Improved Noninvasive Diagnostic Testing for Malignant Hyperthermia Susceptibility from a Combination of Metabolites Determined In Vivo with $^{31}$P-Magnetic Resonance Spectroscopy

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**Background:** Phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) in vivo has been suggested recently as a possible noninvasive diagnostic test in malignant hyperthermia (MH) susceptibility. However, differences between protocols and also within subjects may have led to inconsistent MRS abnormalities reported during and after exercise. The aim of the current study was to detect discriminant abnormalities in the leg muscles using in vivo $^{31}$P-MRS during the rest period.

**Methods:** Fourteen patients shown to be MH-susceptible and 22 patients MH-negative on the basis of in vitro caffeine/halothane contracture tests according to the European MH group protocol were compared to 36 control subjects using in vivo $^{31}$P-MRS during the rest period. A score of MRS combined abnormalities was calculated from a stepwise discriminant function analysis.

**Results:** The MH-susceptible group had a significantly ($P < 0.01$) higher inorganic phosphate (Pi) to phosphocreatine (PCr) (Pi/PCr) value ($0.134 \pm 0.022$) than either the MH-negative ($0.097 \pm 0.016$) or the control ($0.101 \pm 0.017$) group. The MH-susceptible group also exhibited a significantly ($P < 0.01$) higher phosphodiester (PDE) to PCr (PDE/PCr) value ($0.093 \pm 0.050$) than either the MH-negative ($0.034 \pm 0.021$) or the control ($0.029 \pm 0.019$) group. Combining both MRS parameters, 13 of the 14 MH-susceptible patients demonstrated abnormal MRS test results (score value $< 1.65$). Conversely, 21 of the 22 MH-negative patients had normal MRS results (score value $\geq 1.65$). The sensitivity and specificity of this threshold value were $93$ and $95\%$, respectively.

**Conclusions:** This study confirms that $^{31}$P-MRS could be useful for distinguishing noninvasively between MH-susceptible and MH-negative patients if several MRS parameter are combined. Moreover, the present MRS approach appears to be more reliable and easier than that used during exercise. (Key words: Malignant hyperthermia; diagnosis of susceptibility; muscular metabolism. Measurement technique: phosphorus magnetic resonance; spectroscopy.)

BECause the signs are present only during anesthesia, preoperative diagnosis of malignant hyperthermia (MH) susceptibility is difficult. Until now, diagnosis has relied solely on an in vitro contracture test requiring muscle biopsy. Because MH susceptibility is inherited, this invasive method is necessary for investigating members of the family of a known or suspected case of MH. However, positive responses to the contracture test have recently been reported in presumed negative subjects, as well as in patients with various neuromuscular disorders. Therefore, the ultimate goal still remains a noninvasive, sensitive, and specific test that can detect the MH-susceptible patient before anesthesia. In spite of recent progress in molecular genetics, a screening test for the predisposition to MH using this technique is not currently available.

Phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) is a safe and noninvasive technique useful for measuring intracellular pH (pHi) and variations in the concentration of phosphate metabolites, such as phos-
phocreatine (PCr), inorganic phosphate (Pi), phosphomonoesters (PME), phosphodiesters (PDE), and ATP. It has recently been suggested as a possible means of diagnosis of MH susceptibility. Indeed, an MH-susceptible group exhibited a higher muscle Pi/PCr at rest, a slower postexercise recovery of PCr/Pi,8,9 and a marked intracellular acidosis during effort10 than did normal and MH-negative groups. We recently reported that MH-susceptible patients also had a higher PDE/PCr ratio than did MH-negative patients in the leg muscles at rest.11

However, statistical significance between two groups does not imply a reliable method of prediction of group membership. The development of any predictive diagnostic index relies on the use of a more sophisticated statistical technique, such as discriminant function analysis.12 This method calculates a linear combination of parameters having the highest discriminant power. The next step is to show the clinical consequences of choosing a particular cutoff using a graphic method, the receiver operating characteristic (ROC) curve.13 This plots sensibility versus specificity for different cutoffs, indicating all relative frequencies of correct and incorrect decisions.

