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^{31}Phosphorus Magnetic Resonance Spectroscopy Characterization of Muscular Metabolic Anomalies in Patients with Malignant Hyperthermia

Application to Diagnosis

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Background: **Metabolic anomalies are known in skeletal muscles of patients with malignant hyperthermia (MH).**

Methods: **The authors used 31-phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) to compare metabolic changes of the finger flexor muscles recorded throughout two rest-exercise-recovery protocols (each including aerobic or ischemic exercise) in 26 healthy persons and in 13 MH-susceptible (MHS) persons who were unequivocally diagnosed by *in vitro* halothane-caffeine contracture tests on muscle biopsies.**

Results: **No abnormality was observed at rest and during recovery periods. A larger phosphocreatine decrease associated with an early drop of *p*H was noted during the first minute of both exercise periods for MHS patients compared with controls. The early *p*H decrease indicated a disorder affecting glycolytic activation, probably reflecting defects of Ca^{2+} cycling, and provided a sensitivity of 77% for MHS diagnosis. A diagnostic strategy based on the retrospective analysis of 19 selected MR parameters was developed. An MRS score, corresponding to the number of abnormal values among the 19 parameters, was calculated and provided sensitivity and specificity rates of 100%; that is, no false-positive or false-negative results were found. A prospective analysis of 10 new participants further confirmed these findings.**

Conclusions: **These results (1) further confirm that MH is associated with the preexistence of latent muscular disorders; (2) enhance the potential diagnostic capacity of MRS, although it should be tested prospectively on a larger group of participants; and (3) allows the characterization of several abnormal metabolic profiles, in persons with MHS, reflecting the recently described polymorphism of MH. (Key words: Anesthesia; energetics; exercise; muscle metabolism; physiopathology.)**

MALIGNANT hyperthermia susceptibility (MHS) is a genetic predisposition to an anesthetic-induced, life-threatening hypermetabolic state.[1] The pathophysiology of MH is related to abnormal skeletal muscle calcium flux, which is probably due to mutation(s) within the gene(s) encoding for protein(s) of the sarcoplasmic reticulum calcium channel. A recent review suggests that 50% of the MHS was due to mutations in RyR1, the gene encoding for the calcium release channel of the sarcoplasmic reticulum.[2]

Usually MHS is determined by *in vitro* halothane-caffeine contracture tests performed on muscle biopsy specimens. These tests constitute the only recognized diagnostic method in patients thought to have MH. Because *in vitro* contracture tests require a muscle biopsy, 31-phosphorus (^{31}P) magnetic resonance spectroscopy (MRS) has been evaluated as a possible noninvasive alternative method to characterize metabolic anomalies that would be specific for MH.[3-8] Kozak-Reiss *et al.*[3] have reported metabolic abnormalities during the exercise period recorded by MRS on forearm muscle in one MHS patient. Using another experimental protocol, Olgin *et al.*[4] did not identify any metabolic abnormalities during exercise but determined that MHS patients displayed increased values of inorganic phosphate:phosphocreatine ratios of relative concentrations (Pi:PCr) at rest, probably as a result of reduced PCr concentration. They also reported that the kinetics of PCr:Pi recovery after exercise, an index of the mitochondrial function,

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was slowed compared with controls, suggesting that MHS could be coupled to a mitochondrial disease. They proposed that the association of these two parameters provided good specificity and sensitivity for MHS diagnosis.[5] Payen *et al.*[8] reported that the Pi:PCr ratio and phosphodiesters content measured at rest on the gastrocnemius muscle were frequently higher for MHS patients compared with controls, suggesting that parameters measured at rest could be used for MHS diagnosis. Finally, Webster *et al.*[6] reported metabolic anomalies mainly during the exercise period for 11 MHS patients. Those studies clearly indicate that ^{31}P MRS could have a diagnostic value in MH susceptibility. Differences concerning the type of muscles investigated and experimental protocols have made it impossible to formulate so far a unique diagnostic strategy of MH susceptibility based on ^{31}P MRS. Nevertheless, the cited studies indicate a need to investigate how ^{31}P MRS can be used optimally to help diagnose MH.

Methods

Participants

This study was performed on 39 healthy persons (7 women and 22 men) referred to the laboratory for MH diagnosis. All were members of MH families. *In vitro* halothane-caffeine contracture tests were performed on muscle biopsy specimens in accordance with the protocol recommended by the European Malignant Hyperpyrexia Group (see *In Vitro* Contracture Tests).[9] The results were positive for 13 participants (3 women, 10 men; mean age, 40 yr; referred to as participants 27 to 39) who were classified as MHS, whereas 26 participants (4 women, 22 men; mean age, 35 yr; referred to as participants 1 to 26) were MH negative (MHN). The ^{31}P MRS tests were usually performed the day before the participants had the muscle biopsy.

In Vitro *Contracture Tests*

A biopsy was excised from the vastus (n = 8) or biceps brachii (n = 31) muscles under local anesthesia. Biopsies were excised preferentially from biceps because recovery of muscle function was faster for the participants and we failed to observe any difference for the results of *in vitro* contracture tests.‖ The sample was dissected into four bundles approximately 2 cm long and 1 mm wide. Each test was performed in duplicate in accord with the European Malignant Hyperpyrexia Group protocol.[9] Each bundle was placed into an oxygenated (95% oxygen, 5% carbon dioxide) Krebs medium maintained at 37°C. The bundles were stretched to a 2-g predefined tension and were continuously electrically stimulated to produce twitches. Stimulation was performed in the presence of increasing concentrations of caffeine, from 0.5 mM to 4 mM and then more than 32 mM. The second test was performed with 0.5 to 3% vol/vol halothane mixed into the gas flow. A result was considered as positive if a 0.2 g contracture (threshold) appeared for 0.5 or 2 mM caffeine and 0.5–2% vol/vol (0.44 mM) halothane. Participants were recognized as MHS if the results of both contracture tests (halothane and caffeine) were positive.

