NATURA MONTENEGRINA, Podgorica, 2013, 12(2): 471-477

ORIGINAL RESEARCH PAPER

CHANGES IN TESTOSTERONE PROFILE SECRETION IN *Cavia porcellus (Mammalia, Rodentia)* DURING CHRONIC EXPOSURE TO LEAD

Albana MUNGA[1*], Eriona ABAZAJ[2] and Enkeleda OZUNI[3]

1*, 3 Agricultural University of Tirana, Faculty of Veterinary Medicine, Department of Morphofunctional Subjects, Albania. E-mail: albanamunga@yahoo.com

2 Public Health Institute, Department of Parasitology, Tirana, Albania.

Key words:
BLL,
testosterone,
correlation.

SYNOPSIS

The main purpose of this study is to estimate the effect of blood lead level (BLL) in testosterone secretion.

In order to ascertain what reproductive abnormalities occur in experimental animals when exposed to lead, approximately 4 months old animals (*Cavia porcellus*) were treated with intraperitoneal injections of different concentrations of lead acetate in aqueous solutions for 60 days.

According to the data collected, chronic exposure to lead affects greatly the profile of testosterone secretion. There is a strong negative correlation between BLLs and testosterone secretion in two of the doses applied. In very low doses of lead (tolerable daily intake) instead, the secretion of testosterone increases. Pearson's correlation coefficients are as follow; tolerable daily intake dose of lead- r = 0.67011, intermediate dose- r = -0.70361, and near lethal dose- r = -0.82696 (in all cases $\alpha = 0.05$).

INTRODUCTION

The preponderance of evidence is consistent with an indirect effect(s) of lead on the hypothalamic-pituitary axis (i.e., a disruption of gonadotropin secretions), although direct effects on testicular hormonal production are possible (Ng *et al.* 1991).

Rodamilans *et al.,* (1988) assessed serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and steroid binding globulin (SBG) in 23 male lead smelter workers with PbB in the range of 60–80 µg/dL. Comparison with an unexposed group of 20 men (PbB 17 µg/dL) showed that serum LH was significantly increased in the workers and that the magnitude of the effect did not increase with duration of exposure. A significantly lower free testosterone index (testosterone/SBG ratio) in the workers exposed for 1–5 years and significant

changes in serum testosterone (lower), SGB (higher), and free testosterone index (lower) in the workers exposed for >5 years indicated an exposure duration-related effect on serum testosterone.

Other studies of male workers have found different results. Gustafson *et al.,* (1989) found that plasma FSH, plasma LH, and serum cortisol levels were lower in male workers (mean PbB, 39 µg/dL) than in unexposed controls (mean PbB, 4 µg/dL); however, all hormone values were within normal reference limits.

Cullen *et al.,* (1984) found increased serum FSH and LH and borderline low serum testosterone levels in one of seven men with symptomatic occupational lead poisoning and a mean PbB of 87.4 µg/dL. Although serum testosterone concentration was normal in most of these patients, five had defects in spermatogenesis and six had subnormal glucocorticoid production. Serum testosterone levels were significantly lower in groups of male workers with lead poisoning (n=6, mean PbB, 38.7 µg/dL) and lead exposure (n=4, mean PbB, 29.0 µg/dL) than in an unexposed control group (n=9, mean PbB 16.1 µg/dL), but testosterone-estradiol binding globulin capacity and serum levels of estradiol, LH, FSH, and prolactin were normal (Braunstein *et al.,* 1978).

A number of studies have examined the potential association between lead exposure and reproductive parameters in humans. The available evidences suggest that occupational and environmental exposure resulting in moderately high PbBs might result in alterations in sperm and decreased fertility in men. Although the evidence for reduced fertility is not conclusive (Shiau *et al.,* 2004; Bonde & Kolstad 1997), it appears that a threshold for fertility effects in men could be in the PbB range of 30–40 µg/dL.

Studies have shown that sperm quality is affected by occupational exposure to lead. Although there is some variation in the results, most of the available studies suggest that reductions in sperm concentration, indications of adverse effects on sperm chromatin, and evidence of sperm abnormalities may occur in men with mean PbB > 40 µg/dL but not in men with lower PbBs (Alexander *et al.,* 1998a; Lancranjan *et al.,* 1975). The effect of lead was thought to be directly on the testes because tests for changes in gonadotropin secretion were negative. Secretion of androgens by the testes was not affected.

