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Comparison of European and North American Malignant Hyperthermia Diagnostic Protocol Outcomes for Use in Genetic Studies

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Background: Halothane and caffeine diagnostic protocols and an experimental ryanodine test from the North American Malignant Hyperthermia (MH) Group (NAMHG) and the European MH Group (EMHG) have not been compared in the same persons until now.

Methods: The outcomes of the NAMHG and EMHG halothane and caffeine contracture tests were compared in 84 persons referred for diagnostic testing. In addition, the authors assessed the experimental ryanodine protocol in 50 of these persons.

Results: Although the NAMHG and EMHG halothane protocols are slightly different methodologically, each yielded outcomes in close (84–100%) agreement with diagnoses made by the other protocol. Excluding 23 persons judged to be equivocal (marginally positive responders) by the EMHG protocol resulted in fewer persons classified as normal and MH susceptible (42 and 19, respectively) than those classified by the NAMHG protocol (48 and 34, respectively). For the 61 persons not excluded as equivocal, the diagnoses were identical by both protocols, with the exception of one person who was diagnosed as MH susceptible by the NAMHG protocol and as “normal” by the EMHG protocol. The NAMHG protocol produced only two equiv-

ocal diagnoses. Therefore, a normal or MH diagnosis by the NAMHG protocol was frequently associated with an equivocal diagnosis by the EMHG protocol. The time to 0.2-g contracture after the addition of 1 μ M ryanodine completely separated populations, which was in agreement with the EMHG protocol and, except for one person, with the NAMHG protocol.

Conclusions: Overall, the NAMHG and EMHG protocols and the experimental ryanodine test yielded similar diagnoses. The EMHG protocol reduced the number of marginal responders in the final analysis, which may make the remaining diagnoses slightly more accurate for use in genetic studies. (Key words: Anesthetic complications; muscle rigidity; succinylcholine.)

THE North American Malignant Hyperthermia (MH) Group (NAMHG)¹ and the European MH Group (EMHG)² protocols for the diagnosis of MH susceptibility were developed independently. Consequently, they are slightly different, deliberately, because the NAMHG protocol was developed after the EMHG protocol during a period of uncertainty about the best approach. Genetic studies have assumed that either protocol could be used to phenotype MH susceptibility. Although the sensitivities and specificities of both protocols were reported recently,^{3,4} data directly comparing these two protocols are sparse.⁵⁻⁷ Controversy has arisen because of inconsistencies between the outcome of the *in vitro* contracture test and specific mutations in the ryanodine receptor proposed to cause MH.⁸⁻¹³ Previous studies suggested that altering the thresholds of the halothane and caffeine tests required for a positive diagnosis might yield a more reliable linkage.¹⁴ It is premature to assume that the outcome of the contracture test is in error in all cases in which there is discordance with the genetic analysis, and it is important to know the correlation between the results of the NAMHG protocol and those of the EMHG protocol to better evaluate genetic studies in the future.

The NAMHG^{1,7} and EMHG^{4,7} protocols test the *in vitro* contracture response of muscle fiber bundles from biopsy muscle to halothane and, in separate strips, to caffeine.^{1,4,7} Differences between the two protocols are

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most apparent with the halothane test.⁷ The NAMHG protocol specifies that a single concentration of halothane (3%) is administered to a minimum of three fiber bundles.^{3,7} In the EMHG protocol, halothane is added sequentially (0.5, 1, 2%) to two fiber bundles.⁴

Minor differences between the caffeine tests involve the number of strips tested (minimum of three *vs.* two, as with halothane), the specific concentrations of caffeine, and the time of exposure.⁷ A more significant difference between protocols is the threshold for a positive response (0.2 or 0.3 g for the EMHG or NAMHG, respectively) to caffeine (2 mM).

