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Inositol 1,4,5-trisphosphate in blood and skeletal muscle in human malignant hyperthermia†

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Summary

The *in vitro* contracture test (IVCT) is the only available diagnostic method at present for evaluation of malignant hyperthermia (MH) susceptibility. However, the disadvantage of the IVCT is that it is invasive. Several studies suggest that an altered inositol phosphate system is involved in the development of MH. A greater concentration of inositol 1,4,5-trisphosphate (1,4,5-IP_3) was found in MH susceptible (MHS) than in normal (MHN) skeletal muscles. In this study the concentrations of 1,4,5-IP_3 in blood samples and skeletal muscle specimens of identical patients were measured in an attempt to define susceptibility to MH. Muscle biopsies were obtained from 34 patients with clinical suspicion of MH. Patients were first classified as MHS ($n=19$), MHN ($n=8$) or MH equivocal (MHE; $n=7$) by the standard IVCT. For detection of 1,4,5-IP_3 concentrations, blood samples were obtained and an additional muscle specimen was excised. After sample preparation, concentrations of 1,4,5-IP_3 were measured using radioimmunoassay. In blood samples, concentrations of 1,4,5-IP_3 were similar in all individuals tested for MH susceptibility and in control patients not tested for MH susceptibility ($n=44$). In skeletal muscle, 1,4,5-IP_3 concentrations were significantly higher in MHS than in MHE or MHN patients, respectively. Each MHS sample contained more 1,4,5-IP_3 than the highest concentration measured in MHN muscle. Defining arbitrary thresholds for 1,4,5-IP_3 concentration in skeletal muscles in order to discriminate between MHS and MHN status, it was possible to assign three MHE patients to MHS and four to MHN. This study supports the hypothesis that an altered inositol phosphate system might be involved in MH. However, measurement of 1,4,5-IP_3 concentration in a simple blood sample preparation is not reliable for MH susceptibility screening. (*Br. J. Anaesth.* 1997; **78**: 541–547).

Key words

Anaesthetics volatile, halothane. Malignant hyperthermia. Metabolism, inositol phosphate. Muscle skeletal. Screening.

Susceptibility to malignant hyperthermia (MH) in humans is diagnosed by *in vitro* contracture tests (IVCT) using halothane and caffeine on skeletal muscle specimens obtained at open biopsy.[1,2] In 1970 caffeine was introduced as the test drug for MH susceptibility followed by halothane 1 yr later.[3,4] Both caffeine and halothane produce abnormal contracture responses in muscle specimens obtained from MH susceptible (MHS) but not from normal (MHN) individuals. At present, the IVCT using caffeine and halothane is generally accepted as the "gold standard" for diagnosis of MH susceptibility. However, the major disadvantage of the IVCT is that it is invasive. Therefore, it would be desirable to have an accurate, non-invasive, low-cost screening test for MH susceptibility.

Skeletal muscle cells from MHS individuals have an inherited abnormality in the processes that are involved in the excitation–contraction coupling or myoplasmic calcium regulation,[5,6] leading to a rapid and sustained increase in myoplasmic calcium during anaesthetic-induced MH crisis. Intracellular calcium concentrations are controlled by different types of calcium release channels, that is the ryanodine receptor in the terminal cisternae of the sarcoplasmic reticulum (SR), the dihydropyridine receptor in the transverse tubule and the inositol-1,4,5-trisphosphate receptor.[6,7] In pigs, the defect predisposing to MH susceptibility is associated with the ryanodine receptor, possibly because of a specific breeding effect,[8,9] whereas the detailed molecular mechanisms leading to the human MH syndrome are still unknown. Recent studies indicated that a second factor or modulator is required for expression of the MH syndrome in humans.[10] Furthermore, dysfunction in the phosphoinositide pathway and metabolism of free fatty acids have been proposed as underlying causes of the alterations in calcium regulation in MH.[11–13] The second messenger inositol-1,4,5-trisphosphate (1,4,5-IP_3) has a key role in controlling both mobilization of calcium from internal stores and entry of external calcium.[14] As increased concentrations of 1,4,5-IP_3 were found in

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MHS individuals[12] and also in MHS swine,[11] there is an increasing number of reports indicating that an altered inositol phosphate system is involved in the development of MH.[15–18] However, in skeletal muscle the sensitivity of the intracellular Ca^{2+} release mechanism to 1,4,5-IP_3 is highly dependent on experimental conditions, and this had led to conflicting views on the role of the phosphoinositide pathway in muscle.[19]

In this study, we have measured basal concentrations of 1,4,5-IP_3 in blood samples and skeletal muscle specimens to determine if 1,4,5-IP_3 concentration in blood samples from patients with clinical suspicion of MH might be a reliable non-invasive screening test for MH classification. The second aim of this study was to see if a combination of a ryanodine contracture test and measurement of 1,4,5-IP_3 concentrations in skeletal muscle specimens could be helpful in assigning MHE patients to MHS and MHN groups.