‖ Kozak-Ribbens G, Rodet L, Baeta A, Figarella Branger D, Pellissier JF, Cozzone PJ: Comparative Results of halothane/caffeine contracture tests from biceps and vastus muscle biopsies for the diagnosis of MH. Proceedings of the VIIth International Workshop on Malignant Hyperthermia, Hiroshima, Japan, 16–20 July 1994.

^{31}P Magnetic Resonance Spectroscopy

The MR spectra of forearm flexor muscles were recorded at 4.7 T throughout previously described rest-exercise-recovery protocols.[10] Details on experimental conditions are presented in the Appendix.

Exercise Protocol

After 3 min of rest, exercise consisted of finger flexions at 1.5-s intervals for 3 min lifting a 6-kg weight for men and a 4-kg weight for women. The motion amplitude of the sliding weight was approximately 3 cm, and it was kept constant throughout the exercise protocol. There was no duty cycle because work was performed lifting and releasing the weight. Such an exercise has been chosen to ensure PCr consumption greater than 50% of rest level and significant *p*H decrease.[11] Exercise was successively performed under aerobic and ischemic conditions. The protocol under ischemia involved the application of a sphygmomanometer over the upper arm and inflated, just before the start of exercise, above the maximum arterial pressure to achieve circulatory occlusion during the exercise period. At the end of the exercise, the pressure was quickly released. The participant's arm was gently restrained with velcro straps throughout the protocol, which included 20 min of postexercise recovery under fully aerobic conditions. A resting period of at least 1 h was allowed between the two exercise periods. All participants performed both protocols in the same order.

Data Processing

Peak areas of phosphorus metabolite signals including PCr, Pi, adenosine triphosphate (ATP), and phosphomonoesters (mainly corresponding to glucose 6-phosphate and fructose 6-phosphate[12]) were obtained by curve fitting the spectum to Lorentzian line shapes. Relative concentrations of phosphorus metabolites were expressed in relation to PCr content measured at rest (100%). Intracellular *p*H was calculated from the chemical shift of Pi relative to PCr at −2.45 ppm with respect to 85% H_3PO_4.[13] The PCr recovery was fitted to an exponential function as follows[14]:

$$PCr(t) = PCr_{rest} - PCr_{cons} \exp(-k\ t)$$

where *t* is time in min, *rest* and *cons* refer, respectively, to PCr content measured at rest and the amount of PCr consumed measured at end-of-exercise (% of PCr content at rest), and *k* is expressed in $min.^{-1}$ In addition, the initial rate of PCr recovery (Vi) was calculated from the initial slope of the recovery curve:

$$Vi\ (\%\ PCr/min) = k\ PCr_{cons}$$

Statistics

For the sake of clarity, results for all figures are presented as means ± SEM. To provide quantitative information about the distribution of each parameter, values in table 1 are presented as means ± SD. Table 2 summarizes values of metabolic parameters chosen *a priori* according to our previous work.[10]

Several analyses of variance have been performed using general linear models. The procedure (GLM) of SAS software (SAS Institute, Cary, NC), with options REPEATED, LSMEANS, and CONTRAST, were used.

For each group, one-way analysis of variance with repeated measures was used to analyze *p*H time-dependent changes during exercise. An F test was performed to determine the overall effect of time on *p*H changes throughout exercise. Then multiple comparisons procedures (Scheffé contrasts) were used to compare each value of *p*H recorded throughout the exercise period with the resting *p*H value. The same procedure was applied for the analysis of *p*H recovery toward resting values.

Between-group (MHN *vs.* MHS) comparative analyses of metabolites and *p*H time-dependent changes during exercise and recovery were performed using two-way analyses of variance with repeated measures (with time as the repeated variable). Wilk's lambda tests were performed to analyze the effects of time and the interaction between time and group. *Post hoc* repeated comparisons of mean values of *p*H and metabolite concentrations for each min of exercise and recovery between groups were performed (Scheffé contrasts).

Probability values for testing hypotheses were significant at 0.05. When appropriate, sensitivity and specificity were calculated as previously described.[15]

The results obtained retrospectively on participants 1 to 39 were subsequently applied prospectively on a separate group of 10 participants (2 women, 8 men; mean age, 37 yr; referred to as participants 40 to 49).

Results

Figure 1 shows results of *in vitro* contracture tests. Box plots clearly demonstrate the differences between MHS and MHN groups. For the MHS group, contractures recorded with halothane and caffeine were significantly greater than 0.2 g (threshold defined according to the protocol of the European Malignant Hyperpyrexia Group.[9]

Values of metabolic parameters, chosen *a priori* according to our previous work,[10] were measured or calculated from ^{31}P-MR spectra recorded throughout rest-exercise-recovery protocols and compared for the two groups. Figure 2 shows a series of typical MR spectra recorded on the flexor muscles of a healthy volunteer.