Interpretations of the response to halothane and to caffeine differ between the protocols. In the NAMHG protocol, if any one of the three strips exposed to halothane or the three strips exposed to caffeine exhibit a positive response (two-component test), then the person tested is judged to be MH susceptible (MH+), otherwise he or she is considered negative for MH (MH-).^{3,7} In contrast, the EMHG protocol⁴ requires a positive response in one of two strips tested with halothane and a positive response in one of two strips tested with caffeine to be judged MH susceptible (MHS). If the results of the halothane and caffeine tests are negative, then the person is diagnosed as MH nonsusceptible (MHN). If only the halothane or only the caffeine test is positive by the EMHG protocol, then the diagnosis is termed equivocal (MHE), although the person is regarded clinically as MHS. These persons are further subdivided into MHE(h) and MHE(c), depending on whether the positive response was to halothane or to caffeine, respectively.

Recently, the NAMHG and EMHG centers incorporated the use of 1 μ M ryanodine as an investigational test.¹⁵ No values are established for a positive test, but the times to onset of contracture and to reach a tension of 0.2 g have been reported. The ryanodine protocol has not been compared with the outcome of the NAMHG protocol.

Whereas the EMHG has a fixed set of diagnostic thresholds,⁴ the Registry of the NAMHG has suggested a more stringent halothane threshold of 0.7 g for genetic studies (that is, to qualify the specimen as MH+ for analysis of a specific genetic mutation) and a threshold of 0.5 g for clinical diagnosis.³ Significant variability between centers has been shown in multicenter studies with regard to the NAMHG¹⁶ and ryanodine¹⁷ protocols. The current study compares the NAMHG, EMHG, and ryanodine methods for MH diagnosis in a single population.

Methods

This study was approved by the Allegheny University of Health Sciences human studies committee. Eighty-four persons were tested with the NAMHG and EMHG halothane protocols. None of them experienced an "almost certain" episode of MH.¹⁸ Using the more stringent proposed diagnostic criteria for genetic studies (0.7-g halothane response), 48 (16 male, 32 female) persons were diagnosed as MH-, 2 (both male) as MHE (maximum responses to 3% halothane were 0.60 and 0.65 g), and 34 (16 male, 18 female) as MH+ by the NAMHG protocol. The NAMHG MHE group is defined as persons having a positive response only to halothane in the range of 0.5-0.7 g, and these are treated clinically as MH+.³ Participants with known muscle disorders other than MH were excluded from the study. For each participant, 9-11 strips of vastus lateralis muscle were tested, depending on the number of viable preparations isolated from the biopsy specimen. The NAMHG protocol was conducted in the first set of six strips mounted after the biopsy. During this test, three muscle strips were tested to 3% halothane (Halocarbon Laboratories, River Edge, NJ) and the maximum magnitude of contracture within 10 min was recorded. Three muscle strips also were tested with caffeine (free base; Sigma Chemical Co., St. Louis, MO) with twofold increasing increments (0.5, 1, 2, 4, 8, and 32 mM) by the standard protocol.^{3,7} A second set of strips was mounted and two strips were tested with halothane by the EMHG protocol in twofold increasing concentrations (0.5, 1, 2%), holding each concentration for 3 min after reaching the desired concentration of halothane. The small size of the bath (5 ml) allowed rapid equilibration of halothane with the bathing solution, as other investigators demonstrated using a bath of similar size.¹⁹ Finally, one or two strips were tested with ryanodine and, when possible, an additional strip was tested to 3% halothane according to the NAMHG protocol to confirm that the muscle response to halothane was not altered during this period. This additional halothane-treated strip was not used for diagnostic purposes and in no case would the response of the strip have altered the diagnosis, even if it was used. Because the minor differences between caffeine protocols should not alter significantly the diagnostic outcome in most cases, only the caffeine test after the NAMHG protocol was used to complete the studies within 5 h with the small amounts of tissue available.

