Anesthesiology
76:16–27, 1992

Prediction of Malignant Hyperthermia Susceptibility in Low-risk Subjects

An Epidemiologic Investigation of Caffeine Halothane Contracture Responses

Marilyn Green Larach, M.D., F.A.A.P., J. Richard Landis, Ph.D.,† Joan Schaeffer Bunn, B.S.,‡ Marcela Diaz, M.D.§, The North American Malignant Hyperthermia Registry¶*

The most commonly used laboratory test for predicting malignant hyperthermia susceptibility is the caffeine halothane contracture test. However, the specificity and sensitivity of proposed North American diagnostic guidelines for this test have never been evaluated in a large, human study population. Therefore, the authors conducted a multiinstitutional, prospective study of skeletal muscle contracture responses in a subject population at low risk for malignant hyperthermia susceptibility to help determine the specificity of the proposed guidelines. Subjects were selected arbitrarily from a population of patients undergoing surgery unrelated to performance of a diagnostic muscle biopsy. Subjects were admitted to this study and were presumed nonsusceptible if there was no evidence of any of the following malignant hyperthermia risk factors: prior abnormal response to triggering anesthetic agents, myopathy, or family history of malignant hyperthermia susceptibility. The authors suggested rejection of the proposed diagnostic guidelines if an 85% specificity estimate among subjects could not be obtained. The authors analyzed the responses of 1,022 muscle fascicles, derived from 176 subjects, to the following: 1) separate administration of 3% halothane or incremental caffeine concentrations, or 2) the joint administration of 1% halothane and incremental caffeine concentrations. The following contracture results were obtained. First, for individual fascicles, 9.2% exceeded a > 0.7 g threshold for 3% halothane, 15.2% exceeded a ≥ 0.2 g threshold for 2 mM caffeine, 32.4% exceeded a 1-g increase for < 4 mM caffeine, 2.6% had a > 7% maximal increase in tension at 2 mM caffeine, and 63.5% had a "halothane caffeine-specific concentration" at ≤ 1 mM caffeine. Second, the percentages of subjects with 1 or more fascicles exceeding the proposed threshold were as follows: 45.8% for the four-component, 28.8% for the three-component, and 32.7% for the two-component contracture test. Third, the percentages of subjects with 1 or more fascicles exceeding the proposed threshold for both halothane and caffeine were as follows: 9.5% for 3% halothane and 2 mM caffeine, 2.0% for 3% halothane and 7% maximal increase in tension at 2 mM caffeine, and 11.0% for 1% halothane and 2 mM caffeine. Fourth, center-to-center differences were the major source of variation in the rate that subjects exceeded proposed thresholds. These data demonstrate that proposed diagnostic guidelines must be modified to improve specificity estimates before adoption by diagnostic centers. The authors recommend efforts to develop a uniform method for analyzing *in vivo* adverse patient responses to anesthetics and to define contracture *sensitivity* for the patient population susceptible to malignant hyperthermia. (Key words: Malignant hyperthermia: epidemiology; susceptibility. Measurement techniques, caffeine halothane contracture test: specificity. Muscle: skeletal.)

* Director, The North American Malignant Hyperthermia Registry; Assistant Professor of Anesthesia, Pennsylvania State University College of Medicine.

† Professor of Biostatistics, Pennsylvania State University College of Medicine.

‡ Biostatistician, Center for Biostatistics and Epidemiology, Pennsylvania State University College of Medicine.

§ Postdoctoral Fellow in Anesthesia, Pennsylvania State University College of Medicine.

¶ The North American Malignant Hyperthermia Registry: Cleveland Clinic (Hiroshi Mitsumoto, M.D.; Glenn E. DeBoer, M.D.); Hahnemann University (Jeffrey E. Fletcher, Ph.D.; Henry Rosenberg, M.D.); Mayo Clinic (Denise J. Wedel, M.D.); Ottawa Civic Hospital (Gregory Allen, M.D., F.R.C.P.C.); Presbyterian University Hospital (Marshall Millman M.D., Ph.D.); Toronto General Hospital (Beverley A. Britt, M.D., F.R.C.P.C.); Uniformed Services University of Health Sciences (Sheila M. Muldoon, M.D.); University of California at Davis (Gerald A. Gronert, M.D.); University of California at Los Angeles (Jordan D. Miller, M.D.); University of Manitoba (Deepak D. Bose, M.D., Ph.D.; Leena R. Patel, M.D., F.R.C.P.C.); University of Massachusetts Medical Center (Barbara E. Waud, M.D.); University of Nebraska (Dennis F. Landers, M.D., Ph.D.); University of South Florida (Richard F. Kaplan, M.D.); University of Texas Health Science Center (Thomas E. Nelson, Ph.D.); University of Washington (Judy Y. Su, Ph.D.; Lawrence E. Jacobson, M.D.); University of Wisconsin (John F. Kreul, M.D.). Registry Site: Pennsylvania State University College of Medicine; Chairman: Gerald A. Gronert, M.D.; Secretary-Treasurer: Thomas E. Nelson, Ph.D.; Director: Marilyn Green Larach, M.D.

Received from The North American Malignant Hyperthermia Registry, and the Department of Anesthesia and Center for Biostatistics and Epidemiology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania. Accepted for publication August 22, 1991. Supported in part by the American Society of Anesthesiologists; the Department of Anesthesia and Center for Biostatistics and Epidemiology, Pennsylvania State University; the Foundation for Anesthesia Education and Research (Research Starter Grant to Dr. Larach); the Malignant Hyperthermia Association; the Malignant Hyperthermia Association of the United States; and Norwich Eaton Pharmaceuticals, Inc. Presented in part at the Fifth International Malignant Hyperthermia Workshop, Munich, Germany, September 23, 1990; the Annual Meeting of the American Society of Anesthesiologists, Las Vegas, Nevada, October 15, 1990; and the 38th Annual Meeting of the Association of University Anesthesiologists, Philadelphia, Pennsylvania, May 3, 1991.

Address reprint requests to Dr. Larach: Department of Anesthesia, Pennsylvania State University College of Medicine, P. O. Box 850, Hershey, Pennsylvania 17033.

MALIGNANT HYPERTHERMIA (MH) susceptibility is an inherited disorder of skeletal muscle in which commonly used anesthetic medications trigger sustained skeletal muscle hypermetabolism and/or contracture in patients who may have had no symptoms previously. Acute MH reactions are potentially fatal hypermetabolic events[1]; therefore, accurate prediction of preanesthetic MH susceptibility can be lifesaving. Currently, such prediction

depends on accurate analysis of the *in vivo* patient response to anesthesia and/or the *in vitro* muscle response to the caffeine halothane contracture test (CHCT).

Analysis of patients' *in vivo* responses is not standardized or validated. Currently, *in vivo* prediction of MH susceptibility is made with certainty only when a patient has a fulminant MH episode after exposure to known MH-triggering agents. New monitoring modalities may detect early nonspecific metabolic abnormalities that may represent a beginning MH episode.[2] Thus, fulminant MH episodes are seen with less frequency, and early MH episodes may be confused easily with other medical conditions.[3-6] Ethical considerations prevent rechallenging a patient who has had a possible early MH episode with triggering anesthetic medications. Therefore, it is important to develop sensitive and specific laboratory tests for MH susceptibility.

