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Effects of Endocrine Disrupting Heavy Metals on Pituitary and Gonadal Hormones in Normal Weight Automechanics in Ibadan, Nigeria

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ABSTRACT

Association of hypogonadism and visceral obesity (VO) was recently demonstrated in male auto-mechanics occupationally exposed to endocrine disruptors (ED)-lead, cadmium, mercury and arsenic, known to alter the hypothalamic-pituitary-testicular axis. The effects of exposure to these EDs on pituitary and gonadal hormones in normal weight auto-mechanics in Ibadan were investigated. Ninety-nine normal weight male adults without any metabolic syndrome component-elevated VO, blood pressure, tryglycerides, fasting plasma glucose (FPG) and reduced high density lipoprotein cholesterol (HDLC), enrolled into this prospective cross sectional study. They were 50 auto-mechanics, age and anthropometry matched with 49 eugonadic males (occupationally unexposed to EDs) in Ibadan (control). Demography, lifestyle, sexual and reproductive history, anthropometery and blood pressure were obtained by standard methods. Fasting blood (15 mL) obtained was used for biochemical analyses - hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, oestradiol and testosterone) by ELISA; EDs-Lead, cadmium, mercury and arsenic by AAS; FPG, HDLC, triglycerides and oxidative stress (OS) biomarker-total antioxidant capacity (TAC) by spectrophotometry. Data obtained were statistically significant at $P<0.05$. Only 45 (90%) auto-mechanics were eugonadic. EDs except arsenic were significantly higher while libido and TAC level were significantly lower in the auto-mechanics compared with control ($P<0.05$). In automechanics only, lead had an inverse relationship with testosterone ($P= 0.001$) but direct relationship with FSH ($P= 0.013$). LH had a direct relationship with mercury ($P= 0.031$) but indirect relationship with TAC ($P<0.001$). Auto mechanics may be occupational exposed to lead, cadmium and mercury with the induction of oxidative stress and testicular dysfunction.

Keywords: Heavy Metals, Hypogonadism, Metabolic Syndrome, Total Antioxidant Capacity, Testosterone

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INTRODUCTION

Infertility defined as failure to conceive after more than 12 months of unprotected sexual intercourse, is a major clinical problem and affects 10-15% of couples around the world regardless of race or ethnicity (Abarikwu, 2013; Chirputkar and Vaidya, 2015). The contribution of male factor alone to infertility from cases that visited gynecological clinics in Nigeria is 11.1% in Lagos University Teaching Hospital (Adegbola and Akindele, 2013), 27.3% in University College Hospital, Ibadan (Adeniyi *et al*., 2003) and 40% in Amino Kano University Teaching Hospital (Emokpae *et al*., 2007). Although the cause of male infertility is obscure, it has been attributed to genetic and environmental factors (Lalitha *et al*., 2013).

Pituitary and gonadal hormones are essential in the diagnosis of male infertility. Follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and testosterone. Testosterone produced in the Leydig cells is involved in development of reproductive organs and spermatogenesis (McNicholas *et al*., 2003). Testosterone plays a key role in stimulating mitotic and meiotic deoxyribonucleic acid (DNA) synthesis in spermatogonia as well as the induction and maintenance of spermatogenesis. FSH acts directly on the

seminiferous tubules whereas LH stimulates spermatogenesis indirectly via testosterone (Thualfeqar *et al*., 2012).

Oestradiol is involved in the development and maintenance of male fertility. High oestradiol concentration also increases concentration of sex hormone binding globulin (SHBG) (Kalme *et al*., 1999). Prolactin, a 23 Kd hormone, synthesized in the adenohypophyseal lactotrophs, has no known target organ or defined role in male reproduction. However, acute hyperprolactinemia is known to suppress testosterone synthesis and male fertility through prolactin-induced hypersecretion of adrenal corticoids or by inhibiting the secretion of gonadotropin releasing hormone (GnRH) through prolactin receptors on hypothalamic dopaminergic neurons (Gill-Sharma, 2009).

