Male endocrine functions in workers with moderate exposure to lead

T P Ng, H H Goh, Y L Ng, H Y Ong, C N Ong, K S Chia, S E Chia, J Jeyaratnam

Abstract

Evidence for the effect of occupational exposure to lead on the male endocrine system is conflicting. This study evaluated the primary (testicular) and secondary (hypothalamo pituitary testicular) effects of exposure to lead in 122 current lead workers and 49 non-exposed workers. The mean current blood lead concentration was 35·2 (range 9·6–77·4) $\mu g/dl$ in the exposed workers, and 8·3 (range 2·6–14·8) $\mu g/dl$ in the non-exposed workers. Concentrations of plasma luteinising hormone (LH) and follicle stimulating hormone (FSH) were both significantly higher in the exposed workers, but testosterone (T) was not significantly different between the two groups. In older exposed workers, however ($\geqslant$ 40 years), plasma T concentrations were significantly lower, but LH and FSH concentrations were not significantly different. Compared with non-exposed workers, those exposed for less than 10 years had significantly raised LH and FSH and normal T concentrations whereas those exposed for 10 or more years had significantly lower T, and normal LH and FSH concentrations. The concentrations of LH and FSH showed a moderate increase in relation to blood lead concentrations in the range of 10 $\mu g/dl$ to 40 $\mu g/dl$ and thereafter reached a plateau or declined. No apparent trend for plasma T concentrations occurred. No significant difference in prolactin (PRL) concentration was noted. It is concluded that moderate exposure to lead was associated in dose related fashion with small but measurable changes in male endocrine functions that reflected both primary and secondary effects of lead on the testes and the hypothalamo pituitary testicular axis.

Department of Community, Occupational, and Family Medicine
T P Ng, Y L Ng, H Y Ong, C N Ong, K S Chia, S E Chia, J E Jeyaratnam
Department of Obstetrics and Gynaecology, National University of Singapore
H H Goh

Clear evidence exists showing that lead exposure has a deleterious effect on the seminiferous tubules and spermatogenesis in man.[1][2] Case reports of clinically overt lead poisoning[3][4] and controlled studies of male workers[5-7] have shown that exposure to lead is associated with depressed sperm counts and abnormal motility and morphology.

Studies of the effects of lead on concentrations of gonadotrophins and testicular hormones in men have given conflicting results. It is not clear whether lead acts directly on the testicular production of androgens or indirectly through its effects on the hypothalamo pituitary axis production and secretion of gonadotrophins. Suppression of pituitary gonadotrophic secretions was reported in lead intoxicated patients.[3][8] Braunstein et al reported that in 10 lead intoxicated men who complained of impotence, plasma testosterone (T) concentration was reduced, and gonadotrophin and prolactin concentrations were normal, but luteinising hormone (LH) responses to challenge by clomiphene and gonadotrophin releasing hormone were suppressed.[3] On the other hand, Cullen et al found increased follicle stimulating hormone (FSH) and LH and lowered T concentrations in only one of seven patients described.[4] Several studies of lead exposed workers have reported no pituitary or gonadal dysfunction.[5-7]

More recently, studies of lead exposed workers have reported positive evidence of a central effect— that is, a disruption of gonadotrophin secretions. Progressive increase in plasma LH and decrease in plasma T concentrations with no change in FSH was noted among workers exposed to a mean blood lead concentration of 70 $\mu g/dl$ for less than one year (five subjects), one to five years (eight subjects), and more than five years (11 subjects) compared with controls (20 subjects).[9] On the other hand, Gustafson et al recently reported lower plasma FSH and LH concentrations (especially in workers less than 40 years of age) and no difference in T concentrations in 25 workers compared with age matched controls.[10] The levels of lead absorption were moderate (mean blood lead 40 $\mu g/dl$, mean duration (SD) 6·4 (4·1) years). The results in some studies were difficult to interpret for reasons such as small sample sizes, possible confounding effects by age and other factors, and

differences in laboratory methods and quality control of hormonal evaluation.

