

# Non-invasive Evaluation of Malignant Hyperthermia Susceptibility with Phosphorus Nuclear Magnetic Resonance Spectroscopy

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Using *in vivo*  $^{31}\text{P}$  NMR spectroscopy, the authors compared the NMR spectra of the flexor muscles of the forearm from 13 humans characterized as MH susceptible on the basis of *in vitro* caffeine/halothane contracture tests with those from 25 normal controls. The levels of phosphocreatine (PCr), inorganic phosphate (Pi), and ATP during rest, graded exercise, and post-exercise recovery were measured in their forearms. MH susceptible subjects had significantly ( $P < 0.001$ ) higher Pi/PCr values ( $0.222 \pm 0.009$ ) at rest than did normal controls ( $0.140 \pm 0.004$ ). In addition, a significantly ( $P < 0.01$ ) slower post-exercise recovery rate was found in the MH-susceptible group. There was no significant difference between the two groups in the relationship of work rate to Pi/PCr. These data suggest that unchallenged MH susceptible patients can be distinguished from normals using  $^{31}\text{P}$  NMR spectroscopy. The potential use of this technique as a non-invasive tool in determining MH susceptibility is discussed, as well as the possible mechanisms underlying the observed  $^{31}\text{P}$  NMR abnormalities. (Key words: Malignant hyperthermia; biochemistry; detection (diagnosis). Measurement technique: nuclear magnetic resonance.)

MALIGNANT HYPERTHERMIA (MH) is a clinical syndrome characterized by fever, muscle rigidity, increased blood pressure, tachypnea, tachycardia, and acidosis triggered by exposure to inhalational anesthetics and succinylcholine.<sup>1</sup> It is thought that most of these signs are the result of a "hypermetabolic state" of

muscle and the physiological response to the resultant acidosis.<sup>1</sup>

An MH episode can be prevented in MH susceptible patients either by using "non-triggering" anesthetics and/or by pretreating them with dantrolene.<sup>1</sup> However, implicit is the need to know whether or not one is MH susceptible prior to receiving anesthesia. Because the signs are usually present only during anesthesia, pre-anesthetic diagnosis of MH susceptibility is difficult. Suspicion of MH susceptibility is based upon either having signs of an MH episode during anesthesia (*i.e.*, rapid rise in body temperature or muscle rigidity) or being related to a known MH-susceptible person.<sup>1</sup> Since MH is believed to be of autosomal dominant inheritance,<sup>1</sup> the major need for detection is in family members of MH suspected or proven cases. However, confirmation is made only by an *in vitro* contracture test on a muscle sample.<sup>1</sup> The contracture test, while specific, requires a muscle biopsy. The serum creatine kinase (CK) level, a controversial screening test, is not very sensitive and has a high false negative rate.<sup>1-3</sup> Numerous other tests for MH susceptibility have been tried, none of which have met with much success.<sup>1,4</sup>

Phosphorus nuclear magnetic resonance spectroscopy ( $^{31}\text{P}$  NMR) is a non-invasive method for determining intracellular changes in high energy phosphates. This technique has been successfully utilized to evaluate several muscle diseases, primarily metabolic myopathies.<sup>5-12</sup> We employed this new technique of *in vivo*  $^{31}\text{P}$  NMR to determine the levels of phosphocreatine (PCr), inorganic phosphate (Pi), and ATP in muscles of MH-susceptible patients, in the hopes of distinguishing this population from normals.

## Materials and Methods

Thirteen MH-susceptible subjects were tested by  $^{31}\text{P}$  NMR. Twelve had been previously proven susceptible by the halothane contracture test of biopsied muscle, and one had a strong family history and an elevated CK level. A contracture greater than or equal to 0.7 grams with 3% halothane (early protocols used greater than 0.5 g with 0.9% or 1.2%) or 0.3 grams with 2 mM caffeine was considered indicative of MH susceptibility (table 1).<sup>13</sup> Patients with concurrent muscle disease were not included in this study. MH-susceptible subjects