Concerning diagnosis of MH-susceptibility, it recently appeared that neither MRS parameter alone was diagnostically reliable, while an MRS test utilizing more than one parameter was accurate, i.e., Pi/PCr at rest and PCr/Pi postexercise recovery.6 However, differences between protocols and, also, within subjects may explain inconsistent MRS abnormalities observed during and after the exercise period.9 We report a larger follow-up study comparing MRS results obtained during the rest period with the in vitro contracture test. A score of MRS combined abnormalities was calculated to construct a predictive test. Two novel findings are presented here: 1) the importance of variation in the PDE/PCr ratio in leg muscles in the MH-susceptible patients, and 2) the use of a combination of two MRS parameters to obtain a more predictable variable for diagnostic testing.

Materials and Methods

Subjects

Thirty-six patients referred to the Grenoble MH Diagnosis Center were studied. Subjects (20 men and 16 women) were between 15 and 67 yr of age (mean age 33 yr). Muscle biopsy was performed because of a family history of MH (24 patients), symptoms during anes-thesia (6 patients), exertional heatstroke (2 patients), and persistently elevated resting serum creatine kinase levels (4 patients). None of these subjects had any clinical illness or were receiving drug therapy. Thirty-six healthy, normal control subjects (26 men and 10 women) between 20 and 77 yr of age (mean age 31 yr) were studied only by in vivo MRS. Informed consent was obtained from all participants.

Human Muscle Biopsy and Contracture Test

All patients underwent muscle biopsy for contracture test and histologic examination. Open surgical biopsies of the vastus medialis were performed under crural nerve block. The muscle specimens were transferred at room temperature in Krebs-Ringer solution with pH adjusted at 7.4. The contracture test at the Grenoble MH Diagnosis Center was performed according to the recommendations of the European MH Group protocol.14 Separate muscle specimens are exposed to halothane and caffeine, with graded exposure to each one of these agents. The test is considered positive when the muscle specimens produce a sustained increase in baseline tension with amplitude ≥ 0.2 g during both application of ≤ 2% halothane and application of ≤ 2 mm caffeine. The test is equivocal when a contracture is elicited by only one test substance. Other results were deemed MH-negative.

In Vivo $^{31}$P-MRS Study

All patients underwent $^{31}$P-MRS examination the day before the muscle biopsy. Subjects were tested in a 2.35 Tesla 35-cm horizontal bore magnet coupled to a spectrometer (Bruker Spectrospin, Wissenburg, France) with an operating frequency of 40.6 MHz for phosphorus. Subjects were seated on a sliding table. Each leg was studied, one at a time, after being placed on a platform that contained a 6-cm diameter inductively coupled coil below the gastrocnemius muscle. The head of the fibula served as a guide marker to allow reproducible coil positioning. The leg was secured to the platform using straps, with the foot maintained at 10° dorsiflexion and leaning on a pedal.

The $^{31}$P spectra of the gastrocnemius muscle were obtained at rest, after shimming on proton signal. The magnetic field was adjusted for homogeneity so that the line width at half maximum of the water proton was usually < 0.5 ppm (parts per million). Each $^{31}$P spectrum was acquired after completion of 20 single scans (35–55 μs pulse length) at a 20-s pulse repetition rate. This pulse sequence gives fully relaxed spectra.
without reduction of peak intensity because of partial saturation. Spectral width was 6,024 Hz and free induction decays (FID) were obtained in 4K data points (2K complex points).

