Coll. Antropol. **31** (2007) 4: 973–978
Original scientific paper

The Electrophoretic Mobility of Cell Nuclei (EMN) Index and Changes in Acid-Base Homeostasis under Conditions of the Intensive Physical Exercise

Zbigniew Czapla[1], Ewa Szczepanowska[2] and Joachim Cieslik[1]

[1] Department of Human Biological Development, Institute of Anthropology, Adam Mickiewicz University, Poznan, Poland
[2] Department of Human Functional Anatomy and Biometry, University of Szczecin, Szczecin, Poland

A B S T R A C T

The relationship between the Electrophoretic Mobility of Cell Nuclei (EMN) index before and after super-maximal and maximal exercises and changes in physiological parameters associated with acid-base balance of the blood (pH, BE) and lactic acid concentration (LA) were examined in junior (N=33, X=15.6 y.o. SD=1.16 y.), and senior (N=10, X=22.0 y.o. SD=2.70 y.) female rowers. The following parameters: pH, BE and LA changed significantly (p<0.05) from pre- to post-exercise status, while the EMN index changed only in junior female rowers under super-maximal conditions. Correlations between the EMN index and physiological parameters reflect the homeostatic disturbance associated with intensive exercise conditions. The decline in the EMN index appears to depend on the post-exercise changes of an organism's acidity. The results suggest that changes in the EMN index are associated with variation in physiological parameter, i.e. changes in acidity. We conclude that the EMN index reflects acid-base alterations and may be useful in evaluating systematic reactions to stress.

***Key words**: intracellular microelectrophoresis, buccal epithelium cells, EMN index, exercise physiological parameters, acid-base balance, acidity*

Introduction

A study of formation of the EMN (Electrophoretic Mobility of Cell Nuclei) index (ratio of cells with mobile nuclei to cells with non-mobile nuclei) in ontogenesis by the intracellular microelectrophoresis method allows applying the EMN index both as a criterion for evaluation of biological age, as well, as physiological body condition. The Electrophoretic Mobility of Cell Nuclei (EMN) index reflects physiological changes in the body associated with environmental factors potentially harmful to the health of the individual[1–2]. Electrophoretic activity within cell nuclei is assessed by the intracellular microelectrophoresis method[3–5]. The EMN index is used as an indicator of biological age and physiological condition of the body[6–13]. Mean values of the EMN index increase in the progressive phase of ontogenesis, then after achieving an optimum point a (maximal value at 17–18 years old) they regularly decrease with advancing age[1,2,7] (Figure 1). The EMN index was applied for the first time in the estimation of the biological age by the researchers from the Department of Genetics and Cytology, at the University in Kharkov, Ukraine. Their latest works and our results indicate that the intracellular microelectrophoresis method – indication of the EMN index, can be applied also in the medical studies for diagnosing of certain physiological parameters and for the evaluation of the impact of an individual's lifestyle on the biological condition of human body[2,5,9–10,12].

The basis for this interesting physiochemical phenomenon is the mobility of cellular nuclei in a variable electrical field and a change during ontogenesis of the ratio between the numbers of cells with mobile nuclei to the number of cells with non-mobile nuclei. The ratio of the number of cells with mobile nuclei to the number of

Received for publication October 12, 2005

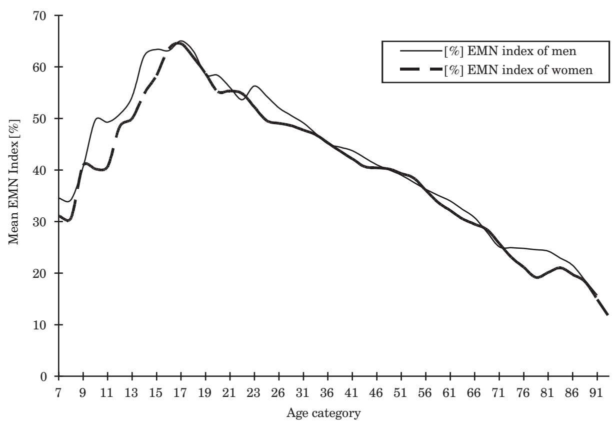

Fig. 1. Formation of mean values of the EMN index in the course of human ontogenesis. Rising lines – progressive phase[7], dropping lines – stable and involution phase[1,2].

cells with non-mobile nuclei (per 100 cells counted by the researcher, with a proper ratio of the size and colour of the nucleus to the size of maintained cytoplasm) allows determining the percentage value of the EMN index. The EMN phenomenon is related to the biochemical composition and physiology of cellular structures and to properties of physical and chemical nature or to electrokinetic and electrostatic properties of nuclei and other cellular structures changing with age[8,14–17].