Rest

At the beginning of each protocol, under normoxia or ischemia, all metabolic parameters were stable, showing that the muscle metabolic state was identical before each test. No significant differences were observed between MHN and MHS participants for *p*H values, PCr:Pi and PCr:ATP (table 1). No measurable signals in the phosphodiesters region (between 0 and 1 ppm) were observed on spectra recorded for MHS participants.

Exercise-induced Metabolic Changes in the Malignant Hyperthermia-negative Group

The PCr and *p*H time-dependent changes recorded throughout exercises (normoxic and ischemic) were qualitatively similar (fig. 3). We address the general metabolic changes associated with exercise, keeping in mind that the magnitude of those modifications was always larger under ischemic conditions, as expected. During the first minute of exercise, no significant *p*H decrease was observed for MHN participants (table 1). Throughout the rest of the exercise, *p*H decreased continuously as a sign

Table 1. Values of Several Metabolic Parameters Measured and Calculated throughout the Rest–Exercise–Recovery Protocols

	Normoxic Protocol		Ischemic Protocol	
	MHS (n = 13)	MHN (n = 26)	MHS (n = 13)	MHN (n = 26)
Rest				
PCr/ATPr	4.1 ± 0.4	3.9 ± 5	4.2 ± 0.5	4.1 ± 5
PCr/Pi	11 ± 4	9.6 ± 2.5	9.4 ± 33	9.5 ± 2
*p*H (units)	6.99 ± 0.04	6.98 ± 0.02	6.97 ± 0.05	6.98 ± 0.02
PMEr (% PCr)	4.5 ± 3	5.0 ± 3	5.2 ± 3	6.5 ± 2
Changes from rest to the first min of exercise				
PCr (% PCr)	−38 ± 21*	−25 ± 10	−50 ± 12*	−40 ± 12
*p*H (units)	−0.10 ± 0.09*	−0.004 ± 0.04	−0.17 ± 0.12*	−0.01 ± 0.05
Changes from rest to end-of-exercise				
PCr (% PCr)	−63 ± 20*	−49 ± 15	−71 ± 14*	−72 ± 15
*p*H (units)	−0.51 ± 0.24*	−0.34 ± 0.2	−0.79 ± 0.22*	−0.58 ± 0.21
PME/PMEr	−6.5 ± 11	3 ± 2	5 ± 4	5 ± 2
ATP/ATPr	85 ± 27	87 ± 12	75 ± 26	79 ± 16
Changes from end-of-exercise to the first min of recovery				
PCr (% PCr)	25 ± 20	18 ± 11	25 ± 13	23 ± 9
*p*H (units)	0.15 ± 0.2	0.07 ± 0.14	−0.05 ± 0.08	0.11 ± 0.2
Changes during the recovery period				
Vi (% PCr/min)	37 ± 26	34 ± 19	24 ± 16	25 ± 12

Values are mean ± SD.

PCr = phosphocreatine; ATP = adenosine triphosphate; PME = phosphomonoesters; Pi = inorganic phosphate.

* Indicates significant difference between MHN and MHS groups ($P < 0.05$).

of anaerobic ATP production. The PCr decrease with respect to time revealed ATP production from PCr hydrolysis, whereas the Pi increase resulted from ATP hydrolysis during muscle contraction. Concerning the amount of PCr hydrolyzed during each minute of exercise, we observed a decrease over time, reaching a minimum at the third minute of exercise. An increase of phosphomonoesters level (phosphomonoesters accumulation) was noted as a result of the imbalance between glycogen phosphorylase and phosphofructokinase activities, as previously described.[12] A slight ATP decrease (about 20%) was observed for MHN participants.

Exercise-induced Metabolic Changes in the Malignant Hyperthermia Susceptible Group

Under both conditions of exercise (normoxic and ischemic), raw values of *p*H and PCr were significantly less for MHS participants, except for the PCr content measured at the end of the ischemic exercise (fig. 3). Whatever the conditions of oxygen availability, a significant *p*H decrease was measured at the onset of exercise in the MHS group (table 1, fig. 3). This decrease in *p*H was significantly larger in MHS participants compared with controls (MHN group; table 1, fig. 3). Taking into account the *p*H changes calculated for each minute of exercise (from the second minute of exercise), no significant difference was reached between groups or for both exercise conditions (normoxia and ischemia). The magnitude of PCr hydrolysis calculated at the beginning of both exercises (aerobic and ischemic) was also significantly larger for the MHS group. Throughout the remaining aerobic and ischemic exercises, the extent of PCr consumption calculated for each minute of exercises was similar in both groups. Finally, magnitudes of PCr changes measured at the end of exercise were significantly different between MHN and MHS groups for both exercises (figs. 3A and 3C, table 1). Phosphomonoesters accumulation and the slight ATP decrease (about 20%) were identical in MHN and MHS participants.

