LABORATORY REPORT

Comparison of Metabolites in Skeletal Muscle Biopsies from Normal Humans and Those Susceptible to Malignant Hyperthermia

M. P. Verburg, M.D., Ph.D.,* B. A. Britt, M.D., F.R.C.P.(C),† F. T. J. J. Oerlemans,‡ B. Scott,§
J. van Egmond, Ph.D.,¶ C. H. M. M. De Bruijn, Ph.D.**

In earlier work on malignant hyperthermia (MH) susceptible pigs the concentration of muscle metabolites differed from that found in normal control pigs. Therefore, in the present study these metabolites were measured in human muscle biopsies to find out whether normal individuals could be discriminated from MH-susceptible persons. Analysis of skeletal muscle metabolites was performed on skeletal muscle obtained from humans (n = 68) being screened to exclude or confirm susceptibility to MH. Three groups were identified based on the reaction pattern of a skeletal muscle sample exposed *in vitro* to caffeine or halothane 1% plus caffeine: 1) MH susceptible (MHS; n = 19); 2) normal humans, (controls; n = 31); and 3) intermediate-reaction type (K-type: n = 18). No significant differences were found in metabolite levels of phosphocreatine (normal, MHS, and K-type: 13.20 vs. 13.74 vs. 14.42 nmol/mg wet weight, respectively), creatine (16.30 vs. 16.94 vs. 15.06 nmol/mg wet weight, respectively), adenosine triphospate (3.75 vs. 3.98 vs. 3.89 nmol/mg wet weight, respectively) and lactate (3.73 vs. 3.65 vs. 3.79 nmol/mg wet weight, respectively). It is concluded that analysis of skeletal muscle metabolites cannot be used as a screening test to confirm or exclude MH susceptibility in humans. (Key words: Hyperthermia: malignant. Muscle, skeletal: metabolites.)

MALIGNANT HYPERTHERMIA (MH) is a pharmacogenetic disease triggered by volatile anesthetics and/or depolar-

* Staff Anesthesiologist, Department of Anesthesia, De Wever Hospital, Heerlen, The Netherlands.

† Associate Professor, Department of Anesthesia, University of Toronto, Toronto, Ontario, Canada.

‡ Research Assistant, Department of Human Genetics, University of Nijmegen, Nijmegen, The Netherlands.

§ Research Assistant, Department of Anesthesia, University of Toronto, Toronto, Ontario, Canada.

¶ Physicist, Department of Anesthesia, University of Nijmegen, Nijmegen, The Netherlands.

** Professor of Physiological Chemistry, Projectgroup Biomedical and Health Care Technology, Eindhoven University of Technology, Eindhoven, The Netherlands.

izing skeletal muscle relaxants.[1] Mortality is still about 10%, in spite of the availability of dantrolene sodium.[2] The incidence of MH crises is estimated to range from 1/15,000 anesthetics in children, to 1/50,000–1/100,000 in adults.[3,4]

The inheritance is thought to be autosomal dominant, with variable expression and reduced penetrance.[1] However, no uniform opinion exists with respect to its inheritance pattern, partly due to the lack of an available mass screening method.

At present the screening method of choice is a pharmacologic *in vitro* test of skeletal muscle, obtained by open biopsy.[3] On exposure to incremental concentrations of caffeine or halothane, a contracture is observed in malignant hyperthermia susceptible (MHS) skeletal muscle at lower concentrations than in control skeletal muscle.[1,5,6] However, the major drawbacks of this test are its invasiveness, expense, and complexity, which make it unsuitable for mass screening.

A possible alternative might be the analysis of skeletal muscle metabolites. Previous reports have shown that it is possible to make a diagnostic biochemical analysis with small muscle biopsies (*e.g.*, needle biopsy) in myopathies.[7,8] As far as MH is concerned, increased concentrations of energy metabolites (*e.g.*, glycolytic intermediates) have been reported in muscle from MH susceptible patients as compared with normal subjects.[9,10]

Studying the porcine MH model, we found that even prior to triggering MH (after induction of anesthesia) susceptible pigs showed a different skeletal muscle metabolite profile compared with control animals.[11] In muscle obtained from MH susceptible pigs a decreased concentration of phosphocreatine (PCr) and an increased concentration of lactate and creatine (Cr) was reported.[11]

