

## Fiber-Type Specific Caffeine Sensitivities in Normal Human Skinned Muscle Fibers

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Caffeine sensitivity was studied in chemically skinned muscle fibers from vastus lateralis muscle obtained by biopsy during reconstructive knee surgery from 15 otherwise healthy young individuals. Muscle fiber type was determined by contracture occurring in strontium (slow-oxidative, type I fiber) or calcium (both type I and type II, fast glycolytic fiber) solutions and in several fibers after contracture testing by ATPase enzyme histochemistry. Caffeine sensitivity (mean  $\pm$  SD), defined as the threshold concentration inducing more than 10% of the maximal tension obtained with a calcium  $3 \times 10^{-5}$  mM solution was  $2.7 \pm 1.3$  mM in 37 type I fibers, whereas it was  $6.9 \pm 2.4$  mM in 61 type II fibers. A paired *t* test showed a significantly increased sensitivity to caffeine in type I fibers ( $P < 0.001$ ) in 13 individuals in whom the two fiber types were identified. The mean ( $\pm$ SD) difference between type I and type II fibers was  $4.1 \pm 1.9$  mM. Type I fibers contracted with greater tension in response to the increasing concentration of caffeine than did type II fibers ( $P < 0.05$ ). These skinned fiber studies showed significantly different caffeine sensitivities between human type I and type II muscle fibers, as previously shown in animal muscles. The findings that human type I muscle fibers have higher caffeine sensitivity than type II muscle fibers should be helpful for the interpretation of the *in vitro* contracture test done in muscle strips containing type I and type II fibers in varying proportions. (Key words: Hyperthermia, malignant; caffeine sensitivity. Muscle, skeletal; fiber type; skinned muscle fiber.)

MALIGNANT HYPERTHERMIA (MH) is a disease of hypermetabolism of skeletal muscle that occurs during anesthesia and can lead to catastrophic results. It would be valuable to identify MH susceptibility (MHS) before surgery because a nontriggering anesthetic could then be administered. The standard laboratory test for MHS is the *in vitro* caffeine-halothane contracture test done in "muscle strips" containing a bundle of muscle fibers.<sup>1,2</sup> Although this technique has been considered to be the most reliable and widely accepted test, it still appears to have an inherent limitation in its sensitivity and specific-

ity.<sup>2-4</sup> In animal studies slow oxidative skeletal muscle containing predominantly type I fibers on enzyme histochemistry is found to have much higher sensitivity to caffeine, halothane, or both than the fast glycolytic muscle, having predominantly type II fibers on enzyme histochemistry.<sup>5-8</sup> The sensitivity found in the cat soleus muscle, which consists of predominantly type I fibers, overlaps that found in muscles obtained from MH-susceptible patients. This suggests that the proportion of the muscle fiber types in a given muscle strip may significantly alter the results of the *in vitro* contracture test. However, the difference between type I and type II muscles found in animal studies has not been confirmed in human muscles. This is in part due to the fact that surgically accessible human skeletal muscles used for the strip technique consist of a complex admixture of type I and type II fibers,<sup>9-11</sup> which precludes the separate study of either fiber type. By using skinned fiber techniques, Takagi *et al.*<sup>12</sup> showed a trend toward higher caffeine sensitivity in human type I fibers. Therefore, we investigated the difference in caffeine sensitivity in human type I and II muscle fibers by the similar technique in a larger number of patients. The results should be helpful in interpreting standard strip caffeine-halothane contracture tests.

### Materials and Methods

#### MUSCLE SPECIMEN

Institutional review board permission was obtained, and a small piece (approximately  $5 \times 5 \times 20$  mm) of the vastus lateralis muscle was obtained by biopsy during reconstructive knee surgery in 15 individuals who were otherwise healthy and had no neuromuscular disease. Halogenated anesthetics were not used. Three patients received succinylcholine.