In this study we evaluated the relation between concentrations of circulating pituitary and testicular hormones in relation to the level of lead absorption and exposure duration in a large group of male lead workers. We also examined possible age related susceptibility with respect to the gonadotoxic effects associated with exposure to lead.

Materials and methods

The study cohort consisted of 122 current male production workers exposed to lead in three lead battery factories, and 49 non-exposed persons— namely, hospital engineering and maintenance workers and technicians. None of the subjects had a history of liver, renal, or other recent or chronic diseases or illnesses.

Environmental air concentrations of lead in the factories were monitored by personal sampling during normal plant operating conditions with personal sampling pumps at a flow rate of 2 l/min and laboratory analysis by graphite atomic absorption spectrophotometry. Time weighted average concentrations of airborne lead among work sections ranged from 0·03 mg/m³ to 0·60 mg/m³ (mean 0·18 mg/m³).

The blood specimens for lead analysis were collected in lead free heparinised polypropylene containers, with precautions taken to prevent contamination as far as possible. Blood lead concentrations were determined by a graphite furnace spectrophotometer (GAAS) with an autosampler. The analyses were carried out in triplicate. The standard deviation for within run precision (based on 15 repetitive measurements) for a blood lead concentration of 8 μg/100 ml was 0·34 and for a blood lead concentration of 80 μg/100 ml was 0·67. The coefficient of variation seldom exceeded 6%. The precision of blood lead analysis in this laboratory over the range of 4 to 80 μg/dl was 0·12 μg/dl. External quality control was carried out in collaboration with the National External Quality Control Assessment Scheme (NEQAS) in the United Kingdom. The mean running variance index score (MRVIS) at the time of analyses was 24–28. Aminolevulinic acid dehydratase (ALAD) enzyme activity in erythrocytes was determined according to the European standardised method.[11]

Blood lead and ALAD estimations were carried out on the lead workers at roughly six month intervals beginning in 1982. The mean number of blood lead estimations among the current workers in the study was 6·2 (SD 4·5; range 1–18).

Blood for hormone assays was collected between 9.00 am and 11.00 am, in containers with EDTA anticoagulants and plasma samples were stored frozen at −20°C until analysis. Testosterone, LH, FSH, and PRL were determined by radioimmunoassay using World Health Organisation matched reagents and standard methods.[12] The hormone assay performance was monitored using three internal

Table 1 *Exposure and effect parameters in study subjects*

Parameters	Non-exposed	Exposed	p Value
No of subjects	49	122	
Age (y):			
Mean (SD)	34·4 (13·4)	32·6 (8·2)	0·40
Range	18–74	17–54	
Years of exposure:			
Mean (SD)		6·0 (5·9)	
Range		0·1–19	
Current blood lead (μg/dl):			
Mean (SD)	8·3 (2·8)	35·2 (13·2)	0·0001
Range	2·6–14·8	9·6–77·4	
Average blood lead (μg/dl):			
Mean (SD)	8·3 (2·8)*	35·1 (12·0)	0·0001
Range	2·6–14·8	9·6–77·4	
ALAD (μM/h/ml RBC):			
Mean (SD)	1·25 (0·25)	0·45 (0·23)	0·0001
Range	(0·78–1·70)	(0·13–1·34)	
T (ng/ml):			
Mean (SD)	7·39 (2·21)	6·74 (2·32)	0·09
Range	2·8–12·6	2·2–13·3	
LH (IU/l):			
Mean (SD)	3·24 (1·61)	4·59 (2·15)	0·0001
Range	0·35–6·6	0·18–10·2	
FSH (IU/l):			
Mean (SD)	1·92 (1·20)	2·52 (1·72)	0·01
Range	0·40–5·5	0·20–9·5	
PRL (mIU/l):			
Mean (SD)	196 (125)	191 (109)	0·60
Range	64–723	58–881	

*Only one sample per subject.

quality assurance pools. The intra- and inter-assay coefficients of variation for T were less than 15% and for LH, FSH, and PRL were less than 10%. The inter-assay coefficients were less than 15%.