Definitive diagnosis of MH susceptibility is desirable to facilitate appropriate medical management of the individual who has had a possible early MH episode. Although MH-susceptible individuals can receive anesthesia safely, this involves special equipment,[7] techniques,[8] and intensive patient monitoring, which are costly and inconvenient, and may increase the risk of intraoperative complications in MH-susceptible patients. Because of diagnostic uncertainty, one major North American children's hospital now uses a MH protocol for 0.5–1% of their patients.[9]

The most promising noninvasive MH susceptibility test is one based on genetic linkage analysis.[10-13] However, more than one gene may be responsible for the expression of MH susceptibility.[14,15] To date, individual research groups cannot demonstrate that they are dealing with phenotypically similar subjects because there is no standardized method for evaluating the *in vivo* response to anesthetics and the sensitivity and specificity of the *in vitro* North American CHCT have not been evaluated.

The *in vitro* CHCT was developed after Kalow *et al.* and Ellis *et al.* observed abnormal skeletal muscle contracture responses to caffeine[16] and halothane,[17] respectively, in survivors of fulminant MH events. For the last 15 yr, the *in vitro* CHCT has been used clinically to predict MH susceptibility in individuals who have not had a fulminant MH episode. At least 2,000 patients have undergone CHCT in North American MH diagnostic centers for diagnosis of their MH susceptibility.**

In 1987, the North American Malignant Hyperthermia Group of MH diagnostic centers standardized techniques for the performance of the *in vitro* North American CHCT and proposed diagnostic guidelines for interpreting CHCT results.[18] The proposed diagnostic guidelines reflected a 1987 consensus based on individual diagnostic center experience. However, before this study, neither the sensitivity nor specificity of the proposed guidelines had been evaluated by multiinstitutional study. The North American Malignant Hyperthermia Registry (Registry) was charged with evaluating whether these proposed guidelines required additional refinement before their adoption by all North American MH diagnostic centers.

We report the Registry's multiinstitutional prospective study of the responses to the CHCT of skeletal muscle derived from a surgical population. Subjects studied were at low risk for MH susceptibility, and biopsies were performed consecutively by 12 United States and Canadian MH diagnostic centers in accordance with the standard North American CHCT protocol.

This study is an analysis of CHCT response in a presumed non–MH-susceptible population. Such a study is informative only for *specificity* or the relative frequency with which a CHCT result will be negative in a presumed non–MH-susceptible population if the proposed diagnostic guidelines were adopted. Also, it will be important to determine the CHCT sensitivity or relative frequency with which a CHCT result will be positive in a MH-susceptible population if the proposed guidelines were adopted. Sensitivity must be determined by examination of clinically diagnosed, MH-susceptible patients and depends on standardization of the analysis of the *in vivo* patient response. These are ongoing Registry research projects. The CHCT should be nearly 100% sensitive because a false-negative diagnosis could result in a fatal MH event. If sensitivity were sufficiently high, most MH experts would accept a CHCT specificity as low as 85% (equivalent to 15% of subjects exceeding proposed thresholds) to avoid the risks of a false-negative diagnosis. An 85% CHCT specificity is comparable to the 85% specificity of elevated creatine phosphokinase-MB enzyme values (but 100% sensitivity) for diagnosis of myocardial infarction.[19]

Materials and Methods

Hypotheses

We tested the following hypotheses by analyzing muscle fascicle contracture responses to halothane and incremental caffeine in the study population. The statistical method constructed upper 95% confidence intervals (with lower limits of zero), with the use of variance estimates incorporating the effects of the multistage clustering of muscle fascicles within subjects within biopsy centers. This was accomplished with the use of the SUDAAN software package.††

** Rosenberg H: Personal communication, January 1991.

†† Shah B: Software for survey data analysis, version 5.4. Research Triangle Park, NC, Research Triangle Institute, 1991.

Hypothesis 1

No more than 15% of all muscle fascicles exceed proposed contracture thresholds for halothane and caffeine.

Hypothesis 2

No more than 15% of all subjects have one or more muscle fascicles exceeding proposed contracture thresholds for a four-, three-, and two-component CHCT. (The four-component test evaluates subject responses to 3% halothane, 2 mM caffeine, "caffeine-specific concentration," and percentage of maximal increase in tension at 2 mM caffeine. The three-component test analyzes responses to 3% halothane, 2 mM caffeine, and percentage of maximal increase in tension at 2 mM caffeine. The two-component test includes contracture responses to 3% halothane and 2 mM caffeine only.)

Hypothesis 3

No more than 15% of subjects have one or more muscle fascicles exceeding proposed contracture thresholds for both halothane and incremental caffeine ("joint agent CHCT").

Hypothesis 4

Individual diagnostic centers are not significant sources of variation in the rate at which subjects exceed proposed thresholds.

Subject Selection and Characteristics

After approval was obtained from the institutional review board, results from all subjects undergoing skeletal muscle biopsy by North American diagnostic centers between October 20, 1987, and February 9, 1990, were reported to the Registry and considered for inclusion in this study. The muscle biopsy results from 7 subjects studied at three MH diagnostic centers were excluded because each of these centers had contributed fewer than 10 subjects. The remaining nine diagnostic centers performing ten or more study biopsies per center contributed a total of 176 study subjects. The number of subjects contributed by each center ranged from 10 to 29. Study subjects were selected arbitrarily from a population of patients undergoing surgery unrelated to performance of a diagnostic muscle biopsy. Subjects were admitted to this study and were presumed to be nonsusceptible to MH if there was no evidence of the following MH risk factors: prior abnormal response to triggering anesthetic agents, myopathy, or family history of MH susceptibility.

Anesthetics administered before harvest of skeletal muscle for use in the CHCT included MH-triggering agents (potent inhalational anesthetics and/or succinylcholine) in 56% (99 of 176; 18 not recorded) of subjects. Subjects' demographics were as follows: age range, 6–89 yr (mean, 54.4 yr); race, predominantly white (151 of 176; 15 not recorded); and sex, 83 male and 91 female subjects (2 not recorded). The mean time interval between muscle excision and test completion was 2.7 h (range, 0.5–9.0 h). Vastus group muscles were tested in 52% of subjects (91 of 176); rectus abdominis muscles were tested in 23% of subjects (41 of 176); and gracilis and nonvastus and/or nonrectus muscles were tested in 25% of subjects (44 of 176).

North American CHCT Technique and Proposed Diagnostic Guidelines

In accordance with the standardized North American protocol,[18] replicate samples of fresh skeletal muscle were stimulated electrically after they were placed in a 37° C tissue bath of Krebs–Ringer's solution buffered with carbogen. Each muscle fascicle was exposed to one of the following test agents: a single dose of 3% (volume/volume) halothane; incremental concentrations of caffeine (0.5, 1, 2, 4, 8, and 32 mM)‡‡; or, optionally, simultaneous exposure to 1% halothane and incremental caffeine concentrations (0.25, 0.5, 1, 2, 4, and 32 mM). One hundred forty-one of 176 subjects had at least two muscle fascicles each exposed to 3% halothane and to incremental concentrations of caffeine. For 6 of 176 subjects, muscle fascicles were exposed only to incremental caffeine with no reported exposure of muscle fascicles to 3% halothane. For 2 of 176 subjects, muscle fascicles were exposed only to 3% halothane with no reported exposure of muscle fascicles to incremental caffeine. Except for the optional simultaneous 1% halothane and caffeine test, each muscle fascicle used for this analysis was exposed to only one test agent.