#### SKINNED FIBER PREPARATION

The skinned fiber contracture test was largely based on the method described by Takagi *et al.*<sup>12</sup> The test was performed immediately after the biopsy; thus, we put the specimen in ice-cold G-2 relaxing solution (table 1), skipping a G-5 relaxing-storage solution, which contains 2.5-fold more EGTA than the G-2 solution.<sup>12</sup> The muscle was chemically skinned in 0.3 mg/dl saponin (Sigma Chemical, St. Louis, Missouri) in G-2 solution, and mi-

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TABLE 1. Constituents of Solutions (mM)

	Relaxing Solution		Ca <sup>2+</sup> Solution		Sr <sup>2+</sup> Solution 3 × 10 <sup>-5</sup>
	G-2	G-0.1	3 × 10 <sup>-7</sup>	3 × 10 <sup>-5</sup>	
ATP-Na <sub>2</sub> *	4	4	4	4	4
Tris*	20	20	20	20	20
Maleate*	20	20	20	20	20
MgSO <sub>4</sub> *	4	4	4	4	4
EGTA*	2	0.1	10	10	10
CaSO <sub>4</sub> *	0	0	1.3	9.36	0
KMS†	101	108	92	80	90
SrCl <sub>2</sub> †	0	0	0	0	0.42

KMS = potassium methanesulfonate.

The chemicals were obtained from Sigma Chemical\* (St. Louis, Missouri) and from Aldrich Chemical† (Milwaukee, Wisconsin).

Modified from Takagi A, Sunohara N, Isihara T, Nonaka I, Sugita

H: Malignant hyperthermia and related neuromuscular diseases: Caffeine contracture of the skinned muscle fibers. Muscle Nerve 6:510-514, 1983.

crodissected into individual muscle fibers in the G-2 solution. The diameter of the muscle fiber was measured on an inverted phase contrast microscope, and only fibers measuring 50–80  $\mu$ m were used for the study. The individual skinned fiber was transferred into a specially designed 1-ml microbath, attached to a Nihon Koden (Tokyo, Japan) force displacement transducer (TB-612 T), and fixed to the end of the bath with double-sided Scotch tape. The muscle contracture was recorded on a Gould (Cleveland, Ohio) carrier amplifier (13-4615-35) and recorder (2200S). The procedure was performed at  $20 \pm 2^\circ$  C and finished within 36 h of the biopsy.

#### CAFFEINE SENSITIVITY TEST

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 to 10% longer than the initial length. At the time when we applied the resting tension, we had no knowledge which fiber turned out to be a type I or type II fiber. Thus, the resting tension was given to all fibers in the same fashion. The threshold concentration (caffeine sensitivity) was determined at the concentration of caffeine (caffeine anhydrate, Fisher Scientific, Fairlawn, New Jersey) inducing tension greater than 10% of the maximal tension of the same fiber obtained with calcium (Ca<sup>2+</sup>)  $3 \times 10^{-5}$  mM solution.

The muscle fiber was loaded with Ca<sup>2+</sup>  $3 \times 10^{-7}$  mM solution; then excess Ca<sup>2+</sup> was washed out once with G-2 and twice with G-0.1 solutions. The contracture of the muscle fiber was examined with 40 mM caffeine solution as the preliminary test (fig. 1). Only fibers inducing tension more than 25 mg were included for the subsequent caffeine sensitivity test. The sequence of Ca<sup>2+</sup>, G-2, and G-0.1 solutions was repeated before the test caffeine solution was given. Caffeine concentration in the bath was increased stepwise by irrigating the tissue bath with 1–15

mM caffeine solutions until the threshold contracture was reached (fig. 1). The compositions of all the solutions used for the study are shown in table 1.

In the last seven cases, the caffeine dose-response curve was studied with at least four different caffeine concentrations. A total of 13 type I and 19 type II muscle fibers were examined.