Statistical analyses were performed using procedures from the Statistical Analysis System (SAS) software.[13] The potential confounding effects of age, smoking, and alcohol on pituitary function were assessed in the control and exposed groups by performing multiple regression analyses of these variables on T, LH, FSH, and PRL. The independent effect of exposure to lead on pituitary and testicular hormones was assessed by using analysis of covariance to adjust for age and smoking as covariates where appropriate in the model.

The data on T and LH were used to construct several indices of gonadal dysfunction as follows: primary hypogonadism was defined as plasma T concentrations below the fifth percentile value of the control subjects in conjunction with LH concentra-

tions exceeding the 95th percentile value. Subjects were considered to have secondary hypogonadism if the plasma T concentration was below the fifth percentile but LH concentration was within the normal range. Finally, a group of compensated primary hypogonadism was defined by taking values of T within the normal range while LH concentration was above the 95th percentile in the unexposed group. Fisher's exact tests were used to evaluate the level of statistical significance for differences in proportions between the groups.

Results

The blood lead concentrations in the occupationally exposed workers indicated a moderate level of lead absorption (table 1). The individual worker's current blood lead concentration corresponded closely with his average blood lead concentration dating back as far as 1982. However, the workers were possibly exposed to higher concentrations of lead before 1982.
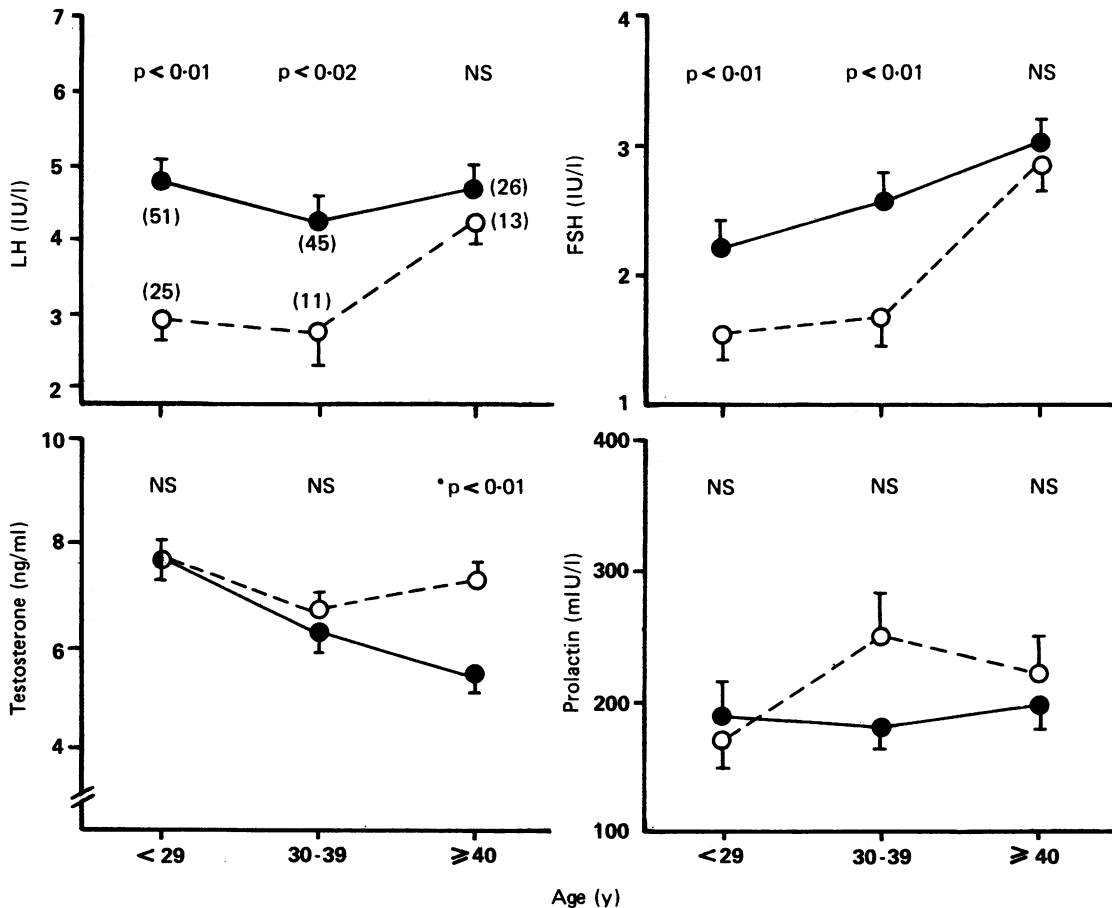

