ORIGINAL ARTICLE

Muscular damage and intravascular haemolysis during an 18 hour subterranean exploration in a cave of 700 m depth

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Objective: To verify presence and severity of muscular and/or intravascular damage during a subterranean exploration of long duration.

Methods: We measured serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) as markers of muscular damage. We also measured haptoglobin as a marker of intravascular haemolysis, and platelets and leucocytes as markers of inflammation.

Results: We found in all the participants an increase in CK, LDH, and platelets and leucocytes (mainly due to neutrophilia and monocytosis), and a decrease in the level of haptoglobin and circulating lymphocytes.

Conclusions: The observed data suggest that continuous effort during long alpine subterranean explorations, environmental conditions, sleep deprivation, multiple impacts on rocks, and compression caused by bindings of the caving harness cause muscle damage, intravascular haemolysis, inflammation response, and immunological changes.

In recent years there has been increasing scientific interest in extreme endurance sport, especially in relation to the biochemical and haematological parameters that generally show marked modifications.^{1,2} We investigated alpine potholing (defined as an extreme sport practised in the mountainous Karst regions, in caves normally characterised by great depth, low temperature, and high humidity) because of its unique environmental characteristics, heavy effort, and particular movement techniques.

Potholers move in dangerous places, in darkness, and absolute isolation, enduring cold temperatures for 20 or more hours, and often during the night (because they need to exit the cave during the day, especially in winter, to check snow conditions, temperature, storms, and the route back to the bivouac). Subterranean exploration is generally slow, especially through narrow winding passages. The faster potholers try to move, the greater the possibility of impact on the rock. The exploration begins with the descent, and must be taken slowly; they cannot risk arriving at the bottom of the cave without sufficient energy for the final and more tiring ascent. Rest periods are limited because of low temperatures.

Considering these specific characteristics of potholing, we tried to verify whether there is muscular and/or intravascular damage during a long subterranean exploration. We measured creatine kinase (CK), the MB isoenzyme of CK (CK-MB), lactate dehydrogenase (LDH), haptoglobin, and some types of leucocyte and platelet.

METHODS

Subjects and physical characteristics

Owing to the high skill level required to perform caving exploration and the considerable number of blood samples necessary for the experiment, only five potholers, between 30 and 45 years of age, were tested; of these, four had been expert potholers for at least 15 years while the other had been involved in this activity for a shorter period of time and at a more amateur level.

Participants underwent a preliminary medical check up and a series of routine blood tests to ascertain that they were in good health. Their height, weight, and skin folds (Harpenden plicametre) were measured, and body mass index and percentage fat mass calculated.

All the volunteers had personal mountain insurance and gave written consent for this study. The experimental protocol was approved by the local ethics committee of the University of Trieste and, during the experiment, all necessary precautions were taken to insure the wellbeing of the participants.

Area of trial and performance test

In order to carry out the experiment an alpine cave was chosen (1930 m depth, internal temperature 2°C, external temperature –15°C) which presented a high level of difficulty. The potholers, each carrying about 8 kg, descended to a depth of 700 m. All knew the way to the cave floor.

The underground exploration began in the early afternoon (1400) and ended the following morning (0800); while underground all the athletes kept on the move apart from a 1 hour rest on the floor of the cave, and did not sleep.

Blood samples

Blood samples were withdrawn by venepuncture from the antecubital vein according to the following protocol: (a) 4 days before the exploration (allowing time for processing analyses similar to those envisaged for the day of the experiment); (b) at 0730 in hospital before starting the experiment, (c) 105 minutes after entering the cave, at 220 m depth (the aim of this sample was to get data at the beginning of the cave exploration, during the descent, and was taken at the first suitable site found, considering the morphology of the cave); (d) on the floor of the cave (–700 m) about 5 hours from the entrance; (e) immediately after exiting the cave, about 18 hours from the entrance; and (f) at 0730 the following day. The potholers had been fasting for about 8 hours before every blood sample except for the first one taken in the cave (5 hours' fasting).

The blood cell count was calculated twice; for the samples before and immediately after the expedition finished. The test tubes used in the cave were placed in a polystyrene container in an insulated box.^{3,4} At the exit, the blood samples were immediately centrifuged to obtain serum and within 2 hours

Abbreviations: CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; LDH, lactate dehydrogenase

it was taken by helicopter to the Clinical Research Laboratories of the Maggiore Hospital of Trieste where all the blood tests were performed.

Blood cells count was measured using a Coulter LH 750 Analyzer (Beckman Coulter, Fullerton, CA, USA), clinical chemistry using an Olympus 5400 (Olympus, Tokyo, Japan), CK-MB using Access 2 (Beckman Coulter) and haptoglobin using a nephelometry technique (Dade-Behring, Marburg, Germany).