The purpose of the present study was to characterize biochemical parameters of muscle energy metabolism in normal and MH susceptible individuals by making use of a relatively simple and rapid analytical system: isotachophoresis (ITP). This is an electrophoretic micromethod that permits separation, identification, and quantification at the picomole level. In ITP, charged sample constituents

are separated under the influence of an electric field. They migrate as a train of contiguous zones in the order of decreasing effective mobilities, enclosed between a leading electrolyte (with highest mobility) and a terminating electrolyte (with lowest mobility). Sample components that under the chosen operational conditions (pH) are uncharged or have effective mobilities outside the range specified by the leading and terminating electrolyte are not separated and consequently not analyzed.[12] This makes the technique specific and versatile. In our previous studies on the porcine MH model we used ITP for rapid muscle metabolite analysis.[11] In the present study we employed the same system for energy metabolites in human muscle biopsies.

Materials and Methods

SAMPLE PREPARATION

After obtaining informed consent and approval by the Committee on Human Experimentation of the University of Toronto, Ontario, Canada, skeletal muscle biopsies were taken from anesthetized patients at the Toronto General Hospital (n = 68).

The standard anesthetic included Innovar® and diazepam for induction, endotracheal intubation and ventilation with oxygen (FI_{O_2}: 0.4), and nitrous oxide, delivered from a vapor-free anesthesia machine. The muscle biopsies for the metabolite studies were taken from the m. vastus lateralis and immediately freeze clamped by a brass clamp precooled in liquid nitrogen. The sample was stored at $-70°$ C. Additional muscle samples were taken to perform the routine *in vitro* contracture test in order to establish MH susceptibility.[5,6] This test was performed according to the description of Britt et al.[6,13]

MHS patients were differentiated from non-MHS (normals) patients according to the protocol of the Department of Anesthesia, Toronto, Canada.[6] The caffeine-specific concentration (CSC), *i.e.*, the dose of caffeine in mM in the absence of halothane required to raise the resting tension of a muscle fascicle by 1 gr, is 4.09 mM or less for MHS patients, and in the presence of 1% halothane it is 1.19 mM or less.

An intermediate group has been identified, showing a normal response to caffeine but an MH response to caffeine plus 1% halothane. The latter group is called the K-type group.[14]

Analysis of skeletal muscle metabolites was performed on the freeze-clamped skeletal muscle, shipped on dry ice from Toronto to Nijmegen, The Netherlands. Before shipping, the samples were coded, and the analyses were performed without knowing whether a sample came from an MH susceptible subject or from a normal subject.

ISOTACHOPHORETIC ANALYSIS

The skeletal muscle metabolites were studied using the isotachophoretic technique.[11,15] A muscle sample was freeze dried and homogenized mechanically. Extraction of muscle metabolites was performed using ice-cold EDTA 1.25 mM (pH 7.6) in 50% methanol at 0° C, for 15 min. The suspension was centrifuged for 1 min at 10,000 × g and the supernatant freeze dried.

To this lyophilized fraction, 200–800 μl of distilled water was added. Analysis was performed by injection of 5 μl of this extract into a commercially available ITP equipment (Tachophor®, LKB Instruments, Bromma, Sweden). Temperature during the measurement was kept at 20° C. The sample zones were monitored by means of UV absorption at 254 nm. Within one run (total analysis time ± 20 min) the following compounds were determined: adenosine triphosphate (ATP), inorganic phosphate (Pi), phosphocreatine (PCr), adenosine diphosphate (ADP), lactate, nicotinamide adenine dinucleotide reduced (NADH), inosine monophosphate (IMP), adenosine monophosphate (AMP), and nicotinamide adenine dinucleotide oxidized (NAD^+) (in order of effective mobility).

FURTHER METHODS

Creatine was measured in a neutralized perchloric acid extract of a muscle specimen, applying the enzymatic system described by Jaworek et al.,[16] with the addition of creatine kinase and ATP.

After all analyses had been performed, the code was broken and the data were submitted to statistical evaluation, using analysis of variance. Statistical significance was assumed if $P < 0.05$.

Results

The muscle biopsies were divided into three groups according to the reaction to caffeine alone and to the combination of halothane 1% plus caffeine. This division is shown in table 1, along with the mean skeletal muscle metabolite concentrations measured in each group. No significant differences were observed between skeletal muscle metabolite concentrations in muscle from normal subjects, MH susceptible subjects, or the K-type reactors.