#### MUSCLE FIBER TYPING

After the caffeine sensitivity test, muscle contracture was tested with strontium (Sr<sup>2+</sup>)  $3 \times 10^{-5}$  mM and Ca<sup>2+</sup>  $3 \times 10^{-5}$  mM solutions. Type I (slow twitch) fibers contract with both Sr<sup>2+</sup> and Ca<sup>2+</sup> solutions, whereas type II (fast twitch) fibers contract only with the Ca<sup>2+</sup> solution<sup>13</sup> (fig. 1). Several type I and type II muscle fibers, classified by Sr<sup>2+</sup> and Ca<sup>2+</sup> response, were removed from the bath and mounted with frozen media. Multiple frozen sections were cut and stained by routine ATPase histochemistry at pH 9.8 to identify the histochemical muscle fiber type.

#### STATISTICAL METHODS

Because several type I and type II muscle fibers were measured in each of the individuals 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. Comparisons between fiber types were done using an unpaired *t* test where the repeat measurements of each type within an individual were first averaged so that a single type I and type II measurement for that individual could be obtained for the paired *t* test. The mean difference and standard deviation between the two fiber types was then obtained.

The relative contracture was found for each fiber by dividing the contracture by the maximal contracture obtained with Ca<sup>2+</sup>  $3 \times 10^{-5}$  mM. The relationship of relative

