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## Haemolysis caused by alterations of $\alpha$ - and $\beta$ -spectrin after 10 to 35 min of severe exercise

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**Abstract** The pathophysiology of exercise related haemolysis is not thoroughly understood. We investigated whether exercise related haemolysis (1) is associated with alterations of red blood cell (RBC) membrane proteins similar to those found in inherited anaemic diseases, (2) can be induced with a non-running exercise mode, (3) is related to exercise intensity, and (4) coincides with indicators of oxidative stress. In ten triathletes [median (P25/P75-percentiles) age: 28.0 (26.3/28.5) years, height: 1.84 (1.78/1.87) m, body mass: 78.5 (74.8/80.8) kg, maximal oxygen uptake: 60.0 (57.3/64.8) ml kg<sup>-1</sup> min<sup>-1</sup>], haptoglobin,  $\alpha$ - and  $\beta$ -spectrin bands, malondialdehyde (MDA) and H<sub>2</sub>O<sub>2</sub>-induced chemiluminescence (H<sub>2</sub>O<sub>2</sub>-Chem) were determined immediately pre- and post-both, a 35 min low intensity and a high intensity cycling exercise [240 (218/253) vs 290 (270/300) W,  $P < 0.05$ ] requiring similar amounts of metabolic energy [28.3 (25.9/29.9) vs 24.9 (18.4/30.5) kJ kg<sup>-1</sup>,  $P > 0.05$ ]. At high exercise intensity haptoglobin [1.10 (0.81/2.53) vs 1.01 (0.75/2.00) g l<sup>-1</sup>] decreased ( $P < 0.05$ ) whilst MDA [2.80 (2.65/3.20) vs 3.13 (2.78/3.31) nmol ml<sup>-1</sup>] and H<sub>2</sub>O<sub>2</sub>-Chem [29.70 (22.55/37.10) vs 37.25 (35.20/52.63) rel. U min] increased ( $P < 0.05$ ), coinciding with the disappearance of the spectrin bands in six out of ten gels. No corresponding changes were found at low intensity exercise. Ten to 35 min of non-running exercise in a regularly used intensity domain causes intra-vascular haemolysis associated with altera-

tions in the RBC membrane proteins similar to those found after in vitro oxidative stress and in inherited anaemic diseases like Sphaerocytosis and Fanconi's anaemia.

**Keywords** Membrane skeleton · Spectrin · Oxidative stress · Training intensity

### Introduction

Symptoms of endurance exercise related haemolysis were first described more than 120 years ago (Fleischer 1881). Meanwhile, numerous research groups have confirmed this observation (Ernst et al. 1988; Gehrman 1966; Gilligan et al. 1943; Hornbostel et al. 1975; Siegel et al. 1979; Ubels et al. 1999; Yoshimura et al. 1980). Top-class endurance athletes do train more than 20 h per week. This may indicate why a cumulative effect of repetitive events with the potential for exercise-induced haemolysis has been considered as a risk factor for compromised iron stores in long distance athletes (Colt and Heyman 1984; Dufaux et al. 1981; Hunding et al. 1981; Resina et al. 1988; Telford et al. 2003).

Mechanical damage to the red blood cells (RBC), especially in the capillaries of the foot during running, has been suggested to be the major cause of exercise-induced haemolysis (Davidson 1964; Dufaux et al. 1981; Ernst et al. 1988; Falsetti et al. 1983; Miller 1990; Poortmans and Haralambie 1979; Telford et al. 2003; Weight et al. 1991). The latter seems to gain evidence based on experimental results of significant haemolysis after only 1 h of running exercise and reports on compromised iron stores especially combined with long distance running and other foot impact sports (Colt and Heyman 1984; Dufaux et al. 1981; Hunding et al. 1981; Resina et al. 1988; Telford and Cunningham 1991). However, other modes of exercise such as swimming (Selby and Eichner 1986) have led to selected observations of intra-vascular haemolysis. The latter finding may raise questions about the traditional interpretation

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of the mechanical damage theory and indicate an increased susceptibility to physical and/or chemical stress based on structural and/or functional alterations of the RBCs rather than compression of capillaries during the foot-strike as the primary reason for exercise related haemolysis. Consequently, a better understanding is required of how a specific training, defined in terms of exercise intensity and duration, may induce haemolysis. This would not only help to prevent frequently observed symptoms of iron deficiency in top-class athletes but possibly also, if not especially, in the steadily growing number of leisure-time athletes. In particular beginners, who very often follow the motto “no pain, no gain” and, therefore, tend to train an even higher percentage of their training volume at high exercise intensities, may profit from a better understanding of factors causing exercise induced haemolysis.