In vitro contracture responses were calculated as the difference between the highest tension observed after test agent exposure and the lowest tension observed just before any increases in tension after drug exposure (low point). The "caffeine-specific concentration" was defined as the caffeine concentration required to produce a 1-g increase above baseline. We identified the caffeine concentration interval in which the "caffeine-specific concentration" occurred (*i.e.*, < 2 mM; ≥ 2 to < 4 mM; ≥ 4 to < 8 mM; or ≥ 8 mM caffeine). "Percentage of maximal caffeine tension" was defined as [(2 mM caffeine contracture tension − low point)/(32 mM caffeine contracture tension − low point)] × 100. The "halothane caffeine-specific concentration" was defined as the caffeine concentration required to produce a 1-g increase above the low point during simultaneous administration of 1% halothane. We identified the caffeine concentration interval in which the "halothane caffeine-specific concentration" occurred (*i.e.*, < 0.25 mM; ≥ 0.25 to < 0.5 mM; ≥ 0.5 to < 1 mM; ≥ 1 to < 2 mM; or ≥ 2 mM caffeine).

‡‡ Center L used an initial caffeine concentration of 0.25 mM.

The proposed North American diagnostic guidelines suggest that a response will exceed normal under the following conditions: 1) more than 0.2–0.7-g contracture after exposure to 3% halothane with the exact value dependent on the individual center; 2) 0.2-g or greater tension at 2 mM caffeine; 3) "caffeine-specific concentration" achieved at less than 4 mM caffeine; 4) percentage of maximal increase in tension greater than 7% above the baseline at 2 mM caffeine (% MAX caffeine); and 5) for those centers performing this test, 1-g or greater contracture after exposure to incremental caffeine (exact concentration currently unspecified) in the presence of 1% halothane. Unlike the European CHCT protocol, which uses the 2 mM caffeine response only, the proposed North American diagnostic guidelines define three different component tests that may be used singly or in combination to measure muscle response to caffeine administration (2 mM caffeine, "caffeine-specific concentration," % MAX caffeine).[18,20] The proposed North American guidelines also suggest that a CHCT result should be interpreted as positive and the individual patient MH susceptible even if only one abnormal contracture response to one test agent in one muscle fascicle is observed. Therefore, if the proposed guidelines were adopted, a North American patient undergoing CHCT might be diagnosed as MH susceptible when only *one* of five component tests shows abnormal results.

Muscle Fascicle Characteristics

All muscle fascicles tested were obtained from muscle that was to be discarded as part of the surgical procedure. The muscle fascicle contracture response was measured on 1,022 muscle fascicles derived from 176 patients with the use of the following test agents: 3% halothane (n = 414), incremental caffeine (n = 427), and 1% halothane with incremental caffeine (n = 181). The mean numbers of replicate fascicles examined per test agent per subject were as follows: 2.35 for 3% halothane (range, 0–4), 2.43 for caffeine (range, 0–4), and 1.03 for 1% halothane and caffeine (range, 0–3). The mean number of replicate fascicles examined per subject was 5.81 (range, 2–9).

The mean wet weight of the muscle fascicles tested was 0.119 g (range, 0.008–0.544), and the mean wet length was 1.75 cm (range, 0.4–4.3). Cross-sectional area (cm^2) was calculated as weight (g)/[1.06 g/cm^3 × length (cm)]. The mean cross-sectional area was 0.07 cm^2 (range, 0.007–0.493), predrug twitch tension was 1.84 g (range, 0.00–19.5), and predrug twitch tension/cross-sectional area was 34.6 g/cm^2 (range, 0.0–520.4).

Sixty-seven muscle fascicles (6.6% of total sample) were excluded from analysis when they either tore before administration of test agents (n = 4), were poorly reactive to electrical stimuli (as verbally described by the individual laboratory, n = 25), or were found to be abnormal histologically (n = 38 fascicles derived from five subjects). Muscle from 67 of 176 study subjects (38.1%) was examined histologically; four diagnostic centers (A, D, E, and I) submitted histologic results for all of their subjects.

Contracture Responses Normalized by Cross-sectional Area

Current protocol stipulates that a fascicle should be 1–2 cm long and 0.1–0.5 cm wide. Cross-sectional area is not specified, even though tension development (contracture) may be proportional to cross-sectional area. We used a standard physiologic method to normalize changes in fiber size and investigated the distribution of 3% halothane, 2 mM caffeine, and % MAX caffeine responses when the tensions were normalized for muscle fascicle cross-sectional area. Normalized tensions were compared with nonnormalized contracture tensions within the framework of a two-way contingency table, with dimensions determined by threshold analysis of the 50th, 75th, and 90th percentiles of each distribution.

Contracture Threshold Data Analysis

Proposed Component Diagnostic Thresholds

With the use of proposed diagnostic guidelines, each subject's CHCT response to any of four component tests was determined. The four component tests were 3% halothane, 2 mM caffeine, "caffeine-specific concentration," and % MAX caffeine. For the following discussion, the rate at which subjects exceed proposed thresholds was calculated with contracture thresholds of more than 0.7 g for 3% halothane; 0.2 g or more for 2 mM caffeine; less than 4 mM caffeine for "caffeine-specific concentration"; and more than 7% MAX caffeine. The optional halothane caffeine-specific concentration test was not used to determine a subject's response because the North American Malignant Hyperthermia Group has not agreed on the appropriate caffeine concentration at which it should be measured.

Additional analysis included determination of each subject's CHCT response with "caffeine-specific concentration" eliminated (three-component test) and "caffeine-specific concentration" and % MAX caffeine eliminated (two-component test). For each diagnostic center, the total and mean number of contracture responses exceeding proposed thresholds for each subject were analyzed.

If proposed diagnostic guidelines were followed, a subject would be classified as MH susceptible even if only one contracture response exceeding proposed thresholds for one required test was observed. Clinically, within each diagnostic center, a diagnosis of MH susceptibility may

be made even if some required tests have been omitted. Therefore, the rate at which subjects exceeded proposed thresholds was determined for two groups: all subjects in whom at least one component test was administered (n = 176) and the subject subset in whom all component tests had been administered to each subject (n = 153 for four-component test, n = 153 for three-component test; and n = 168 for two-component test).

Specificity estimates were calculated with the following formula: 100 × number of subjects with true-negative results/(number of subjects with true-negative results + number of study subjects with results exceeding threshold)]. For this analysis, we assumed that all study subjects were nonsusceptible to MH. If we assume that there is an annual incidence of MH susceptibility of 1/14,000 in the surgical population,[21] the expected number of MH susceptible study subjects would be 1.3.