Statistical analysis

Due to the small size of the sample ($n = 5$) a one way non-parametric Fisher's randomisation test was used to assess the increase in total CK and LDH concentrations between subsequent blood samples. Considering that for the first four blood samples, the differences between successive measurements in total CK and LDH of each potholer are always positive and that the number of possible binary answers is equal to 32 (2^5), the probability of obtaining such a result is 3.1% for each pair, which was the lowest allowed by the test. Consequently, observed differences were considered significant. The only exception was the LDH concentration between samples 3 and 4, for which the increase, although present, was not significant because of the marked fall observed for just one potholer.

The same test was applied to values for leucocytes, neutrophils, monocytes, and platelets collected before and immediately after the exploration. The test was not performed on haptoglobin because only three measurements were available, but data showed a clear decrease in haptoglobin concentration during the exploration and a sudden rise the following day.

RESULTS

According to the results of the haematological analysis and medical check up, all the potholers were in good health and none had any indication of musculoskeletal disorders. Table 1 shows their general physical characteristics.

In all of the potholers, a significant increase in the levels of serum CK (fig 1A) and LDH (fig 1B) was observed and the maximum peak was reached at the end of the ascent. The day after the exploration the serum levels of CK and LDH were also markedly over the beginning values.

CK-MB serum values increased during the subterranean movement and reached the maximum level at the exit, ranging from 25.8 to 32.8 $\mu\text{g/l}$. A marked drop in serum haptoglobin (fig 1C), even considering its biological variability,⁵ was observed as early as the point on the floor of the cave; the lowest value was reached at the exit of the cave, and it had almost returned to its initial values the following day after it almost rose to initial values. Haptoglobin values dropped when subjects were on the floor of the cave and immediately after they ascended, and values of one of them dropped under the typical reference interval.³ Only three subjects were tested. Values for leucocytes, neutrophils, and monocytes showed a significant increase between samples collected before and soon after the exploration. This was also

Figure 1 Serum levels of (A) creatine kinase, (B) lactate dehydrogenase, and (C) haptoglobin of tested potholers during a very difficult subterranean exploration lasting 18 hours. 1, on the outside at 0730; 2, in the cave at 105 minutes after entering; 3, on the floor of the cave (-700 m) 5 hours after entering; 4, at the exit of the cave 18 hrs after entering; 5, at 0730 the following day. Only three potholers had their haptoglobin tested.

true for platelets, although a slight decrease was observed in potholer number 5; nevertheless, the rise in platelets was substantially significant ($p \leq 6.3\%$). Finally, a decrease in lymphocytes (except for one potholer) was found (table 2). The least expert subject showed greater modification in the markers than did the other potholers.

DISCUSSION

The analysis of the principal body measurements showed that the subjects were of average weight with the exception of one, who was underweight. The body mass index and body

Table 1 Characteristics of the potholers

Subject no.	Height (m)	Weight (kg)	BMI kg/m ²	Sum of the folds (mm)	Fat body mass (kg)	Body fat (%)
1	1.96	81	21.1	34	16.5	20.4
2	1.75	75	23.6	31	13.9	19.1
3	1.78	77	24.5	40	16	21.4
4	1.82	78	23.5	34	15.9	20.4
5	1.80	80	24.7	35	16.6	20.8

BMI, body mass index.

Table 2 Pre/post caving exploration changes in leucocytes, neutrophils, monocytes, lymphocytes and platelets

Subject no.	Levels of markers in samples before and after the caving exploration ($\times 10^3 \mu\text{l}$)									
	Leucocytes		Neutrophils		Monocytes		Lymphocytes		Platelets	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	6.2	19.41	2.74	16.32	0.82	1.86	2.34	1.1	208	246
2	5.24	12.97	2.89	9.29	0.53	1.2	1.69	1.22	188	230
3	6.41	11.77	3.49	9.26	0.69	1.17	2.1	1.29	204	218
4	5.45	12.98	3.11	10.11	0.4	0.92	1.73	1.88	197	228
5	5.84	10.38	3.2	7.97	0.44	0.89	1.98	1.39	209	204

fat percentage of the participants were consistent with men accustomed to intense sporting activity.^{6,7}