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TABLE 1. Contracture Results and History of MH-susceptible Subjects and Biopsied Controls

| Subject           | Contracture Tension (grams) |                 | Indication for Biopsy           |
|-------------------|-----------------------------|-----------------|---------------------------------|
|                   | 3% Halothane                | 2 mMol Caffeine |                                 |
| MHS 1             | 2.4                         | 0               | Suspicious episode, elevated CK |
| MHS 2             | 1.3                         | 0               | Family history                  |
| MHS 3             | 1.8                         | 0.1             | Masseter muscle rigidity        |
| MHS 4             | 2.8                         | 0.1             | Family history                  |
| MHS 5             | 1.6                         | 0.8             | Family history                  |
| MHS 6             | 0.75 (1.2%)                 | 0.8             | MH episode                      |
| MHS 7             | 3.6 (1.2%)                  | 0.5             | Family history                  |
| MHS 8             | —                           | —               | Family history, elevated CK     |
| MHS 9             | 2.0 (0.9%)                  | 1.5             | Family history                  |
| MHS 10            | 1.3                         | 0               | MH episode                      |
| MHS 11            | 2.2                         | 0               | Family history                  |
| MHS 12            | 1.1                         | 0               | Elevated CPK                    |
| MHS 13            | 2.5                         | 0.2             | Family history                  |
| MH <sup>-</sup> 1 | 0.2                         | 0               | Family history                  |
| MH <sup>-</sup> 2 | 0.4                         | 0               | Family history                  |

MHS = MH susceptible subject; MH<sup>-</sup> = non-MH susceptible control proven by biopsy.

were between the ages of 13 and 50 yr; eight were men and five were women. Control subjects (15 men and 10 women) were normal, healthy, non-athletes between

the ages of 18 and 50 yr. Two of these controls, relatives of the patients, were proven non-susceptible to MH by the contracture test. Signed consent was obtained from all subjects.

The basic protocols and techniques used in our laboratory for testing human arm muscle by NMR have been detailed in previous publications.<sup>14,15</sup> Only a brief description will be presented here.

NMR spectra of the wrist flexor muscles were obtained using a double-tuned, 4 cm diameter, two-turn surface coil. Subjects were tested in our 1.9 tesla, 30-cm bore magnet coupled to a spectrometer (Oxford Instrument—TMR 32) with an operating frequency of 32.5 MHz for phosphorus. Radiofrequency pulses of 45  $\mu$ sec duration (90° flip angle with an estimated tissue penetration of 0.65 cm) were delivered every 5 s for NMR acquisition. This pulsation pattern gives partially saturated spectra of 81% for PCr, 86% for Pi, and 91% for  $\beta$ -ATP of fully relaxed spectra. The short interpulse delay (5 s) was used to quickly average the rapidly changing concentrations of metabolites during exercise and recovery.

Spectra were obtained during rest, graded exercise, and post-exercise recovery. Resting spectra were averages of 72 scans over a 6-min period. Following collection of resting data, each subject began an exercise protocol. This consisted of wrist flexions of 0.5-s duration to repeatedly push a handle connected to a Cybex ergometer once every 5 s, just prior to NMR sampling.<sup>14</sup> First, the subject's maximum torque against the ergometer was determined. Each subject then progressively exercised at 20%, 40%, and 60% of their maximum torque for 6 min at each level to obtain a steady state. Spectra were measured during the last 2 min of each exercise level (24 scans). At the end of exercise, recovery was followed for 3 min in 1-min blocks.

The spectra were multiplied by 10 Hz line broadening, Fourier transformed, phased, and plotted with an x-y plotter. A common baseline for all peaks was determined and peak areas for Pi, PCr, and  $\beta$ -ATP were measured by triangulation to obtain ratios of Pi/PCr, ATP/PCr, ATP/Pi, and ATP/(PCr + Pi). Wilcoxon's rank sum test was used for statistical analysis of population differences. Values are given as mean  $\pm$  SEM.

## Results

Typical <sup>31</sup>P NMR spectra at rest from an MH susceptible patient and a normal control are illustrated in figure 1. There are similar peaks in both spectra. However, the Pi to PCr ratio is higher in the MH-susceptible patient. The distributions of Pi/PCr values for the MH-susceptible patients and normal controls are plotted in figure 2. From this figure, it can be seen that