Patients and methods

The study was approved by the local Ethics Committee and written informed consent was obtained from patients and their parents, if appropriate. We studied 34 patients from 19 families, 23 adults and 11 children, aged 7–44 yr, with a clinical suspicion of MH. Before starting the investigation, a personal and family history was obtained from all patients. Before operation, blood samples for measurement of 1,4,5-IP_3 concentration were obtained from all patients, and also from 44 control subjects without a history of MH.

MUSCLE BIOPSIES

Adult muscle biopsies were obtained under regional anaesthesia (3-in-1 nerve block) with 1% prilocaine 40 ml. Biopsies in children were excised under general anaesthesia. Patients received midazolam 0.1 mg kg^{-1} orally, 2 h before induction of anaesthesia. Anaesthesia was induced with alfentanil 50 μg kg^{-1} i.v. followed by propofol 2–2.5 mg kg^{-1}. Before laryngoscopy and tracheal intubation, vecuronium 0.1 mg kg^{-1} was given i.v. A continuous infusion of propofol ⩾150 μg kg^{-1} min^{-1} and 66% nitrous oxide in oxygen was used for maintenance of anaesthesia. Two or three muscle bundles were excised carefully from the vastus lateralis. The fresh specimens were placed in a container filled with carboxygenated Krebs–Ringer solution of the following composition (mmol $litre^{-1}$): NaCl 118.1; KCl 3.4; $CaCl_2$ 2.5; $MgSO_4$ 0.8; KH_2PO_4 1.2; $NaHCO_3$ 25.0; and glucose 11.1, and transported to our laboratory. Apart from these specimens, an additional muscle sample (weight 200–400 mg) was excised for measurement of 1,4,5-IP_3 concentration. Before cutting the fibres, the muscle specimen was clamped, and immediately after excision the samples were frozen in liquid nitrogen and stored at −80°C.

IN VITRO CONTRACTURE TEST

The muscle bundles were dissected into 6–8 strips (length 15–25 mm; width 2–3 mm; weight 100–250 mg). All tests were performed within 5 h after biopsy. The muscle strips were suspended in an 80-ml organ bath perfused with Krebs–Ringer solution bubbled with carbogen continuously, temperature was kept constant at 37°C and pH was adjusted to 7.4. Isometric tension was amplified by a Gould THE (Cleveland, OH, USA) and recorded on a Gould polygraph TA 2000 (Cleveland, OH, USA) using a Fleck TF 3V force displacement transducer (Mainz, Germany). The muscles were stimulated electrically to achieve a supramaximal response by a Grass Stimulator SD 9 (Quincy, MA, USA) using a square wave with a duration of 1 ms and a frequency of 0.2 Hz. An initial baseline tension of 20 mN was applied. Two specimens were investigated simultaneously.

Patients were classified first as MHS, MHN or MHE (MH equivocal) by the IVCT with caffeine and halothane, according to the procedure of the European Malignant Hyperthermia Group (EMHG).[1] The IVCT gave halothane and caffeine thresholds for each patient as follows: MHS = muscle contractures ⩾2 mN (corresponding to ⩾0.2 g in the original version of the procedure) at a caffeine concentration of 2.0 mmol $litre^{-1}$ or less *and* at a halothane threshold concentration of 0.44 mmol $litre^{-1}$ or less; MHN = muscle contractures ⩾2 mN at a caffeine concentration of 3.0 mmol $litre^{-1}$ or more *and* at a halothane threshold concentration greater than 0.44 mmol $litre^{-1}$; MHE = patients with an abnormal contracture response either to caffeine (MHEc) or halothane (MHEh).

ANALYSIS OF INOSITOL 1,4,5-TRISPHOSPHATE CONCENTRATIONS

Frozen muscle samples were powdered in a liquid nitrogen cooled dismembranator (Braun Melsungen, Germany) and the wet weight of the samples was measured. Subsequently, muscle and blood samples were mixed with 0.2 volume ice-cold 20% perchloric acid and kept on ice for 20 min. Proteins were sedimented by centrifugation at 2000 *g* for 15 min at 4°C. Supernatants were adjusted to a pH of 7.5 by addition of 10 N KOH and kept ice-cold. Precipitated $KClO_4$ was sedimented and removed by centrifugation. Samples were neutralized by adding 300 μl of a 1:1 (v/v) of Freon (1,1,2-trichlorotrifluoroethane) and tri-n-octylamine, followed by vigorous mixing of the separate phases on a vortex mixer. After centrifugation for 1 min at 2000 *g*, three phases were obtained. A 400-μl portion of the upper phase, which contained the neutralized sample, was removed for subsequent analysis. After addition of the assay buffer (D-myo-[^{3}H] inositol-1,4,5-trisphosphate) and 1,4,5-IP_3 binding protein, samples were incubated for 15 min and centrifuged. After decanting and discarding the supernatant, the pellets were resuspended and 2 ml of scintillant added to each tube. Subsequently, radioactivity was measured in a β-scintillation counter. For calculation of the results a standard curve, generated by plotting the percent B/B_0 as a function of $\log_{10}$ 1,4,5-IP_3 concentration was used.