This phenomenon is related to metabolic processes occurring on the surface of the cell nucleus[18–20] and also to cellular physicochemical properties[8,11,13,17]. It is dependent on the rate of metabolic process in nucleus and changes of its structure as a result of changes in ion composition and its charge – directly or indirectly related to the processes of transcriptive activity of DNA[13,17]. The electro-negatively charged nucleus moves in compliance with the change of the imposed variable magnetic field to electrodes, towards to the anode. The decrease of pH of plasma picks out anions and changes the permeability of cellular membranes in the direction of diminishing homeostatic imbalance inside and outside the cellular milieu. As a consequence, hydrogen ions penetrate into the internal milieu of cell. In these conditions the percentage of cell nuclei changes depends on age[8,13]. Biophysical studies suggest that the EMN index reflects the structural and functional condition of the nuclei of buccal epihelium cells associated with age and physiological features[9,16]. A higher negative nucleus cell charge has been observed in cancerous cells and intensively, it means rapidly, dividing cells[19–21].

The physiological conditions of the body changes in genetically defined limits are determined by homeostatic mechanisms specific for individual structures and functions. One of these mechanisms is the acid-base balance. Regular sport training can shift the level and direction of this balance: on one hand, more effectively eliminating acid products of metabolism and energy conversion, and on the other hand, buffering blood volume extension[22]. It is an expression of adaptation to the demands of rigorous training due to a more efficient metabolism that permits attainment of achievements under extreme conditions of exercise in trained as compared to untrained individuals.

The purpose of this study is to evaluate the relationship between changes in the physiochemical characteristics of buccal epithelium cells measured by the EMN index and physiological parameters of the acid-base balance (pH, BE and LA) in the body during super-maximal and maximal exercises. We attempted to answer the questions: is the EMN index a marker of changes in acid-base homeostasis in response to stress associated with physical exercise? And, does the EMN index change in relation to the degree of acidity attributed to the different intensities of physical exercise?

Material and Methods

Two groups of female rowers: 33 juniors (X=15.6 y.o. SD=1.16 y.) and 10 seniors (X=22.0 y.o. SD=2.70 y.) were subjected to the study. Training experience was smaller in the junior female group as compared to the senior female group, X=2.6 y. (SD=1.24 y.) versus X=8.0 y (SD=3.22 y.), respectively. Two types of exercise tests on Concept II (Morisville, USA) rowing ergometer were administered to all rowers:

1. Super-maximal – the incremental exercise test of the highest intensity, in the shortest period of time, during 2000 m. Under these conditions, intensity reached super-maximal levels, i.e. above maximal oxygen uptake (VO_2max).
2. Maximal – the incremental exercise test of the intensity up to VO_2max. This condition consisted of rowing with starting load of 50 W for juniors and 80 W for seniors. Exercise intensity was increased every 3 minutes by 30 W up to VO_2max. The starting load differentiation for junior and senior female rowers was based on their level of training experience.

Arterial blood from the fingertip was drawn 3 minutes before and 3 minutes after the exercise test to measure acid-base balance parameters (pH and BE) by a blood gas analyzer (AVL 995Hb, USA) and lactic acid concentration (LA) by the enzymatic method (Boehringer-Manheim, Germany).

The buccal epithelium cells were collected before and just after the exercise test. These cells were used to evaluate their physicochemical cellular feature changes as specific for electrophoretic mobility of nuclei expressed by the EMN index before and after each exercise test. Disposable, sterile spatulas were used to collect the epithelial samples. It was a non-invasive, bloodless and painless procedure. The cells were suspended in 0.09% NaCl solution and were observed under the microscope, magnified 200 times, on a special plate, in a variable electric field of 20–30 V voltage and 0.1 mA intensity and frequency of changes of electric field within 1–2 Hz. Electrophoresis was carried out with the use of the Biotest apparatus constructed especially for this type of tests[5–6].