In a previous study we could demonstrate structural alterations of the RBC skeleton after long lasting exercise (Jordan et al. 1998). It can be only speculated whether the latter may be interpreted as an exercise induced acute effect on RBC membrane proteins similar to the chronic defects of  $\alpha$ - and  $\beta$ -spectrin observed in haemolytic diseases such as inherited Sphaerocytosis and Elliptocytosis (Bichis and Huber 2000; Eber 1991; Guiliani et al. 1999; Kanzaki et al. 1991; Straface et al. 2000; Tse and Lux 1999). Comparable changes in  $\alpha$ - and  $\beta$ -spectrin have been previously shown after oxidative stress in vitro (Snyder et al. 1983). Free radical production large enough to overwhelm the antioxidant defense system of the human body has been found especially after high intensity or very long duration exhaustive exercise (Cooper et al. 2002). Therefore, the present study tested the hypotheses that in highly trained endurance athletes haemolysis (1) is associated with alterations of RBC membrane proteins similar to those found in anaemic diseases, (2) can be systematically induced with a non-running exercise mode, (3) is related to exercise intensity, and (4) coincides with indicators of oxidative stress.

## Methods

### Subjects

Ten healthy male triathletes with fitness levels, training volumes and related weekly energy expenditure typical of athletes of national caliber (Table 1; Burke and Read 1987; Thompson et al. 1995; Van Erp-Baart et al. 1989) participated in the present study. None of them were smoking, under pharmacological or specific dietetic treatment. Written informed consent, conforming to internationally accepted policy statements on the use of human subjects as approved by the local ethics committee, was obtained from every participant prior to the beginning of the experiments after explanation of the nature and risks involved in participation.

**Table 1** Subjects' characteristics

	(X (P25/P75))
Age (years)	28.0 (26.3/28.5)
Height (m)	1.84 (1.78/1.87)
Body mass (kg)	78.5 (74.8/80.8)
Max. oxygen uptake ( $\text{ml kg}^{-1} \text{min}^{-1}$ )	60.0 (57.3/64.8)
Max. power (W)	410 (392/428)
Swimming training ( $\text{km week}^{-1}$ )	4.0 (3.9/4.6)
Cycling training ( $\text{km week}^{-1}$ )	180 (178/200)
Running training ( $\text{km week}^{-1}$ )	40 (33.8/40.0)
Energy cost of training ( $\text{kJ week}^{-1}$ )	29,600 (26,378/32,743)

All subjects were asked to avoid alcohol and coffee as well as strenuous activity 24 h before the exercise tests. The training was unchanged with almost identical training sessions and resting periods at given days of subsequent weeks during the experimental period. Every subject provided a training diary which enabled the estimation of the individual weekly energy cost of training based on generally accepted assumptions about the energy cost of locomotion per meter distance in swimming, cycling and running (Beneke and di Prampero 2001; Di Prampero 1986).