Such a pulse length (35–55 μs) corresponded to the best compromise between the signals from high-energy phosphorus compounds and from immobile phosphat metabolites, especially membrane phospholipids. However, their signals could still have affected the accuracy of muscle metabolite measurements. Therefore, a convolution difference was applied to the FID before Fourier transformation using 15-Hz and 200-Hz exponential line broadenings (LB) to remove the spectral wide component because of immobilized phosphat metabolites. An automated phase correction was applied to the biggest peak, i.e., PCR. Spectral analysis was based on the integration of the Pi, PDE, PCR, and βATP peaks using the UXNMR software. The horizontal baseline was drawn through the mean value of the noise determined at the low (+9 to +11 ppm) and high (−19 to −21 ppm) field portions of the spectrum. Areas were defined between the lowest points of the baseline for each peak. The Pi area was usually defined between +5.7 and +4.1 ppm, PDE between +3.6 and +2.2 ppm, PCR between +1.4 and −1.4 ppm, and βATP between −14.8 and −17.2 ppm. Results were expressed as relative concentrations: Pi/PCR, PDE/PCR, βATP/PCR, and βATP/PCR+Pi. The pHı was calculated from the automated measure of chemical shift (δ) of Pi relative to that of PCR using pHı = 6.75 + log[(δ − 3.27)/(5.69 − δ)]. Both legs were successively examined in all patients and results expressed the mean of values obtained from each leg.

The reliability of MRS measurements within a given test session, as well as between days, was tested in nine control subjects and one MHS patient (n = ten individuals) over two repeated experiments on the same leg with at least a 6-day interval (n = 20 spectra). Each experiment was measured twice at 24-h intervals by the same investigator using the procedure described above (n = 40 measurements). The reliability of MRS repeated measurements (within-day) and experiments (between-day) was estimated by concordance coefficient (κ) values ranged from 0 to 1. This coefficient was calculated from residual and interindividual variances. A κ value near 1 means a good concordance between two measurements (or two experiments). The variability of MRS parameters between the 36 control subjects was expressed by the coefficient of variation [CV = (SD/mean) * 100].

**Statistical Analysis**

Data were expressed as mean ± SD. The resting MRS parameters of MH-susceptible versus MH-negative or control were tested separately using the two-tailed unpaired Student's t test after applying Bonferroni's correction for multiple comparisons. A stepwise discriminant analysis was performed to select the MRS parameters having the highest discriminant power. A new variable, Y, was defined as the combined linear function of these MRS parameters. The coefficients were determined by the Fisher's method. An individual score of combined MRS abnormalities was then calculated. Sensitivity, specificity, and predictive values of this score were determined in the usual way. A receiver operating characteristic (ROC) curve of sensitivity versus specificity was plotted to show the consequences of choosing a particular cutoff. It should be noted that the Y variable was determined from the 36 biopsied patients only, and then applied to the 36 controls.

**Results**

The contracture tests revealed that 10 patients were MH-susceptible (MHS), 22 patients MH-negative (MHN), and 4 patients MEQ for halothane (MHEH) (table 1). None were MH-equivocal for caffeine. Because MH-equivocal patients are clinically regarded as MH-susceptible, they were then classified in the MH-susceptible group for the MRS data analysis. None of the patients had specific myopathy on histological examination of muscle specimens.

Typical fully relaxed 31P spectra obtained at rest from an MH-susceptible and an MH-negative patient are shown in figure 1. There are similar peaks on both spectra. However, the Pi/PCR and PDE/PCR ratios are measurably higher in the MH-susceptible patient. For the three groups (MH-susceptible, MH-negative, and

Table 1. *In Vitro* Halothane/Caffeine Contracture Test Results in the 36 Patients

<table>
<thead>
<tr>
<th>Indication for Biopsy</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHS</td>
</tr>
<tr>
<td>Family history of MH</td>
<td>8</td>
</tr>
<tr>
<td>Symptoms during anesthesia</td>
<td>1</td>
</tr>
<tr>
<td>Exeretional heatstroke</td>
<td>0</td>
</tr>
<tr>
<td>Elevated serum creatine kinase</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
</tr>
</tbody>
</table>