These findings indicate that lead can act directly on the testes to cause depression of sperm count and peritubular testicular fibrosis, reduced testosterone synthesis, and disruption of regulation of LH (Braunstein *et al.,* 1978; Cullen *et al.,* 1984; Rodamilans *et al.,* 1988).

SUBJECT AND METHODS

The purpose of this study is to estimate the effect of BLL in testosterone secretion. 40 individuals of the species *Cavia porcellus* were selected. They were all

sexually mature males with body weight between 400 and 550 g (approximately 4 months old) clean of infective disease. They were divided into 4 groups (1 control group and three manipulated groups).

Administration of the lead was made in three different doses, TDI (tolerably daily intake), LD_{50}-5% (to avoid the death till the end of the experiment) and an intermediate dose. The doses were injected intraperitoneally (in the lower abdominal quadrant) in form of aqueous solution of lead acetate. In this route the volume injected can reach 10-15 ml for adult guinea pigs (Beynon & Cooper, 1991). The doses applied were TDI 0.0036 mg Pb/kgbw/day (RIVM report 711701 025, 2001), LD_{50}-5% 4.9558 mg Pb/kgbw/day and the intermediate dose 2.61 mg Pb/kgbw/day. The experiment lasted 60 days and blood samples were collected every week via cardiac puncture (Beynon & Cooper, 1991).

All blood samples were analyzed for lead content with the technique of Atomic Absorption Spectrometry (AAS), and testosterone concentration with ELISA method. The spectrometer used was type Varian, Spectr-200 with limit of detection for cadmium 0,006 ppb (µg/L). These examinations were made at the laboratory of Analytic Chemistry, Institute of Food Safety and Veterinary, Tirana, Albania. Hormonal examinations were made with Elecsys 2010 system (detection limit for testosterone 0.069 nmol/l), and realized in medical laboratory “Intermedica” Tirana, Albania.

RESULTS AND DISCUSSIONS

During 60 days of manipulation, blood samples were collected every week and tested for total blood lead concentration with AAS and serum testosterone concentration. The gross data were transformed in mean values for each group, organized in tables and illustrated in graphics as below. The point is to get a clear picture what happens with testosterone secretion in males while treated with different doses of lead.

As expected in the animals of control group (Tab.1 and Fig. 1), there was found no lead or very small traces of lead in blood, and testosterone secretion is maintained pretty steady during 8 weeks of experimentation.

Table 1. Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in control group.

Week	0	1	2	3	4	5	6	7	8
BLL	0	0	0	0	0.002	0.0025	0	0	0
Testosterone	6.05	7.28	6.82	6.22	6.91	6.55	6.76	7.18	7.64

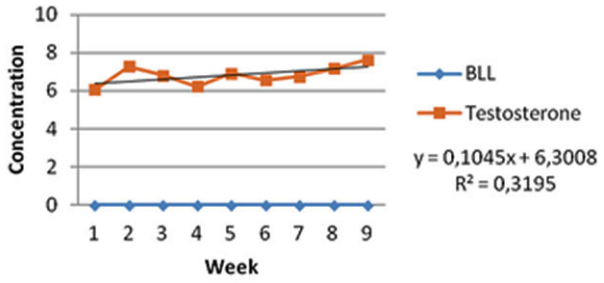

Figure 1:
Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in control group and linear regression line for changes in testosterone secretion.

Obviously did not result any statistical connection between blood lead concentration and testosterone profile secretion.

Table 2 and figure 2 present the data of the group treated with tolerable daily intake dose. Surprisingly this dose, which is considered to have no important adverse effects for the organism, can induce a significant and almost constant increase in testosterone secretion reflected by relatively high levels of serum testosterone.

Table 2. Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in TDI group.

Week	0	1	2	3	4	5	6	7	8
BLL	0	0	0	0.006	0.2001	0.222	0.175	0.17	0.118
Testosterone	6.08	10.47	12.76	16.43	17.88	18.5	20.45	23.74	27.34

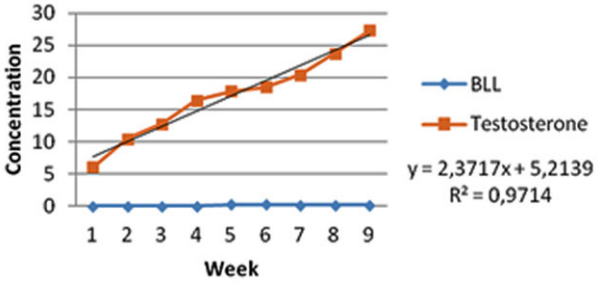

Figure 2:
Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in TDI group and linear regression line for changes in testosterone secretion.