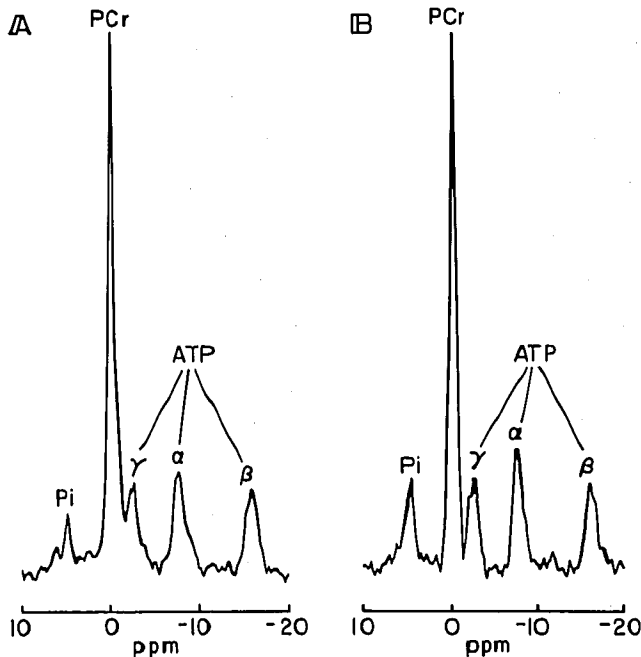
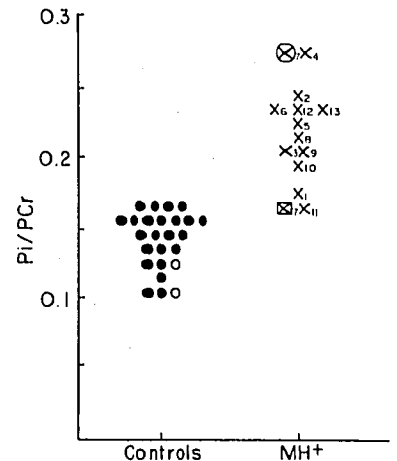


FIG. 1. <sup>31</sup>P NMR spectra of forearm muscles obtained during rest. Spectrum A is from a normal control and spectrum B from an MH-susceptible subject. The different peaks represent relative amounts of phosphorus nuclei from different high-energy phosphate compounds, as labeled. The y-scale has been adjusted so that the PCr peak heights in A and B are equal. Notice that the Pi/PCr is greater in B.

there are two distinct Pi/PCr populations; the higher values being the MH susceptible subjects (average  $0.222 \pm 0.009$ ) compared with lower values for normal controls (average  $0.140 \pm 0.004$ ). The two populations are significantly different with a  $P < 0.001$ . A resting Pi/PCr of 0.175 or greater was seen in the affected persons only, while all persons with a resting Pi/PCr less than 0.160 were normal. The values from only two MH-susceptible patients overlap with the control population. One of these (⊗) with a value of 0.160 is that of the only patient in the study who chronically receives daily oral dantrolene to control muscle cramps. When she was restudied after 2 weeks free of dantrolene, her Pi/PCr value had risen to 0.270 (⊗).

An increased Pi/PCr as seen in the MH-susceptible patients at rest can be due to one of three reasons: 1) an increased Pi, 2) a decreased PCr, or 3) an increased Pi level and a concomitant decreased PCr level. The ratios ATP/Pi and ATP/PCr are useful in determining which one of the above reasons are responsible for the increased Pi/PCr in the MH susceptible patients. When ATP is constant, suggested by figure 3a, these ratios essentially indicate absolute differences in Pi and PCr, respectively. Figures 3b and c show that there is a significantly ( $P < 0.01$ ) decreased ATP/Pi and an elevated ATP/PCr in the MH-susceptible group. By comparing the ratios for each individual MH-susceptible patient numbered in figures 2 and 3 to each corresponding control mean, one can see that individual consistency is maintained among the ratios, as described for the over-

FIG. 2. Distribution of resting Pi/PCr values for normal controls (circles) and MH-susceptible subjects (x). The MH-susceptible data points are numbered for comparison to table 1 and figure 3. The one MH-susceptible patient with a Pi/PCr value of 0.160 (⊗) was obtained while she was receiving daily oral dantrolene. She was later restudied 2 weeks after being free from dantrolene (⊗). Notice that this distribution clearly defines two almost distinct populations ( $P < 0.001$ ). Also notice that the two control subjects proven non-MH susceptible by biopsy (○) fall within the range of normal controls.