Recovery

Figure 4 shows recovery profiles of PCr and *p*H recorded under aerobic conditions after both types of exercises. At recovery, post-exercise acidosis was noted in the MHN group (figs. 4B, 4D). This additional acidosis, accounting for proton production associated with PCr resynthesis, was always recorded except after the ischemic exercise

Table 2. Metabolic Parameters and Corresponding Thresholds Used in Calculation of Specificity and Sensitivity of the MRS Test

Parameter		Normoxic Protocol	Ischemic Protocol
Rest			
1 (% PCr)	PME content	13	10
2 (*p*H units)	*p*H	6.96	6.99
Changes from rest to the first min of exercise			
3 (% PCr)	PCr	−40	−50
4 (*p*H units)	*p*H	−0.09	−0.08
Changes from the first to the second min of exercise			
5 (% PCr)	PCr	−31	−25
6 (*p*H units)	*p*H	−0.3	−0.6
Changes from the second to the third min of exercise			
7 (% PCr)	PCr	−24	−19
8 (*p*H units)	for *p*H	−0.45	−0.57
9 (relative to PME level at rest)	for PME	6.5	8
Changes from rest to the third min of exercise			
10 (% of ATP level at rest)	ATP	0.73	0.55
11 (% PCr)	PCr	−69	−87
12 (*p*H units)	*p*H	−0.74	−0.90
13[a] (% PCr/min)	initial rate of PCr decrease	59	71
Changes from the third min of exercise to the first min of recovery			
14 (% PCr)	for PCr	9.5	9
15 (*p*H units)	for *p*H	−0.30	−0.45
Changes from the fifth min of recovery to the 20th min of recovery			
16 (% PCr)	for PCr	75	64
17 (*p*H units)	for *p*H	0.55	0.70
18 (relative to PME level at rest)	for PME	3.5	4.5
Changes during the recovery period			
19 (% PCr/min)	for PCr	10.5	8

Selection of 19 metabolic parameters and corresponding thresholds used in the calculation of specificity and sensitivity of the MHS test. Normal values of thresholds correspond to minimal values for parameters P 10,15,16,17, and 19 and maximal values for all other parameters. % PCr are calculated with respect to the PCr level measured at rest (100%).

performed by the MHS group. Although *p*H values recorded at the first and second minute of aerobic exercise were significantly different between both groups, the extent of additional acidosis calculated as the difference between two consecutive points was not statistically different. The initial rate of PCr recovery, calculated from the monoexponential fit, did not reveal any differences between the MHN and MHS groups (figs. 4A, 4C). In both groups, the kinetics of recovery were faster for higher end-of-exercise *p*H values, as expected.[11]

This analysis shows that anomalies recorded in the MHS group were mainly associated with *p*H and PCr time-dependent changes, whereas no significant differences were recorded for phosphomonoesters or ATP profiles. Neither the rest nor the recovery periods provided any metabolic information about a possible specific disorder of MHS muscle.

Application to Malignant Hyperthermia Susceptible Diagnosis: A Retrospective Analysis

At the beginning of both exercises, an early intracellular acidosis associated with a larger extent of PCr consumption was systematically observed in the MHS group. For these two parameters, threshold values were calculated as follows: First, for each parameter and for each protocol, a "real" confidence interval (not issued from statistical analysis) was determined to encompass at least 96% of the recorded values in the MHN group, meaning that 25 of the

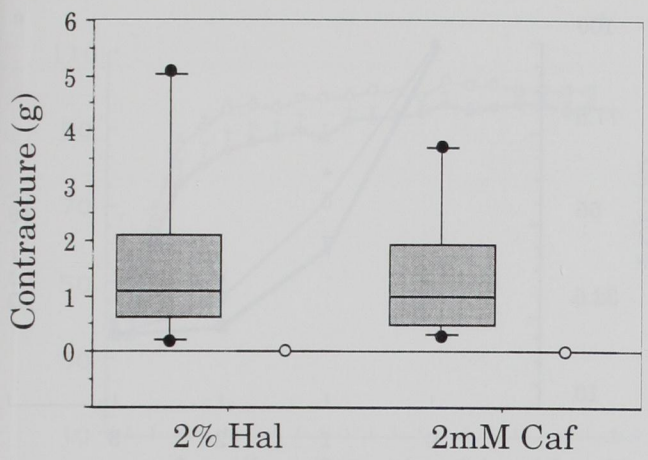

Fig. 1. Results of *in vitro* contracture tests expressed as contracture (g) for 2% halothane (Hal) and 2 mM caffeine (Caf). Tests have been performed in 13 MHS participants (*black circles*) and in 26 controls (*empty circles*, all superimposed) according to the European Malignant Hyperthermia Group guidelines.[9]

26 MHN participants were properly classified. Second, a parameter value was considered abnormal with reference to the value of the corresponding threshold (chosen as the lower or higher limit of the confidence interval, depending on the parameter; see table 2). Finally, considering both protocols, a parameter value was considered abnormal when it was found out of the confidence interval for at least one protocol. As shown in tables 3 and 4, 10 of 13 patients in the MHS group displayed an abnormal *p*H decrease at the beginning of one or both exercises, whereas only 2 of 26 MHS participants had this abnormality. Therefore, three MHS participants had false-negative results and two MHN participants had false-positive results. Accordingly, considering the intracellular acidosis recorded at the onset of both exercises (aerobic and ischemic), the sensitivity value was 77% and specificity was 93%.

Considering these low values, we tried to perform a retrospective analysis based on additional parameters. To formulate a diagnostic test, we focused on distinct anomalies in the time courses of various metabolic parameters and selected 19 parameters among the 24 usually measured or calculated for each participant (table 2).[10] These parameters were chosen *a priori* to properly describe metabolic changes associated with the rest-exercise-recovery protocols.[10] For each parameter, a metabolic threshold was determined for the MHN group and classified as normal or abnormal, as described before. Then for each participant (MHN and MHS), a score was calculated (MRS score). This score corresponds to the number of abnormal values recorded