The criteria for a positive diagnosis by the NAMHG protocol were a contracture response greater than or

Table 1. Agreement between Contracture Thresholds of the North American MH Group (NAMHG) Halothane and Caffeine Protocols and the European MH Group (EMHG) Halothane Protocol

NAMHG Protocol Criteria				Published Sensitivity (%) ³	Agreement with EMHG Protocol [*]
Contracture to Halothane 3%		Contracture to Caffeine 2 mM			
Comparing the positive response					
≥0.7 g	or	≥0.3 g		88	30/34 (88%)
≥0.5 g	or	≥0.3 g		97	31/36 (86%)
Comparing the negative response				Published Specificity (%) ³	
<0.7 g	and	<0.3 g		81	42/50 (84%)
<0.5 g	and	<0.3 g		78	42/48 (88%)

* The number of subjects with a contracture threshold of ≥ 0.2 g to halothane $\leq 2\%$ for comparing the positive response, or < 0.2 g for comparing the negative response, by the EMHG protocol divided by the total number of subjects meeting the indicated NAMHG protocol criteria.

equal to 0.7 g (genetic study threshold) or ≥ 0.5 g (clinical diagnosis threshold) to 3% halothane in any one of the three strips tested, or a caffeine contracture greater than or equal to 0.3 g in any of the three strips tested. The criteria for the EMHG protocol were a minimum contracture of 0.2 g to 2% halothane or less and to 2 mM caffeine or less.

The ryanodine test was performed according to the protocol first standardized by the EMHG and later adopted by the NAMHG. In this test, high purity ($> 99\%$ by high-pressure liquid chromatography) 1 μM ryanodine (Calbiochem, La Jolla, CA) was added to the bath, and the times to onset of contracture and to a baseline tension of 0.2 g were recorded. There are no specific criteria for a positive diagnosis of MH by the ryanodine test. However, the results of Wappler *et al.*¹⁵ suggest that the best separation of MHS and MHN participants tested with a 1 μM concentration of ryanodine occurs at the time to 0.2-g contracture. Using this criterion, the ranges for the time from ryanodine addition to a 0.2-g contracture were 2–17.7 min for the MHS group and 22–46 min for the MHN group.¹⁵ The times to onset and to 0.2-g contracture were selected for analysis of the ryanodine contracture results in the current study. When two muscle strips were tested, only the strip with the more rapid responses was used in the analysis.

Results

Because comparisons were made between responses of muscle to various agents during a 5-h period, we wanted to determine whether time was a significant factor in the analysis. For 17 MH+ participants, an additional muscle strip was tested to 3% halothane using the

NAMHG protocol at the same time (4.64 ± 0.54 h [$n = 17$]; mean \pm SD) as the tests with halothane using the EMHG protocol and ryanodine test were conducted to compare with the set used for the NAMHG protocol (2.58 ± 0.35 h [$n = 17$]). The twitch height decreased from 3.59 ± 2.98 g ($n = 17$) to 1.63 ± 2.39 g ($n = 17$). However, there was no difference ($P = 0.431$; paired two-tailed t test) in the response of this fourth strip (1.20 ± 1.1 g [$n = 17$]) and the mean response of the three strips tested by the NAMHG protocol (1.03 ± 0.69 g [$n = 17$]). These findings suggest that there was no significant bias introduced related to testing time and supports testing for as long as 5 h from the time of the biopsy, as recommended by the NAMHG¹ and EMHG⁴ protocols.

The outcome of the halothane test for each protocol was compared with the diagnostic outcome of the opposite protocol to determine whether the two methods of halothane testing reliably measure the same outcome. Using the suggested genetic study thresholds for the NAMHG protocol (≥ 0.7 g to 3% halothane or ≥ 0.3 g to 2 mM caffeine), the EMHG halothane test corresponded with a diagnosis of MH+ by the NAMHG protocol in 88% of the participants (table 1).