"Joint Agent" Diagnostic Thresholds

According to the European CHCT protocol, an individual is classified as MH susceptible only if he or she demonstrates contracture responses exceeding a threshold of 0.2 g or greater to administration of *both* halothane and caffeine. Analogous to this European approach, we determined the rate at which North American study subjects exceeded the following "joint agent" thresholds: 3% halothane contracture greater than 0.7 g *and* 2 mM caffeine contracture 0.2 g or greater; 3% halothane contracture greater than 0.7 *and* greater than 7% MAX caffeine; and 1% halothane contracture greater than 0.2 g *and* 2 mM caffeine contracture 0.2 g or greater. If a subject exceeded threshold for only one of the two joint agents, then the response was designated "equivocal."

Sources of Variation in the Rates That Fascicles and Subjects Exceed Proposed Thresholds

Muscle Fascicle Mean Contracture Responses

Some CHCT laboratory methods differ among diagnostic centers. Methods that differ include the following: the return of muscle fascicles to optimal length after relaxation, performance of liquid-phase halothane assay, and method for adding incremental caffeine concentrations to the bath solution. The significance of these CHCT method differences among centers was evaluated with the use of a general linear mixed-effects model as described below.

Sources of variation in mean contracture tension were investigated by including diagnostic center and replicate muscle fascicles within subjects as random effects. Subject age, sex, pre-CHCT anesthetic agents, wet weight, length, cross-sectional area, time between muscle excision and test completion (elapsed time), pre-agent twitch height normalized for cross-sectional area effects, predrug tension, and return to optimal length after relaxation, presence of liquid-phase halothane assay, and caffeine addition method were investigated for potential inclusion in the predictive model.

Rate at Which Study Subjects Exceed Proposed Thresholds

To determine the specificity estimates for the CHCT diagnostic guidelines, differences in the proportion of 176 subjects identified as exceeding one or more proposed thresholds for the four-, three-, and two-component CHCT were evaluated with the use of the standard chi-squared tests, and the magnitudes of various effects were estimated as odds ratios. Factors examined included the following: diagnostic center, age, sex, muscle type, and pre-CHCT anesthetic agents. Since specificity estimates may be increased falsely because all component tests were not performed in each subject, we also estimated CHCT specificity for the subject subset exposed to all CHCT component tests. Differences between diagnostic centers in the proportion of subjects diagnosed as exceeding threshold after exposure to all component tests also were evaluated with the use of standard chi-squared tests. With the use of the proposed new "joint agent" CHCT, differences between diagnostic centers in the proportion of subjects exceeding thresholds were evaluated with the use of standard chi-squared tests. A P value < 0.05 was considered significant.

Results

Contracture Responses Normalized by Cross-sectional Area

Alternate definitions of MH susceptibility, such as normalizing contracture response by cross-sectional area, were investigated. Normalizing for cross-sectional area did not appear to alter the distribution of contracture tension responses. A two-way contingency table of percentile distribution of absolute *versus* normalized contracture tensions showed a strong dominance of the main diagonal for 3% halothane, 2 mM caffeine, and % MAX caffeine responses (with 79%, 88%, 88% of all tensions, respectively, on the main diagonal).

Component CHCT Threshold Analysis

Rate at Which Muscle Fascicles Exceed Proposed Thresholds

The rate at which fascicles exposed to 3% halothane exceeded a proposed contracture threshold of > 0.2 g was 25.6% (range, 2.6–50.0%; n = 414 fascicles). Increasing the threshold to > 0.7 g decreased the overall

rate to 9.2% (range, 0–26.0%; n = 414). When the proposed diagnostic threshold was increased to > 1.0 g, then the overall rate decreased to 6.0% (range, 0–15.1%; n = 414). The rate at which fascicles exposed to 2 mM caffeine exceeded a proposed threshold of ≥ 0.2 g was 15.2% (range, 0–37.7%; n = 427). If the proposed diagnostic threshold was increased to ≥ 0.5 g at 2 mM caffeine, then the rate decreased to 6.1% (range, 0–22.6%; n = 427). The rate at which fascicles exceeded a proposed threshold of > 7% MAX caffeine was 2.6% (range, 0–10.0%; n = 379). The rate at which fascicles exceeded a proposed "caffeine-specific concentration" threshold at < 4 mM caffeine was 32.4% (range, 0–75.0%; n = 426). If the proposed diagnostic concentration for "caffeine-specific concentration" were decreased to < 2 mM caffeine, then the overall rate would decrease to 2.8% (range, 0–13.5%; n = 426). The rate at which fascicles exposed to both 1% halothane and incremental caffeine exceeded a proposed "halothane caffeine-specific concentration" threshold of ≤ 1 mM caffeine was 63.5% (range, 0–84.8%; n = 181). If the diagnostic concentration for halothane caffeine-specific concentration were lowered to ≤0.5 mM, the rate would decrease to 23.2% (range, 0–41.3%; n = 181). If the diagnostic concentration were decreased to ≤ 0.25 mM, then the rate would decrease to 7.2% (range, 0–13.0%; n = 181).

Hypothesis 1 states that no more than 15% of all muscle fascicles exceed proposed contracture thresholds for halothane and caffeine. One-sided 95% confidence intervals indicate that hypothesis 1 should be accepted for the proposed North American diagnostic thresholds of > 0.7 g contracture for 3% halothane (0, 12.3%) and > 7% MAX caffeine (0, 4.2%). Hypothesis 1 should be rejected for the proposed North American diagnostic thresholds of ≥ 0.2 g contracture at 2 mM caffeine (0, 18.4%) and "caffeine-specific concentration" at < 4 mM caffeine (0, 37.0%).

Rate at Which Study Subjects Exceed Proposed Thresholds

Triggering anesthetic agents were used in 56% of surgical procedures, and no subjects had adverse anesthetic reactions. Data will be presented on the rate that partially and completely tested subjects exceeded proposed thresholds.

Of all subjects tested with the four-component CHCT, 47.7% exceeded proposed thresholds for at least one of the component tests (range, 0–95.2% for each diagnostic center; n = 1,647 fascicles derived from 176 subjects). Twenty-three of 176 subjects were not tested for response to either 3% halothane, 2 mM caffeine, % MAX caffeine, or "caffeine-specific concentration." Among the 153 fully tested subjects, 45.8% of subjects exceeded proposed thresholds (range, 0–100%; n = 1,483 fascicles). Mean number of muscle fascicles per subject was 9.7 (range, 7.6–11.8). Zero percent of the subjects from Center C exceeded proposed thresholds (mean of 11.8 muscle fascicles per subject in 12 subjects). One hundred percent of the subjects from Center H exceeded proposed thresholds (mean of 7.6 muscle fascicles per subject in 7 subjects) (table 1).