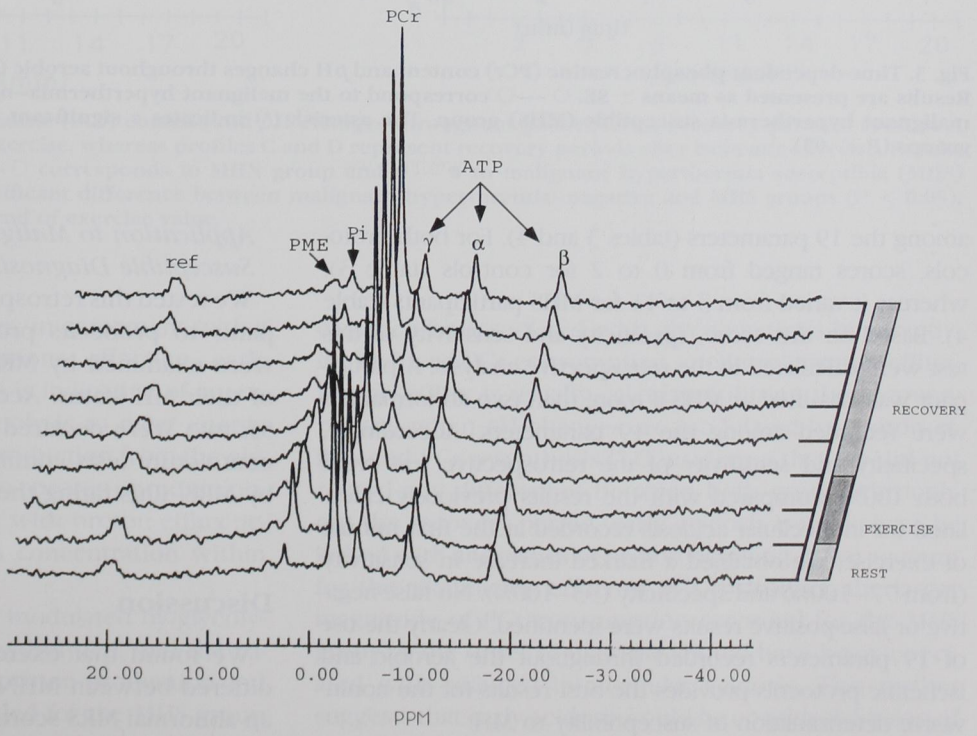

Fig. 2. A typical series of ^{31}-phosphorus magnetic resonance spectra recorded from flexor muscles of an healthy volunteer throughout a rest-exercise-recovery protocol, as described in the Appendix. Assignments are ref, reference compound (phenylphosphonic acid) contained in a micropipette positioned at the surface coil center to accurately monitor global changes in spectral intensity; PME, phosphomonoesters; Pi, inorganic phosphate; PCr, phosphocreatine; and α, β, γ ATP: α, β, γ phosphate groups of ATP. Each spectrum has been recorded in 1 min. For the sake of clarity, one spectrum is presented at rest, three throughout the exercise period, and five throughout the recovery period. Vertical axis represents signal intensity and horizontal axis refers to the chemical shifts expressed in ppm (*i.e.*, the frequency of the various signals).