MH = malignant hyperthermia; MHS = MH-susceptible; MHEH = MH-equivocal for halothane; MHN = MH-negative.
The distributions of Pi/PiCr, PDE/PiCr, and MRS score in the 36 biopsied patients and the 36 control subjects are shown in figure 2. Some values from MH-susceptible patients overlapped with those from MH-negative and control subjects. The thresholds having the highest sensitivity and specificity for each MRS parameter (arrows) led to five patients with false-positive results and two patients with false-negative results using Pi/PiCr; and two patients with false-positive results and five patients with false-negative results using PDE/PiCr. Conversely, 13 of the 14 MH-susceptible patients were well classified for an MRS score < 1.65. A score value ≥ 1.65 allowed classification of 21 of the 22 MH-susceptible patients. Thus, there was one patient with a false-negative result (score value = 1.76) and one patient with a false-positive result (score value = 1.58) using the MRS score. In reference to the contracture test results, the MRS sensitivity and specificity of this threshold value were 93 and 95%, respectively. Applying the MRS score to the control group, 28 of the 36 control subjects were well classified. The score value of each of the eight MRS abnormal-defined subjects was more than 1.55.

Receiver operating characteristic (ROC) curves of sensitivity versus specificity were plotted along different MRS score values, as well as different Pi/PiCr and PDE/PiCr values, for the 36 biopsied patients (fig. 3). The combination of both MRS parameters clearly enhanced the sensitivity versus specificity for different score cutoffs regarding that of one MRS parameter. For a sensitivity of 100%, the specificity of the MRS score was 50%, while that of Pi/PiCr and PDE/PiCr decreased to 32% and 18%, respectively. In the same way, for a specificity of 100%, the sensitivity of the MRS score was 71% versus 79% and 50% for Pi/PiCr and PDE/PiCr, respectively.

Finally, the reliability of MRS repeated measurements and experiments is shown in table 3. The concordance coefficients between two measurements were > 0.9 for all parameters. A good concordance (κ > 0.8) between two experiments was also observed for Pi/PiCr and PDE/PiCr. The variability of MRS parameters between the 36 normal patients (CV) was 16% for Pi/PiCr, 12% for ATP/PiCr, 9% for pH, and 50% for PDE/PiCr ratio because of its very low value.

**Table 2. Resting Phosphorus MRS Data of the Three Groups**

<table>
<thead>
<tr>
<th>MRS Parameters</th>
<th>MHS Patients (n = 14)</th>
<th>MHN Patients (n = 22)</th>
<th>Control Subjects (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi/PiCr</td>
<td>0.134 ± 0.022*</td>
<td>0.097 ± 0.016</td>
<td>0.101 ± 0.017</td>
</tr>
<tr>
<td>PDE/PiCr</td>
<td>0.093 ± 0.056*</td>
<td>0.034 ± 0.021</td>
<td>0.029 ± 0.019</td>
</tr>
<tr>
<td>pH</td>
<td>7.09 ± 0.07†</td>
<td>7.05 ± 0.04</td>
<td>7.06 ± 0.04</td>
</tr>
<tr>
<td>ATP/PiCr</td>
<td>0.243 ± 0.031</td>
<td>0.226 ± 0.023</td>
<td>0.232 ± 0.021</td>
</tr>
<tr>
<td>ATP/Pi + PiCr</td>
<td>0.214 ± 0.025</td>
<td>0.206 ± 0.021</td>
<td>0.211 ± 0.019</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SD. MRS = magnetic resonance spectroscopy; MHS = malignant hyperthermia-susceptible; MHN = malignant hyperthermia-negative; Pi = inorganic phosphate; PDE = phosphodiester; PiCr = phosphocreatine; PDE = phosphocreatine; pH = intracellular pH.

* MHS patients versus MHN patients and control subjects; P < 0.01 (Student’s t test).
† MHS patients versus MHN patients and control subjects; P < 0.05 (Student’s t test).