The rate at which subjects exceeded proposed thresholds decreased to 31.8% when caffeine-specific concentration was eliminated from the diagnostic criteria to produce a three-component CHCT (range, 0–76.2% for each diagnostic center; n = 1,220 fascicles derived from 176 subjects). Twenty-three of 176 subjects were not tested for response to either 3% halothane, 2 mM caffeine, or

TABLE 1. Caffeine Halothane Contracture Test Response in Completely Tested Subjects

Center	Number of Subjects	Number (%) of Subjects Exceeding Threshold for the Four-component CHCT*†	Number (%) of Subjects Exceeding Threshold for the Three-component CHCT*†	Number (%) of Subjects Exceeding Threshold for the Two-component CHCT*†
A	10	2 (20.0)	1 (10.0)	1 (10.0)
C	12	0 (0.0)	0 (0.0)	0 (0.0)
D	18	13 (72.2)	7 (38.9)	7 (38.9)
E	13	6 (46.2)	5 (38.5)	5 (38.5)
F	23	14 (60.9)	8 (34.8)	8 (34.8)
G	19	3 (15.8)	1 (5.3)	1 (5.3)
H	7‡	7 (100.0)	4 (57.1)	15 (71.4)
I	27	11 (40.7)	5 (18.5)	5 (18.5)
L	24§	14 (58.3)	13 (54.2)	13 (52.0)
Pooled	153¶	70 (45.8)	44 (28.8)	55 (32.7)

CHCT = caffeine halothane contracture test.
* A subject exceeds proposed diagnostic thresholds even if only one fascicle exceeds threshold for one component test.
† Center-to-center differences in the proportion of subjects diagnosed as exceeding threshold were highly significant, $P < 0.01$ (χ^2 test).
‡ Two-component CHCT subject number increased to 21.
§ Two-component CHCT subject number increased to 25.
¶ Two-component CHCT subject number increased to 168.

% MAX caffeine. The rate among the 153 subjects who had at least one muscle fascicle per subject tested for response to 3% halothane, 2 mM caffeine, and % MAX caffeine was 28.8% (range, 0–57.1%; n = 1,109 fascicles). Center C tested a mean of 8.8 muscle fascicles per subject in 12 subjects and had no subjects exceed proposed thresholds. In contrast, 57.1% of subjects tested at Center H had muscle fascicles exceeding proposed thresholds (mean of 5.4 muscle fascicles per subject in 7 subjects) (table 1).

When 176 subjects were studied for their responses to 3% halothane and 2 mM caffeine alone (two-component CHCT), 31.3% of subjects exceeded proposed thresholds (range, 0–71.4%; 841 fascicles with a mean of 4.8 fascicles per subject). Eight subjects were tested incompletely; in the 168 subjects tested completely, 32.7% exceeded proposed thresholds (range, 0–71.4%; 824 fascicles with a mean of 4.9 fascicles per subject) (table 1).

Hypothesis 2 states that no more than 15% of all subjects have one or more muscle fascicles exceeding proposed contracture thresholds for a four-, three-, and two-component CHCT. One-sided 95% confidence intervals demonstrate that hypothesis 2 should be rejected for the four-component (0, 58.8%), three-component (0, 39.7%), and two-component (0, 45.6%) CHCT (fig. 1).

"Joint Agent" Threshold Analysis

A "joint agent" threshold (3% halothane contracture > 0.7 g *and* ≥ 0.2 g contracture at 2 mM caffeine) decreased the rate at which subjects exceeded proposed "joint" thresholds to 9.5% (range, 0–28.0%; n = 824 fascicles derived from 168 subjects) with an "equivocal" response rate of 23.2%. Centers A, C, and G all had a 0% rate. Center L had the highest rate of 28.0% (table 2). Only 2.0% (range, 0–7.7%; n = 545 fascicles derived from 153 subjects) of subjects exceeded a proposed new "joint agent" threshold of 3% halothane contracture > 0.7 g *and* > 7% MAX caffeine. This proposed "joint" threshold yielded the lowest "equivocal" response rate of 14.4%. Centers A, C, D, G, H, and L all had 0% of subjects exceeding the proposed thresholds. In contrast, Center E had 7.7% of subjects exceeding the proposed thresholds (table 3). Eleven percent (range, 0–32%; n = 402 fascicles derived from 91 subjects) of all subjects exceeded a proposed "joint agent" threshold of 1% halothane contracture > 0.2 g *and* ≥ 0.2 g contracture at 2 mM caffeine. A 23.1% equivocal response rate was noted. Only six centers tested subjects with the optional test of 1% halothane.

Hypothesis 3 states that no more than 15% of subjects have one or more muscle fascicles exceeding proposed contracture thresholds for both halothane *and* incremental caffeine. One-sided 95% confidence intervals indicate that hypothesis 3 should be accepted for 3% halothane *and* % MAX caffeine (0, 3.5%) and should be rejected for 3% halothane *and* 2 mM caffeine (0, 15.2%) and 1% halothane *and* 2 mM caffeine (0, 22.8%) (fig. 2).

Sources of Variation

Muscle Fascicle Mean Contracture Response

Subject-to-subject differences contributed to the major source of variation in the distribution of mean contracture responses to both 3% halothane and 2 mM caffeine. Subjects contributed to 62.6% of total variance in 3% halothane contracture responses and 57.9% of total variance in 2 mM caffeine responses. However, individual muscle fascicle variation contributed to 61.1% of total variance in % MAX caffeine responses.

Differences between responses obtained from alternate muscle types were investigated. Mean contracture re-

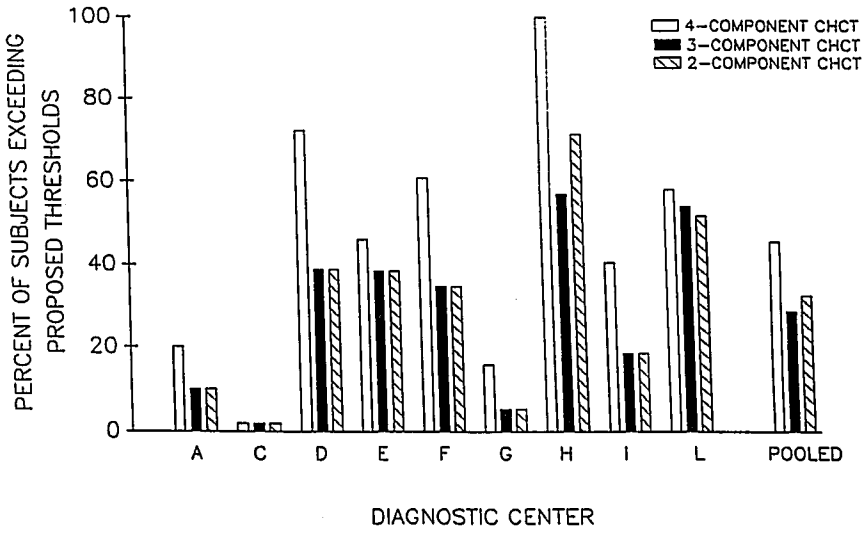

FIG. 1. Rate at which study subjects exceed proposed thresholds for the caffeine halothane contracture test (CHCT) component tests. The three-component (3% halothane, 2 mM caffeine, and percent maximum caffeine) and two-component (3% halothane, 2 mM caffeine) CHCT reduced the percentage of subjects exceeding proposed diagnostic thresholds. All subjects presented had results on all component tests. Center-to-center differences in the proportion of subjects exceeding proposed thresholds were highly significant ($P < 0.01$).