Figure 1   Mean testosterone and gonadotrophin concentrations in exposed (●) and non-exposed (○) workers. Bars denote standard deviations. No of subjects in parentheses.

Twenty eight (23%) of the workers had been exposed for 10 or more years at their jobs. The occupationally exposed and non-exposed groups were comparable in respect of age. There were 20 (41%) current smokers (mean pack-years 7·7; SD 11·5) in the non-exposed group, and 67 (55%) current smokers (mean pack-years 8·5; SD 6·8) in the exposed group. The differences were not statistically significant. There were few regular alcohol drinkers (more than once weekly) within the non-exposed (4·8%) and exposed (8·1%) groups.

Analyses of the relation of the level of each hormone with age among the unexposed men showed that increasing age was significantly associated with increases in FSH ($r = 0.44$, $p = 0.01$) and LH concentrations ($r = 0.35$, $p = 0.01$) but not with T or PRL concentrations. Smoking was significantly associated with decreased concentrations of PRL ($p < 0.05$).

Compared with non-exposed men, the lead workers showed somewhat lower but statistically non-significant concentrations of T, significantly raised concentrations of LH and FSH, and no significant differences in PRL concentrations (table 1). These differences were not likely to be confounded by age.

Low values of T concurrent with high concentrations of LH (primary hypogonadism) were seen in two of the exposed (1·6%) and in none of the non-exposed subjects. Reduced T and normal LH concentrations (secondary hypogonadism) were noted in 17 (13·9%) of the exposed subjects and two (4·1%) of the non-exposed subjects ($p = 0.05$). Raised LH values with normal T concentrations (compensated primary hypogonadism) were found in 29 (23·8%) of the exposed and three (6·1%) of the non-exposed subjects ($p < 0.05$).

Figure 1 shows the differences in concentrations of T, LH, FSH, and PRL between the two groups subdivided into three age groups. Although the concentrations of T in the exposed and non-exposed subjects were not significantly different in the group as a whole and in the younger subjects, it was significantly lower in the exposed group aged 40 years and above ($p < 0.01$). On the other hand, LH and FSH concentrations in the exposed group were significantly higher in the younger but not in the older age group. Prolactin concentrations did not show any age-specific differences between the groups.

Further analysis entailed dividing the exposed subjects into two subgroups that were defined by their duration of employment (less than 10 years and 10 or more years). The concentrations of T, LH, FSH, and PRL in each group were adjusted for age and smoking. As shown in fig 2, LH and FSH concentrations were both significantly increased in conjunction with normal concentrations of T in the group with less than 10 years of exposure. By contrast, the group with long duration of lead exposure (10 years or
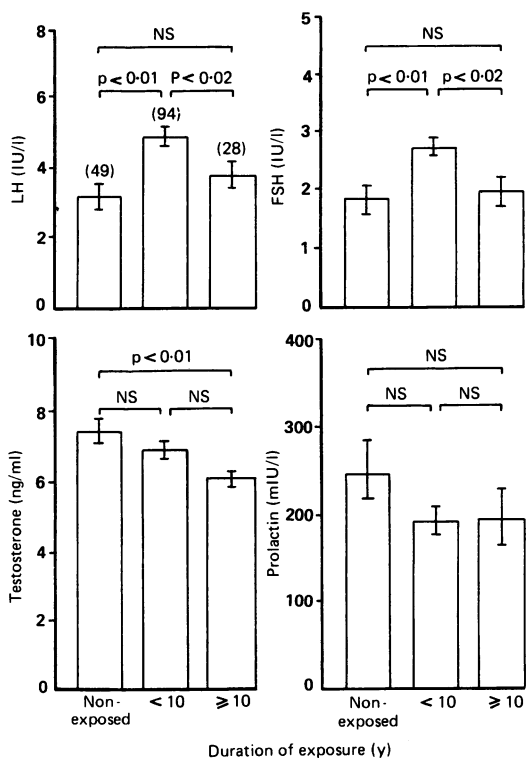