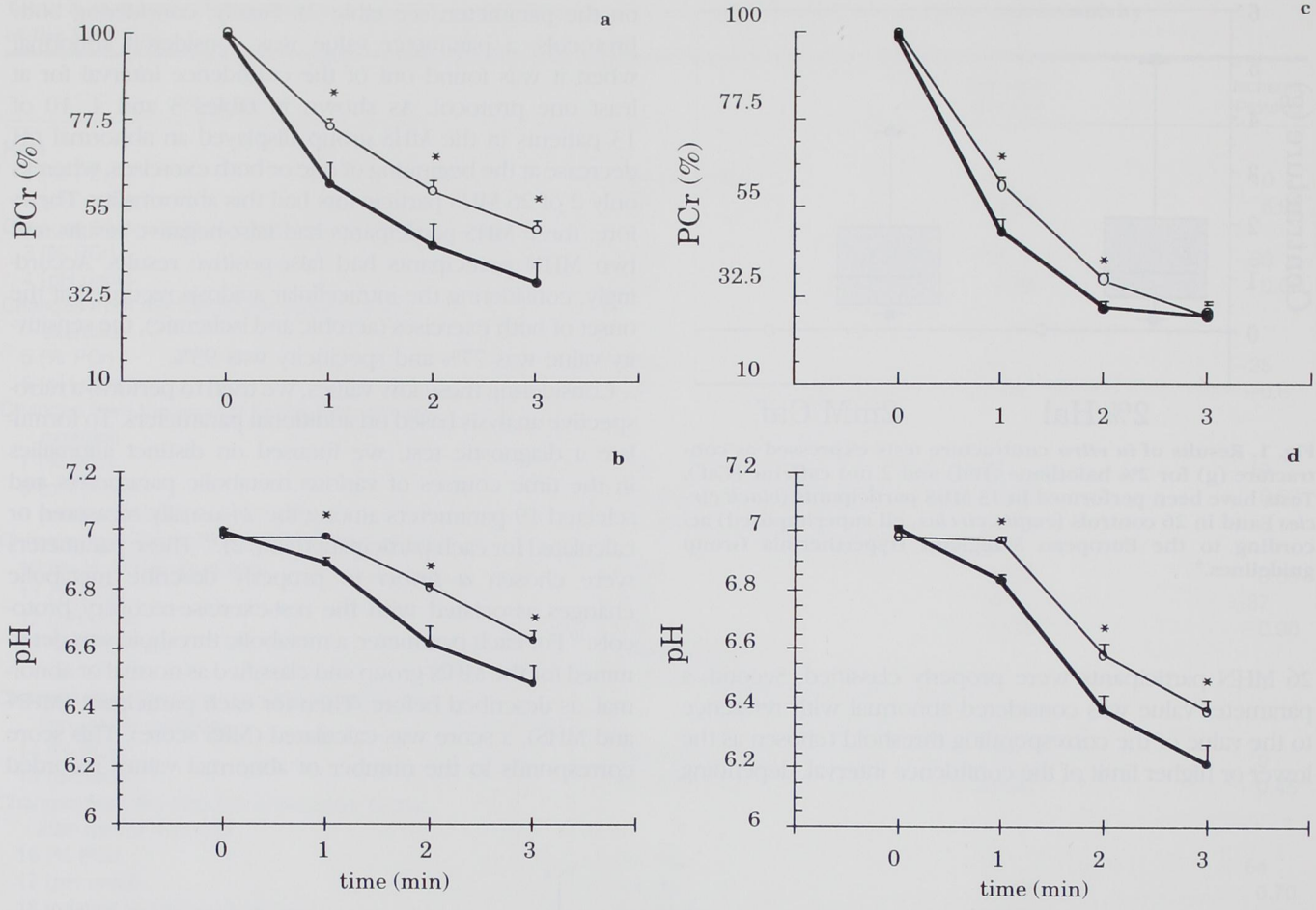

Fig. 3. Time-dependent phosphocreatine (PCr) content and *p*H changes throughout aerobic (*A, B*) and ischemic (*C, D*) exercises. Results are presented as means ± SE. ○ ---- ○ correspond to the malignant hyperthermia–negative (MHN) group and ● ---- ● to malignant hyperthermia susceptible (MHS) group. The asterisk (*) indicates a significant difference between MHN and MHS groups ($P < .05$).

among the 19 parameters (tables 3 and 4). For both protocols, scores ranged from 0 to 2 for controls (table 3), whereas it varied from 3 to 11 for MHS participants (table 4). Based on this score, specificity and sensitivity of this test were calculated in the retrospective analysis. A participant was classified as MHS if more than two abnormalities were recorded among the 19 parameters. Accordingly, specificity and sensitivity of the retrospective test were both 100%. Compared with the results previously calculated for intracellular acidosis recorded at the first minute of exercise, we obtained a marked increase in sensitivity (from 77–100%) and specificity (93–100%). No false-negative or false-positive results were identified. Clearly the use of 19 parameters recorded throughout the aerobic and ischemic protocols provides the best results for the noninvasive determination of susceptibility to MH.

Application to Malignant Hyperthermia Susceptible Diagnosis: Prospective Analysis

We tested this retrospective analysis in 10 new participants to probe its prospective value. These persons were examined by MRS without previous knowledge of their MH status. According to the MRS score (table 5), two were declared MHS and eight MHN. *In vitro* contracture tests confirmed the 10 diagnoses obtained by MRS, illustrating the potential of the MRS method.

Discussion

We found that exercise-induced metabolic changes differed between MHN and MHS participants and that an abnormal MRS score can indicate MH susceptibility.