TABLE 2. 3% Halothane *and* 2 mM Caffeine "Joint Agent" Contracture Test Response in Completely Tested Subjects

Center	Number of Subjects	Number of Subjects within Threshold for Both Component Tests	Number of Subjects Exceeding Threshold on Both Component Tests	Percent Subjects*† Exceeding Threshold
A	10	9	0	0
C	12	12	0	0
D	18	11	2	11.1
E	13	8	2	15.4
F	23	15	2	8.7
G	19	18	0	0
H	21	6	2	9.5
I	27	22	1	3.7
L	25	12	7	28.0
Pooled	168	113	16	9.5

n = 824 fascicles derived from 168 subjects.
* A subject exceeds threshold only if at least one fascicle exceeds threshold for 3% halothane (contracture > 0.7 g) *and* one fascicle exceeds threshold at 2 mM caffeine (contracture ≥ 0.2 g).
† Center-to-center differences in the proportion of subjects diagnosed as exceeding threshold were highly significant, $P < 0.01$ (χ^2 test).

sponse to 3% halothane (vastus 0.24 g *vs.* nonvastus 0.27 g; $P = 0.78$), 2 mM caffeine (vastus 0.08 g *vs.* nonvastus 0.16 g; $P = 0.88$), and % MAX caffeine (vastus 1.0% *vs.* nonvastus 0.6%; $P = 0.60$) did not differ significantly. A few muscle fascicle characteristics were statistically significant at the 5% level for contracture response to 3% halothane (wet weight, cross-sectional area, elapsed time, predrug tension) and to 2 mM caffeine (elapsed time), but none of these muscle fascicle characteristics were significant after adjusting for differences in centers and subjects within centers. Moreover, the explanatory models using the muscle fascicle characteristic effects alone accounted for only 5.9% of the variation in the contracture responses to 3% halothane and only 3% of the variation in the responses to 2 mM caffeine in contrast to more than 80% of the variation when adjustments for centers and subjects within centers were included.

Some center CHCT method differences were associated with highly significant variations in 3% halothane and 2 mM caffeine mean contracture responses ($P < 0.01$; general linear model). Significant differences in contracture responses were found between centers, depending on whether or not the center returned muscle fascicles to their optimal length after stress relaxation, drained the caffeine bath before adding the next caffeine concentration, and measured the liquid halothane concentration. Subject-to-subject differences were still significant even after adjustment for center CHCT method differences.

Rate at Which Study Subjects Exceed Proposed Thresholds

Center-to-center differences in the proportion of 176 subjects exceeding the proposed four-component CHCT thresholds were highly significant, ranging from a low of

TABLE 3. 3% Halothane *and* Percent Maximum Caffeine "Joint Agent" Contracture Test Response in Completely Tested Subjects

Center	Number of Subjects	Number of Subjects within Threshold for Both Component Tests	Number of Subjects Exceeding Threshold on Both Component Tests	Percent Subjects*† Exceeding Threshold
A	10	9	0	0
C	12	12	0	0
D	18	15	0	0
E	13	9	1	7.7
F	23	19	1	4.4
G	19	19	0	0
H	7	6	0	0
I	27	24	1	3.7
L	24	15	0	0
Pooled	153	128	3	2.0

n = 545 fascicles derived from 153 subjects.
* A subject exceeds threshold only if at least one fascicle exceeds threshold for 3% halothane (contracture > 0.7 g) *and* one fascicle exceeds 7% maximum caffeine.
† Center-to-center differences in the proportion of subjects diagnosed as exceeding threshold were significant, $P = 0.04$ (χ^2 test).

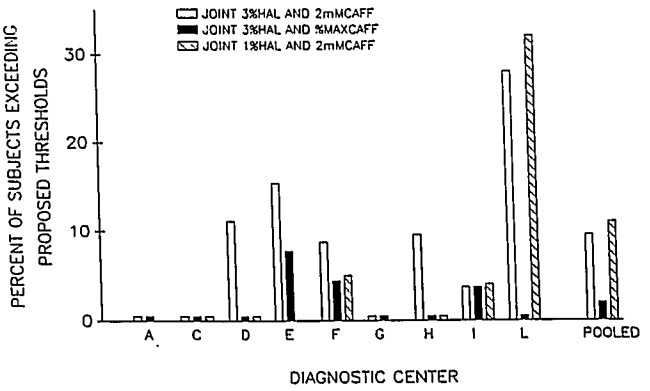

FIG. 2. Rate at which study subjects exceed proposed "joint agent" caffeine halothane contracture test (CHCT) thresholds. "Joint agent" tests (3% halothane *and* 2 mM caffeine; 3% halothane *and* percent maximum caffeine; 1% halothane *and* 2 mM caffeine) markedly reduced the percentage of subjects exceeding proposed diagnostic thresholds relative to any of the component CHCT tests. All subjects presented were tested for both components of the joint test. Center-to-center differences in the proportion of subjects exceeding thresholds were highly significant for: 3% halothane *and* 2 mM caffeine ($P < 0.01$); 3% halothane *and* percent maximum caffeine ($P = 0.04$); and 1% halothane *and* 2 mM caffeine ($P = 0.01$).

0% to a high of 95.2% ($P < 0.01$). Factors examined that were not significant for the four-component CHCT after adjusting for center-to-center variation were the following: subject age (decrease of 10-yr odds ratio = 0.98; $P = 0.87$), subject sex (male-to-female odds ratio = 0.78; $P = 0.49$), muscle type (nonvastus *vs.* vastus odds ratio = 0.55, $P = 0.16$; nonrectus *vs.* rectus odds ratio = 1.34, $P = 0.53$), and pre-CHCT anesthetic agents (non–MH-triggering *vs.* MH-triggering agents odds ratio = 1.39; $P = 0.47$).

Center-to-center differences in the proportion of subjects exceeding proposed thresholds for the two- and three-component CHCT were highly significant ($P < 0.01$). Factors that were not significant after adjusting for center-to-center variation were subject age, sex, muscle type, and pre-CHCT anesthetic agent administration.

Center-to-center differences in the proportion of subjects exceeding proposed thresholds were highly significant for the two-, three-, and four-component CHCT when the responses of the subject subset exposed to all of the specified single agent component tests were analyzed. The proportion of subjects exceeding proposed new "joint agent" thresholds (3% halothane *and* 2 mM caffeine, $P < 0.01$; 1% halothane *and* 2 mM caffeine, $P = 0.01$; 3% halothane *and* % MAX caffeine, $P = 0.04$) were significantly different between diagnostic centers.

Hypothesis 4 states that individual diagnostic centers are not significant sources of variation in the rate at which subjects exceeded proposed thresholds. These data demonstrate that hypothesis 4 should be rejected.