Figure 2 Adjusted mean testosterone and gonadotrophin concentrations in exposed and non-exposed workers by duration of exposure. Bars denote standard deviations. Mean age (SD) 34·4 (13·4), 30·3 (7·5), and 40·2 (5·3) years for non-exposed group and groups exposed < 10 y and ≥ 10 y respectively.

more) had significantly lower LH and FSH concentrations, which were not significantly different from those seen in the non-exposed. Testosterone in the long exposure group was significantly lower, however, when compared with the non-exposed. No statistically significant differences in PRL concentrations were seen among the groups.

The concentrations of these hormones were finally related to the current blood lead concentrations (table 2 and fig 3). The concentrations of LH and FSH appeared to show a gradual increase with higher concentrations of mean blood lead from 10 μg/dl, reached a peak at about 40 μg/dl, and thereafter reached a plateau or declined. No clear trends were seen for T or PRL concentrations.

Discussion
Some degree of compensated primary hypogonadism is to be expected in active and healthy older men.[14-17] This was reflected by the association of increasing age with higher FSH and LH concurrent with normal T

Table 2   Exposure and effect parameters by current blood lead concentrations

Parameters	Current blood lead concentration (μg/dl)					
	<15	15–	25–	35–	45–	55–
No of subjects	58	19	34	25	28	7
Age (y):						
Mean	33·7	33·6	33·5	34·0	31·7	27·4
SD	12·8	7·7	7·5	9·9	8·2	3·5
Range	18–74	21–45	18–46	17–54	17–48	20–30
Years of exposure:						
Mean	0·5	5·8	7·6	5·6	5·8	3·5
SD	2·0	6·8	6·4	5·9	5·7	3·3
Range	0·0–9	0·1–19	0·1–19	0·1–18	0·1–19	0·2–9·0
Current blood lead (μg/dl):						
Mean	8·7	21·3	29·9	38·7	48·8	61·3
SD	2·0	2·2	2·9	2·8	3·2	7·6
Range	2·6–14·8	17·0–24·9	25·7–34·9	35·0–44·7	45·2–54·9	55·6–77·4
Average blood lead (μg/dl):						
Mean	9·0	23·0	32·5	37·6	46·7	52·9
SD	3·8	5·8	6·3	4·3	5·8	12·2
Range	2·6–22·9	11·6–33·8	23·6–51·6	26·7–44·3	31·2–56·9	39·2–77·4
ALAD (μM/h/ml of RBC):						
Mean	1·20	0·61	0·49	0·41	0·26	0·21
SD	0·28	0·19	0·14	0·13	0·06	0·07
Range	0·47–1·70	0·30–1·02	0·23–0·79	0·22–0·77	0·15–0·37	0·13–0·30
T (ng/ml):						
Mean	7·2	6·6	6·7	6·9	7·0	6·5
SD	2·2	3·0	2·3	2·3	2·3	1·3
Range	2·6–12·6	2·4–12·9	2·2–11·8	4·2–13·3	3·6–12·7	5·0–9·1
LH (IU/l):						
Mean	3·5	4·4	4·6	5·1	4·2	4·2
SD	1·9	1·8	2·1	2·1	2·3	2·6
Range	0·4–9·4	1·6–7·1	0·7–9·4	0·7–9·5	0·2–10·2	1·2–9·1
FSH (IU/l):						
Mean	2·0	2·3	2·6	2·7	2·3	2·7
SD	1·4	1·2	1·7	2·2	1·0	3·0
Range	0·4–7·7	0·9–4·7	0·7–8·0	0·7–9·5	0·2–4·6	0·7–9·3
PRL (mIU/l):						
Mean	194	175	218	188	173	187
SD	117	58	158	90	99	80
Range	64–723	76–269	58–881	83–483	77–519	98–343