### Exercise protocol

Each subject performed first an incremental load test on an electrodynamically braked cycle ergometer (Type 380, Elema Schönander, Germany) for the determination of maximal oxygen uptake and for the prediction of the workloads corresponding to the blood lactate concentration (BLC) of  $3.0 \text{ mmol l}^{-1}$  (low exercise intensity) and  $4.0 \text{ mmol l}^{-1}$  (high exercise intensity), respectively. The incremental load test started with a load of 100 W and was increased by 50 W every third min. It was finished at the individual's maximal power output indicated by volitional fatigue after strong vocal encouragement. After that the order of the subsequent prolonged constant workload tests at low and high exercise intensity was randomized with a break of one week between the tests. Feasibility of the predicted prolonged workload exercise intensity was examined based on the dynamic behaviour of the BLC (Beneke 1995, 2003b; Beneke and von Duvillard 1996). Low intensity exercise was confirmed if after an initial increase in BLC, a steady state or a decrease of the BLC was observed during the subsequent testing period. Such a test was terminated after 35 min though it generally could have been sustained for a significantly longer period of time. If during prolonged constant workload the initial increase in BLC progressed more or less continuously throughout the test, the corresponding workload was hardly sustainable for 35 min and confirmed as high exercise intensity. Energy consumption during the two prolonged workload tests was calculated using the oxygen uptake and the increase in BLC during each test as measures of aerobic and anaerobic metabolic rate (Beneke 2003a, 2003c; Beneke and Meyer 1997).

## Oxygen uptake and blood lactate measurements

The oxygen uptake was measured continuously throughout each test (COSMED K2 gas analyser, Cosmed, Italy). The maximal oxygen uptake was defined as the highest oxygen uptake averaged throughout a 30 s time segment of the final min of the test. For the analysis of the BLC (Ebio plus, Eppendorf, Germany) 20  $\mu$ l of capillary blood were drawn from the hyperemic ear lobe before and every third minute during the incremental test and after every fifth minute during the 35 min prolonged constant workload test as well as immediately after termination of each test.

## Collection and processing of venous blood

Measures of haemoconcentration and haemolysis, alterations of the RBC skeleton, oxidative stress related degradation of proteins and lipid peroxidation was determined from venous blood. Before and immediately after each test, 10 ml of venous blood was collected into a heparin coated Vacutainer (Vacutainer, Becton Dickinson, USA) from an antecubital vein without occlusion with the subject in seated position. The tube was wrapped to protect the blood from light. Blood was immediately spun in a refrigerated centrifuge (Centrifuge RC-5, DuPont, Germany, 10 min at 1,000 $\times$ g at 4°C), then plasma transferred into pointed tubes and again centrifuged before being divided into small aliquots and immediately stored at -80°C until analysis.

The remaining blood cells were suspended with isotonic phosphate buffer (PBS, pH 7.4), centrifuged and the supernatant including buffy-coat carefully aspirated. This washing process was repeated four times. The remaining RBCs were haemolysed in 30 ml hypotonic Hepes-buffer (10 mOsm, pH 8.8) with added 1 mM EDTA and 0.1 mM PMSF. After 20 min the solution was spun at 30,000 rpm for 20 min at 4°C. The supernatant was carefully removed. The latter steps were also repeated three times. Then the white pellet was re-suspended with 250 $\mu$ l SDS sample buffer and a spatula Dithioerythrit. After that proteins were denatured for 10 min at 95°C and shaken for 30 min. Insoluble particles were sedimented by spinning at 15,000 rpm. Ghosts were stored at -80°C until analysed in a protein electrophoresis.

## Analysis of indicators of haemoconcentration and haemolysis

Haematocrit (HCT) and total protein concentration (TP) were analysed to calculate haemoconcentration after exercise using the micro haematocrit- (Hettich centrifuge, Germany) and the Biuret method (Automatic Analyser 747, Hitachi, Japan). As a measure of haemolysis haptoglobin (Norpartigen NR OSL 02, Behring, Germany) was analysed by radial immune diffusion method.

## Electrophoresis of RBC membrane proteins

Alterations of the RBC skeleton were qualitatively detected based on the visibility of  $\alpha$ - and  $\beta$ -spectrin in a protein electrophoresis. For the electrophoresis ready-to-use gels (ExcelGel SDS gradient 8–18, Pharmacia, Sweden), ExcelGel SDS buffer stripes (Pharmacia, Sweden) and the MULTIPHOR II Electrophoresis Unit (LKB Instrument GmbH, Germany) were used. Firstly, a sample volume of 3 $\mu$ l was directly applied on the gel and then the electrophoresis (600 V, 50 mA, 30 W, 15°C) was carried out over 75 min. The SDS Page Low Molecular Weight Standard (Bio-Rad Laboratories, USA) served as standard and the Bio-Rad Silver Stain Kit (Bio-Rad Laboratories, USA) was used for staining. A reduction or loss of spectrin-bands after exercise was considered as a positive test result.