As shown in the figure above there is a positive statistical connection between BLL and testosterone secretion with Pearson's correlation coefficient r = 0.67011 (α = 0.05). These results are consistent with some studies that report increase of testosterone secretion in workers exposed chronically to lead compounds (Ng *et al.*, 1991), but inconsistent with many other studies. This may be due to increase of gonadotropin secretion (especially LH) in organism exposed to low doses of lead (Ng *et al.*, 1991) combined with no adverse effects of these levels of lead in the ultrastructure of testes as we found earlier in our studies (Munga & Xhaxhiu 2008). There is a completely different picture in groups treated with intermediate and near-lethal (LD_{50}-5%) doses. The data are gathered in tables 3 and 4, and graphically

illustrated in figures 3 and 4. In both these groups there is an important accumulation of lead in blood associated with a significant decrease in testosterone secretion.

Table 3: Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in Intermediate dose group.

Week	0	1	2	3	4	5	6	7	8
BLL	0	0.682	0.76	1.129	1.382	1.661	1.975	1.972	1.998
Testosterone	6.52	1.72	1.58	1.4	1.65	1.76	1.73	1.67	0.99

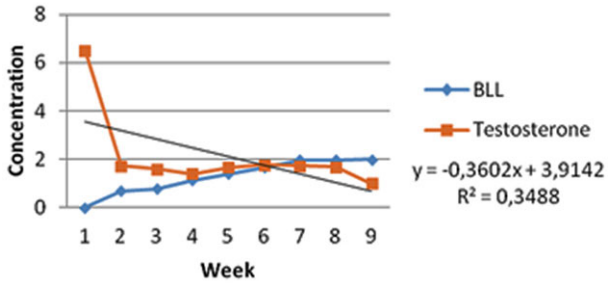

Figure 3: Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in Intermediate dose group and linear regression line for changes in testosterone secretion.

As it can be seen in the figure above, initially there is a severe decrease of testosterone secretion. The linear regression line shows the overall tendency of lead induced suppression in testosterone secretion and correlation coefficient results relatively high, r = -0.70361 (α = 0.05).

Table 4: Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in LD_{50}-5% dose group.

Week	0	1	2	3	4	5	6	7	8
BLL	0	0.732	0.851	1.169	1.96	2.012	2.136	2.495	2.626
Testosterone	6.81	2.57	2	1.23	1.18	1.43	1.44	0.54	0.63

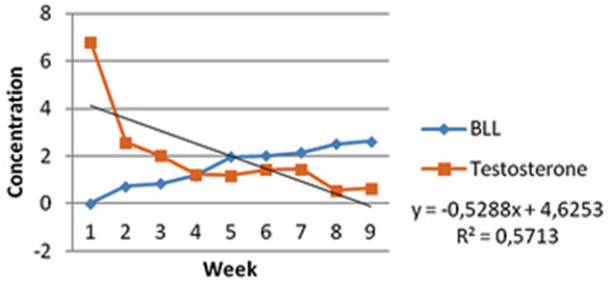

Figure 4: Lead accumulation in blood (ppm) and testosterone serum concentration (nmol/l) during 8 weeks of manipulation in LD_{50}-5% dose group and linear regression line for changes in testosterone secretion.

A strong negative correlation is found in LD_{50}-5% group too (r = -0.82696 for α = 0.05). It is presented a similar picture with a more pronounced decrease of testosterone secretion during the first weeks of treatment with these high doses of lead. These results match with those of many other authors who found moderate decreases in testosterone secretion due to lead poisoning (Cullen *et al.,*1984), or significant decrease of testosterone levels (Braunstein *et al.,* 1978) accompanied with oligospermia and testicular lesions.

The reasons of these strong negative correlations according to many authors and supported by the results of our study may be the direct effects of lead in testicular morphologic parameters including damages in seminiferous epithelium, Sertoli cells and basal lamina (Foster *et al.,* 1998; Wildt *et al.,* 1983; Rodamilans *et al.,* 1988; Batra *et al.,* 2001; Adhikari *et al.,* 2001).

