The change of blood Pb levels of basketball players after strenuous exercise

Seyfi SAVAș¹, Ömer Şenel¹, İlyas OKAN¹ & Hüseyin Çelikkan²

1. Gazi University, School of Physical Education and Sports, Ankara, Turkey

2. General Directorate of Mineral Research and Exploration, Ankara, Turkey

Correspondence to:	Ömer Şenel Gazi Üniversitesi, Beden Eğitimi Spor Yüksek ,Teknik Okullar, 06500, Ankara, TURKEY EMAIL: maksu@gazi.edu.tr
Submitted: 2006-11-2	23 Accepted: 2007-03-10
Key words:	blood Pb level; strenuous exercise; basketball; stripping voltammetry

Neuroendocrinol Lett 2007; 28(2):187–190 PMID: 17435664 NEL280207A14 © 2007 Neuroendocrinology Letters www.nel.edu

Abstract This study is related to the investigation of the Pb levels in blood of the 12 healthy University male basketball players before and after a strenuous training session by the use of square wave anodic stripping voltammetry. Although the results do not show acute lead intoxication it is obvious that blood lead levels show significant increase after training sessions. The average increase in blood lead levels is 297%. This increase is largely due to increased respiration rate during the training period.

INTRODUCTION

Heavy metal pollution is a serious health threatening environmental problem we face during normal flow of our lives. For instance the passage of blood Pb levels over $125 \,\mu$ g/dL causes deaths due to lead intoxication. Similarly blood lead levels of $100 \,\mu$ g/dL in adults and $80 \,\mu$ g/dL in children cause serious damage in brain and kidneys (ATSDR, 1992; 1997). It is also reported that lead levels of $30-40 \,\mu$ g/dL in blood effect the nervous system resulting neurological symptoms (ATSDR, 1997; EPA, 2006). It is known that elevated levels of lead in human blood have diversified adverse effects on human metabolism.

One of the major sources of lead in atmosphere is the exhaust fumes coming from cars (Sezgin *et al.* 2003; Gibson and Farmer, 1986.) Apart from that the combustion of fuels such as coal also makes great contribution to the emission of lead into the atmosphere. The main source of the bioaccumulation of lead in human body is food. However environmental factors also play an important role in this process. If we consider that the lead levels in playing grounds are well over 1000 ppm the children are much more prone to the bioaccumulation of this metal in their bodies (Hamamcý *et al.*·1997).

There are so many studies related to the determination of heavy metals such as lead by the use of spectrophotometric (Willis, 1999; Cida et al. 2001) and electrochemical (Locatelli and Torsi, 2003 Kaličanin et al. 2004) methods. Electrochemical methods with their very low detection limits are very suitable for heavy metal determination. The techniques such as anodic stripping polarography or voltammetry which contains preconcentration step may be suitable for the determination of heavy metals as low as 10⁻⁷ M by increasing the accumulation time. The detection limits of the stripping methods may be lowered by the use of square wave of differential pulse methods. Jyothi et al. (2003) reported the determination of Cd, Pb, Cu and Zn by the use of stripping techniques without any interference. The way of digestion of the sample is as important as the method employed. Generally the digestion of samples is carried out with the use

Seyfi Savaş, Ömer Şenel, İlyas Okan & Hüseyin Çelikkan

of micro wave apparatus in acidic solvents to eliminate any organic residue which may interfere with the analysis (Somer and Ünal, 2004; Nedeltcheva *et al.* 2005).

The sportsmen are also adversely effected by the amount of heavy metals present in atmosphere and training ground. This effect is aggravated in training time due to increased rate of metabolism. This study is related to the investigation of the Pb levels in blood of the 12 healthy university male basketball players before and after a strenuous training session using square wave anodic stripping voltammetry. The changes in blood levels are tabulated in Table 2. This procedure was repeated after six weeks training period. The players were subjected the same type of diet during this period. The results displayed that the Pb levels in blood was increased after the training session in spite of carefully controlled hygienic conditions of the playing court.

Table.1 The electrochemical analysis conditions of square wave
stripping voltammetry.

Deposition potential	-1.250 V
Deposition time	150 s
Scan rate	2 mV
Amplitude	25 mV
Frequency	30 Hz
Rest time	15 s
Stirring rate	350 rpm

MATERIAL AND METHODS

Chemicals and Solutions

All the chemicals used in the digestion of samples, preparation of buffer solutions and standard additions were of analytical grade (suprapure quality). 10^{-2} , 10^{-3} and 10^{-4} M Pb, Cu and Zn solutions were prepared from the dilutions of 0.1 M Pb(NO₃)₂, Cu(NO₃)₂ and Zn(NO₃)₂ (Merck) stock solutions. The blood samples taken from the players were digested with nitric acid (Riedel). All the solutions were prepared with de-ionized water.