## Analysis of indicators of oxidative stress

The analysis of malondialdehyde (MDA) and H<sub>2</sub>O<sub>2</sub>-induced chemiluminescence (H<sub>2</sub>O<sub>2</sub>-Chem) served as measures of oxidative stress related lipid peroxidation and degradation of proteins, respectively. The analysis of MDA is based on its reaction with two molecules of thiobarbiturate acid to a red coloured matter, which can be measured by fluorescence photometry. MDA was analysed according to the method published by Schimke and Papias (1986). Oxidative denaturation and modification of proteins is associated with an increase in H<sub>2</sub>O<sub>2</sub>-Chem. Spontaneous chemiluminescence is of rather low intensity and its measurement; therefore, requires specific technical prerequisites (Popov et al. 1989). Addition of H<sub>2</sub>O<sub>2</sub> amplifies the chemiluminescence. A chemiluminometer according to Popov et al. (2001) was applied to measure the time course of the H<sub>2</sub>O<sub>2</sub>-Chem.

## Statistics

All data are reported as median and P25/P75-percentiles. Friedman-Test and Wilcoxon-Test were used for the analysis of repeated measures. Cross-tabs, Chi-square statistics and Fisher Exact Test were applied for the analysis of differences between observed and expected counts. The significance level was set at alpha  $P < 0.05$ .

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## Results

Low and high intensity prolonged constant workload tests resulted in different ( $P < 0.05$ ) duration, oxygen uptakes, and levels of BLC (Table 2). However, no difference ( $P > 0.05$ ) in mechanical or metabolic energy was found (Table 2).

All tests at low but none at high exercise intensity showed a steady state of the BLC. The increase in BLC

**Table 2** Measures of performance and energy metabolism

	Low intensity (X (P25/P75))	High intensity (X (P25/P75))
Power (W)	240 (218/253)	290 *(270/300)
Duration (min)	35 (35/35)	26.3 *(20.6/31.3)
Mechanical energy (kJ kg <sup>-1</sup> )	6.4 (5.8/6.9)	5.8 (4.3/7.1)
Intensity (% of max. oxygen uptake)	62.0 (56.0/70.0)	78.5 *(69.7/82.1)
BLC (mmol l <sup>-1</sup> )	3.2 (2.7/3.8)	8.2 *(6.0/10.2)
Metabolic energy (kJ kg <sup>-1</sup> )	28.3 (25.9/29.9)	24.9 (18.4/30.5)

\*Significant difference to low exercise intensity,  $P < 0.05$

contributed to 0.5 (0.3/0.6) % to the total energy cost of the low intensity prolonged constant workload test, which was less ( $P < 0.01$ ) than the contribution of 1.7 (0.9/3.0) % at the high intensity condition.

Steady state or non-steady state conditions of the BLC could be proven as an independent factor for exercise induced changes of the RBC membrane skeleton ( $P < 0.01$ ). At the high exercise intensity the haptoglobin concentration decreased ( $P < 0.05$ ). This coincided with an increase in MDA ( $P < 0.05$ ) and H<sub>2</sub>O<sub>2</sub>-Chem ( $P < 0.05$ ) and the disappearance of the spectrin bands in 6 out of 10 gels. No changes in haptoglobin and membrane proteins were found at the low exercise intensity.

Low and high intensity exercise showed a similar increase ( $P < 0.05$ ) in TP, which indicated almost identical levels of haemoconcentration with 6.4 (4.0/11.3) % at the low and 7.5 (6.1/12.3) % at the high exercise intensity ( $P > 0.05$ ; Table 3). Also the changes in HCT were not different between the two tests ( $P > 0.05$ ; Table 3).

