in parentheses (in one MHN patient, type II fibers were not found).

* Statistically different from type II within group of MHS patients ($P < .01$, paired t test).

† Statistically different from MHS ($P < .001$).

‡ Statistically different from type II within group of MHN patients ($P < .001$, paired t test).

§ Statistically different from type I MHN fibers ($P < .05$, unpaired t test).

Discussion

The main finding of the current study is that caffeine sensitivity was significantly higher in both type I and type II fibers from MHS patients compared with muscle fibers from MHN patients. This increase in caffeine sensitivity was significantly higher in type II than in type I fibers. A significant difference was found between the caffeine thresholds of type II MHS fibers and type I MHN fibers. These results have an important practical implication for the interpretation of the standard *in vitro* contracture test used for diagnostic procedure. If a given muscle bundle from a MHS patient happens to contain more type II fibers, the standard contracture test could not give a false-negative result.

Our study uses a skinned fiber preparation as it allows rapid application and removal of particular agents, which influences the myoplasmic calcium concentra-

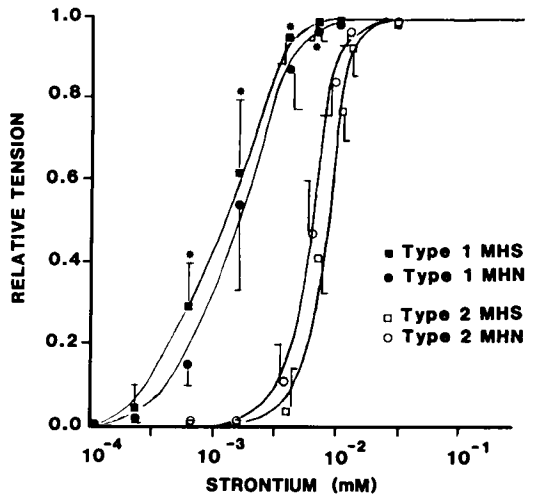

Fig. 3. Changes in tension (expressed as relative tension) to increasing concentrations of strontium for histochemically identified type I and II fibers from the human vastus lateralis muscle. The smooth curves were fitted by Hill's equation. Means (±SD) were obtained from 6 malignant hyperthermia-susceptible (MHS) (filled square) and 6 malignant hyperthermia-nonsusceptible (MHN) (filled circle) type I fibers and from 15 MHS (open square) and 9 MHN (open circle) type II fibers. Significant differences between muscle fiber type were found at strontium 6.3×10^{-4}, 1.6×10^{-3}, and 4.0×10^{-3} mM. No significant difference was found between MHN and MHS fibers in a given group of muscle fiber type.

tion.[12,13] Such preparation has been used to release Ca^{++} from sarcoplasmic reticulum (SR) with caffeine reported to directly activate Ca^{++} release mechanisms.[18–20] The technique uncouples T-tubules from SR structures, and this has been verified in electron micrographs.[14] However, abnormalities have been observed in the dihydropyridine receptors (DHPRs) of T-tubules from MHS pigs as well as in the ryanodine receptor of the SR.[21,22] It also is known that these DHPRs act as (or in fact are) the voltage sensors controlling Ca^{++} release from the SR[23–25] and that these two structures are juxtaposed. Hence, such uncoupling of T-tubules DHPRs from the SR Ca^{++} release channels might well be expected to alter the behavior of the SR in response to whatever stimulating technique is used to release Ca^{++}. This may explain the overlap in some individual patient fibers' responses between MHS and MHN muscle leading to the fact that the results observed in the single fiber caffeine test will not carry over quantitatively to the caffeine contracture test as employed clinically. However, a positive correlation in the MHS group was found between caffeine thresholds of muscle bundles and type I alone and type I plus II caffeine sensitivities. This finding suggests that the caffeine effects at the single muscle fiber level may reflect the cut MH muscle cell's greater contracture sensitivity to caffeine, a method used to diagnose MH. Hence, the present results may be helpful in interpreting standard muscle bundle caffeine contracture tests.

The difference in caffeine sensitivities between MHS and MHN fibers is consistent with a previous report using both mechanical skinning of the membrane and saponin treatment to eliminate the sarcolemma.[7] The range of the threshold caffeine concentrations for MHS and MHN fibers found in our study overlaps that found by others using a similar skinning and storage technique.[8,14] Moreover, our results are in agreement with

Table 5. Concentration of Strontium for Half-Maximal Activation (Sr 50) and Hill Coefficients of Fiber Type from Malignant Hyperthermia Susceptible (MHS) and Malignant Hyperthermia Nonsusceptible (MHN) Muscles

	Muscle Fiber Type	Sr 50 (mM)	Hill Coefficient
MHS	I (n = 6)	$1.3 \times 10^{-3} \pm 0.7 \times 10^{-3}$	1.15 ± 0.35
	II (n = 15)	$7.7 \times 10^{-3} \pm 2.7 \times 10^{-3}$*	1.91 ± 0.21*
MHN	I (n = 6)	$1.5 \times 10^{-3} \pm 0.7 \times 10^{-3}$	1.09 ± 0.21
	II (n = 9)	$6.5 \times 10^{-3} \pm 0.8 \times 10^{-3}$*	2.01 ± 0.44*

Values are mean ± SD; n = number of fibers tested.

* Significant changes ($P < .05$) *versus* type I muscle fiber.