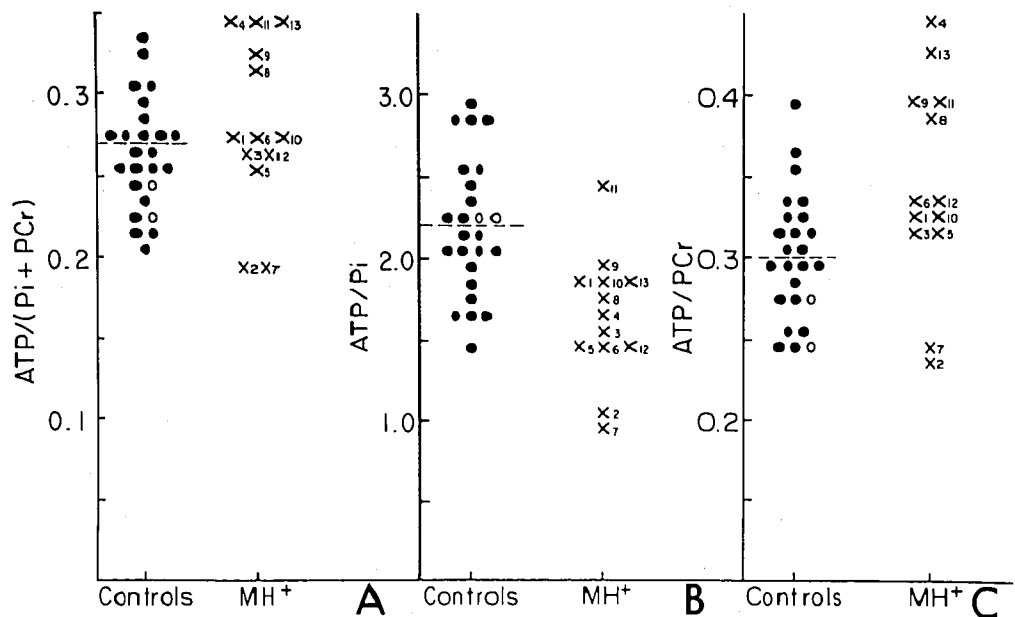


all population. This suggests that the Pi/PCr increase is due to both a reduction in PCr and an increase in Pi.

Analysis of absolute ATP levels by NMR is complicated by the fact that there are no real internal standards in a <sup>31</sup>P NMR spectra for interpersonal comparison. However, the overall relation between ATP levels and the sum of PCr + Pi is maintained in the patient group (fig. 3a), indicating an equal level of ATP in both groups.

By measuring the chemical shift of Pi, one can determine the intracellular pH from <sup>31</sup>P spectra.<sup>14-16</sup> No dif-

FIG. 3. Distributions of resting ATP/(Pi + PCr), ATP/Pi and ATP/PCr for normal controls (circles) and MH-susceptible subjects (x). The data points for the MH-susceptible group are consistently numbered in accordance with table 1 and figure 2. The dashed lines represent the mean values for the control group. No significant difference in distribution between controls and MH-susceptible subjects is seen in ATP/(Pi + PCr) ratios (A). However, B shows significantly ( $P < 0.01$ ) lower ATP/Pi ratios in the MH-susceptible group, while C shows significantly ( $P < 0.002$ ) higher ATP/PCr in the MH-susceptible group, compared to normals.



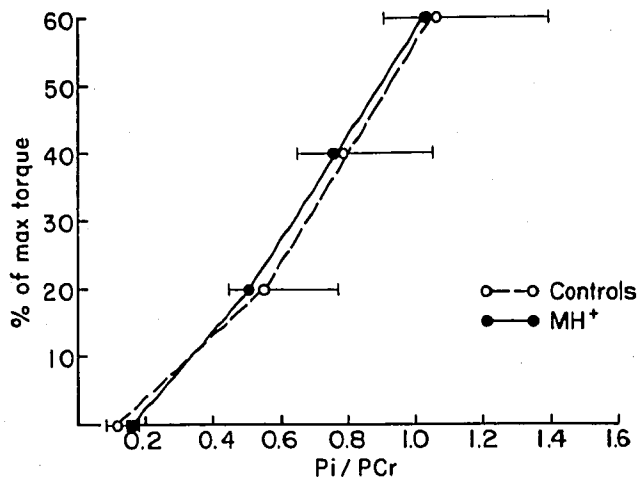


FIG. 4. Exercise transfer functions for a control group (open circles and broken line,  $n = 19$ ) and MH-susceptible group (circles and solid line,  $n = 7$ ). The y-axis represents the percent of maximum torque obtained on a Cybex ergometer during repeated wrist flexion of 0.5-s durations. (Maximum torque was determined for each individual.) The x-axis represents Pi/PCr values obtained during the last 2 min of exercise at each level. Values are mean  $\pm$  SEM. Notice that there is no difference in the transfer functions of the two groups.

ference was found in the pH at rest between normals and MH-susceptible subjects. The pH was 7.0 for both groups at rest.