Using slightly less stringent clinical criteria for the NAMHG protocol (≥ 0.5 g to halothane or ≥ 0.3 g to caffeine), two participants formerly classified as MHE (maximum halothane contractures of 0.60 g and 0.65 g) were now included as MH+ in the comparison. The agreement between diagnosis by the NAMHG protocol and the outcome of the EMHG halothane protocol decreased only slightly with these less stringent criteria (table 1). As with the positive response, there was very little difference between the genetic and clinical criteria

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Table 2. Agreement between Contracture Thresholds of the EMHG Halothane and Caffeine Protocols and the NAMHG Halothane Protocol

EMHG Protocol Criteria			
Contracture to Halothane \leq 2%		Contracture to Caffeine 2 mM	Published Sensitivity (%)
Comparing the positive response \geq 0.2 g	and	\geq 0.2 g	99
Comparing the negative response $<$ 0.2 g	and	$<$ 0.2 g	93.6
			Published Specificity (%)
			Agreement with NAMHG Protocol*
			18/19 (95%)
			42/42 (100%)

* The number of subjects with a contracture threshold of \geq 0.7 g to halothane 3% for comparing the positive response, or $<$ 0.7 g for comparing the negative response, by the NAMHG protocol divided by the total number of subjects meeting the indicated EMHG protocol criteria.

for the NAMH and EMHG halothane protocols when the negative response was evaluated (table 1).

The percentage of the cases in which the NAMHG halothane test would be positive or negative using the EMHG diagnosis criteria⁴ ranged from 95–100% (table 2). However, if the halothane response was positive and the caffeine response was negative (*i.e.*, MHE[h]), only 56% of these 18 equivocal patients were also positive by the NAMHG halothane protocol (data not shown). Overall, although they support strong agreement between the MH+ and MHS diagnoses and between the MH– and MHN diagnoses, these findings suggest that it is impossible to predict whether MHE(h) results by the EMHG protocol will be positive or negative by the NAMHG halothane protocol.

The diagnoses by the NAMHG protocol for the 84 participants were compared with those by the EMHG

protocol in table 3. The number of participants meeting the NAMHG genetic study thresholds is indicated with the number of participants for the clinical diagnosis thresholds in parentheses, indicating the two persons whose diagnoses were altered. Whether the clinical diagnosis or genetic study guidelines are used, it is clear that a substantial number of persons diagnosed as MH– or MH+ by the NAMHG protocol are MHE by the EMHG protocol. We eliminated the participants diagnosed as MHE to determine whether there was good agreement between the diagnosis of MHN or MHS by the EMHG protocol and MH– and MH+ by the NAMHG protocol (table 3, bottom). The 19 MHS participants according to the EMHG protocol were all MH+ (100%) by the NAMHG protocol. All but one of the 41 MHN participants according to the EMHG protocol were MH– by the NAMHG protocol. For the person diagnosed as MH+

Table 3. Comparison of Diagnosis as MH+, MHE, and MH– by the North American MH Group (NAMHG) Protocol with Diagnosis as MHS, MHE [MHE(h) and MHE(c)], and MHN by the European MH Group (EMHG) Protocol

NAMHG Diagnosis	EMHG Diagnosis				NAMHG Totals
	MHN	MHE(h)	MHE(c)	MHS	
Includes MHE diagnosis					
MH ⁻	40	6	2	0	48
MHE	1 (0)	1 (0)	0	0	2 (0)
MH ⁺	1 (2)	11 (12)	3	19	34 (36)
EMHG Totals	42	18	5	19	
		EMHG Diagnosis			
		MHN	MHS		NAMHG Totals
Excludes MHE diagnosis					
MH ⁻		40	0		40
MH ⁺		1	19		20
EMHG Totals		41	19		

Numbers of subjects in each category are indicated. For example, in the first row the 48 subjects diagnosed as MH– by the NAMHG protocol consisted of 40 MHN, 6 MHE(h), 2 MHE(c), and 0 MHS by the EMHG protocol. Values in parentheses indicate where the numbers of subjects are changed when the threshold for a positive response by the NAMHG halothane protocol is lowered from 0.7 g to 0.5 g. The bottom half of the table excludes those subjects diagnosed as MHE by the EMHG protocol.