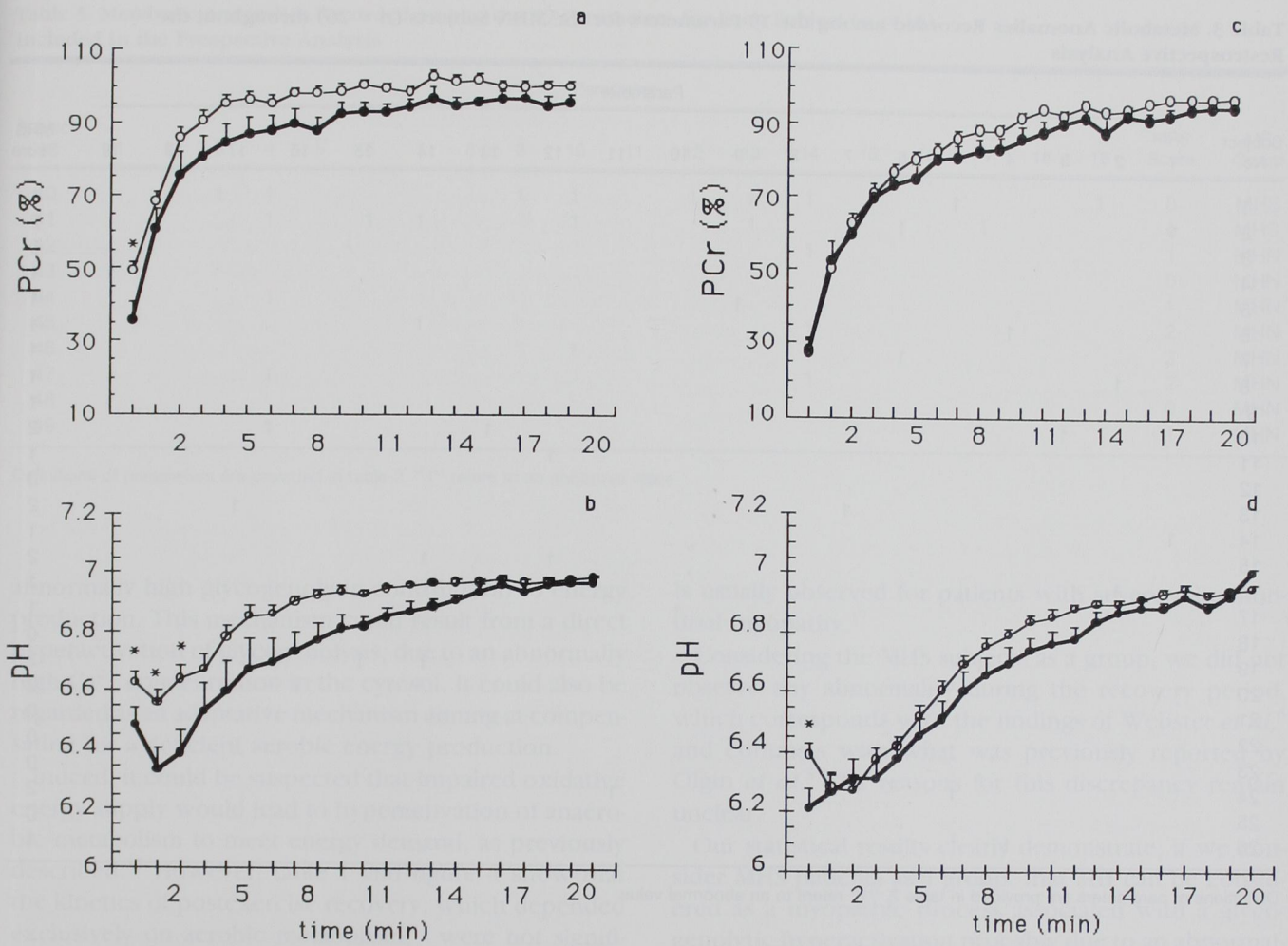

Fig. 4. Time-dependent phosphocreatine (PCr) content and *p*H changes throughout post-exercise recovery periods. Profiles A and B were recorded after aerobic exercise, whereas profiles C and D represent recovery periods after ischemic exercise. Results are presented as means ± SE. ○ ---- ○ corresponds to MHN group and ● ---- ● to malignant hyperthermia susceptible (MHS) group. The asterisk indicates a significant difference between malignant hyperthermia–negative and MHS groups ($P < 0.05$). The first point corresponds to the end of exercise value.

The statistical analysis identifies, in agreement with a previous study,[6] several deviations affecting early changes of intracellular *p*H. This is indicative of an excessive contribution of glycogenolysis to meet energy demand. During exercise, ATP production from the glycolygenolytic pathway leads to proton production, whereas PCr hydrolysis together with proton efflux accounts for a decrease in proton concentration within muscle cells.[16]

Consequently, *p*H changes are modulated by glycolysis activity, the amount of PCr hydrolyzed, and the capacity of muscle cells to handle proton production and elimination. Early acidosis recorded for the MHS group can then be associated with glycogenolysis hyperactivity, reduced PCr consumption, or altered proton efflux. Proton efflux is usually calculated during the recovery period, when *p*H changes are modulated by proton efflux and PCr resynthesis.[16] Considering that we did not record any differences between both groups throughout the recovery period, early acidosis is unlikely to be linked with anomalies of proton handling. If we account for the alkalinizing effect of PCr hydrolysis, the larger magnitude of PCr consumption recorded for the MHS group at the onset of exercise should have been associated with reduced intracellular acidosis. This further suggests that early acidosis could be considered a sign of

Table 3. Metabolic Anomalies Recorded among the 19 Parameters for the MHN Subjects (n = 26) throughout the Restrospective Analysis

Subject No.	Parameter																			MRS Score
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
1					1															1
2	1					1														2
3																				0
4																			1	1
5									1											1
6				1																1
7						1														1
8		1																		1
9																			1	1
10			1										1							2
11												1								1
12																				0
13							1										1			2
14	1																			1
15												1		1						2
16		1																		1
17										1										1
18																				0
19														1	1					2
20										1						1				2
21																				0
22																				0
23																				0
24					1						1									2
25																				0
26				1																1

Definitions of parameters are provided in table 2. "1" refers to an abnormal value.

Table 4. Metabolic Anomalies Recorded among the 19 Parameters for the MHS Subjects (n = 13) throughout the Retrospective Analysis

Subject No.	Parameter																			MRS Score
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
27				1											1		1			3
28			1							1		1	1				1			5
29			1	1			1	1		1	1	1					1			8
30			1	1		1			1		1	1	1					1		8
31			1	1		1			1	1	1	1	1			1	1	1		11
32				1					1				1	1			1		1	6
33				1	1		1			1			1		1		1			7
34			1	1	1	1					1	1				1		1	1	9
35			1	1		1							1	1						5
36	1	1	1								1		1				1			6
37		1			1							1					1		1	5
38			1	1					1		1		1				1	1		7
39			1	1		1			1	1	1		1	1		1				9

Definitions of parameters are provided in table 2. "1" refers to an abnormal value.

Table 5. Metabolic Anomalies Recorded among the 19 Parameters for Each Subject (n = 10) Included in the Prospective Analysis

Subject No.	Parameter																			MRS Score	MR Group
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19		
40			1	1					1	1		1	1	1					1	8	MHS
41				1		1	1			1			1				1			6	MHS
42														1						1	MHN
43																				0	MHN
44				1																1	MHN
45							1							1						2	MHN
46										1				1						2	MHN
47				1										1						2	MHN
48																				0	MHN
49				1																1	MHN

Definitions of parameters are provided in table 2. "1" refers to an abnormal value.

abnormally high glycogenolytic contribution to energy production. This mechanism could result from a direct hyperactivation of glycogenolysis, due to an abnormally high Ca^{2+} concentration in the cytosol. It could also be regarded as an adaptative mechanism aiming at compensating for a deficient aerobic energy production.