TABLE 1
PRE- AND POST-EXERCISE ACID-BASE BALANCE PARAMETERS IN JUNIOR AND SENIOR FEMALE ROWERS AND RESULTS OF STUDENT T-TEST

Type of exercise	Group		pH		Student t-test value	BE (mmol/L)		Student t-test value	LA (mmol/L)		Student t-test value
			rest	exerc		rest	exerc		rest	exerc	
Super-maximal	juniors	X	7.375	7.141	24.91**	–1.3	–17.5	32.68**	1.5	13.0	–32.64**
		SD	0.012	0.066		1.4	3.1		0.3	2.3	
	seniors	X	7.374	7.121	5.58*	–1.9	–18.8	6.58**	1.8	12.9	–9.95**
		SD	0.013	0.090		1.7	3.7		0.2	2.1	
maximal	juniors	X	7.384	7.225	22.44**	–1.2	–12.7	27.53**	1.2	8.7	–25.15**
		SD	0.018	0.056		1.3	3.3		0.3	2.4	
	seniors	X	7.375	7.175	10.57**	–0.7	–15.0	16.41**	1.3	9.4	–11.09**
		SD	0.028	0.071		1.2	3.8		0.4	2.5	

*$p<0.05$, **$p<0.01$, X– mean, SD – standard deviation, pH – determination of acidity, BE – basis excess, LA – lactic acid

The analysis was carried out with STATISTICA 6.1 software using the Students t-test. Calculations included changes of the EMN index, and also pH, BE and LA concentrations before (pre-) and after (post-) the exercise test, and delta scores (Δ) between them, too. Correlations between values at rest and after exercise test were also calculated. Significance was set at a level of $p \leq 0.05$. The study was approved by the Regional Commission for Research Ethics at the Karol Marcinkowski Medical University in Poznan, Poland.

TABLE 2
PRE- AND POST-EXERCISE VALUES OF THE EMN INDEX IN JUNIOR AND SENIOR FEMALE ROWERS AND RESULTS STUDENT T-TEST

Type of exercise	Group		EMN (%)		Student t-test value
			rest	exerc	
Super-maximal	juniors	X	41.52	35.11	2.90**
		SD	20.13	19.08	
	seniors	X	48.50	42.25	0.56
		SD	8.38	22.27	
maximal	juniors	X	43.96	43.98	–0.02
		SD	16.56	16.09	
	seniors	X	46.00	47.71	–0.48
		SD	17.80	16.40	

**$p<0.01$, EMN index – Electrophoretic Mobility of Cell Nuclei index

Results

Resting status – pre-exercise (rest) and exercise – post-exercise (exerc) parameters describing acid-base balance of both groups of female rowers are presented in Table 1. Corresponding values for the EMN index are presented in Table 2. Changes in pH, BE and LA from the pre- to post-exercise statuses are significant ($p \leq 0.01$) in junior and senior female rowers under super-maximal and maximal conditions (Table 1).

Pre- and post-exercise the EMN index values differ significantly only among junior female rowers under super-maximal conditions (Table 2).

A comparison of delta (Δ) scores (differences) between pre- and post-exercise are summarized in Table 3. Delta scores for pH and BE differ significantly between junior and senior female rowers only under maximal conditions. Corresponding delta scores for super-maximal condition are not different between junior and senior female rowers. Delta scores for LA and the EMN index do not differ between junior and senior female rowers for maximal and super-maximal conditions.

Correlations between the EMN index and pH, BE and LA values at pre- and post-exercise are summarized in Table 4A. Correlations are significant for these three acid-base balance parameters and the EMN index only among junior female rowers under resting conditions. Corresponding correlations for senior rowers are reasonably similar but because of the small sample they do not reach significance. The correlations for delta scores between pre- and post-exercise statuses are not statistically significant.

Correlations under maximal exercise test conditions are summarized in Table 4B. Only one correlation is significant between LA concentration pre-exercise and the EMN index post-exercise in junior female rowers ($r=-0.33$). Correlations for senior female rowers tend to be stronger but because of the small sample size they are probably not statistically significant.