(by the NAMHG protocol) and as MHN (by the EMHG protocol), one strip had a halothane contracture of 0.7 g (others were 0.2 and 0.4 g) and one strip had a caffeine contracture of 0.1 g (others were both 0.0 g). No additional strips were tested by the NAMHG protocol. Both strips tested by the EMHG halothane protocol exhibited 0.1-g contractures at 2% halothane. Specimens from this participant were not tested with ryanodine. The histologic report revealed no specific muscle disorder but some minor type II fiber atrophy. The participant (a 75-yr-old man) was referred for problems experienced by his son during anesthesia suggestive of MH (temperature elevation to 40.6°C and heart rate increase to 160 beats/min). Two grandchildren were tested previously and had maximum contractures to 3% halothane of 0.9 g (an 8-yr-old girl) and 3.7 g (a 15-yr-old boy). Overall, the clinical status of this participant is uncertain, and he could be truly MHS or have had a false-positive result by the NAMHG protocol. Recently, we determined that this man is heterozygous for a dihydropyridine receptor mutation associated with MH,²⁰ as is his grandson, who was diagnosed as MH+ and is the only other family member to be screened for mutations (S. L. Stewart and J. E. Fletcher, manuscript in preparation). If this mutation truly causes MH, then this may be a false-negative diagnosis by the EMHG protocol.

When the threshold for the halothane response by the NAMHG protocol was decreased to 0.5 g, as suggested for clinical diagnosis,³ one additional mismatch occurred between the NAMHG and EMHG protocols (see the value in parentheses for MH+/MHN in table 3). This participant had one 0.65-g contracture to 3% halothane (others were 0.15 and 0.10 g). The contractures to 2 mM caffeine were 0.05, 0.00, and 0.00 g. No additional strips were tested using the NAMHG protocol. The two strips tested using the EMHG halothane protocol had no contractures (0.00 and 0.00 g) at 2% halothane. The times to onset and 0.2-g contracture to 1 μ M ryanodine were 59 and 86 min, respectively. The participant's father died after an appendectomy for unclear reasons, even after autopsy. Therefore, the true MH status of this person cannot be established conclusively, and the outcome of the NAMHG diagnostic test could be truly MH+ or a false-positive result.

Because we conducted only the caffeine test with the NAMHG protocol (three strips tested), it is of interest to determine whether the diagnostic outcome would be altered significantly if only two strips were tested by the EMHG protocol. For 7 of 24 participants (29%) with a positive response to caffeine (all MHS or MHE[c] participants),

only one of the three strips tested was positive. Therefore, we assume that approximately one third of the seven (approximately 2.3 participants, or approximately 10% of 24) may not have been positive if only two strips were tested, as required for the EMHG protocol. In agreement, 9 of 33 (27%) participants tested positive only to one strip tested by the NAMHG halothane protocol, suggesting that 9% may not have been positive if only two strips had been tested.

Fifty of 84 participants had sufficient viable muscle to allow testing with ryanodine and by the EMHG (9 MHS, 8 MHE, 33 MHN) and NAMHG (13 MH+, 2 MHE, 35 MH-) protocols. The best agreement between either the NAMHG or the EMHG diagnosis and the onset of the ryanodine contracture was achieved using 12 min to separate the groups (fig. 1). Only 2 of 14 MH+ participants by the NAMHG protocol had a time to onset longer than 12 min (fig. 1A). These two participants were MHE(h) by the EMHG protocol, had 0.7- and 1.5-g contractures to 3% halothane by the NAMHG protocol, and the times to onset of ryanodine contractures were 12.4 and 13.8 min, respectively. All but one MH- participant (10.2 min) had a time to onset longer than 12 min (fig. 1A), and this person also was classified as MHN by the EMHG protocol (fig. 1B). Using 16 min to separate the groups for the time to reach a 0.2-g contracture, a diagnosis in only one MH+ participant (0.7 g to 3% halothane) did not correspond with the diagnosis by the NAMHG protocol (fig. 2A). All MHN (the shortest time to 0.2-g contracture was 16.7 min) and MHS (the longest time to 0.2-g contracture was 15 min) participants were separated by the time to reach a 0.2-g contracture using the EMHG protocol (fig. 2B). The MHE participants by the EMHG protocol were divided equally by the 16-min line (fig. 2B).