Table 5: Pearson's correlation coefficients for testosterone secretion changes according to lead accumulation in blood for different doses applied.

Dose applied	Pearson's coef. (r)
Control	-0.1259
TDI	0.67011
Int. dose	-0.70361
LD_{50}-5%	-0.82696

CONCLUSIONS

Chronic lead exposure has significant effects in serum testosterone levels in males of *Cavia porcellus*.

The effects appear different for different doses of lead applied.

Low doses (TDI) of lead can induce the increase of testosterone secretion with significant positive correlation (r = 0.67).

High doses of lead (intermediate and LD_{50}-5% doses) have the opposite effect on testosterone secretion causing the reduction of serum testosterone secretion. It results a strong negative correlation between high levels of BLL and testosterone secretion (r = -0.7036 for Int. dose, and r = -0.827 for LD_{50}-5% dose).

REFERENCES:

Adhikari. N., Sinha, N., and Narayan. R., 2001.: Lead-induced cell death in testes of young rats. - *Journal of Applied Toxicology,* 21: 275-277.

Alexander. BH., Checkoway. H., and Faustman. EM., 1998a.: Contrasting associations of blood and semen lead concentrations with semen quality among lead smelter workers. - *American Journal of Industrial Medicine,* 34: 464-469.

Baars. AJ., Theelen. RMC., Janssen. PJCM., Hesse. JM., van Apeldoorn. ME., Meijerink. MCM., Verdam. L., and Zeilmaker. MJ., RIVM report 711701 025, 2001.: Re-evaluation of human-toxicological maximum permissible risk levels. - *Netherlands National Institute for Public Health and the Environment,* 66-70.

Batra. N., Nehru. B., and Bansal. MP., 2001.: Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. - *Journal of Applied Toxicology,* 21: 507-512.

Beynon. P H., and Cooper. JE., 1991.: Manual of exotic pets, New edition, pp: 51-62.

Bonde. JP., and Kolstad. H., 1997.: Fertility of Danish battery workers exposed to lead. - *International Journal of Epidemiolog,.* 26(6): 1281-1288.

Braunstein. GD., Dahlgren. J., and Loriaux. DL., 1978.: Hypogonadism in chronically lead-poisoned men. – *Infertility,* 1: 33-51.

Cullen. MR., Kayne. RD., and Robins. JM., 1984.;Endocrine and reproductive dysfunction in men associated with occupational inorganic lead intoxication. - *Archives of Environmental Health,* 39: 431-440.

Foster. WG., Singh. A., and McMahon. A., 1998.: Chronic lead exposure effects in the Cynomolgus monkey (Macaca fascicularis) testis. - *Ultrastructural Pathology,* 22(1): 63-71.

Gustafson. A., Hedner. P., and Schutz. A., 1989.: Occupational lead exposure and pituitary function. - *International Archives of Occupational and Environmental Health,* 61: 277-281.

Lancranjan. I., Popescu. HI., and Gavanescu. O., 1975.: Reproductive ability of workmen occupationally exposed to lead. - *Archives of Environmental Health,* 30: 396-401.

Munga. A., and Xhaxhiu. D., 2008.: Lead effects in testicular morphologic parameters in guinea pig. - *Preceedings International Conference on Biological and Environmental Sciences*. 26-28 September, 69-75.

Ng. TP., Goh. HH., and Ong. HY., 1991.: Male endocrine functions in workers with moderate exposure to lead. - *British Journal of Industrial Medicine,* 48: 485-491.

Rodamilans. M., Osaba. MJ., and To-Figueras. J., 1988.: Lead toxicity on endocrine testicular function in an occupationally exposed population. - *Human Toxicology,* 7:125-128.

Shiau. CY., Wang. JD., and Chen. PC., 2004.: Decreased fecundity among male lead workers. -*Occupational and Environmental Medicine,* 61:915-923.

Wildt. K., Eliasson. R., and Berlin. M., 1983.: Effects of occupational exposure to lead on sperm and semen. In: Clarkson TW, Nordberg GF, Sager PR, Reproductive and developmental toxicity of metals. - *Proceedings of a Joint Meeting, Rochester, NY. New York, NY: Plenum Press*. 279-300.

Received: 14 June 2013.