Other P values are not significant.
Discussions

The major finding of this study was that combined $^{31}$P-MRS abnormalities improve noninvasive diagnostic testing for MH susceptibility. A score of MRS abnormalities was established from two MRS parameters hav-
Table 3. Variations in Phosphorus MRS Parameters between Two Repeated Measurements as Well as Two Repeated Experiments in Nine Control Subjects and One MHS Patient

<table>
<thead>
<tr>
<th>MRS Parameters</th>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Concordance Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mes 1</td>
<td>Mes 2</td>
<td>Mes 1</td>
</tr>
<tr>
<td>Pi/PcR</td>
<td>0.105</td>
<td>0.104</td>
<td>0.103</td>
</tr>
<tr>
<td>PDE/PcR</td>
<td>0.038</td>
<td>0.036</td>
<td>0.032</td>
</tr>
<tr>
<td>ATP/PcR</td>
<td>0.242</td>
<td>0.238</td>
<td>0.234</td>
</tr>
<tr>
<td>pH</td>
<td>7.070</td>
<td>7.074</td>
<td>7.069</td>
</tr>
</tbody>
</table>

Values are expressed as means of MRS parameters in nine control subjects and one MHS patient.

The concordance coefficient (k) was calculated from interindividual and residual variances for each MRS parameter.

MRS = magnetic resonance spectroscopy; MHS = malignant hyperthermia-susceptible; Exp = experiment; Mes = measurement; Pi = inorganic phosphate; PDE = phosphodiester; ATP = adenosine triphosphate; pH = intracellular pH.

to discriminate the MH-susceptible patients. Therefore, the rest period was worth evaluating.

The MRS abnormalities reported here confirm those reported in previous studies. Malignant hyperthermia-susceptible patients had a higher resting PDE/PcR in leg muscles, confirming our preliminary results. Moreover, they had a significantly higher resting Pi/PcR ratio, as described by Olgin et al., although some disagreement exists in the exact Pi/PcR ratios between these different studies. This may be because of their not fully relaxed spectra, because T1 relaxation times for Pi and PcR are different. This also may be caused by the use of convolution difference technique in the current study, because this method removes the spectral wide component that could overestimate the intensity of the smallest peaks, thus enhancing the value of the Pi/PcR ratio.

However, statistical differences between two groups do not imply a reliable method for prediction of normal or abnormal character for one individual. The use of the discriminant analysis, which calculates a linear combination between parameters, improves their predictive potential. All MH-susceptible patients did not exhibit both a high Pi/PcR and a high PDE/PcR. However, a combination of both parameters was necessary to better distinguish between MH-susceptible and MH-negative patients, as shown in figure 2. Moreover, the determination of resting Pi/PcR and PDE/PcR ratios was in good agreement within repeated measurements, as well as repeated experiments, which reinforces their predictive interest. It should also be mentioned that both MRS parameters were not assigned to a similar weight in the determination of the MRS score. Indeed, the Pi/PcR ratio remains the most important predictive factor, but the PDE/PcR ratio could enhance the sensitivity of the MRS score for low Pi/PcR values. Such factors as the age of the patient could affect the weight of PDE/PcR ratio within a group, as previously reported. No obvious difference in age was noted between our MH-susceptible and MH-negative patients. Such factors will be tested in a larger MH population.

The one false-negative patient was a 21-year-old man. He had normal Pi/PcR (0.096) and PDE/PcR (0.061) ratios, and his contracture test produced definite results. The muscular biopsy was performed because of a family history of MH. His mother has been classified MH susceptible with true MRS abnormalities. It is unclear whether this represents a false-negative MRS test or a false-positive contracture test. The molecular genetic analysis for the predisposition to MH could be useful for elucidation of this point.