RYANODINE CONTRACTURE TEST

After the IVCT, surplus muscle specimens were used in the ryanodine contracture test for diagnosis of MH susceptibility, as described recently.[20] Ryanodine was added as a bolus to the organ bath in order to obtain a concentration of 2 μmol litre^{-1}. The development of contracture after ryanodine administration was characterized by attainment of three phases: (1) start of contracture (min); (2) time (min) when contracture reached 2 mN; and (3) time (min) when contracture reached 10 mN. With the results for MHS and MHN after ryanodine 2 μmol litre^{-1}, we arbitrarily defined time intervals to assign MHE patients to either the MHS or MHN group, respectively.

The following chemicals were used: caffeine (Sigma, Deisenhofen, Germany), halothane (Hoechst, Frankfurt, Germany), ryanodine (purity >99% by HPLC; Calbiochem, La Jolla, USA). All substances were prepared freshly, dissolved in pre-warmed and pre-gassed Krebs–Ringer solution at 37 °C and added directly to the organ bath. Halothane was added to carbogen from a Dräger vaporizer (Lübeck, Germany); the concentration of halothane was measured with an anaesthetic gas monitor (Normac, Datex, Helsinki, Finland). Bath concentration of halothane was measured by gas chromatography.[21]

STATISTICAL ANALYSIS

Data are presented as median, range and SD (patients characteristics). Values were analysed using the Kruskal–Wallis and Mann–Whitney non-parametric tests to determine differences between groups. Results were considered significant if $P<0.05$.

Results

Nineteen patients were classified as MHS, eight as MHN and seven as MHE, according to the IVCT criteria of the European MH Group. Patient characteristics did not differ significantly (see table 1), but because one MHE patient had a creatine kinase (CK) concentration of 1800 iu litre^{-1}, CK values at rest were significantly greater in the MHE (315.7 (615.5) iu litre^{-1}) than in the MHS (61.6 (28.3) iu litre^{-1}) and MHN (55.6 (27.1) iu litre^{-1}) groups.

In simple blood sample preparations, 1,4,5-IP_3 concentrations were similar in all individuals tested for MH susceptibility and in control patients (fig. 1). Concentrations of 1,4,5-IP_3 in the MHS group ranged from 4.3 to 11.6 (median 8.7) pmol/tube. In the MHE group, values were 5.1–12.5 (median 9.9) pmol/tube. Median 1,4,5-IP_3 concentrations in the MHN group (5.5 pmol/tube) were smaller than those in the other groups. However, the range (4.0–11.0 pmol/tube) did not differ significantly from concentrations in the MHS and MHE groups. The control population showed a homogeneous distribution with a minimum of 3.4 and a maximum of 12.0 pmol/tube, which were similar to the results of the MH patients.

In skeletal muscles from the same patients, there were significant differences in 1,4,5-IP_3 concentrations (fig. 2). The median in the MHS group (6.2 pmol/mg ww) was significantly higher than that in the MHN group (1.7 pmol/mg ww). The ranges in both groups also showed marked differences: 1,4,5 IP_3-concentrations in the MHN group were 0.2–2.8 pmol/mg ww compared with 2.9–12.8 pmol/mg ww in the MHS group. Concentrations of 1,4,5-IP_3 in MHE individuals were between those of the MHS and MHN subjects, and were significantly different

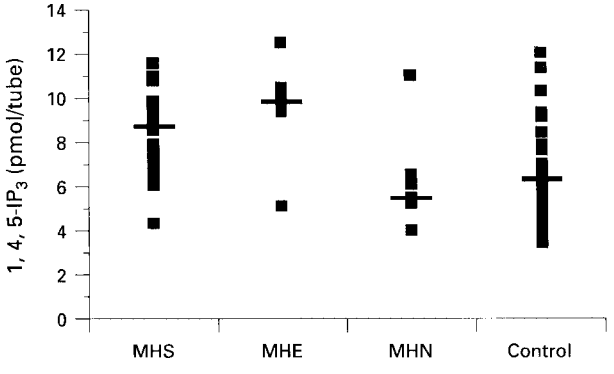

Figure 1 Median (—) and individual (■) concentrations of inositol 1,4,5-trisphosphate (1,4,5-IP_3) measured in blood samples from MHS ($n=19$), MHE ($n=7$) and MHN ($n=8$) patients, and in controls ($n=44$).

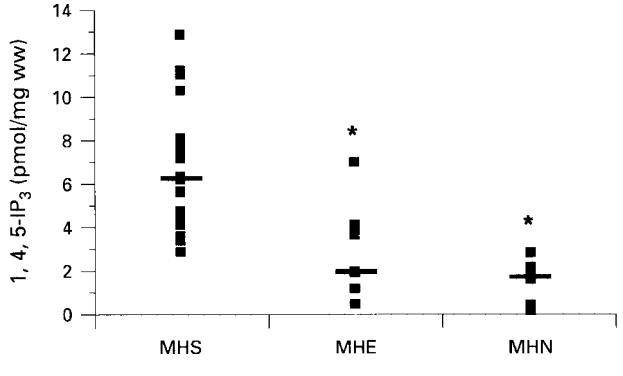

Figure 2 Median (—) and individual (■) concentrations of inositol 1,4,5-trisphosphate (1,4,5-IP_3) measured in skeletal muscle specimens from MHS ($n=19$), MHE ($n=7$) and MHN ($n=8$) patients. *$P<0.05$ compared with MHS.