Discussion

The presented results confirm the utility of pH, BE and LA as proper indicators of changes in an organism's

TABLE 3
CHANGES OF DIFFERENCE PRE-POST-EXERCISE (Δ) IN ACID-BASE BALANCE PARAMETERS AND THE EMN INDEX AND RESULTS OF STUDENT T-TEST FOR JUNIOR AND SENIOR FEMALE ROWERS

Type of exercise	Group		Δ pH	Student t-test value	Δ BE (mmol/L)	Student t-test value	Δ LA (mmol/L)	Student t-test value	Δ EMN (%)	Student t-test value
Super-maximal	juniors	X	–0.252	–0.53	–16.19	–0.42	11.51	–0.38	–6.09	0.00
		SD	0.062		3.287		2.34		12.82	
	seniors	X	–0.234		–16.95		11.05		–6.06	
		SD	0.090		5.149		2.22		22.82	
maximal	juniors	X	–0.158	–2.41*	–11.52	–2.86**	7.55	0.69	0.06	0.38
		SD	0.055		3.297		2.36		8.55	
	seniors	X	–0.200		–14.29		8.05		1.39	
		SD	0.070		3.258		2.71		8.69	

* p<0.05, ** p<0.01, X – mean, SD – standard deviation, BE – basis excess, LA – lactic acid, EMN index– Electrophoretic Mobility of Cell Nuclei index

TABLE 4A
CORRELATIONS BETWEEN ACID-BASE PARAMETERS AND THE EMN INDEX PRE- AND POST- SUPER-MAXIMAL EXERCISE TEST IN JUNIOR AND SENIOR FEMALE ROWERS

Feature	Super-maximal exercise test: EMN rest		EMN exerc		Δ EMN	
	juniors	seniors	juniors	seniors	juniors	seniors
pH rest	0.5212**	0.6812	0.4323**	0.5754	–0.1715	–0.8265
pH exerc	–0.0126	–0.1588	–0.0584	–0.4465	–0.0692	–0.3885
BE rest	0.4646**	0.3647	0.3415*	–0.1576	–0.2200	–0.2879
BE exerc	0.0095	–0.1475	–0.0669	–0.4025	–0.1115	–0.3494
LA rest	–0.3416*	0.1745	–0.3620*	0.6286	–0.0052	0.5514
LA exerc	–0.1238	0.4399	–0.0056	0.1365	0.1784	–0.0251

*p<0.05, **p<0.01, pH – determination of acidity, BE – basis excess, LA – lactic acid, EMN – Electrophoretic Mobility of Cell Nuclei index

acid-base balance caused by physical exercise. Both super-maximal and maximal exercises significantly influence on pH, BE and LA in female rowers (Table 1). On the other hand (Table 2), the EMN index changes significantly only under super-maximal exercise conditions. The decrease of mean values of the EMN index after (post-) to before (pre-) exercise is observed. Therefore, the difference of –6.41% for junior and –6.25% for senior female rowers is noticed. Similar result lower mean value of the EMN index after exercise test is observed among students of Olomouc University, Czech Republic[10].

Super-maximal exercise test produces significant changes in the physicochemical features of buccal epithelium cells and it is evident in the EMN index only in junior female rowers. The direction of (negative) change from pre- to post-exercise is the same in senior female rowers but the difference between mean values are weak (not statistically significant). No changes in the EMN index are observed under maximal exercise conditions. Examination of the delta scores indicates significant differences between junior and senior female rowers only for pH and BE under maximal exercise conditions. Junior female rowers represent smaller changes in pH and BE. Corresponding delta scores for LA and the EMN index do not differ between junior and senior female rowers under maximal exercise conditions but the magnitude of the changes are lower in junior rowers. Delta scores for all four parameters (pH, BE, LA and the EMN index) are reasonably similar and do not differ between junior and senior female rowers under super-maximal conditions.

Senior group achieves VO_2max at higher oxygen consumption values than junior group while at the same time senior group shows a greater acid reaction after the exercise test. Changes in LA concentration are connected with LA compensation by the bicarbonate buffer in the blood. From the workload at the anaerobic threshold, the correlation of pH, BE, and LA are not linear. It suggests that under conditions of exercise test of super-maximal and maximal intensity the acid-base blood parameters (pH and BE) balance shows greater changes in the base group, neutralizing the increase hydrogen positive ions in exercise test more than LA concentration. On the

TABLE 4B
CORRELATIONS BETWEEN ACID-BASE PARAMETERS AND THE EMN INDEX PRE- AND POST- MAXIMAL EXERCISE TEST IN JUNIOR AND SENIOR FEMALE ROWERS