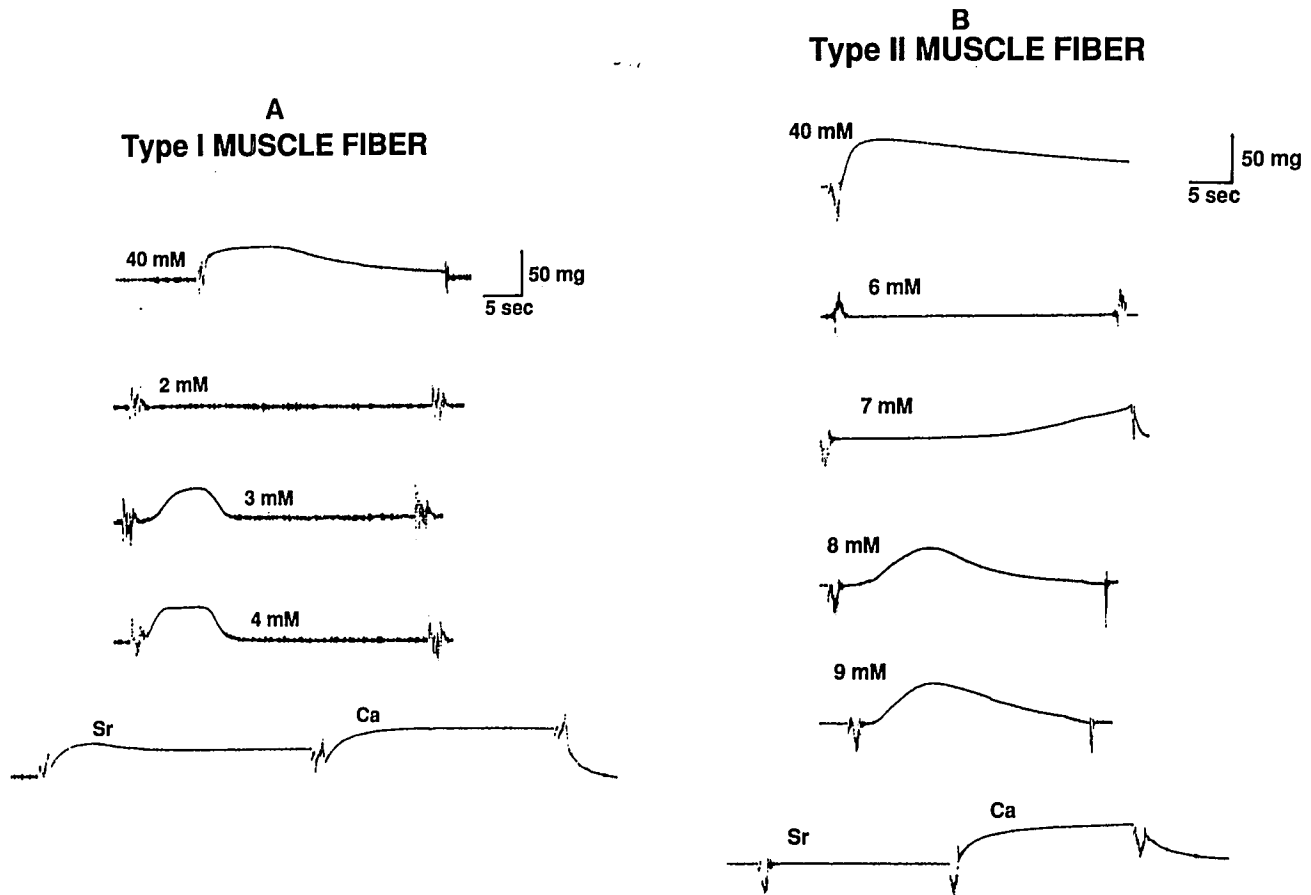


FIG. 1. A typical example of type I fiber contracture (A) shows the preliminary contracture with 40 mM caffeine solution, no contracture with 2 mM caffeine, the threshold contracture with 3 mM caffeine concentration, and increased tension with higher concentration of caffeine. Type I fibers show contracture with both  $\text{Sr}^{2+}$  and  $\text{Ca}^{2+}$ . In contrast, the type II fiber (B) begins contracture at a higher caffeine concentration (7 mM in this case) and increases its tension with higher caffeine concentrations. Type II fibers reveal contracture only with  $\text{Ca}^{2+}$  solution.

contracture to caffeine concentration was obtained for each fiber using linear regression. The resulting slopes were summarized for each fiber type by the mean slope and its standard deviation. Comparison between fiber types was done using an unpaired *t* test where mean slopes for each fiber type were first obtained for each individual.

### Results

From 15 individuals, 37 type I and 61 type II muscle fibers were studied. The mean ( $\pm$ SD) caffeine sensitivity, measured as the threshold concentration, was  $2.7 \pm 1.3$  mM in all type I fibers and  $6.9 \pm 2.4$  mM in all type II fibers, as shown in figure 2. In 13 individuals from whom both fiber types were sampled a significant difference ( $P < 0.001$ ) in caffeine sensitivity was found when type I fibers ( $2.8 \pm 1.0$  mM) were compared with type II fibers ( $6.9 \pm 1.8$  mM).

Three fibers classified as type I by the  $\text{Sr}^{2+}/\text{Ca}^{2+}$  test were found on ATPase histochemistry frozen sections and

they were lightly stained, indicating that they were type I fibers according to the histochemistry muscle fiber typing.<sup>9</sup> Five type II fibers classified by the  $\text{Sr}^{2+}/\text{Ca}^{2+}$  test were found on frozen sections and darkly stained by the same technique, which was consistent with histochemical type II fibers.

The relationship between relative contracture tension and caffeine concentrations is shown in figure 3 for representative type I and type II fibers. The mean ( $\pm$ SD) slope of all type I muscle fibers was  $0.37 \pm 0.21$ , whereas that of all type II fibers was  $0.24 \pm 0.15$ . In seven patients from whom both type I and II fibers were tested, the slope was significantly steeper for type I fibers ( $0.36 \pm 0.12$ ) compared with type II fibers ( $0.23 \pm 0.07$ ) ( $P < 0.05$ ).