Discussion

A major advantage of the ITP analysis of skeletal metabolites is the small amount of biopsy material needed. A muscle sample of 10–25 μg provides satisfactory and reproducible separation of a considerable number of essential metabolites with the same analysis.[15]

The results we obtained with ITP for metabolite values are comparable to values obtained using other analytical

TABLE 1. Skeletal Muscle Metabolites in Malignant Hyperthermia Susceptible (MHS) Humans, Non-MH Susceptible (normal) Humans, and Those Belonging to an Intermediate Group (K-type)

Parameter	Normals (n = 31)	K-type (n = 18)	MHS (n = 19)	p Value
CSC	5.59 ± 0.24	5.61 ± 0.31	2.21 ± 0.21	
(C + H)SC	1.87 ± 0.15	0.90 ± 0.05	0.62 ± 0.06	
ATP	3.75 ± 0.74	3.89 ± 0.43	3.98 ± 0.61	0.44
ADP	0.14 ± 0.09	0.13 ± 0.08	0.11 ± 0.05	0.28
AMP	0.05 ± 0.04	0.04 ± 0.04	0.03 ± 0.02	0.18
IMP	0.14 ± 0.13	0.09 ± 0.07	0.09 ± 0.07	0.18
NAD⁺	0.13 ± 0.10	0.11 ± 0.10	0.12 ± 0.09	0.81
PCr	13.20 ± 3.33	14.42 ± 3.85	13.74 ± 3.72	0.51
Cr	16.03 ± 4.93	15.06 ± 3.88	16.94 ± 3.84	0.43
Ct/(Cr + PCr)	0.54 ± 0.06	0.51 ± 0.06	0.55 ± 0.09	0.17
Lactate	3.73 ± 1.67	3.79 ± 1.92	3.65 ± 1.74	0.97
PCr/Lactate	4.41 ± 2.36	4.68 ± 2.28	4.61 ± 2.43	0.91
Pi	7.42 ± 1.92	7.24 ± 1.40	6.97 ± 1.61	0.66

CSC: Caffeine-specific concentration of caffeine to raise the baseline tension with 1 g, in the absence of halothane; (C + H)SC: caffeine + halothane specific concentration of caffeine to raise the baseline tension with 1 g, in the presence of 1% v/v halothane. ATP = adenosine triphosphate; ADP = adenosine diphosphate; AMP = adenosine

monophosphate; IMP: inosine monophosphate; NAD⁺: Nicotinamide adenine dinucleotide oxidized; PCr: Phosphocreatine; Cr: creatine. Pi: inorganic phosphate.

All values are expressed in nmol/mg wet weight, mean value ± SD.

techniques and with muscle biopsies taken by a biopsy needle. Essen *et al.*[17] reported values of ATP (3.92 ± 0.35 nmol/mg wet weight); PCr (10.17 ± 1.09 nmol/mg wet weight); and lactate (3.33 ± 0.41 nmol/mg wet weight). However, a major criticism of the analysis of skeletal muscle metabolites by means of needle biopsy is the excessively long time needed for freezing the sample.[18] Freezing of skeletal muscle samples should occur within 100 ms to avoid breakdown of PCr during the freezing process.[18] The best way to do this is to put a liquid nitrogen cryoprobe into the muscle mass. However, for ethical reasons this was not feasible as the mass to be frozen will be much larger than that needed for a needle biopsy sample. Furthermore, chemical irritation may occur due to the liquid nitrogen. In addition, changes in skeletal muscle metabolite levels can be detected by using the conventional needle biopsy sample.[15]

Therefore, in the present study we chose the open biopsy technique because: 1) it does not damage the tissue; 2) blood supply to the sample is maintained until immediately before freezing; and 3) it ensures no contamination by nonmuscle tissue, such as fat or fascia.

Our previous studies on the porcine MH model showed that on the basis of skeletal muscle analysis, a differentiation could be made between MH susceptible and control pigs.[11] Furthermore, studies by other workers on humans susceptible to MH suggest the presence of preexistent abnormalities in skeletal muscle metabolites.[9] In contrast to the findings from our porcine study[11] and the study by Isaacs and Heffron,[9] no significant differences were measured in skeletal muscle metabolites in MHS muscle and normal muscle. However, in the study of Isaacs and Heffron,[9] the muscle biopsies were not freeze clamped.