Feature	Maximal exercise test					
	EMN rest		EMN exerc		Δ EMN	
	juniors	seniors	juniors	seniors	juniors	seniors
pH rest	0.0434	0.3901	0.0225	0.7443	0.0052	0.5215
pH exerc	–0.0654	0.3520	–0.0252	0.1613	–0.1203	–0.5009
BE rest	–0.1279	0.2795	–0.2080	0.4170	–0.0304	0.0038
BE exerc	–0.0934	0.3054	–0.0523	0.1116	–0.1100	–0.4508
LA rest	–0.2649	–0.3578	–0.3319*	–0.8350	–0.0391	–0.5671
LA exerc	0.1144	–0.3940	0.0091	–0.2280	–0.0279	0.4936

*p<0.05, **p<0.01, pH – determination of acidity, BE – basis excess, LA – lactic acid, EMN – Electrophoretic Mobility of Cell Nuclei

other hand, the EMN index does not show statistically significant changes. It can reflect an effective metabolic mechanism of stress compensation during intensive exercise in those examined female rowers[23–25]. The idea to take under the consideration all examined subjects in regard to the training experience (a division into junior and senior female rowers) can show differences in metabolic adaptation process during practicing sport. It justifies depth of metabolic changes as result of intensive physical exercises.

Assuming that the disturbance in acid-base homeostasis is a result of the accumulation of acid metabolic products, the EMN index, as well as pH, BE, and LA parameters, reflect this disturbance[22,26–28]. This inference is suggested by the decrease in the EMN index in super-maximal exercise (Table 2) and the high correlations between the EMN index and the physiological parameters under super-maximal exercise conditions in junior rowers (Table 4A). The relationship between the EMN index and indicators of disturbance in acid-base balance highlight the influence of intensive exercise test as a significant metabolic stressor producing physiochemical changes of the buccal epithelium cells. The EMN index can thus be an indicator of disturbance in acid-base homeostasis caused by intensive physical exercise.

Conclusions

1. Changes in the EMN index after maximal and super-maximal exercise tests reflect metabolic changes of acid character on the cells structure level.
2. The EMN index can be used as an indicator of disturbance in acid-base balance homeostasis because it correlates with pH, BE and LA.
3. The EMN index describes the physiological state of the body at rest and under intensive physical exercise conditions.
4. The EMN index can be used to evaluate the influence of exercise test metabolic stress on the physiochemical properties of cells.

Acknowledgements

We would like to thank Professor Robert M. Malina for helpful adjustment of language in this article.

REFERENCES

1. CZAPLA Z, Variability and Evolution, 8 (2000) 135. — 2. CZAPLA Z, CIESLIK J, Medical Review. Scripta Periodica, (III) 3 (2000) 24. — 3. SHAKHBAZOV VG, NABOKOV AL, COLUPAEVA TV, Avtorskoe svidetelstvo Nr 1169614. MKI A 61 B 10/00. No 3355951. Bjul. No 28 (1985) — 4. CZAPLA Z, CIESLIK J, Gerontol Pol, 12 (4) (2004) 45. — 5. SHAKHBAZOV VG, COLUPAEVA TV, NABOKOV AL, Laboratornoe Delo, 7 (1986) 404. — 6. SHAKHBAZOV VG, GRIGOREVA HH, COLUPAEVA TV, Fiziol Cheloveka, 22 (1996) 71. — 7. CIESLIK J, KACZMAREK M, KALISZEWSKA-DROZDOWSKA MD, Dziecko Poznanskie 90 (Bogucki, Wydawnictwo Naukowe, Poznan, 1994). — 8. SHCKORBATOV YG, SHAKHBAZOV VG, COLUPAEVA TV, RUDENKO AO, L'Eurobiologiste, 28 (218) (1995) 25–253. — 9. CZAPLA Z, Appraisal of changeability of the EMN index with regard to lifestyle on the basis of daily examination. In: Riegerova J, (Eds.): Methodological Aspects of Researches in the field of EMN (Electrophoretical Mobility of Nuclei). (Palacky University, Olomouc, 1998). — 10. RIEGEROVA J, PRIDALOVA M, BRAZINOVA K, Observing the electrokinetic qualities of the buccal epithelium cell Nuclei of sportsmen before and after physical strain. In: Riegerova J, (Eds.), Methodological Aspects of Researches in the field of EMN (Electrophoretical Mobility of Nuclei) (Palacky University, Olomouc, 1998). — 11. SHAKHBAZOV VG, SHCKORBATOV YG, COLUPAEVA TV, Mech Ageing Dev, 99 (3) (1997) 193. — 12. SHAKORBATOV YG, COLUPAEVA TV, SHAKHBAZOV VG, PUSTOVOJ PA, Fiziol Cheloveka, 21 (2) (1995) 93. — 13. SHCKORBATOV YG, SHAKHBAZOV VG, BOGOSLAVSKY AM, RUDENKO AO, Mech. Ageing Dev, 83 (1995) 87. — 14. CZAPLA Z, CIESLIK J, Prz Antropol-Anthropol Rev, 61 (1998) 93. — 15. CZAPLA Z, Phenomenon of electrophoretic mobility of cell nuclei (EMN) as a consequence of biological and physical properties of the cell. In: Riegerova J (Eds): Methodological Aspects of Researches in the field of EMN (Electrophoretical Mobility of Nuclei, Palacky University, Olomouc, 1998). — 16. SHCKORBATOV YG, Naturwissenschaften, 86 (1999) 452. — 17. SHCKORBATOV YG, GRIGORYEVA NN, SHAKHBAZOV VG, GRABINA VA, BOGOSLAVSKY AM, Bioelectromagnetics, 19 (7) (1998) 414. — 18. MAEKAWA A, Nagoya J Med Sci, 13 (1967) 215. — 19. KISHIMOTO S, LIBERMAN L, J Cell Biol, 25 (1965) 103. — 20. MAYHEW E, NORDLING S, J Cell Physiol, 68 (1968) 78. — 21. AMBROSE EJ, JAMES AM,