Discussion

The Registry conducted a multiinstitutional, prospective study of the contracture responses of skeletal muscle derived from surgical patients at low risk for MH susceptibility. The responses of biopsy specimens from many subjects exceeded the proposed diagnostic thresholds for the North American CHCT. If one assumes there were zero MH susceptible individuals (see below) within the study population, then the reported rates at which subjects exceeded proposed thresholds would be false-positive rates, and the data would indicate a 54.2% CHCT specificity for a subject undergoing all four component tests (n = 1,483 fascicles derived from 153 subjects). If the proposed guidelines were altered by deleting the "caffeine-specific concentration" component test, then the CHCT specificity estimate would increase to 71.2% (n = 1,109 fascicles derived from 153 subjects). Modifying the CHCT to a two-component test (3% halothane *and* 2 mM caffeine) would yield a CHCT specificity estimate of 67.3% (n = 824 fascicles derived from 168 subjects).

Critique of Methods

Since the incidence and prevalence of MH susceptibility within North America are unknown, we cannot predict how many undiagnosed MH-susceptible individuals might have been present in the study population. Study subjects were selected arbitrarily from a population of patients undergoing surgery unrelated to performance of a diagnostic muscle biopsy. Subjects were admitted to this study and were presumed to be nonsusceptible to MH if there was no evidence of any of the following MH risk factors: prior abnormal response to triggering anesthetic agents, myopathy, or family history of MH susceptibility. The annual incidence of MH susceptibility in North America has been estimated to be 1/14,000.[21] Given this annual incidence, the expected number of study subjects who might be MH susceptible would be 1.3 (0.7% of the study population). Thus, our CHCT specificity estimates are likely to be representative of those found in a 100% non-MH susceptible population. It was necessary to use clinical diagnostic criteria alone to define the low-risk patient population because there are no accepted diagnostic tests for MH susceptibility other than the test being examined in this study, *e.g.*, the CHCT.

Potentially, the CHCT results may have been confounded by the use of histologically or functionally abnormal muscle because histologic examination was performed with the use of adjacent muscle from only 38.1% of subjects. We excluded 7.5% of examined muscle (n = 5 subjects) because of significant histologic abnormalities (*e.g.*, target-core fibers, diffuse type I and type II atrophy, and diffuse fibronecrosis).

Our current method for identifying "caffeine specific

concentration" differs from that used by most MH diagnostic centers for clinical testing purposes because we identified the caffeine concentration interval at which a 1-g contracture occurred. The advantage of our method was that we could accurately determine the rate at which "caffeine specific concentration" exceeded the proposed threshold concentration of 4 mM caffeine without relying on one of several mathematical functions used by diagnostic centers for prediction of a hypothetical "caffeine specific concentration." Our method does not require prediction of the exact shape of the variable response between tension and incremental caffeine administration. However, our method did not allow us to predict the exact caffeine concentration that would yield an 85% "caffeine specific concentration" specificity estimate. Before we can recommend a highly sensitive and adequately specific "caffeine specific concentration," we will need to validate an appropriate mathematical function for predicting "caffeine specific concentration."

Sources of Variation in the Rate Fascicles Exceed Proposed Thresholds

Subjects and diagnostic centers contributed the major sources of variation in the distribution of mean contracture responses among fascicles to test agents. In addition, several muscle fascicle characteristics were significant predictors of contracture increments, but these explained only a minimal percentage of the total variation. None of these characteristics was significant after adjusting for subject effects.

Although many aspects of CHCT laboratory methods have been standardized in the current North American protocol, a few have not and may have been responsible for some of the observed diagnostic center variation. Factors that have been standardized include the following: CHCT indications; subject minimum weight; preferred muscle site; time interval between CHCT and possible MH reaction; anesthetic regimen before CHCT; dantrolene avoidance before CHCT; Krebs–Ringer's transportation media; maximum allowable time between muscle excision and CHCT completion; muscle fascicle dissection method; muscle fascicle length and width; performance of a length tension curve; CHCT bath preparation and configuration; bath solution composition, *p*H, and temperature; halothane gas concentration measurement method; electrical stimulation method; test agent concentrations; positive contracture thresholds; minimum number of control CHCT examinations that must be performed before diagnostic center establishment; and uniform CHCT data reporting to the Registry.[18]

One source of diagnostic center variation was whether the diagnostic center returned muscle fascicles to optimal length after stress relaxation. Three of the nine diagnostic centers did not return fascicles to optimal length when stress relaxation was observed. Contractures reported for such fascicles may not have been isometric. Unfortunately, additional analysis cannot be performed because the identity of these fascicles is unknown.

Since performance of a liquid-phase halothane assay and the method in which caffeine was added to the bath solution individually correlated with mean contracture responses to both halothane and caffeine, halothane assay performance and caffeine addition method may be markers of other unidentified diagnostic center characteristics. Additional research is necessary to identify these unexamined center and subject characteristics and standardize them within the CHCT protocol to permit more uniform MH susceptibility prediction across all diagnostic centers.

Sources of Variation in the Rate Subjects Exceed Proposed Thresholds

Center-to-center differences in the rate at which subjects exceeded proposed thresholds were highly significant. No other examined variables were significant.

Unlike Melton *et al.*, who found higher 3% halothane and 2 mM caffeine contractures in fascicles excised from rectus abdominis rather than vastus muscle, we did not find a significant difference in either mean contracture responses or overall percentage of subjects exceeding proposed thresholds.[22] Data from the subjects studied by Melton *et al.* are included in this multiinstitutional study. We found that the differences in responses between muscle types identified by Melton *et al.* in their diagnostic center were not significant when examined across multiple MH diagnostic centers. Various factors, such as surgical excision techniques, may be responsible for these differences, which require additional study.

Potential Modifications of the North American Diagnostic Guidelines

Alternate methods for analyzing contracture response by standardizing for cross-sectional area were investigated. However, appropriate thresholds can be selected only after our study results are combined with those from a population of MH susceptible patients who have experienced a definitive clinical MH episode.

According to the European standards,[20] a subject must demonstrate an abnormal response to *both* halothane and caffeine to have a positive CHCT response. If an abnormal response to only one test agent is observed, then the subject's CHCT response is designated "equivocal," pending additional investigation of family members. In a review by Ording,[23] the European CHCT responses of 73 control subjects obtained from the European MH Group were presented. Five of 73 control subjects had a positive response to only one test agent; their CHCT responses were

designated as MH "equivocal" for research purposes but MH susceptible for clinical management. No control subjects had positive responses to both test agents. For the European MH Group, use of the "equivocal" response category permitted the European CHCT to reach a specificity of 100% rather than the 93.2% specificity that would have been obtained without the "equivocal" response category.

If the proposed diagnostic guidelines of the North American protocol were modified in a similar fashion, then the "joint agent" threshold of > 0.7 g at 3% halothane and > 7% MAX caffeine would improve the specificity estimate to 98.0%. However, this "joint agent" threshold should not be adopted for MH susceptibility prediction until CHCT *sensitivity* for the proposed component and "joint agent" CHCT thresholds are determined. Future research should evaluate the sensitivity of proposed CHCT component and "joint agent" thresholds so that appropriate thresholds can be recommended that will be *both* highly sensitive and adequately specific.