Table 6. Correlation Coefficients for Fiber Type Caffeine Sensitivity and Halothane and Caffeine Thresholds for *In Vitro* Test Recommended by the EMHG from 15 Malignant Hyperthermia Susceptible (MHS) and 16 Malignant Hyperthermia Nonsusceptible (MHN) Patients

	N	Covariate Caffeine Sensitivity (fiber type)	Halothane Threshold	Caffeine Threshold
MHS	15	I	0.14	0.53*
		II	0.02	0.41
		I + II	0.08	0.54*
MHN	16	I	—	0.23
		II	—	0.15
		I + II	—	0.12

EMHG = European Malignant Hyperpyrexia Group.

* $P < .05$.

studies demonstrating a hypersensitive calcium release mechanism in response to caffeine in isolated SR.[26–29]

Our findings, on human muscle, are consistent with previous studies demonstrating, in some animal species, differences in caffeine sensitivities between muscle fiber type using bundles of muscle predominantly or exclusively composed of a given muscle fiber type from rats,[30,31] cats,[32] chickens,[33] and MHS pigs.[34] A previous study on chemically skinned muscle fiber from rabbits also demonstrated a higher caffeine sensitivity in type I fiber.[15]

Our results are in agreement with studies on human skinned muscle fiber. The mean (±SD) caffeine sensitivities for both type I and type II fibers from normal patients closely overlap that found by Takagi *et al.*[7] using both mechanically and chemically skinned fibers from normal biceps brachii muscles. These authors also demonstrated that normal muscle fibers with high Sr^{++} sensitivity had lower caffeine threshold than fibers with low Sr^{++} sensitivity. However, the fiber types were not confirmed using standard histochemical staining. The caffeine concentrations we found were approximately 3–4 mM higher for both normal fiber types than those described by Mitsumoto *et al.*[9] Our method differed mainly by the use of (1) a storage-relaxing solution containing glycerol, (2) EGTA for skinning the fiber, and (3) higher calcium concentrations during the caffeine sensitivity testing. In addition, we used the Hill model for comparison of the binding of Sr^{++} between MHS and MHN muscle fiber types. The Hill coefficients that characterize the steepness of the sensitivity curves at the concentration of Sr^{++} for half-maximal activation were similar in the two groups of patients for a given muscle fiber type. Our results indicate that the muscle type of a single MHS fiber, as well as normal muscle, can be determined by the activation pattern with Sr^{++}.

Previous studies on MH susceptible human skinned muscle fiber are also consistent with our results.[7,8] It was observed in small series of MHS patients that type II MHS fibers developed a striking increase in caffeine sensitivity. Hence the difference between muscle fiber types from a given MHS patient was not significant. This led some authors to suggest that type II MHS fibers could be the most affected in MH.[8] However, these findings on type II caffeine hypersensitivity also may be explained by a more richly developed SR in type II fiber.[1] The primary defect responsible for MH has been identified in abnormalities in the mechanism of calcium release from isolated MHS skeletal muscle SR.[26–29,35] Hence, type II caffeine hypersensitivity also could be due to quantitative difference of SR without necessarily implicating a fiber-type specific defect responsible for MH.

Our results indicate that muscle fiber type may influence the results of the *in vitro* caffeine contracture test in normal muscle but not in MH muscle. Abnormal responses to these tests have been observed in several myopathic and neuropathic disorders, but the interpretation remains controversial.[36–39] Type I predominance is seen often in the myopathies, whereas type II atrophy seems to be nonspecific and secondary to various diseases in which muscle activity is reduced.[1] Consequently, we agree with Mitsumoto *et al.*[9] that "positive" caffeine contracture test in these neuromuscular disorders may be related to an increase in type I distribution and/or type I abnormal caffeine sensitivity in a given muscle bundle. However, most of the abnormal responses with muscle bundles were found with halothane alone.[37,38] Since no positive correlation was found between halothane thresholds in muscle bundles and fiber-type caffeine sensitivities in skinned muscles, our results cannot be helpful in the interpretation of an abnormal halothane test.

Salviati *et al.*[8] have found that caffeine reactivity of type II fibers is more sensitive than that of type I fibers for discriminating patients with MH susceptibility from normal patients. Takagi *et al.*[7] also have shown the same results, but one false-negative result was obtained in a survivor of MH. Our study clearly documents that in MHS the reactivity of type II skinned fibers to caffeine is enhanced more than reactivity of type I skinned fibers. However, the increase in caffeine reactivity of the muscle strip with MHS retains the greatest diagnostic sensitivity.[40]

If our results in skinned individual fibers may be extrapolated to muscle strips as employed in the contracture test, an MHS patient is extremely unlikely to have a false-negative result solely related to high abnormal type II fibers contained in a given muscle strip. We cannot exclude the possibility of a positive response to the caffeine contracture test related to a high increase proportion of type I fibers contained in a muscle bundle from a MHN patient. However, there is generally a mixed proportion of fiber types within the human vastus lateralis muscle, and only few muscles show type I predominance greater than 70%.[2,37] Consequently, the validity of the *in vitro* caffeine contracture test performed on muscle strips containing type I and type

II fibers in varying proportions is strengthened by the analysis of fiber-type caffeine specificity in MH susceptible muscle. Because of a possible overlap in certain individual patient fiber responses, the caffeine skinned muscle fiber technique cannot be validated for diagnostic purpose.

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