concentrations in normal subjects in the present study. Furthermore, as with earlier reports,[18][19] smoking was found to be associated with decreased concentrations of PRL. Therefore, all analyses of the effects of lead on the pituitary and testicular hormones were carried out after adjusting for possible confounding effects due to age and smoking

Contrary to some earlier reports[5][7] we found that exposure to lead had an effect on testicular function. As a group, lead exposed workers had significantly higher FSH and LH and similar T concentrations compared with those in the non-exposed group. Raised LH and FSH concentrations are sensitive indicators of Leydig and Sertoli cell failure. In many clinically apparent hypogonadism syndromes, they are almost invariably raised even when T concentrations are normal.[20][21] Lead may adversely affect testicular function by reducing the production of T or by decreasing testicular sensitivity to gonadotrophic actions, leading to compensatory increases in FSH and LH concentrations.

Our results are at variance with those of Gustafson et al[10] who found reduced FSH and LH concentra-

tions. The authors stated that Sertoli cell damage was likely even at the moderate level of lead exposure studied, but their results were inconsistent with this statement, as this should lead to a high instead of the low concentration of FSH found. They suggested that lead may exert dual effects, that is, damage of the Sertoli cells and the hypothalamo pituitary structures similar to that induced by chronic alcoholism. An effect based on such an analogy, however, appears too profound to be credible, considering the moderate lead exposure (mean blood lead 40 μg/dl) and relatively short duration of exposure (6·4; SD four years).

Similar results to ours in respect of raised LH concentrations were reported by Rodamilans et al.[9] They found no increase in FSH concentration, however, but a reduction in T concentration in workers exposed to high concentrations of lead, which was related to duration of exposure. The mean blood lead concentration was 70 μg/dl but few appeared to have been exposed for more than 10 years. The investigators considered that the disruption of the hypothalamo pituitary testicular axis in
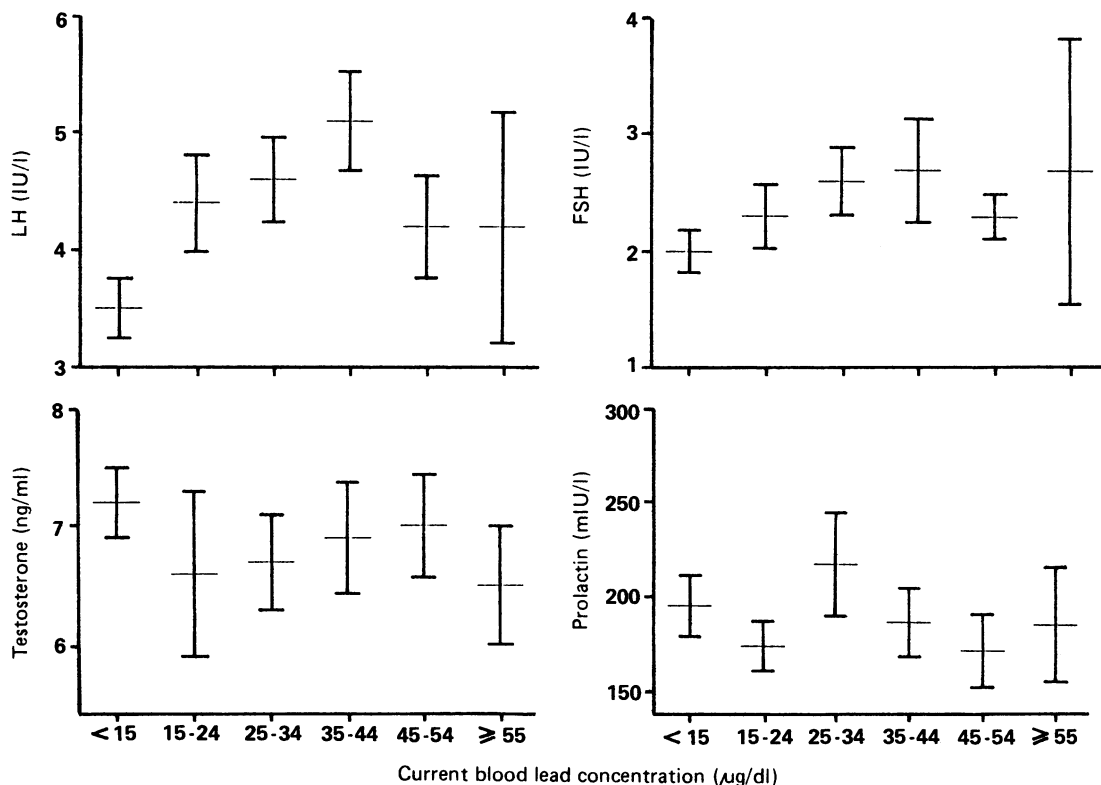
Figure 3   Mean testosterone and gonadotrophin concentrations by current blood lead concentrations. Bars denote standard errors.