## Discussion

The important new findings in this study were that haemolysis could be induced with a non-running exercise mode and that this was associated with alterations in the RBC membrane proteins qualitatively similar to those found in inherited anaemic diseases like for example Sphaerocytosis and Fanconi's anaemia. Since our subjects performed two bouts of cycle exercise, which required the same mechanical and metabolic

energy but different exercise intensities and duration, and because we could only identify an exercise-induced haemolysis after the short-lasting higher intensity condition, it seems likely that exercise-induced haemolysis is related to exercise intensity.

Evidence for exercise intensity as a decisive factor for exercise induced haemolysis seems to be supported not only by the observation that steady state or non-steady state conditions of the BLC could be identified as an independent factor for changes of the RBC membrane skeleton ( $P < 0.01$ ) but also by the finding of haemolysis only at the higher exercise intensity. Additionally, the present results clearly indicate that the exercise intensity, which caused haemolysis, is not suitable for high training volumes. Eight out of ten trained endurance athletes, well adapted to a significant amount of regular training (Table 1), were unable to sustain this higher exercise intensity for a period of 35 min. In fact, prolonged constant workload tests causing a continuous increase in the BLC until test termination, comparable to that observed under the high exercise intensity condition (Table 3), normally lead to exhaustion after significantly less than 30 min (Beneke 2003b). Up to 15% of the total training volume of top-class athletes consists of high intensity training bouts comparable to the high intensity condition of the present experiment (Föhrenbach 1991; Hartmann et al. 1989).

The occurrence of haemolysis after cycling clearly does not support the traditional so called "mechanical damage theory", which suggests mechanical damage to the RBCs, especially in the capillaries of the foot during running, as the primary cause of exercise induced destruction of RBCs (Davidson 1964; Dufaux et al.

**Table 3** Measures of haemoconcentration and haemolysis, oxidative stress related degradation of proteins and lipid peroxidation

	Low exercise intensity (X (P25/P75))		High exercise intensity (X (P25/P75))	
	Pre	Post	Pre	Post
Total protein (g l <sup>-1</sup> )	74.5 (70.1/75.8)	79.4 <sup>***</sup> (76.7/82.8)	71.2 (68.5/76.3)	78.3 <sup>***</sup> (76.0/82.5)
HCT (%)	45.3 (42.4/46.1)	46.8 <sup>***</sup> (43.9/48.0)	44.2 (42.9/47.0)	45.6 <sup>***</sup> (44.0/49.0)
Haptoglobin (g l <sup>-1</sup> )	1.30 (0.91/1.81)	1.49 <sup>a</sup> (0.93/2.24)	1.10 (0.81/2.53)	1.01 <sup>a*</sup> (0.75/2.00)
MDA (nmol ml <sup>-1</sup> )	3.05 (2.55/3.48)	3.11 <sup>a</sup> (2.65/3.60)	2.80 (2.65/3.20)	3.13 <sup>a*</sup> (2.78/3.31)
H <sub>2</sub> O <sub>2</sub> -Chem (rel. U min)	45.29 (28.73/49.94)	35.65 (31.20/55.10)	29.70 (22.55/37.10)	37.25 <sup>*</sup> (35.20/52.63)

<sup>a</sup>Corrected for haemoconcentration based on total protein changes

\*Significant difference to pre exercise of same intensity ( $P < 0.05$ )

\*\*Significant difference to pre exercise of other intensity ( $P < 0.05$ )



1981; Ernst et al. 1988; Falsetti et al. 1983; Miller 1990; Poortmans and Haralambie 1979; Telford et al. 2003; Weight et al. 1991). Also other factors considered as potential reasons for intra-vascular haemolysis such as dehydration and haemoconcentration (Smith et al. 1995; Szygula 1990) cannot explain the in vivo changes of the RBC membrane skeleton and occurrence of haemolysis observed in the present study. Both, the high and the low intensity exercise test resulted in almost identical levels of haemoconcentration (Table 3).