Taking the samples

The blood samples were taken from the players before and after the training periods at the beginning and end of six week training period. The bloods of the participants were taken by the paramedics with the approval of the ethical committee using sterilized syringes. The samples had been kept in a refrigerator till they were used.

Digestion of the samples

The digestion procedure was carried out in microwave apparatus after taking1 mL of blood and adding 2.5 mL HNO₃ on it. The microwave were kept at 160 °C for five minutes, 190 °C, 100 °C and 80 °C ten minutes each. The totally digested samples were diluted to 10 mL with the addition of de-ionized water (16.8 M Ω).

Voltammetric procedure

The trace elements analyses of the samples were carried out by the use of square wave stripping voltammetry under the conditions given in Table 1. The electrochemical analysis were performed computer controlled CHI660B model potentiostat and BAS CGME hanging

Table 2. The Pb level in players' blood before the training session and after the training session.

	Before the training session		After the training session		Statistics	
Samples	Mean value (μg Pb/dL blood)	Standard deviation	Mean value (μg Pb/dL blood)	Standard deviation	Difference	Percentage change
Sample 1	1.04	0.194	7.56	0.68	6.52	627
Sample 2	17.18	5.067	10.67	2.23	-6.51	-38
Sample 3	1.49	0.328	7.53	3.52	6.04	406
Sample 4	1.43	0.071	15.27	2.78	13.84	968
Sample 5	5.41	0.528	5.91	0.84	0.49	9
Sample 6	1.12	0.107	4.17	0.96	3.05	273
Sample 7	5.85	0.743	11.57	1.75	5.72	98
Sample 8	2.86	0.050	17.31	2.60	14.45	505
Sample 9	4.48	0.478	10.41	0.70	5.93	132
Sample10	9.06	0.590	11.57	1.77	2.51	28
Sample11	6.83	0.271	15.63	1.45	8.80	129
Sample12	10.88	0.679	20.82	1.61	9.94	91

mercury drop electrode. The working electrode was 100µm capillary mercury electrode and the counter and reference electrodes were a Pt wire and Ag/AgCl (3 M NaCl) electrodes. The residual oxygen in the system was removed by purging Ar gas with spectrophotometric purity. The peak potential for Pb²⁺ was -0.47 V (Ag/AgCl) under the conditions stated in Table 1 (Figure 1).

Analytic procedure

0.5 mL of the samples which had been previously made up to 10 mL were taken into the cell and 2 mL acetic acidacetate buffer was added to it. The solution was stirred for 2 minutes before the stripping process. The final voltammogram is given in Figure 1. The voltammograms obtained after three standard additions of $20\,\mu\text{L}$ 10^{-4} M Pb solutions were superimposed upon each other. The resulting voltammogram was displayed in Figure 1. The increase in the Pb peak at -0.47 V was evaluated according to standard addition method. The procedure was repeated three times for each and the average value of the amount of Pb in each sample was computed. The blank study revealed that the amount of metal coming from nitric acid and de ionized water was negligible. That was why the amount of Pb found in the blank sample was subtracted from the amount obtained for the samples. The results are tabulated in Table 2 in µg Pb/dL blood together with standard deviations.

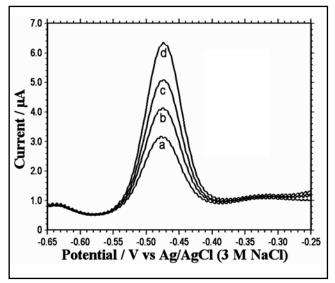


Figure 1. Anodic square wave voltammograms of Pb²⁺ peak located at -0.47 a) 0.5 mL sample +2 mL acetic acid-acetate buffer b) Addition of 20 μ L 10⁻⁴ M Pb²⁺ c) Addition of 40 μ L 10⁻⁴ M Pb²⁺ d) Addition of 60 μ L 10⁻⁴ M Pb²⁺.

Tab	le 3. Statistical	evaluation	of the blood Pb le	vels

RESULTS AND DISCUSSION

As seen from Table 2 except for those obtained for sample 2 all the results indicate significantly increased Pb²⁺ levels in blood after training sessions. This increase of 279% may be attributed increased contact of players with the basketball and atmosphere of the playing ground with increasing breathing rate. The atmospheric lead content of Ankara is given officially as 0.5 ppm by the General Directorate of Environment and Forestry.