Discussion

Based on direct comparisons conducted in one laboratory, the outcomes of the technically different NAMHG and EMHG halothane protocols compare favorably with one another, and the final diagnoses by the NAMHG and EMHG halothane and caffeine testing protocols are similar but not identical. Furthermore, an experimental ryanodine test compared favorably with the outcomes of the NAMHG and EMHG protocols.

Good agreement (84–100%) occurred between the halothane response after one protocol and diagnosis by the other protocol. Therefore, although the halothane

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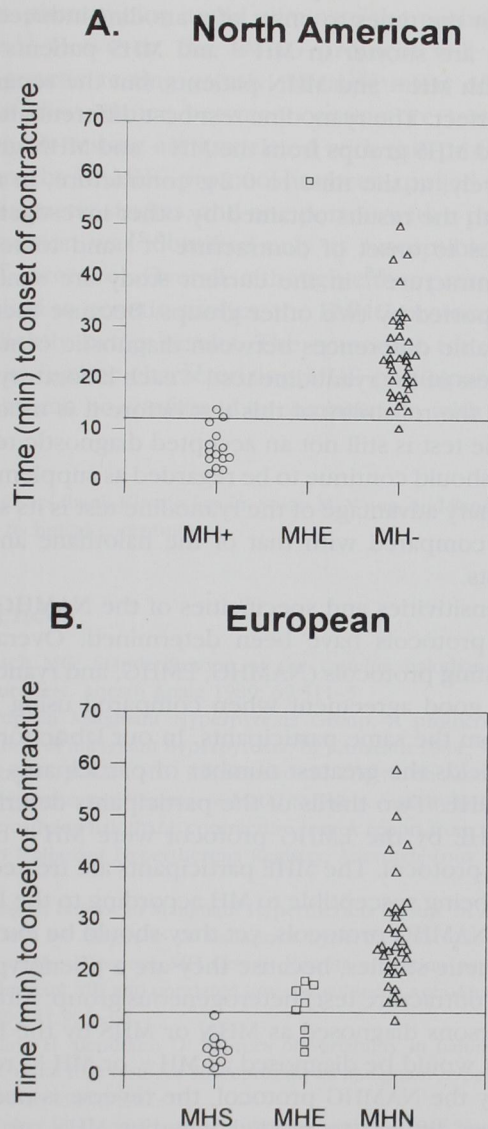


Fig. 1. The time to onset of ryanodine contractures in participants diagnosed by halothane and caffeine testing. The diagnoses were determined by (A) the North American Malignant Hyperthermia Group protocol using the recommended threshold for genetic studies (0.7-g contracture to 3% halothane or \geq 0.2-g contracture to 2 mM caffeine)³ or (B) the European Malignant Hyperthermia Group protocol and the time to onset of contracture to 1 μ M ryanodine was recorded. A line is drawn at 12 min to indicate the best separation of the malignant hyperthermia-susceptible and malignant hyperthermia-negative participants.

protocols differ in methods and thresholds, they reach similar diagnoses. The halothane test is only one component of the diagnostic test. The final diagnosis depends on the interpretation of this test and the caffeine test. By the NAMHG protocol, a 0.2-g contracture threshold for 2 mM caffeine results in a sensitivity of 84% and a

specificity of 79%, whereas a 0.3-g contracture threshold for caffeine has a sensitivity of 69% and a specificity of 87%.³ The use of a two-component test (0.5-g contracture to halothane or 0.3-g contracture to caffeine) for the NAMHG protocol results in an overall sensitivity of 97%