The concept of using the exercise transfer function for interpersonal comparisons of muscle performance is described elsewhere.<sup>16</sup> In brief, it is a function relating work rate and the biochemical response, Pi/PCr. The transfer functions of seven MH susceptible patients and 19 controls, who successfully performed the entire protocol, are illustrated in figure 4. No difference was found between the transfer functions of the MH-susceptible subjects and the controls. Moreover, there was no difference in the pH changes during exercise between the groups, as detected by NMR.

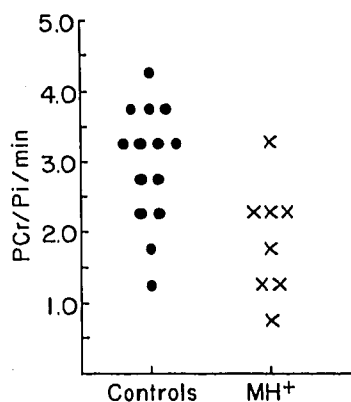


FIG. 5. Distributions of post-exercise recovery rates for MH-susceptible subjects (x) and normal controls (circles). Recovery rates were calculated from the slopes of the linear relationship of PCr/Pi versus time for the first 2 min after exercise. The two populations are significantly different ( $P < 0.01$ ), with a slower recovery rate (smaller PCr/Pi/min slope) in the MH-susceptible subjects.

The post-exercise recovery of PCr/Pi plotted against time has linear characteristics for the initial 2 min, and its rate can be calculated from this slope.<sup>5,6</sup> Figure 5 shows the population distributions of recovery rates for the MH-susceptible and control groups. The two population distributions are significantly different ( $P < 0.01$ ). A slower mean recovery rate can be seen in the MH-susceptible subjects ( $1.833 \pm 0.245$  PCr/Pi/min) compared to the normal controls ( $2.960 \pm 0.227$  PCr/Pi/min).

## Discussion

Our results suggest that MH-susceptible patients can be distinguished from normals on the basis of a simple <sup>31</sup>P NMR measurement of muscle Pi/PCr at rest. Those subjects with a Pi/PCr above 0.175 had been shown to be MH susceptible by contracture tests, whereas normal patients had a Pi/PCr below 0.160. These data suggest the potential use of <sup>31</sup>P NMR for detecting MH susceptibility; however, a larger, properly blinded study is needed to further establish the role of <sup>31</sup>P NMR in the diagnosis of MH susceptibility.

The advantages of <sup>31</sup>P NMR over muscle biopsy are that the NMR test is non-invasive and painless, and can be accomplished in less than 20 min. Although our series did not include patients who had MH with other associated myopathies,<sup>4</sup> a high ratio of Pi/PCr at rest has been found in patients with several muscle disorders in other studies (table 2). Therefore, since similar increases in Pi/PCr are seen in other diseases, the NMR test is not specific for MH. However, as with any clinical test, interpretation must include the history and physical exam to determine the pre-test probability of a given disease (Baye's Theorem).<sup>17</sup> An asymptomatic patient with a family history of MH and a high Pi/PCr is most likely to be MH susceptible, rather than having one of the other diseases mentioned in table 2, all of which have associated symptoms. Using a lower limit of 0.175 (Pi/PCr) in figure 2, above which is a positive result for MH susceptibility and, below, negative, one can determine a sensitivity of 85% and a predictive value of 100% in our NMR test. These values are only an estimate, since a more meaningful determination must be made from a larger, properly blinded prospective study, currently under way. Specificity cannot be accurately assessed in our study, since the test population was a preselected group of known MH-susceptible patients.