Table 1 Patients characteristics (mean (SD) [range]). *$P<0.05$ compared with MHN

	MHS ($n=19$)	MHE ($n=7$)	MHN ($n=8$)
Age (yr)	27 [7–44]	30 [7–45]	19 [9–30]
Sex (M/F)	11/8	4/3	3/5
Weight (kg)	59 (19.2) [24–85]	64 (20.3) [25–87]	59 (17.4) [26–85]
Height (cm)	163 (18.4) [128–190]	169 (19.8) [130–189]	162 (15.3) [125–178]
Creatine kinase (iu litre^{-1})	61.6 (28.3) [18–106]	315.7 (615.5) [26–1800]*	55.6 (27.1) [27–110]

Table 2 Contractures after bolus administration of ryanodine 2 μmol litre^{-1} in skeletal muscle specimens of malignant hyperthermia susceptible (MHS), equivocal (MHE) and non-susceptible (MHN) patients. Hal. thresh. = threshold tension for halothane; Caff. thresh. = threshold tension for caffeine. OT = onset time of contracture; 2 mN = contracture reaches 2 mN; 10 mN = contracture reaches 10 mN. ⋆($P<0.05$) compared with MHS

Patient No.	Hal. thresh. (mmol litre^{-1})	Caff. thresh. (mmol litre^{-1})	Muscle twitch (mN)	OT (min)	2 mN (min)	10 mN (min)
MHS						
1	0.44	1.0	42.0	1.6	6.0	10.7
2	0.22	2.0	63.0	3.0	7.2	13.0
3	0.22	1.0	29.0	2.5	4.7	7.6
4	0.22	1.0	51.0	3.4	6.9	9.1
5	0.22	1.0	37.0	1.1	3.8	9.5
6	0.11	1.0	25.0	0.8	2.0	3.3
7	0.44	1.5	22.0	3.2	10.5	17.6
8	0.44	2.0	99.0	3.2	8.3	13.4
9	0.22	2.0	43.0	1.0	2.8	12.9
10	0.44	2.0	52.0	1.7	8.7	17.8
11	0.44	2.0	56.0	1.2	7.3	17.7
12	0.44	2.0	23.0	2.5	10.0	14.0
13	0.44	2.0	47.0	2.3	8.8	15.6
14	0.44	2.0	32.0	3.2	9.6	14.4
15	0.44	1.5	15.0	2.4	8.9	15.0
16	0.22	1.0	25.0	3.0	8.0	12.3
17	0.11	0.5	61.0	1.9	3.6	5.4
18	0.44	2.0	49.0	3.5	9.8	12.6
19	0.22	1.5	36.0	3.0	9.2	17.5
Median	0.44	1.5	42.0	2.5	8.0	13.0
MHE						
1	—	1.5	22.0	1.3	5.8	9.8
2	—	2.0	17.0	12.5	18.0	23.3
3	—	2.0	54.0	3.8	11.4	18.2
4	0.44	4.0	31.0	2.0	6.8	14.1
5	0.44	32.0	62.0	3.1	5.9	9.2
6	—	2.0	48.0	11.6	17.1	23.5
7	—	1.5	35.0	4.7	15.8	23.4
Median	—	2.0	35.0	3.8⋆	11.4	18.2
MHN						
1	—	3.0	24.0	6.6	11.8	18.0
2	—	3.0	28.0	8.6	15.1	26.7
3	—	3.0	53.0	7.9	12.1	18.3
4	—	3.0	59.0	5.8	14.8	19.7
5	—	3.0	75.0	5.7	17.9	30.1
6	—	3.0	47.0	5.3	13.0	20.0
7	—	3.0	54.0	8.8	16.2	25.8
8	—	3.0	21.0	6.1	12.4	19.9
Median	—	3.0	30.5	6.4⋆	13.9⋆	20.0⋆

from the MHS results. Individual data for three MHE patients varied from 3.7 to 7.0 pmol/mg ww, corresponding to the range of values for MHS, and four values varied from 0.5 to 2.0 pmol/mg ww, corresponding to MHN.

Individual data for contracture testing using ryanodine 2 μmol litre^{-1} are presented in table 2. Contracture development after ryanodine administration was enhanced significantly in MHS compared with MHN muscles. Median values for MHE muscles were always between those of MHS and MHN.