### Discussion

Our study shows that the caffeine sensitivity was significantly increased in type I muscle fibers compared with

# CAFFEINE SENSITIVITIES IN HUMAN TYPE I & II MUSCLE FIBERS BY SKINNED-FIBER TECHNIQUE

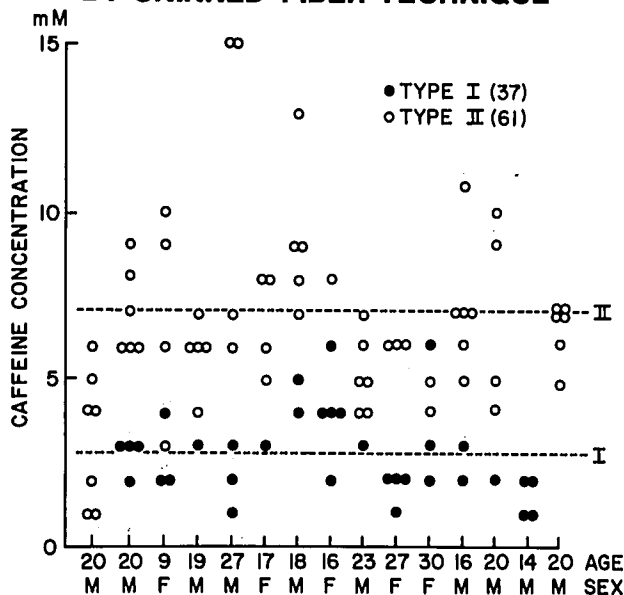


FIG. 2. The caffeine concentration that induced 10% of the maximal contracture with  $\text{Ca}^{2+} 3 \times 10^{-3}$  (the threshold) is shown with a circle for each muscle fiber. Type I fibers show a significantly increased caffeine sensitivity at caffeine concentration  $2.7 \pm 1.3$  mM (broken line I shows the mean) compared with type II fibers at  $6.9 \pm 2.4$  mM (broken line II indicates the mean). Age and sex (M or F) are shown for each patient.

type II fibers. However, the concentrations we found were approximately 3–4 mM lower for both fiber types than those described by Takagi *et al.*<sup>12</sup> The main difference in our methods was that we did not use the storage-relaxing solution (G-5) containing much higher EGTA because we began the test immediately following biopsy. In addition, our muscle specimens were all obtained from the vastus lateralis, whereas Takagi *et al.*<sup>12</sup> studied biceps brachii muscles. None of our muscle fibers was mechanically skinned. These differences in methods could have caused higher caffeine sensitivities in both type I and type II fibers. However, the range of the threshold concentration (caffeine sensitivity) found in our studies closely overlaps with that found by Britt *et al.*<sup>14</sup> Moreover, the range of the caffeine sensitivities we found was also within that found with strip muscle contracture tests in our MH laboratory (Mitsumoto H, DeBoer GE: unpublished observation) and by others.<sup>2,14</sup>

Recently Ruff<sup>15</sup> demonstrated that contracture of skinned human type I fibers is significantly more sensitive to  $\text{Ca}^{2+}$  ion concentration when compared with type II fibers. His results support our observation that human type I fibers are more sensitive to caffeine than type II

fibers because it is well known that caffeine stimulates  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum and induces muscle contracture.<sup>16</sup> However, it remains to be clarified whether the direct effect of caffeine on rat myofilament demonstrated by Wendt and Stephenson<sup>17</sup> is different between type I and type II fibers.

Halothane sensitivity was not tested in our system because the halothane concentration could not be reliably controlled in a 1-ml tissue bath.<sup>18</sup> This also appears to be the case even for the strip test.<sup>19</sup> Nevertheless, human type I fibers may also be more sensitive to halothane than type II fibers because caffeine sensitivity has been found to parallel halothane sensitivity in the muscle strip test in animal studies.<sup>5,8</sup>

Our conclusion that type I muscle fiber has a high caffeine sensitivity probably has an important practical implication for the interpretation of the *in vitro* contracture test. If a given muscle strip happens to contain more type I fibers, the contracture test could give a false-positive result. Analysis for fiber type proportion in each muscle strip may provide additional critical information for MHS; it could modify interpretation of the contracture test and decrease the number of false-negatives or false-positives.