PCr is the first metabolite to decrease in the event of an increased energy demand or decreased production. In the MH samples this was not the case, which supports the hypothesis that skeletal muscle metabolism is not abnormal in MH susceptible humans before the MH episode is precipitated. This statement is further supported by a recent study wherein PCr and ATP changes in pigs prior to and during an MH crisis[19] were measured using a nuclear magnetic resonance (NMR) technique. On induction of the MH crisis with succinylcholine, a drop occurred in PCr levels, whereas prior to the administration of succinylcholine no significant differences in PCr levels were measured. Perhaps the observed differences in pig muscle metabolites[11] might be due to the increased vulnerability to manipulation of pig muscle compared with human muscle.

In summary, these results suggest that measuring skeletal muscle metabolites from a muscle biopsy is not useful in predicting MH susceptibility. The use of NMR should be further evaluated as a noninvasive screening method to detect abnormalities in skeletal muscle metabolism and may turn out to be useful in predicting MH susceptibility.

References

1. Gronert GA: Malignant hyperthermia. ANESTHESIOLOGY 53:395–423, 1980
2. Britt BA: Malignant hyperthermia. Int Anesthesiol Clin 17:VII–X, 1979
3. Nelson TE, Flewellen EH: Current concepts. The malignant hyperthermia syndrome. N Engl J Med 309:416–418, 1983
4. Britt BA, Kalow W: Malignant hyperthermia: A statistical review. Can Anaesth Soc J 17:293–315, 1970
5. Kalow W, Britt BA, Terreau ME, Haist C: Metabolic error of

muscle metabolism after recovery from malignant hyperthermia. Lancet ii:895–898, 1970

6. Britt BA, Frodis W, Scott E, Clements M-J, Endrenyi L: Comparison of the caffeine skinned fibre tension (CSFT) test with the caffeine–halothane contracture (CHC) test in the diagnosis of malignant hyperthermia. Can Anaesth Soc J 29:550–562, 1982

7. Edwards RHT, Young A, Wiley CM: Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. N Engl J Med 302:261–271, 1980

8. Edwards RHT, Harris RC, Jones DA: The biochemistry of muscle biopsy in man, Clinical Applications. Recent Advances in Clinical Biochemistry, vol 2. Edited by Alberti KGMM, Price CP. Edinburgh, Churchill Livingstone, 1981, pp 243–269

9. Isaacs H, Heffron JJA: Morphological and biochemical defects in muscle of human carriers of the malignant hyperthermia syndrome. Br J Anaesth 47:475–481, 1975

10. Ellis FR, Halsall PJ, Allam P, Hay E: A biochemical abnormality found in muscle from unstressed malignant hyperpyrexia susceptible humans. Biochem Soc Trans 12:357–358, 1984

11. Verburg MP, Oerlemans FTJJ, Van Bennekom CA, Gielen MJM, De Bruijn CHMM, Crul JF: In vivo induced malignant hyperthermia in pigs. I. Physiological and biochemical changes and the influence of dantrolene sodium. Acta Anaesthesiol Scand 28, 1–8, 1984

12. Everaerts FM, Beckers JL, Verheggen ThPEM: Isotachophoresis: Theory, Instrumentation and Applications. Amsterdam, Elsevier, 1976

13. Britt BA, Endrenyi L, Kalow W, Peters L: The adenosine triphosphate (ATP) depletion test: Comparison with the caffeine contracture test as a method of diagnosing malignant hyperthermia susceptibility. Can Anaesth Soc J 23:624–635, 1975

14. Nelson TE, Flewellen FH: Spectrum of susceptibility to malignant hyperthermia. Anesth Analg 62:545–552, 1983

15. Oerlemans FTJJ, Van Bennekom CA, De Bruijn CHMM: Metabolite profiling of human muscle extracts by isotachophoresis. J Inherited Metab Dis 4:109–110, 1981

16. Jaworek D, Gruger W, Bergmeyer HU: Methoden der Enzymatische Analyse II. Edited by Bergmeyer HU. Weinheim, Verlag Chemie, 1974, pp 2178–2181

17. Essen B, Hagenfeldt L, Kayser L: Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man. J Physiol (Lond) 265:489–506, 1977

18. Kretzschmar KM, Wilkie DR: A new approach to freezing tissues rapidly. J Physiol (Lond) 202:66P–67P, 1969

19. Roberts JT, Burt T, Gouylai L, Chance B, Sreter F, Ryan J: Immediate uncoupling of high energy phosphorylation in muscle of malignant hyperthermic swine determined non-invasively by whole body 31P nuclear magnetic resonance (abstract). ANESTHESIOLOGY 59:A230, 1983