Table 3 summarizes the results of the IVCT, 1,4,5-IP_3 concentrations in skeletal muscle and the ryanodine contracture test in MHE patients. In an attempt to assign MHE muscles to either the MHS or MHN group, we arbitrarily denned thresholds for 1,4,5-IP_3 concentrations in skeletal muscles of $\geqslant$2.9 pmol/mg ww for MHS and $\leqslant$2.8 pmol/mg ww for MHN. Thus an assignment of three MHE patients to the MHS group was possible. MHE patient Nos 1, 3, 6 and 7 were defined as MHN. Using the range of values for MHS and MHN according to the ryanodine contracture test, we arbitrarily denned intervals to categorize MHE muscles as either MHS or MHN, as described recently.[20] Three MHE muscles showed enhanced contracture development and were classified as MHS. In four muscles a marked delay in contracture development occurred and these were allocated to the MHN group. In five

Table 3 Results of *in vitro* contracture tests with halothane and caffeine (IVCT), measurement of inositol 1,4,5-trisphosphate (1,4,5-IP_3) concentration in skeletal muscles and the ryanodine contracture test (RCT) using ryanodine at a concentration of 2 μmol litre^{-1} in malignant hyperthermia equivocal (MHE) patients

Patients No.	IVCT	1,4,5-IP3 (pmol/mg ww)	RCT
1	MHE	MHN (0.5)	MHS
2	MHE	MHS (4.1)	MHN
3	MHE	MHN (2.0)	MHN
4	MHE	MHS (3.7)	MHS
5	MHE	MHS (7.0)	MHS
6	MHE	MHN (1.2)	MHN
7	MHE	MHN (1.9)	MHN

of seven MHE muscles, consistent results in both tests were observed. The same MH classification was possible using measurement of 1,4,5-IP_3 concentrations and ryanodine contracture testing.

Discussion

We have found that 1,4,5-IP_3 concentrations in skeletal muscle specimens were significantly higher in MHS than in MHN patients. In contrast, 1,4,5-IP_3 concentrations in blood samples of the same patients and controls did not differ. Thus it was possible to differentiate between MHS and MHN individuals by measurement of 1,4,5-IP_3 concentrations in skeletal muscle specimens but not in simple blood sample preparations. Furthermore, we have demonstrated that a combination of a ryanodine contracture test and measurement of 1,4,5-IP_3 concentrations in skeletal muscle specimens could be helpful in assigning MHE patients to MHS and MHN groups.

For evaluation of susceptibility to MH, only the *in vitro* contracture tests with halothane and caffeine have been shown to be reliable in distinguishing between MHS and MHN individuals. Human skeletal muscle contracture testing for diagnosis of MH susceptibility seems to be highly sensitive.[22] However, the specificity of the IVCT was estimated as 80–85%.[23] For the first time in 1993 four cases were described of presumably false-negative contracture responses in muscle specimens from patients with a clinical history of MH.[24] However, there is considerable debate about these cases because the authors did not use the EMHG test procedure; in two cases there was no clinical evidence of MH and muscle histology and neurological examination were not performed in all patients. Four additional cases were presented recently of false-negative IVCT results in juvenile patients surviving MH episodes during general anaesthesia.[25] The authors suggested that the age of the patients might have affected the contracture results. However, whereas in newborn MHS piglets decreased responsiveness to halothane compared with mature animals has been observed,[26] age dependence of MH in humans has not been demonstrated until now.

The caffeine contracture test produces false-negative results in approximately 15% of MH susceptible patients. Moreover, both caffeine and the halothane tests were shown not to be specific for MH, as positive IVCT results were observed in some individuals with other muscle disorders.[27] Contracture test results in control patients suggested a false-positive rate of approximately 4% using the European[28] and 9% using the North American MH procedures.[23] The hypothesis that MH is caused by an abnormal ryanodine receptor gene focused interest on developing an alternative test for diagnosis of MH using ryanodine. *In vitro* studies of ryanodine-induced contractures showed that in muscle specimens of MHS patients, contractures developed significantly earlier than in specimens from MHN individuals.[20 29–32] Although the results of contracture testing with ryanodine in conjunction with the standard halothane and caffeine contracture tests could improve diagnosis of MH susceptibility,[33] the ryanodine contracture test is also not specific for MH. Moreover, the disadvantage of contracture testing with halothane, caffeine and ryanodine is that it is invasive, time consuming and expensive.

Since the identification of the ryanodine receptor gene, thought to be responsible for MH,[34 35] it has been assumed that MH susceptibility could be detected using a genetic test based on DNA analysis. Molecular genetic studies have shown that a single point mutation in the ryanodine receptor gene (C1843-T mutation) is frequently correlated with MH in swine.[9] However, the corresponding mutation in the human genom (C1840-T mutation) was detected first in only one of 35 MH families.[36] At present, the frequency of this single point mutation in the human ryanodine receptor gene has been calculated to be less than 10%.[10 37] Moreover, mutation screening of the ryanodine receptor gene in MHS individuals has led to the identification of seven other mutations to date.[38] Furthermore, genetic heterogeneity has been reported in MH[39] and several additional MHS loci on chromosomes 17, 7 and 3.[40–42] DNA-based testing in MH would be non-invasive, accurate and less expensive, but there is little probability that a universal genetic test for MH susceptibility in humans will be easily developed because of heterogeneity, increasing number of identified mutations in MH and association with muscle cell deficiencies induced by other neuromuscular diseases.