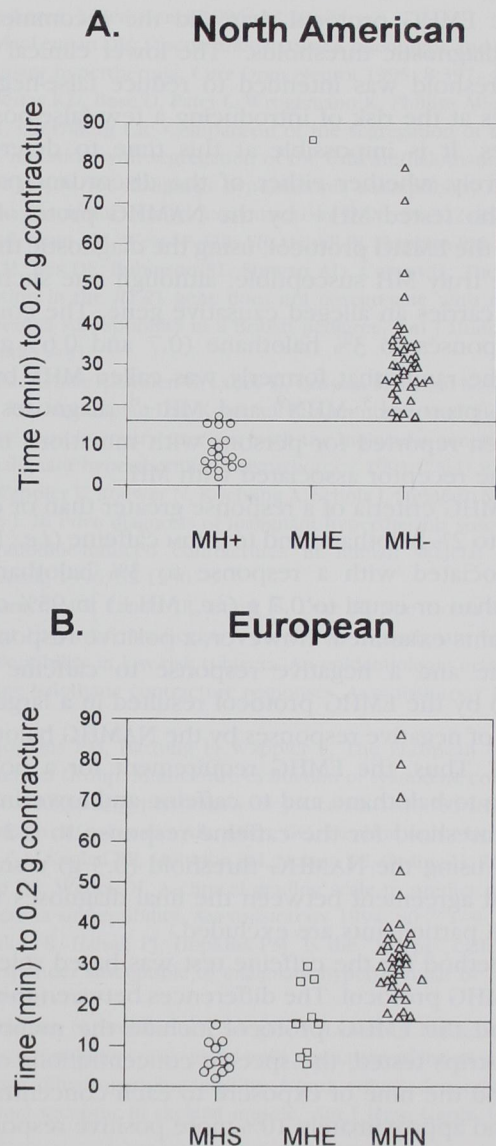


Fig. 2. The time to a 0.2-g contracture to ryanodine in participants diagnosed by halothane and caffeine testing. The diagnoses were determined by (A) the North American Malignant Hyperthermia Group protocol using the recommended threshold for genetic studies (0.7-g contracture to 3% halothane or \geq 0.2-g contracture to 2 mM caffeine)³ or (B) the European Malignant Hyperthermia Group protocol and the time to 0.2-g contracture to ryanodine 1 μ M was recorded. A line is drawn at 16 min to indicate the best separation of the malignant hyperthermia-susceptible and malignant hyperthermia-negative participants.

and a specificity of 78%.³ The EMHG protocol supports the 0.2-g threshold in the caffeine test for maximizing sensitivity and specificity (99 and 93.6%, respectively).⁴ Regarding the final diagnosis, use of the 0.7-g threshold for the NAMHG halothane protocol, as suggested for genetic studies,³ resulted in slightly better agreement with the EMHG protocol than did the recommended clinical diagnostic thresholds.³ The lower clinical diagnosis threshold was intended to reduce false-negative diagnoses at the risk of introducing a few false-positive diagnoses. It is impossible at this time to determine conclusively whether either of the discordant participants who tested MH+ by the NAMHG protocol and MHN by the EMHG protocol, using the diagnostic thresholds, are truly MH susceptible, although one such participant carries an alleged causative gene. The contracture responses to 3% halothane (0.7 and 0.65 g) are within the range that formerly was called MHE by the NAMHG protocol.³ MHN⁹ and MH-⁸ diagnoses also have been reported for persons with mutations in the ryanodine receptor associated with MH.