The present study confirms previous observations that high intensity exercise may lead to an increase in free radical production large enough to overwhelm the antioxidant defense system of the human body (Cooper et al. 2002). Increases in MDA and H<sub>2</sub>O<sub>2</sub>-Chem seem to serve as indicators of oxidative stress related damage of both, lipids and proteins. Reduction of unsaturated fatty acids of the RBC membrane (Sumikawa et al. 1993), impaired membrane plasticity (Ernst et al. 1988; Galea and Davidson 1983) and increased fragility (Röcker et al. 1983; Smith et al. 1995; Yoshimura et al. 1980) have been described after long lasting competitive endurance events. The presently observed alterations in the gel electrophoresis of RBC membrane proteins in terms of  $\alpha$ - and  $\beta$ -spectrin after the higher-intensity exercise were quite similar to those induced by oxidants in vitro (Snyder et al. 1983).

In the present study, the combination of changes in spectrin bands with occurrence of haemolysis does support previous suggestions of an inter-relationship between haemolysis and the loss of membrane skeleton material indicated by structural changes of the RBC membrane skeleton observed with scanning electron microscopy after a Marathon race (Jordan et al. 1998). In this mechanism we see some similarities to inherited changes of RBC membrane skeletal proteins, which cause major disturbances of mechanical properties as well as cell shape, and can lead to increased intra-vascular haemolysis (Agre et al. 1982). Alterations of spectrin together with genetically impaired enzymatic anti-oxidative capacity (Besso 2000) and increased susceptibility for cross-linking reagents play a key role in Fanconi's anaemia (Mathur et al. 2000). Defects of  $\alpha$ - and  $\beta$ -spectrin but also of ankyrin, band 3 and protein 4.2 have been identified as specific components of the inherited Sphaerocytosis and Elliptocytosis (Bichis and Huber 2000; Eber 1991; Guiliani et al. 1999; Kanzaki et al. 1991; Straface et al. 2000; Tse and Lux 1999).

In conclusion, 10–35 min non-running exercises in an intensity domain regularly applied to endurance athletes of any calibre can cause intra-vascular haemolysis associated with in vivo alterations in the RBC membrane proteins, especially in the  $\alpha$ - and  $\beta$ -spectrin, similar to those found in inherited anaemic diseases like Sphaerocytosis and Fanconi's anaemia. We suppose these structural and possibly also functional alterations of the RBCs resulting in an increased susceptibility of the cells to physical and/or chemical stress rather than

compression of capillaries during the foot strike as the primary contributor to exercise related haemolysis.