Activation of G protein-linked receptors led to the dissociation of the G protein in the subunits G_α and $G_{\beta\gamma}$, both of which can activate different phospholipase C (PLC) enzymes.[14] In energy-requiring transducing mechanisms, PLC hydrolyses the lipid precursor phosphatidylinositol 4,5-bisphosphate to stimulate formation of both diacylglycerol (DAG) and 1,4,5-IP_3, which binds to an 1,4,5-IP_3 receptor to mobilize stored Ca^{2+}. It is believed that abnormal regulation of intracellular Ca^{2+} concentration within skeletal muscle is the process of primary significance in MH episodes. Furthermore, malfunctions in the phosphoinositide cycle and free fatty acid metabolism were shown to be associated with MH susceptible muscle.[11–13 16–18] Basal concentrations of 1,4,5-IP_3 in skeletal muscles of MHS swine[11] and humans[12] were found to be significantly higher than in MHN. It was demonstrated that successive additions of 1,4,5-IP_3 induced short pulses of calcium release.[43] Moreover, microinjection of 1,4,5-IP_3 increased intracellular Ca^{2+} in intact skeletal muscle from MHS swine with higher potency and efficacy than in muscles from MHN swine.[15] Dantrolene decreased resting intracellular Ca^{2+} concentration and prevented l,4,5-IP_3-induced increase in intracellular Ca^{2+}. These results suggest that skeletal muscle fibres from MHS have a higher sensitivity to l,4,5-IP_3-induced regulation of intracellular Ca^{2+}, which could play an important role in MH induction. In the presence of halothane, l,4,5-IP_3-concentration increased to a higher extent in MHS than in MHN muscles.[11] Moreover, it has been shown that halothane challenge caused an increase in the second messengers 1,4,5-IP_3 and 1,3,4,5-IP_4 in MHS but

not in MHN swine.[18] Treatment with dantrolene reduced the increase to values close to basal concentrations. To date, it is not clear if the alterations in phosphoinositide metabolism are primary in MH induction or of a secondary nature. ATP at low concentrations enhances the ability of 1,4,5-IP_3 to open Ca^{2+} channels in the SR membrane. It has been hypothesized that the lower the ATP concentration, possibly as a result of the hypermetabolic state of MH, and the higher the 1,4,5-IP_3 concentration, the more Ca^{2+} is released.[17] It has been speculated therefore that measurement of Ca^{2+} or 1,4,5-IP_3 concentrations, or both, in muscle but also in non-muscle cells, could be used for diagnosing MH susceptibility.[44–46] However, the differences in these investigations were not reliable in determining susceptibility to MH until now.[46] Moreover, in accordance with our data from simple blood sample preparations, no differences in 1,4,5-IP_3 concentrations in human red blood cells were observed between MHS and MHN.[47]

In summary, this study supports the hypothesis that an altered inositol phosphate system might be involved in MH. One might speculate that the differences between skeletal muscles might also exist in other cell types, including blood cells. Further investigations are required to develop an accurate, reliable, low-cost and non-invasive screening test. Such a test might be measurement of 1,4,5-IP_3 concentration in different blood components or cell types, or methods of detecting genetic defects in 1,4,5-IP_3 metabolism.