Takagi *et al.*<sup>12</sup> have shown that type II fibers are more sensitive than type I fibers for discriminating patients with MHS from normals. Britt *et al.*<sup>14</sup> have concluded that the skinned fiber technique is slightly less sensitive than strip testing for detecting MHS. However, they did not analyze

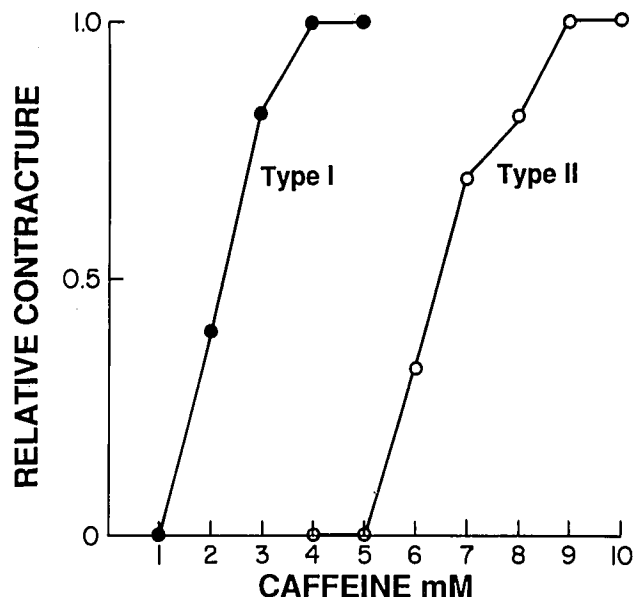


FIG. 3. The relative tension (the tension induced at a given concentration of caffeine solution normalized by the maximal tension obtained with  $\text{Ca}^{2+} 3 \times 10^{-3}$  mM solution) was plotted for a representative type I fiber and a type II fiber.

the fiber type in skinned muscle fibers separately, as Takagi *et al.*<sup>12</sup> did. It is possible that the selective type II skinned fiber study is equally or even more sensitive than the muscle strip technique for diagnosing MHS. However, we certainly agree with Britt *et al.*<sup>14</sup> that the skinned fiber technique is much more laborious and less practical than the strip technique.

Abnormal sensitivity to the caffeine-halothane contracture test has been demonstrated in several neuromuscular diseases, such as central core disease, Duchenne muscular dystrophy, and myotonic dystrophy.<sup>4,20,21</sup> MH episodes have been reported in patients with these disorders. However, the incidence of MH episodes in these neuromuscular diseases appears to be much lower than would be expected if every patient with these diseases has MHS. Although all these patients should be managed as if they had MHS, the interpretation of a positive contracture test in these neuromuscular diseases remains controversial.<sup>3,4</sup>

Skinned fibers from patients with Duchenne muscular dystrophy are found to be intermediate between type I and type II fibers using  $\text{Sr}^{2+}/\text{Ca}^{2+}$  activation.<sup>22</sup> Also, the caffeine sensitivity of degenerating and regenerating muscle fibers appears to be greater than that of healthy muscle fibers.<sup>12,22</sup> Muscle histology in central core disease is characterized by marked type I fiber predominance, and disfigured core formation occurs only in type I muscle fibers.<sup>9</sup> Duchenne muscular dystrophy may also have type I fiber predominance, whereas myotonic dystrophy has type I fiber atrophy.<sup>9</sup> These unique changes occurring in these neuromuscular diseases might be related to the abnormal caffeine sensitivity in type I muscle fibers. Therefore, we cannot absolutely exclude the possibility that a "positive" contracture test in these neuromuscular diseases may be solely related to abnormal type I fibers contained in a given muscle strip.

Thus, there seems to be significant evidence that muscle fiber type influences the results of the caffeine contracture test. Further prospective studies in individuals with MHS certainly seem indicated to compare fiber type specific caffeine sensitivity in skinned fibers and caffeine-halothane sensitivity in muscle strips that are also histochemically fiber typed.

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