The basis for the observed differences in Pi/PCr is unclear. The measured difference may be a result of either true concentration differences of the metabolites or to changes in the characteristics of the metabolites (or their binding to proteins) in diseased muscle that

alter their magnetic properties and, thus, affect their measurement by NMR with the pulse pattern used in this study. If relaxation times (T1) are changed, the measurement of Pi and PCr may be altered without any actual change in the concentrations of metabolites. However, such findings have not been reported in the literature for the diseases studied by NMR to date. Moreover, in our study, the saturation correction factors (percentage of fully relaxed spectra measured) for PCr, Pi, and  $\beta$ -ATP were each the same in both the normal controls and the MH-susceptible patients ( $81\% \pm 3\%$  for PCr,  $86\% \pm 4\%$  for Pi, and  $91\% \pm 2\%$  for  $\beta$ -ATP). These were obtained by comparing fully relaxed spectra obtained with a 20-s interpulse delay with saturated spectra obtained with a 5-s delay. This indicates that the relaxation times for each of the metabolites measured are the same for the two populations, and that the Pi/PCr differences in rest are due to concentration differences.

An elevated resting Pi/PCr may be due to several causes: 1) muscle fiber type distribution abnormalities, 2) active muscle damage, 3) membrane abnormalities leading to selective Pi accumulation or PCr loss by the cell, and 4) metabolic abnormalities. Type I fibers (oxidative, red fibers) have a higher Pi/PCr than type II fibers (glycolytic, white fibers), at least in animals.<sup>18,19</sup> An unusually high relative number of type I fibers may result in a higher Pi/PCr. A study of the fiber type distributions in leg muscles of MH susceptible patients showed no relationship between fiber type and positive contracture response.<sup>20</sup> Although this study characterized the histology of leg muscles and the present study measured Pi/PCr in arm muscles, it is unlikely that the observed differences in Pi/PCr are due to fiber type differences. There was no clinical evidence of active muscle damage at the time of this study, nor was there histologic evidence of such in leg muscles at the time of biopsy. Furthermore, active necrosis in MH susceptibles has not been found between episodes.<sup>1,4</sup>

Several authors have suggested the existence of a cell membrane defect in muscle of MH susceptible patients.<sup>1,4</sup> A leaky membrane is often invoked as the explanation for elevated serum CK levels observed in some of these patients.<sup>21</sup> As mentioned above, figure 3a indicates a constancy of ATP, while figures 3b and c show an increase in Pi and a decrease in PCr, respectively. Thus, the observed increase in resting Pi/PCr is not due to cell membrane selectivity (*i.e.*, leak) to one of these metabolites. Biochemically, in a closed system (*i.e.*, with an intact membrane), Pi and PCr change in opposite directions, while ATP may remain unchanged. Therefore, changes in Pi/PCr are more evident than changes in either ATP/Pi or ATP/PCr, which is shown

TABLE 2. Muscle Disorders in which High Pi/PCr Ratios have been Recorded

|   |
|---|
| 1. Mitochondrial myopathies <sup>5</sup>  |
| 2. Muscular and myotonic dystrophies <sup>7-9</sup>   |
| 3. Metabolic myopathies (glycolytic defects, lipid storage diseases) associated with secondary atrophy <sup>6,8</sup> |
| 4. Polymyositis <sup>8</sup>  |
| 5. Hypothyroid myopathy <sup>10</sup>   |
| 6. Advanced denervating muscle disorders <sup>8,11</sup>  |
| 7. Muscle injury <sup>12</sup>  |

in figures 2 and 3. This suggests that the difference in resting Pi/PCr is due to a metabolic abnormality, rather than a membrane leak.

The biochemical significance of this ratio (Pi/PCr) has been discussed in previous publications.<sup>14-16</sup> In short, it is an indicator of the energy state of a tissue, which, in muscle, is controlled by oxidative metabolism. Raised Pi/PCr in muscle signifies, in principle, a lowering of the phosphate potential ( $\text{ATP}/\text{ADP} \times \text{Pi}$ ).<sup>14-16</sup> Since the resting state is a steady state of ATP breakdown and ATP synthesis, the increased resting Pi/PCr, and, thus, the lowering of the phosphate potential, is due to an impairment of ATP synthesis or increased ATP breakdown.