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References

1. The European Malignant Hyperpyrexia Group. A protocol for the investigation of malignant hyperpyrexia (MH) susceptibility. *British Journal of Anaesthesia* 1984; **56**: 1267–1269.
2. Larach MG for the North American Malignant Hyperthermia Group. Standardization of the caffeine halothane contracture test. *Anesthesia and Analgesia* 1989; **69**: 511–515.
3. Kalow W, Britt BA, Terreau ME, Haist C. Metabolic error of muscle metabolism after recovery from malignant hyperthermia. *Lancet* 1970; **2**: 895–898.
4. Ellis FR, Harriman DGF, Keaney NP, Kyei-Mensah K, Tyrrell JH. Halothane induced muscle contracture as cause of hyperpyrexia. *British Journal of Anaesthesia* 1971; **43**: 721–722.
5. Carrier L, Villaz M, Dupont Y. Abnormal rapid Ca^{2+} release from sarcoplasmic reticulum of malignant hyperthermia susceptible pigs. *Biochimica et Biophysica Acta* 1991; **1064**: 175–183.
6. MacLennan DH, Phillips MS. Malignant hyperthermia. *Science* 1992; **256**: 789–794.
7. Spät A, Bradford PG, McKinney JS, Rubin RP, Putney JW. A saturable receptor for ^{32}P-inositol-1,4,5-trisphosphate in hepatocytes and neutrophils. *Nature (London)* 1986; **319**: 514–516.
8. Fill M, Coronado R, Mickelson JR, Vilven J, Ma J, Jacobson BA, Louis CF. Abnormal ryanodine receptor channels in malignant hyperthermia. *Biophysical Journal* 1990; **50**: 471–475.
9. Fujii J, Otsu K, Zorzato F, DeLeon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 1991; **253**: 448–451.
10. Fletcher JE, Tripolitis L, Hubert M, Vita GM, Levitt RC, Rosenberg H. Genotype and phenotype relationships for mutations in the ryanodine receptor in patients referred for diagnosis of malignant hyperthermia. *British Journal of Anaesthesia* 1995; **75**: 307–310.
11. Foster PS, Gesini E, Claudianos C, Hopkinson KC, Denborough MA. Inositol 1,4,5-trisphosphate phosphatase deficiency and malignant hyperpyrexia in swine. *Lancet* 1989; **ii**: 124–127.
12. Scholz J, Troll U, Schulte am Esch J, Hartung E, Patten M, Sandig P, Schmitz W. Inositol-1,4,5-triphosphate and malignant hyperthermia. *Lancet* 1991; **337**: 1361.
13. Fletcher JE, Tripolitis L, Erwin K, Hanson S, Rosenberg H, Conti PA, Beech J. Fatty acids modulate calcium-induced calcium release from skeletal muscle heavy sarcoplasmic reticulum fractions: Implications for malignant hyperthermia. *Biochemistry and Cellular Biology* 1990; **68**: 1195–1201.
14. Berridge MJ. Inositol trisphosphate and calcium signalling. *Nature (London)* 1993; **361**: 315–325.
15. Lopez JR, Perez C, Linares N, Allen P, Terzic A. Hypersensitive response of malignant hypothermia-susceptible skeletal muscle to inositol 1,4,5-trisphosphate induced release of calcium. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1995; **352**: 442–446.
16. Scholz J, Troll U, Sandig P, Schmitz W, Scholz H, Schulte am Esch J. Biochemical changes in malignant hyperthermia susceptible swine: cyclic AMP, inositol phosphates, α_1-, β_1- and β_2-adrenoceptors in skeletal and cardiac muscle. *Acta Anaesthesiologica Scandinavica* 1993; **37**: 575–583.
17. Steinmann CML. A possible role for inositol 1,4,5-trisphosphate (IP_3) in malignant hyperthermia. *Medical Hypotheses* 1993; **42**: 190–192.
18. Tonner PH, Scholz J, Richter A, Löscher W, Steinfath M, Wappler F, Wlaz P, Hadji B, Roewer N, Schulte am Esch J. Alterations of inositol polyphosphates in skeletal muscle during porcine malignant hyperthermia. *British Journal of Anaesthesia* 1995; **75**: 467–471.
19. Foster PS. The role of phosphoinositide metabolism in Ca^{2+} signalling of skeletal muscle cells. *International Journal of Biochemistry* 1994; **26**: 449–468.
20. Wappler F, Roewer N, Köchling A, Scholz J, Steinfath M, Schulte am Esch J. *In vitro* diagnosis of malignant hyperthermia susceptibility with ryanodine-induced contractures in human skeletal muscles. *Anesthesia and Analgesia* 1996; **82**: 1230–1236.
21. Zbinden AM, Frei FJ, Funk B, Thomsen DA, Westenskow D. Determination of the partial pressure of halothane (or isoflurane) in blood. *British Journal of Anaesthesia* 1985; **57**: 796–802.
22. Larach MG. Should we use muscle biopsy to diagnose malignant hyperthermia susceptibility? *Anesthesiology* 1993; **79**: 1–4.
23. Larach MG, Landis JR, Bunn JS, Diaz M. Prediction of malignant hyperthermia susceptibility in low-risk subjects. *Anesthesiology* 1992; **76**: 16–27.
24. Isaacs H, Badenhorst M. False-negative results with the muscle caffeine halothane contracture testing for malignant hyperthermia. *Anesthesiology* 1993; **79**: 5–9.
25. Wedel DJ, Nelson TE. Malignant hyperthermia—diagnostic dilemma: false-negative contracture responses with halothane and caffeine alone. *Anesthesia and Analgesia* 1994; **78**: 787–792.
26. Wedel DJ. The effect of age on the development of MH in susceptible piglets. *Anesthesiology* 1994; **81**: A426.
27. Lehmann-Horn F, Iaizzo PA. Are myotonias and periodic paralyses associated with susceptibility to malignant hyperthermia? *British Journal of Anaesthesia* 1990; **65**: 692–697.
28. Ørding H, for the European Malignant Hyperthermia Group. Experience with the European protocol for contracture testing. *Journal of Neurological Sciences* 1990; **98S**: 522.
29. Hopkins PM, Ellis FR, Halsall PJ. Ryanodine contracture: a potentially specific in vitro diagnostic test for malignant hyperthermia. *British Journal of Anaesthesia* 1991; **66**: 611–613.
30. Lenzen C, Roewer N, Wappler F, Scholz J, Kahl J, Blank M, Rumberger E, Schulte am Esch J. Accelerated contractures after administration of ryanodine to skeletal muscle of malignant hyperthermia susceptible patients. *British Journal of Anaesthesia* 1993; **71**: 242–246.