Prior Estimates of CHCT Specificity in North America

Before the 1987 North American Malignant Hyperthermia Group CHCT standardization, several North American diagnostic centers used a CHCT protocol in which muscle fascicles were exposed to 1% halothane instead of 3% halothane. For example, using this protocol and a diagnostic threshold of > 0.5 g for 1% halothane and > 0.4 g for 2 mM caffeine, Rosenberg and Reed observed a zero positive rate among 12 control subjects tested at a single diagnostic center.[24] This diagnostic center subsequently performed the CHCT according to the 1987 protocol and submitted these data for inclusion in our current study. The performance of Center C in our study is comparable to that previously reported by Rosenberg and Reed.

Relationship of These Findings to Clinical Testing

This study does not report individual diagnostic center CHCT specificity, because individual diagnostic centers have not adopted the proposed North American CHCT diagnostic guidelines for clinical testing purposes. Although individual diagnostic centers use the standard North American CHCT laboratory method, they currently diagnose MH susceptibility by using individually determined diagnostic thresholds for two or more selected CHCT component tests. For clinical testing, each center currently selects its own component tests and diagnostic thresholds by analyzing the contracture responses of a presumed non–MH susceptible population who had biopsies performed at their own center. Currently, the sensitivity and specificity of each center's CHCT depends on the experience of each individual center. It is hoped that diagnostic guidelines for clinical testing also can be standardized with the use of the results of this study and similar future epidemiologic studies on CHCT sensitivity.

Significance

Our study demonstrates that no more than 15% of all muscle fascicles tested in a population at low risk for MH susceptibility exceeded the proposed diagnostic thresholds of > 0.7 g contracture for 3% halothane and > 7% MAX caffeine (hypothesis 1). Therefore, we propose that hypothesis 1 should be accepted for these particular diagnostic thresholds. Our data indicate that more than 15% of all subjects have one or more muscle fascicles exceeding proposed contracture thresholds for a four-, three-, and two-component CHCT (hypothesis 2); therefore, we propose that hypothesis 2 should be rejected. We found that no more than 15% of subjects had one or more muscle fascicles exceeding proposed contracture thresholds for both 3% halothane *and* % MAX caffeine ("joint agent" CHCT) (hypothesis 3); thus, we propose that hypothesis 3 be accepted for this "joint" test. Finally, we established that individual diagnostic centers were significant sources of variation in the rate at which subjects exceeded proposed thresholds (hypothesis 4); therefore, we propose that hypothesis 4 should be rejected.

These data demonstrate that the proposed diagnostic CHCT guidelines must be modified to improve specificity estimates before adoption by the diagnostic centers. The authors recommend continuing those studies in progress that are seeking to develop a uniform method for analyzing *in vivo* adverse patient responses to anesthetics and to define CHCT *sensitivity* for the MH susceptible patient population. These studies will help facilitate both improved clinical diagnosis of MH and the future development of noninvasive molecular genetic and biochemical tests for the prediction of MH susceptibility by more precisely defining susceptible and nonsusceptible individuals.

The authors thank Gerald A. Gronert, M.D., Henry Rosenberg, M.D., and Barbara E. Waud, M.D., for reviewing all study subject CHCT data. They also thank Tracy Nichols and Geoffrey A. Orr, Ph.D., for Registry database programming and design; David R. Larach, M.D., Ph.D., and Julien F. Biebuyck M.D., D. Phil., for their helpful suggestions; and Pam Myers for manuscript preparation.

References

1. Kolb ME, Horne ML, Martz R: Dantrolene in human malignant hyperthermia. ANESTHESIOLOGY 56:254–262, 1982
2. Brownell AKW: Counselling of malignant hyperthermic susceptible individuals, Malignant Hyperthermia. Edited by Britt BA. Boston, Martinus Nijhoff, 1987, pp 309–323
3. Larach MG, Rosenberg H, Larach DR, Broennle AM: Prediction of malignant hyperthermia susceptibility by clinical signs. ANESTHESIOLOGY 66:547–550, 1987

4. Hackl W, Mauritz W, Schemper M, Winkler M, Sporn P, Steinbereithner K: Prediction of malignant hyperthermia susceptibility: Statistical evaluation of clinical signs. Br J Anaesth 64: 425–429, 1990
5. Britt BA: Malignant hyperthermia. Can Anaesth Soc J 32:666–677, 1985
6. Rosenberg H: Clinical presentation of malignant hyperthermia. Br J Anaesth 60:268–273, 1988
7. McGraw TT, Keon TP: Malignant hyperthermia and the clean machine. Can J Anaesth 36:530–532, 1989
8. Harrison GG: Dantrolene—dynamics and kinetics. Br J Anaesth 60:279–286, 1988
9. Lerman J, Relton JES: Anaesthesia for malignant hyperthermia susceptible patients, Malignant Hyperthermia. Edited by Britt BA. Boston, Martinus Nijhoff, 1987, pp 369–392
10. MacLennan DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt BA, Worton RG: Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature 343:559–561, 1990
11. McCarthy TV, Healy JMS, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K: Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12-13.2. Nature 343:562–564, 1990
12. MacKenzie AE, Allen G, Lahey D, Crossan M, Nolan K, Mettler G, Worton RG, MacLennan DH, Korneluk R: A comparison of the caffeine halothane muscle contracture test with the molecular genetic diagnosis of malignant hyperthermia. ANESTHESIOLOGY 75:4–8, 1991
13. Fujii J, Otsu K, Zorzato F, De Leon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH: Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253:448–451, 1991
14. Levitt RC, Nouri N, Jedlicka AE, McKusick VA, Marks AR, Shutack JG, Fletcher JE, Rosenberg H, Meyers DA: Evidence for genetic heterogeneity in malignant hyperthermia susceptibility. Genomics 11:543–547, 1991
15. Gillard EF, Otsu K, Fujii J, Khama VK, DeLeon S, Derdemezi J, Britt BA, Duff CL, Worton RG, MacLennan DH: A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. Genomics 11:751–755, 1991
16. Kalow W, Britt BA, Terraeau ME, Haist C: Metabolic error of muscle metabolism after recovery from malignant hyperthermia. Lancet 2:895–898, 1970
17. Ellis FR, Harriman DGF, Keaney NP, Kyei-Mensah K, Tyrrell JH. Halothane-induced muscle contracture as a cause of hyperpyrexia. Br J Anaesth 43:721–722, 1971
18. Larach MG, for The North American Malignant Hyperthermia Group: Standardization of the caffeine halothane muscle contracture test. Anesth Analg 69:511–515, 1989
19. Galen RS, Reiffel JA, Gambino SR: Diagnosis of acute myocardial infarction. JAMA 232:145–147, 1975
20. The European Malignant Hyperpyrexia Group: A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. Br J Anaesth 56:1267–1269, 1984
21. Britt BA, Kalow W: Malignant hyperthermia: A statistical review. Can Anaesth Soc J 17:293–315, 1970
22. Melton AT, Martucci RW, Kien ND, Gronert GA: Malignant hyperthermia in humans—standardization of contracture testing protocol. Anesth Analg 69:437–443, 1989
23. Ording H: Diagnosis of susceptibility to malignant hyperthermia in man. Br J Anaesth 60:287–302, 1988
24. Rosenberg H, Reed S: In vitro contracture tests for susceptibility to malignant hyperthermia. Anesth Analg 62:415–420, 1983