If we take the fact that the players are at their adolescent ages into account we can expect that this level of lead accumulation may cause neurological complication in future. It was also reported that $10-15 \mu g/dL$ Pb in blood may cause neurobehavioral complications in children (WHO, 1996). Pb may cause anemia and other complications. Although clinical manifestations may occur at elevated concentrations its biological effects may be observed at much lower concentrations (Cope, 2004).

CONCLUSION

If we take the fact that 95% of lead in human body accumulates in bones (Cope, 2004), the significant increase in the lead levels of blood after the training session can be attributed to the environmental condition exposed during that period. Therefore the hygiene and cleanliness of the training grounds and materials are of great importance to obviate chronic lead intoxication in people doing sports. The training grounds have to be constructed outside the metropolitan regions away from the city atmosphere with heavy metal pollution. Although the results do not show acute lead intoxication it is obvious that blood lead levels showed a significant increase after training sessions. The average increase in blood lead levels is 297%. As seen in Table 3 the Pb level in blood increased from 5.69±4.99 (µg Pb/dL blood) to 11.54 \pm 4.96 (µg Pb/dL blood) after the training session. This difference is highly significant at p<0.01 significance level. This three folds increase is largely due to increased respiration rate during the training period. This is an issue which should be seriously addressed.

ACKNOWLEDGEMENT

We are grateful to Prof. Dr. M. Levent AKSU for the chemical evaluations and collecting the electrochemical data.

	Mean (μg Pb/dL blood)	SD	X1-X2	SD of Mean Differences	t-value	Percentage change
Before the training session (n=12)	5.69	4.99	5.05	5.86	-3.447**	102.81
After the training session (n=12)	11.54	4.96	-5.85			

Neuroendocrinology Letters Vol. 28 No. 2 2007 • Article available online: http://node.nel.edu

REFERENCES

- 1 ATSDR (1992). Case studies in environmental medicine, lead toxicity. Agency for toxic substances and disease registry, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.
- 2 ATSDR (1997). Toxicological profile for Lead (Update). Draft for public comment. Agency for toxic substances and disease registry, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.
- 3 EPA; http://www.epa.gov/ttn/atw/hlthef/lead.html#ref1 [20 May 2006]
- 4 Sezgin N, Ozcan HK, Demir G, Nemlioglu S, Bayat C (2003). Determination of heavy metal concentrations in street dusts in Istanbul E-5 highway. *Environment International.* 29: 979–985.
- 5 Gibson MG, Farmer JG (1986). Multi-step chemical extraction of heavy metals from urban soils. *Environ Pollut B.* **11**:117–135.
- 6 Hamamcý C, Gumgum B, Akba O, Erdogan S (1997). Lead in urban street dust in Diyarbakýr, Turkey. Fresenius Environ Bull. 6: 430–447.
- 7 Willis JB (1999). The early days of atomic absorption spectrometry in clinical chemistry. *Spectrochimica Acta Part B.* **54**: 1971– 1975.
- 8 Cida BP, Boiab C, Pomboc L, Rebeloc E (2001). Determination of trace metals in fish species of the Ria De Aveiro (Portugal) by electrothermal atomic absorption spectrometry. *Food Chemistry*. **75**: 93–100.

- 9 Locatelli C, Torsi G (2003). Analytical procedures for the simultaneous voltammetric determination of heavy metals in meals. *Microchemical Journal*. **75**: 233–240.
- 10 Kaličanin BM, Nikolić RS, Marjanović NJ (2004). Application of potentiometric stripping analysis with constant inverse current for determining soluble lead in human teeth. *Analytica Chimica Acta*. **525**: 111–114.
- 11 Jyothi NVV, Mouli PC, Reddy SRJ (2003). Determination of zinc, copper, lead and cadmium in some medicinally important leaves by differential pulse anodic stripping analysis. *J Trace Elem Med Biol.* **17**: 79–83.
- 12 Somer G, Ünal Ü (2004). A new and direct method for the trace element determination in cauliflower by differential pulse polarography. *Talanta*. **63**: 323–328.
- 13 Nedeltcheva T, Atanassova M, Dimitrov J, Stanislavova L (2005). Determination of mobile form contents of Zn, Cd, Pb and Cu in soil extracts by combined stripping voltammetry. *Analytica Chimica Acta.* **528**: 143–146.
- 14 WHO (1996). Trace Elements in Human Nutrition and Health, World Health Organization, Belgium. pp. 200.
- 15 Cope WG, Leidy RB, Hodgson E (2004). Classes of toxicants: Use of classes. In: A Textbook of Modern Toxicology. Hodgson E. (Ed.) 3rd edition. Wiley, Canada. pp. 51.