It has been postulated that the mitochondria of MH patients are compromised in their ability to produce ATP.<sup>22</sup> Several investigators have demonstrated a decreased capacity for respiration in mitochondria from muscle of MH susceptible individuals, even when not exposed to any triggering agent.<sup>1</sup> Gronert and Heffron showed a 40-60% decrease in respiratory activity during state 3 (active respiration) in MH-susceptible swine.<sup>23</sup> Other investigators have, however, found mitochondrial respiration unaltered in MH.<sup>4</sup> Britt *et al.*, in studying mitochondria from MH-susceptible humans, found no significant difference in mitochondrial performance as compared to non-susceptible patients.<sup>21</sup> The effective test for ATP synthesis *in vivo* under the work condition (during active respiration) is provided by figure 4, where the MH-susceptible population available to us showed no significant difference compared with controls. While it is obvious that a larger population should be studied by this method, our existing data suggest that a much smaller difference in energy state exists between MH-susceptible and normal individuals during steady-state exercise than in the resting condition. This suggests that ATP synthesis is not greatly impaired in unchallenged human MH-susceptible subjects *in vivo*, at least during exercise.

The alternative explanation for the observed increased resting Pi/PCr is that of an increased rate of ATP breakdown by some ATPase in the cell, due either

to increased intrinsic activity and/or increased levels of an activator substance (i.e.,  $\text{Ca}^{++}$ ). The most likely candidate is a  $\text{Ca}^{++}$ -dependent ATPase. Cheah and Cheah have found higher rates in some  $\text{Ca}^{++}$ -dependent ATPases in muscle of MH-susceptible pigs.<sup>24</sup> In addition, Lopez *et al.* showed a higher resting cytoplasmic free  $\text{Ca}^{++}$  concentration in muscle of both MH-susceptible swine and humans when compared to normals.<sup>25-27</sup> Because cytoplasmic concentrations of  $\text{Ca}^{++}$  are normally high during exercise, and, thus,  $\text{Ca}^{++}$ -dependent ATPase is actively breaking down ATP, the differences between the two groups may not be evident during exercise. In addition, the one MH-susceptible patient we studied who was receiving dantrolene, which attenuates calcium release into the cytoplasm,<sup>1,28</sup> had a normal resting Pi/PCr (0.160). When she was restudied after 2 weeks free of dantrolene, her Pi/PCr value was higher than normal (0.270) and reached the level of the others in the MH-susceptible group (fig. 2). This explanation is also consistent with the recovery data presented in figure 5. Since recovery requires cessation of contraction, and, thus, a decrease in energy utilization, it is dependent on  $\text{Ca}^{++}$  removal from the cytoplasm. If the mechanism for removal of  $\text{Ca}^{++}$  from the cytoplasm is effected, then the recovery rate will be slowed, as we observed. Condrescu *et al.* and others report that  $\text{Ca}^{++}$  uptake capacity is significantly lower in sarcoplasmic reticulum vesicles prepared from MH-susceptible patients as compared to normals.<sup>27,29</sup>

This is also consistent with the general hypothesis of MH. It is thought that the MH episode is due to increased and uncontrolled release of intracellular  $\text{Ca}^{++}$  into the cytoplasm.<sup>1,4,30</sup> The increased  $\text{Ca}^{++}$  augments the turnover of ATP, which is normally replenished by PCr and oxidative phosphorylation. In an *in vivo* study previously conducted in our laboratory, a rapid rise in Pi/PCr was observed with <sup>31</sup>P NMR during an MH crisis in MH-prone swine (Roberts, Burt, Gouylai, Chance, Screter, and Ryan: unpublished observations). Galloway and Denborough showed a similar change in an *in vitro* study using <sup>31</sup>P NMR and biopsied swine muscle.<sup>31</sup> Thus, it seems that, in an MH episode, the utilization of high energy phosphates exceeds their production and PCr falls drastically with a concomitant rise in Pi.

Further research is needed to determine the exact basis of the observed differences in resting Pi/PCr. The evidence thus far points to a metabolic defect. However, what is most important and exciting is that there is a difference in the unchallenged MH-susceptible patient detectable by <sup>31</sup>P NMR. For this reason, <sup>31</sup>P NMR has the potential to be useful as a non-invasive tool in detecting MH susceptibility. However, until this study is

confirmed and carried out in a larger number of patients in a blinded manner, contracture testing is still employed to diagnose MH susceptibility.

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