Indeed, it could be suspected that impaired oxidative energy supply would lead to hyperactivation of anaerobic metabolism to meet energy demand, as previously described.[17] However, table 1 and figure 4 show that the kinetics of postexercise recovery, which depended exclusively on aerobic metabolism,[11] were not significantly different between the two groups, thereby excluding any aerobic deficiency. This observation clearly confirms that the early metabolic changes affecting *p*H are to be linked to an abnormally high activation of glycogenolysis. Malignant hyperthermia sensititivity is associated, in pigs and humans, with impaired Ca^{2+} release from sarcoplasmic reticulum,[1,18,19] and this early acidosis can be considered a result of a higher calcium level-induced glycogenolysis hyperactivation.

In contrast to previous findings, we did not record any significant abnormality either at rest or during recovery.[4,5,8] However, in those previous studies, maximal voluntary contraction was usually measured just before the exercise, and this could account for a PCr decrease (increase of the Pi:PCr ratio). In this case, abnormalities observed at rest could correspond to a higher sensitivity of MHS participants to PCr hydrolysis during moderate exercise (leading to a temporary Pi:PCr increase) rather than a permanent increase in the Pi:PCr ratio at rest, as is usually observed for patients with severe mitochondrial myopathy.[17]

Considering the MHS subjects as a group, we did not observe any abnormality during the recovery period, which corresponds with the findings of Webster *et al.*[6] and contrasts with what was previously reported by Olgin *et al.*[5] The reasons for this discrepancy remain unclear.

Our statistical results clearly demonstrate, if we consider MHS patients as a group, that MH can be considered as a myopathic process associated with a glycogenolytic hyperactivation probably due to an abnormality of calcium cycling. The sensitivity calculated from *p*H changes during the first minute of both exercise periods was not entirely satisfactory because it detected only 10 of 13 MHS participants. The MRS score, corresponding to the sum of deviations recorded throughout normoxic and ischemic protocols, provides a better characterization because 100% sensitivity was reached both in the retrospective and the prospective analyses. However, we could question the specificity of the metabolic abnormalities detected. Early intracellular acidosis coupled to large PCr depletion has been observed in several cases of metabolic myopathies, including Brody's disease (deficiency of Ca^{2+} ATPase of sarcoplasmic reticulum) while early *p*H decrease has been noted in postviral infection syndrome.[20,21] We must emphasize that only an asymptomatic patient with a family history of MH and for whom a significant MRS score is recorded, is most likely to be MH susceptible. In addition, MRS is not advocated for screening the general popula-

tion but rather for testing a distinct group of persons who are at risk because of a family history of undesirable MH events. Specificity should then be regarded as the capacity of the test to exclude MH susceptibility for a participant considered at risk and not for any participant selected randomly in the general population. The variability of MRS score, ranging from 3 to 11 for MHS patients, indicated a heterogeneity of metabolic profiles that corresponded to the multiple possible defects leading to MH. Indeed, abnormalities of calcium cycling within muscle cells might result from mutation in genes encoding the Ca^{2+} release channel or from other proteins that participate in the excitation-contraction process. The MHS locus has been found primarily on humans chromosome 19q, but a growing number of non-chromosome 19q MHS families has been reported,[22,23] including a localization on chromosome 3[24] and chromosome 7.[25] In addition, we must keep in mind that the MHS phenotype has been identified from *in vitro* contracture tests in patients with other neuromuscular diseases.[2]

We found that the selection of appropriate MRS parameters recorded throughout protocols including rest, exercise and recovery periods enable us (1) to noninvasively detect MH susceptibility and (2) to identify MHS as the manifestation of a latent myopathic process. The variability of MRS score illustrates the various abnormal metabolic profiles that can be recorded for MHS patients and corresponds with the multigenic origin of MHS and the possible association of MHS with other myopathies.[26] The diagnostic capacity of this score was tested prospectively in 10 new participants (two MHS and eight MHN) who were properly classified. The diagnostic potential of this MRS score must be evaluated in a larger group of persons.

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Appendix

All magnetic resonance spectroscopy experiments were conducted on a Bruker-Biospec 47/30 spectrometer equipped with a horizontal magnet (bore diameter, 30 cm). To record ^{31}-phosphorus magnetic resonance (^{31}P MR) spectra of forearm flexor muscles, the participants sat in a chair positioned by the magnet and inserted their arm horizontally into the magnet bore to position the flexor digitorum superficialis muscle over a 50-mm diameter, double-tuned (1-H, ^{31}P) surface coil. The forearm was placed approximately at the same height as the shoulder to ensure a good venous return and was gently restrained with velcro straps to minimize movements throughout the exercise period. Optimization of the field homogeneity was done by monitoring the 200.14-MHz signal from the muscle water and fat proton. ^{31}P MR data were acquired at 81.14 MHz after 55-μs radiofrequency pulses applied at 2-s intervals. Pulsing conditions were as follows: repetition time, 2 s; spectral width, 10,000 Hz; collection of 4 10^3 data points. Spectra were time averaged over 1 min (32 scans) and sequentially recorded during 3 min of rest, 3 min of exercise, and 20 min of recovery. A 15-Hz line broadening function was applied before Fourier transformation. A micropipette filled with a solution of phenylphosphonic acid was positioned at the surface coil center to accurately monitor global changes in spectral intensity. A typical series of spectra recorded from a healthy volunteer is presented in figure 2.