31. Wappler F, Roewer N, Lenzen C, Köchling A, Scholz J, Steinfath M, Schulte am Esch J. High-purity ryanodine and 9,21-dehydroryanodine for in vitro diagnosis of malignant hyperthermia in man. *British Journal of Anaesthesia* 1994; **72**: 240–242.
32. Hartung E, Koob M, Anetseder M, Schoemig P, Krauspe R, Hogrefe G, Engelhardt W. Malignant hyperthermia (MH) diagnostics: a comparison between the halothane-caffeine- and ryanodine-contracture-test results in MH susceptible, normal and control muscle. *Acta Anaesthesiologica Scandinavica* 1996; **40**: 437–444.
33. Hopkins PM, Ellis FR, Halsall PJ. Comparison of in vitro contracture testing with ryanodine, halothane and caffeine in malignant hyperthermia and other neuromuscular disorders. *British Journal of Anaesthesia* 1993; **70**: 397–401.
34. MacLennan DH, Duff C, Zorzato F, Fujii J, Philipps MS, Korneluk RG, Frodis W, Britt BA, Worton RG. Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. *Nature (London)* 1990; **343**: 559–561.
35. McCarthy TV, Healy JMS, Heffron JA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K. Localisation of the malignant hyperthermia susceptibility locus to human chromosome 19q 12–13.2. *Nature (London)* 1990; **343**: 562–564.
36. Gillard EF, Otsu K, Fujii J, DeLeon S, Khanna VK, De Leon S, Derdemezi J, Britt BA, Duff CL, Worton RG, MacLennan DH. A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. *Genomics* 1991; **11**: 751–755.
37. Steinfath M, Singh S, Scholz J, Becker K, Lenzen C, Wappler F, Köchling A, Roewer N, Schulte am Esch. C1840-T mutation in the human skeletal muscle ryanodine receptor gene: frequency in northern German families susceptible to in vitro contracture response. *Journal of Molecular Medicine* 1995; **73**: 35–40.
38. Steinfath M, Scholz J, Singh S, Wappler F. Welche Bedeutung haben Genotypveränderungen in der Diagnostik der malignen Hyperthermie? *Anästhesiologie, Intensivmedizin, Notfallmedizin, Schmerztherapie* 1996; **31**: 1–10.
39. Levitt RC, Nouri N, Jedlicka AE, McKusick VA, Marks AR, Shutack JG, Fletcher JE, Rosenberg H, Meyers DA. Evidence for genetic heterogeneity in malignant hyperthermia susceptibility. *Genomics* 1991; **11**: 543–547.
40. Levitt RC, Olckers A, Meyers S, Fletcher JE, Rosenberg H, Isaacs H, Meyers DA. Evidence for the localization of a malignant hyperthermia susceptibility locus (MHS2) to human chromosome 17q. *Genomics* 1992; **14**: 562–566.
41. Iles DE, Lehmann-Horn F, Scherer SW, Tsui LC, Olde Weghuis D, Suijkerbuijk RF, Heytens L, Mikala G, Schwartz A, Ellis FR, Stewart AD, Deufel T, Wieringa B. Localization of the gene encoding the α_2/δ-subunits of the L-type voltage-dependent calcium channel to chromosome 7q and analysis of the segregation of flanking markers in malignant hyperthermia susceptible families. *Human Molecular Genetics* 1994; **3**: 969–975.
42. Sudbrak R, Procaccio V, Klausniter M, Curran JL, Monsieurs K, Van Broeckhoven C, Ellis FR, Heytens L, Hartung EJ, Kozak-Ribbens G, Heilinger D, Weissenbach J, Lehmann-Horn F, Mueller CR, Deufel T, Stewart AD, Lunardi J. Mapping of a further malignant hyperthermia susceptibility locus to chromosome 3q13.1. *American Journal of Human Genetics* 1995; **56**: 684–691.
43. Meyer T, Stryer L. Transient calcium release induced by successive increments of inositol 1,4,5-trisphosphate. *Proceedings of the National Academy of Sciences USA* 1990; **87**: 3841–3845.
44. Klip A, Britt BA, Elliott ME, Walker D, Ramlal T, Pegg W. Changes in cytoplasmic free calcium caused by halothane. Role of the plasma membrane and intracellular Ca^{2+} stores. *Biochemistry and Cellular Biology* 1986; **64**: 1181–1189.
45. Klip A, Britt BA, Elliott ME, Pegg W, Frodis W, Scott E. Anaesthetic-induced increase in ionised calcium in blood mononuclear cells from malignant hyperthermia patients. *Lancet* 1987; **ii**: 463-466.
46. Ørding H. Diagnosis of susceptibility to malignant hyperthermia in man. *British Journal of Anaesthesia* 1988; **60**: 287–302.
47. Foster PS, Gesini E, Denborough MA. Inositol 1,4,5-trisphosphate metabolism in red blood cells from individuals susceptible to malignant hyperthermia. *Lancet* 1993; **342**: 986–987.