## References

- Agre P, Orringer EP, Bennet V (1982) Deficient red-cell spectrin in severe, recessively inherited spherocytosis. *N Engl J Med* 306:1155–1161
- Beneke R (1995) Anaerobic threshold, individual anaerobic threshold, and maximal lactate steady state in rowing. *Med Sci Sports Exerc* 27(6):863–867
- Beneke R (2003a) Maximal lactate steady state concentration (MLSS): experimental and modelling approaches. *Eur J Appl Physiol* 88:361–369
- Beneke R (2003b) Methodological aspects of maximal lactate steady state – implications for performance testing. *Eur J Appl Physiol* 89:95–99
- Beneke R (2003c) Experiment and computer aided simulation – complementary tools to understand exercise metabolism. *Biochem Soc Trans* 31(6):1263–1266
- Beneke R, di Prampero PE (2001) Mechanische und metabolische Belastung beim Radfahren – eine Analyse aus physiologischer und biomechanischer Sicht. *Dtsch Z Sportmed* 51(1):29–33
- Beneke R, Meyer K (1997) Walking performance in and economy in chronic heart failure patients pre and post exercise training. *Eur J Appl Physiol* 75:246–251
- Beneke R, von Duvillard SP (1996) Determination of maximal lactate steady-state response in selected sports events. *Med Sci Sports Exerc* 28(2):241–246
- Besso F (2000) Fanconi anaemia. *Nippon Rinsho* 58(7):1467–1472
- Bichis M, Huber AR (2000) Hereditary diseases of erythrocyte membrane: from clinical aspects to underlying genetical and molecular mechanisms. *Ann Biol Clin (Paris)* 58(3):277–289
- Burke LM, Read RSD (1987) Diet patterns of elite Australian male triathletes. *Phys Sportsmed* 15(2):140–155
- Colt E, Heyman B (1984) Low ferritin levels in runners. *J Sports Med Phys Fitness* 24:13–17
- Cooper CE, Vollaard NBJ, Choueiri T, Wilson MT (2002) Exercise, free radicals and oxidative stress. *Biochem Soc Trans* 30(2):280–285
- Davidson RJ (1964) Exertional haemoglobinuria: a report on three cases with studies on the haemolytic mechanism. *J Clin Path* 17:536–540
- Di Prampero PE (1986) The energy cost of human locomotion on land and in water. *Int J Sports Med* 7:55–72
- Dufaux B, Hoederath A, Streitberger I, Hollmann W, Assmann G (1981) Serum ferritin, transferrin, haptoglobin and iron in middle and long distance runners, elite rowers, and professional racing cyclists. *Int J Sports Med* 2:43–46
- Eber SW (1991) Disorders of the membrane skeleton of erythrocytes in hereditary spherocytosis and elliptocytosis: significance of the molecular defect for pathogenesis and clinical severity. *Klin Padiatr* 203(4):284–295
- Ernst E, Sturmvoll M, Magyarosy I (1988) "Sportanämie" – Wo hin verschwinden die Erythrozyten? *Dtsch Z Sportmed* 39:476–480
- Falsetti JL, Burke ER, Feld RD, Frederick EC, Ratering C (1983) Hematological variations after endurance running with hard soled and air cushioned shoes. *Phys Sports Med* 11:118–127
- Fleischer R (1881) Über eine neue Form von Hämoglobinurie beim Menschen. *Berl Klin Wschr* 18:691–695
- Föhrenbach R (1991) Leistungsdiagnostik, Trainingsanalyse und –steuerung bei Läuferinnen und Läufern verschiedener Laufdisziplinen. Hartung-Gorre Verlag, Konstanz
- Galea GA, Davidson RJ (1983) Haematological and haemorheological changes in marathon runners. *Clin Hemorheol* 3:320
- Gehrmann G (1966) Mechanische Hämolyse. *Dtsch Med Wschr* 91:1846–1850