The one false-positive result occurred in a 43-year-old woman. She had a normal PDE/PcR ratio (0.045) but a slightly elevated Pi/PcR ratio (0.119). Her son underwent biopsy because of symptoms during anesthesia and was classified as MH-equivalent for halothane. However, her MRS score value (1.58) was similar to those of eight MRS abnormal-defined control subjects. Intermediate MRS score values do not, thus, allow distinction between MH-susceptible patients and others.

An algorithm should be proposed from these observed MRS results, assuming that no conclusive rule can be made from the one MHS false-negative patient. For a low MRS score value (e.g., < 1.5), subjects would be classified as MH-susceptible; this accounts for 10 of our 14 MH-susceptible patients. Conversely, subjects having a high MRS score (e.g., ≥ 1.65) would have a good chance of being classified as MH negative, as were 21 of our 22 MH-negative patients. For an intermediate MRS score value, no final conclusion can be made from MRS results exclusively. In our study population, four patients fell into this category (three MH-susceptible, one MH-negative), requiring another test for the MH diagnosis (i.e., the contracture test). Such a procedure could also increase the probability of finding a positive contracture test in patients having an undefined MRS test.

These MRS abnormality are nonspecific for MH. An elevated resting PDE/PcR ratio has been also reported in leg muscles in other myopathies. This abnormality has not yet been observed in forearm muscles in MH-susceptible patients. A difference in fiber type...
composition between leg and forearm muscles could explain this discrepancy, as previously reported in normal subjects.\textsuperscript{23} Glycerophosphorylcholine (GPC), which is the normally occurring phosphodiesters, is more abundant in fiber of type 1 (e.g., in soleus muscle).\textsuperscript{24,25} Having the longest $T_1$, relaxation time of the phosphate metabolites,\textsuperscript{26} the GPC peak needs fully relaxed spectra to be detected in the leg muscles. Nevertheless, this does not explain why PDE/PCr is higher in MH-susceptible patients, because no difference in fiber type composition has been observed between MH-susceptible and MH-negative patients.\textsuperscript{27} An in vitro MRS study from peripheral acid muscle extracts is currently being performed in our laboratory to identify the nature of PDE peak and its significance in MH. Although it may regulate membrane phospholipid composition,\textsuperscript{28} the exact role of PDE in muscle remains unclear.

An increased resting Pi/PCr ratio has also been reported in patients with several muscle disorders,\textsuperscript{21,23,29,30} as well as in normal patients after muscle injury.\textsuperscript{31} The increased resting Pi/PCr ratio was assumed to be caused by an increased rate of ATP breakdown.\textsuperscript{32,33} In MH, this could reflect defects in calcium cycling.\textsuperscript{34} Therefore, MRS abnormalities lack specificity for the MH genetic defect. Because the pretest probability of the MH-susceptibility (disease prevalence) is probably smaller than that of other neuromuscular disorders in the general population, the MRS test may be useful neither for mass screening in the general population nor for patients with neuromuscular symptoms suggesting myopathy. However, when the disease prevalence is high, as seen in a population of patients with a family or personal history of MH, the MRS could be useful as a diagnostic test. Indeed, assuming that the disease prevalence is 0.5 in family medical history, the probability that disease exists when the MRS test result is positive (positive predictive value) reaches 0.95 following Bayes' Theorem. Conversely, the probability that no disease exists when the MRS test result is negative (negative predictive value) is 0.95. Such high predictive values illustrate the medical interest of MRS testing in MH. An additional tool of the MH phenotype could also be provided. Because genetic MH-susceptibility is heterogeneous,\textsuperscript{31,35} it seems helpful to combine MRS and contracture tests with genetic analysis to obtain the strongest diagnostic statement.

In this study, we have shown that $^{31}$P MRS may be an accurate noninvasive tool of diagnosis MH susceptibility if MRS parameters are combined. The MRS examination during the rest period appears easier and more reliable than those during and after exercise. A prospective study is, however, necessary to confirm these results. In some cases, the MRS test does not truly distinguish between MH-susceptible and MH-negative patients. Improvement of the accuracy of the MRS test requires a preselected population.

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References


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