The moderate increase in CK and LDH sarcoplasmic enzymes at the beginning of the exploration and the subsequent marked increase during the ascent suggest that this type of strenuous activity causes muscle damage, which is in accordance with previous studies of endurance sports.⁸⁻¹⁰ This is particularly evident during the ascent stage, when the intensity and duration of exercise,^{8,9} and the effects of impacts¹¹⁻¹³ against the rock, are harder than during the descent. While descending a rope is a straightforward task, ascending involves multiple "frog-like" movements* and a consequent high expenditure of energy.¹⁴ Subhorizontal progression and vertical climbing are equally taxing, particularly when carried out in narrow winding passages, because of continuous pushing of the thigh and forearm against the rock walls. It is not surprising that the potholers reported pain in almost all muscular areas, in particular the lower limbs, the back of the thighs, the large dorsal muscles, the back muscles of the neck, the large upper buttock muscle, and the fore flexors of the arms. Pain was also present the following day, and high values of CK and LDH were also noted. CK-MB also increased, but the ratio of CK-MB to CK did not exceed levels suggestive of myocardial ischaemia.¹⁵

A significant increase in the absolute numbers of total leucocytes, due to neutrophilia and monocytosis, but not in lymphocytes, was observed in all the athletes; this suggests, in accordance with previous data,^{16,17} that not only catecholamines and cortisol were responsible,¹⁸ but also the inflammatory response caused by tissue injury,^{8,19} the result of strenuous exercise and numerous impacts against rocks. It is well known that tissue damage is followed by invasion and activation of inflammatory cells triggered by agents released by damaged muscle tissue. The products resulting from cellular degradation and the acidosis that is almost always present in the skeletal muscles when they are being used, recruit for chemotaxis numerous neutrophils that promote inflammation and attract macrophages.^{20,21}

It should be noted that metabolism and cold temperatures can also contribute to immune response to physical exercise.²² Cold in particular can also influence muscle capacity to generate force;²³ in our opinion this situation can adversely effect movement and progression, and can consequently make already established muscle damage worse because of the increased frequency of falls and imperfect movement

control. The fact that potholers also move during the night and do not sleep must be taken into consideration; it is likely that sleep deprivation can contribute to the changes of white blood cells.^{24,25}

We also found an increase in platelet levels, as described in other endurance sports,¹⁷ which suggest that they are involved in the inflammatory response.²⁶

Finally we measured a fall in serum values of haptoglobin that reflects the presence of intravascular haemolysis,^{27,28} particularly during the ascent. This is probably because during the descent the athletes are not tired and move more fluidly, with fewer impacts on the rock, than when they ascend. It should be emphasised that the subjects, especially the less expert one, had several areas of bruising on the body. It is also likely that the compression caused by the bindings of the climbing harness contributes to haemolysis; the upper part of the thigh is bound very tightly and this duly affects blood circulation in the lower limbs. Further investigation is needed in this area but it seems reasonable to think that potholing is a sport with marked trauma, and that potholers need a minimum of 2 weeks of recovery before performing another long subterranean descent to avoid establishing anaemia.²⁹ Serum values of haptoglobin rapidly return to normal levels and this is consistent with the hypothesis, recently evaluated by other authors,²⁹ that there is an increased rate of synthesis following the inflammatory response. In other words, our results suggest that the changes in haptoglobin concentrations may reflect a composite reaction to opposing influences: haemolysis and inflammatory response.

CONCLUSIONS

Analysis of our data shows that potholing is a particularly intensive and traumatic endurance sport. In some cases, this prolonged activity can induce (a) destruction of the skeletal muscle because of physical activity and muscle damage and (b) intravascular haemolysis. This situation seems to develop from a state of local inflammation to one of general inflammation, which corresponds to the first phase of tissue repair.²⁰ This condition will result in a greater resistance of the muscle^{8,20} to this kind of activity, thus inducing muscular adaptation to this particular athletic discipline. Finally, our results highlight the importance of good climbing and movement technique and experience to limit impact on rocks and consequent traumatic intravascular and/or muscular damage.

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* To ascend, climbers use a bolt belayed rope, and wear a caving sitting harness and chest straps. In order to climb back up a rope they use two belays, a ventral one and one attached to the hand by means of a stirrup on the foot. The technique consists of raising the hands with the belay, and then the legs in the stirrup, and then pushing down with the legs so that the ventral belay slides down until the legs are completely extended. In this way, "frog after frog", the potholers reach the exit.

What is already known on this topic

- We performed a search in PubMed, the Cochrane Library, and Pascal (2005), using the search criteria "potholer/s", "potholing", "spelunker/s", "spelunking", "caving" and "speleology" without finding any data on this topic
- There were some data on histoplasmosis (a lung disease caused by a fungus found, among other places, in certain caves), cave rescue difficulties, and geological cave characteristics

What this study adds

- In this first article, we add new information on the biochemical and haematological modifications during long duration and deep subterranean exploration
- In particular, we focused on muscle damage, inflammatory response, and intravascular haemolysis caused by the particular movements used (both with ropes and narrow passages) and extreme environmental conditions

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