- Gilligan DR, Altschulte MD, Katersky EM (1943) Physiological intravascular hemolysis of exercise, hemoglobinuria following cross-country runs. *J Clin Invest* 22:859–869
- Guiliani AL, Bigoni B, Veronesi M, Manservigi R, Mischiati C, Berti G, Zavagli G, Ricci G (1999) Membrane protein pattern in hereditary spherocytosis in five subjects from north-east Italy obtained by SDS-PAGE using N'N'-diallyltartardiamide. *Eur J Haematol* 63(5):302–305
- Hartmann U, Mader A, Petersmann G, Grabow V, Hollmann W (1989) Verhalten von Herzfrequenz und Laktat während ruderspezifischer Trainingsmethoden. *Dtsch Z Sportmed* 40(6):200–212
- Hornbostel H, Kaufmann W, Siegenthaler W (1975) Pathophysiologie und Diagnostik hämolytischer Anämien. *Dtsch Med Wschr* 100:1400–1402
- Hunding A, Jordal R, Paulev PE (1981) Runner's anemia and iron deficiency. *Acta Med Scand* 209:315–318
- Jordan J, Kiernan W, Merker HJ, Wenzel M, Beneke R (1998) Red cell membrane skeletal changes in marathon runners. *Int J Sports Med* 18:1–4
- Kanzaki A, Wada H, Yawata Y (1991) Cytoskeleton anomalies in disorders of red cell membrane proteins. *Rinsho Ketsueki* 32(6):573–579
- Mathur R, Chowdhury MR, Singh G (2000) Recent advances in chromosome breakage syndromes and their diagnosis. *Indian Pediatr* 37(6):615–625
- Miller BJ (1990) Haematological effects of running: a brief review. *Sports Med* 9:1–6
- Poortmans JR, Haralambie G (1979) Biochemical changes in a 100 km run: proteins in serum and urine. *Eur J Appl Physiol* 40:245–254
- Popov I, Lewin G, Gäbel W, von Baehr R (1989) Local and systemic effects of organ hypoxia detected by chemiluminescence and photochemiluminescence. *Biomed Biochem Acta* 49:297–300
- Popov I, Volker H, Lewin G (2001) Photochemiluminescent detection of antiradical activity. V. Application in combination with the hydrogen peroxide-initiated chemiluminescence of blood plasma proteins to evaluate antioxidant homeostasis in humans. *Redox Rep* 6(1):43–48
- Resina AL, Gatteschi Giamberardino MA, Rubenni MG, Trabassi E, Troni MG (1988) Comparison of RBC indices and serum iron parameters in trained runners and control subjects. *Haematologica* 73:449–454
- Röcker L, Laniado M, Kirsch K (1983) The effect of physical exercise on plasma volume and red blood cell mass. In: Dunn CDR (ed) *Current concepts in Erythropoiesis*. Wiley, Chichester, pp 245–277
- Schimke I, Papies B (1986) Einige methodische Aspekte der Bestimmung Thiobarbitursäureaktiver Substanzen im Plasma. *Z Med Lab Diag* 27:71–76
- Selby GB, Eichner ER (1986) Endurance swimming, intravascular hemolysis, anaemia and iron depletion. *Am J Med* 81:791–794
- Siegel AJ, Hennekens CH, Solomon HS, Van Boeckel B (1979) Exercise-related hematuria. *JAMA* 241(4):391–392
- Smith JA, Kolbuch-Braddon M, Gillam I, Telford RD, Weidemann MJ (1995) Changes in the susceptibility of red blood cells to oxidative and osmotic stress following submaximal exercise. *Eur J Appl Physiol* 70:427–436
- Snyder LM, Leb L, Piotrowsky J, Sauberman N, Liu SC, Fortier NL (1983) Irreversible spectrin-haemoglobin cross linking in vivo: a marker for red cell senescence. *Br J Haematol* 53:379–384
- Straface E, Masella R, Principe DD, Franceschi C, Korkina LG, Zatterale A, Pagano G, Malorni W (2000) Spectrin changes occur in erythrocytes from patients with Fanconi's anemia and their parents. *Biochem Biophys Res Commun* 273(3):899–901
- Sumikawa K, Mu Z, Inoue T, Okochi T, Yoshida T, Adachi K (1993) Changes in erythrocyte membrane phospholipid composition induced by physical training and physical exercise. *Eur J Appl Physiol Occup Physiol* 67(2):132–137
- Szygula Z (1990) Erythrocytic system under the influence of physical exercise and training. *Sports Med* 10:181–197
- Telford RD, Cunningham RB (1991) Sex, sport and body-size dependency of hematology in highly trained athletes. *Med Sci Sports Exerc* 23:788–794
- Telford RD, Sly GJ, Hahn AG, Cunningham RB, Bryant C, Smith JA (2003) Footstrike is the major cause of hemolysis during running. *J Appl Physiol* 94(1):38–42
- Thompson JL, Manore MM, Skinner JS, Ravussin E, Spraul M (1995) Daily energy expenditure in male endurance athletes with differing energy intake. *Med Sci Sports Exerc* 27(3):347–354
- Tse WT, Lux SE (1999) Red blood cell membrane disorders. *Br J Haematol* 104(1):2–13
- Ubels FL, van Essen GG, de Jong PE, Stegeman CA (1999) Exercise induced macroscopic haematuria: run for a diagnosis? *Nephrol Dial Transplant* 14(8):2030–2031
- Van Erp-Baart AM, Saris WH, Brinkhorst RA, Vos JA, Elvers JW (1989) Nationwide survey on nutritional habits in elite athletes. *Int J Sports Med* 10(Suppl1):S3–S10
- Weight LM, Byrne M, Jacobs P (1991) Haemolytic effects of exercise. *Clin Sci* 81:147–52
- Yoshimura H, Inoue T, Yamada T, Shiraki K (1980) Anemia during hard physical training and its causal mechanism with special reference to protein nutrition. *Wld Rev Nutr Diet* 35:1–86