The EMHG criteria of a response greater than or equal to 0.2 g to 2% halothane and to 2 mM caffeine (*i.e.*, MHS) was associated with a response to 3% halothane of greater than or equal to 0.7 g (*i.e.*, MH+) in 95% of the participants examined. However, a positive response to halothane and a negative response to caffeine (*i.e.*, MHE[h]) by the EMHG protocol resulted in a large percentage of negative responses by the NAMHG halothane protocol. Thus, the EMHG requirement for a positive response to halothane and to caffeine and lowering the EMHG threshold for the caffeine response to 0.2 g instead of using the NAMHG threshold (0.3 g) results in excellent agreement between the final diagnoses when the MHE participants are excluded.

The method for the caffeine test was based solely on the NAMHG protocol. The differences between this protocol and the EMHG protocol include the number of muscle strips tested, the specific concentrations of caffeine, and the time of exposure to each concentration. We found approximately 10% more positive responders when the results from three strips were used rather than those from two strips. Studies directly comparing the outcomes of the NAMHG and EMHG caffeine protocols are still required to test the effects of increasing the number of strips from two to three and to account for the different caffeine concentrations and times of exposure. A large multicenter study might be useful in this regard and to establish interlaboratory differences.

The results of the current and previous studies^{15,17,21}

show that the times to onset of ryanodine-induced contractures are shorter in MH+ and MHS patients compared with MH- and MHN patients, but the separation is not perfect. The ryanodine test best differentiated the MH+ and MHS groups from the MH- and MHN groups, respectively, at the time to 0.2-g contracture, in agreement with the results obtained by other investigators.¹⁵ The times to onset of contracture^{15,21} and to reach a 0.2-g contracture¹⁵ in the current study are similar to those reported by two other groups. Because there are considerable differences between diagnostic centers in the success of the ryanodine test,¹⁷ each laboratory must establish the reliability of this test before it is used. The ryanodine test is still not an accepted diagnostic test for MH and should continue to be regarded as supplemental. The primary advantage of the ryanodine test is its simple method compared with that of the halothane and caffeine tests.

The sensitivities and specificities of the NAMHG³ and EMHG⁴ protocols have been determined. Overall, all three testing protocols (NAMHG, EMHG, and ryanodine) were in good agreement when compared using specimens from the same participants. In our laboratory, the EMHG yields the greatest number of participants classified as MHE. Two thirds of the participants determined to be MHE by the EMHG protocol were MH+ by the NAMHG protocol. The MHE participants are treated clinically as being susceptible to MH according to the EMHG and the NAMHG protocols, yet they should be excluded from genetic studies, because they are a phenotypically (by the contracture test) heterogeneous group. Although most persons diagnosed as MHN or MHS by the EMHG protocol would be diagnosed as MH- or MH+, respectively, by the NAMHG protocol, the reverse is not true. It is more difficult to predict whether MH- or MH+ persons are MHN or MHS, because a significant number would be MHE. Several (three in the current study) of these MHE participants also exhibited marginal responses to ryanodine.

The EMHG protocol eliminates a substantial number of MHE persons from diagnostic studies, and these are typically those with marginal responses. The price of reducing the sample size should be compensated to some extent by a slightly more reliable diagnosis, as supported by the differences in estimated sensitivity and specificity for these protocols.^{3,4} Thus, routine use of the EMHG protocol may reduce slightly the possibility of a false-positive or false-negative diagnosis when the MHE group is eliminated. With the small number of family members involved in many linkage studies, even a single

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error in phenotyping could eliminate the probability of linkage.¹¹ However, the EMHG protocol will not improve genetic testing greatly, because many of the reports of discordance between contracture test results and genetic testing are associated with this protocol. In addition, the NAMHG protocol has been applied successfully to genetic studies, and one person with a proposed MH mutation was identified only as susceptible by the NAMHG protocol. Overall, although either protocol can be applied to genetic studies, the EMHG protocol might yield slightly better results. The ryanodine test may be useful in further characterizing MHE participants if its reliability can be confirmed in combination with genetic studies.

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