LOWICK JHB, Nature, 177 (1956) 576. — 22. MARKIEWICZ K, CHOLEWA M, Acta Physiol Pol, 30 (5–6), suppl. 19 (1979) 91. — 23. WASSERMAN K, WHIPP BJ, KOVAL SN, BEAVER WL, J Appl Physiol, 35 (2) (1973) 236. — 24. CHWALBINSKA-MONETA J, Koncepcja progu anaerobowego (CMiK PAN, Warszawa, 1993). — 25. CHWALBINSKA-MONETA J, KRYSZTOFIAK K, ZIEMBA A, NAZAR K, KACIUBA-USCILKO H, Eur J Appl Physiol, 73 (1–2) (1996) 117. — 26. KORZUN EI, SHAKHBAZOV V G, MAILIAN EC, COLUPAEVA TV, KOVALENKO EA, Pato Fiziol, 6 (1985) 63. — 27. DWYER J, BYBEE J, Med Sci Sports Exerc, 15 (1983) 72. — 28. DOMBOVY D, BONNEKAT HW, WILLIAMS TJ, STAATS BA, Med Sci Sports Exerc, 19 (2) (1987) 111.

Z. Czapla

Department of Human Biological Development, Institute of Anthropology, Adam Mickiewicz University, ul. Umultowska 89, 61-614 Poznan, Poland
e-mail: czapla@amu.edu.pl

ELEKTROFORETSKA MOBILNOST JEZGRE STANICE (EMN INDEKS) I PROMJENE U KISELO-BAZIČNOJ HOMEOSTAZI U UVIJETIMA INTENZIVNE FIZIČKE AKTIVNOSTI

S A Ž E T A K

Razlike u elektroforetskoj mobilnosti jezgre stanice (EMN indeks), prije i poslije super-maksimalne i maksimalne aktivnosti u fiziološkim parametrima povezanim sa kiselo-bazičnim balansiranjem pH krvi i koncentracijom laktične kiseline, bile su ispitivane na mlađim (N=33, X=15.6 godina, SD=1.16y.), i starijim (N=10, X=22.0 godina SD=2.7y) ženskim veslačicama. Slijedeći parametri; pH, BE i LA značajno su se mijenjali ($p<0.05$) od prije do poslije vježbenog statusa, dok su se EMN index i fiziološki parametri reflektirali na narušavanje homeostaze u uvijetima intenzivne akztivnosti. Opadanje EMN indeksa ovisilo je o post-vježbenim promjenama kiselosti organizma. Rezultat sugerira da su promjene u EMN indeksu povezane sa varijacijama fizioloških parametara i promjenom kiselosti. Zaključili smo da se EMN index reflektira na kiselo-bazične promjene te da bi mogao biti koristan u detektiranju stresa.