men chronically exposed to lead is due, in the first stage, to a direct testicular toxicity affecting T synthesis, with additional toxicity at the hypothalamus or pituitary after longer periods of exposure. This hypothesis, however, does not appear to be adequately supported by their own results. A second stage toxic effect on the hypothalamo pituitary axis would lead to a decrease and not an increase in LH secretion. It would appear that their results could best be explained solely by a toxic effect of lead primarily at the level of the testes.

In the present study, we found that men occupationally exposed to lead for less than 10 years had high FSH and LH and normal testosterone, whereas men exposed for 10 or more years had normal FSH and LH and low T concentrations. These findings could be explained by a direct toxic action of lead on the testes leading to reduced production of T. In men with less chronic exposure to lead, the intact hypothalamo pituitary axis was capable of compensating the loss by increasing FSH and LH secretions, hence T production was brought back to normal. In men more chronically exposed to lead, however, a secondary effect might have rendered

the hypothalamo pituitary axis incapable of compensating for the reduced production of T and hence FSH and LH secretions would be reduced to normal, with consequent reduction of T to concentrations below normal. The effects of lead appeared to be dose dependent, varying both with the intensity and duration of exposure.

We believe, therefore, that exposure to lead produces a complex series of interacting hormonal changes reflecting damage first at the testicular and then at the hypothalamo pituitary level. Most of the lead workers, particularly those who were younger with less exposure, showed compensated primary hypogonadism that may be reversible if exposure ceases. Those who were older with more chronic exposure appeared to show secondary hypogonadism. Whether this is likely to be reversible is uncertain. Judging by the endocrine results, however, damage at both levels are neither extensive nor profound at moderate levels of exposure to lead.

It would appear that the testes are very sensitive to the toxic insults of lead, even at low or moderate exposure. In the study by Lancranjan *et al*, even men with "physiological" levels of lead absorption (blood

lead concentration (SD) 23 (14) μg/dl) among technicians and office workers who worked in a polluted environment appeared to show higher rates of impaired spermatogenesis, although the results were statistically non-significant.[5] In this respect the raised LH and FSH concentrations found in association with mean blood lead concentration of 20 to 40 μg/dl is noteworthy. Some of these men have worked for up to 19 years, however, and may have been exposed to higher concentrations of lead in the past. If confirmed by subsequent studies, this finding has significant implications for the reproductive health of working populations with prolonged exposure to low concentrations of environmental lead.

Requests for reprints to: Dr Ng Tze Pin, Department of Community, Occupational and Family Medicine, National University of Singapore, Lower Kent Ridge, Singapore 0511.

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