There is an increasing risk of chemical toxicity associated with increasing industrialization especially in developing countries where there is improper handling of these chemicals (Anetor *et al*., 2009). In Nigeria, auto-mechanics are found in places called mechanic village where they carry out repair and servicing of motor vehicles. Their activities include repairs of brakes and steering, repair of engines, spray painting, recharging of batteries, welding, soldering and electrical wiring. The wastes generated from them include gasoline, diesel, spent engine oil and paint, resulting in the release of heavy metals to the environment (Aloysius *et al*., 2013).

Spent engine oil and solvents form one of the most hazardous waste generated in a mechanic village as they contain heavy metals to which the auto-mechanics are exposed (Obini *et al*., 2013). Thus, auto-mechanics are exposed to heavy metals- lead, cadmium, arsenic and mercury (Anetor *et al*., 2009). Arinola and Akiibinu in 2006 observed significantly higher levels of metals in auto-mechanics when compared with controls. The poor handling and transport of these heavy metals especially in developing countries could disrupt endocrine functions (Anetor *et al*., 2009).

Endocrine disrupting chemicals are exogenous substances that cause adverse health effects in an intact organism or its progeny secondary to changes in endocrine function (Saalu and Osinubi, 2009). They can interfere with the synthesis, secretion, transport, binding or elimination of hormones in the body (Bolawa *et al*., 2014). Thus, heavy metals could adversely affect male reproductive system by disrupting the gonadal-endocrine axis or the spermatogenesis process (Jorge *et al*., 2008). The hypothalamic-pituitary-gonadal axis can be affected by heavy metals either directly or indirectly. Heavy metals induce modifications of neurotransmitter in the central nervous system (CNS) and impair the hypothalamic release of gonadotropin-releasing hormone (Bolawa *et al*., 2014).

Lead's main influence on male reproduction probably occurs by altering the reproductive hormonal axis and the hormonal control on spermatogenesis, rather than by a direct toxic effect on the seminiferous tubules of the testes (Mohsen *et al*., 2011). It is a powerful disruptor of adrenal steroidogenesis, inhibiting synthesis and activity of progesterone and 17-hydroxyprogesterone. High levels of exposure to lead also have inhibitory effects on testosterone and 17β-oestradiol.

Cadmium disrupts steroidogenesis by interfering with the biosynthesis of androgens, oestrogen and progesterone (Georgescu *et al*., 2011). It may bind both oestrogen and androgen receptor. It activates oestrogen receptor through an interaction with the hormone-binding domain of the receptor. It also affects gonadal function and secretory pattern of prolactin (Bolawa *et al*., 2014). Mercury and arsenic are also oestrogen mimics (Johnson *et al*., 2003). Mercury-based compounds disrupt steroidogenesis, including sex hormone synthesis (Georgescu *et al*., 2011). Arsenic is a potent endocrine disruptor, altering gene regulation by the closely related glucocorticoid, mineralocorticoid, progesterone, and androgen steroid receptors even at low concentrations (Davey *et al*., 2007).

Significant hypogonadism has been reported in auto-mechanics occupationally exposed to heavy metals. Although these were men with normal weight, they had increased measures of visceral adiposity compared with control (Okoli *et al*., 2015). Increased visceral obesity is a component of metabolic syndrome (MS) - a cluster of metabolic disorders including dyslipidaemia, hypertension, increased visceral adiposity, increased blood pressure and elevated fasting plasma glucose that are risk factors for the development of cardiovascular disease and type 2 diabetes mellitus. Hypogonadism was also recently associated with MS (Fabian *et al*., 2016). Obesity has also been shown to adversely affect male fertility through peripheral conversion of testosterone to oestrogen and the inhibition of hypothalamic-pituitary-gonadal axis (Hammond *et al*., 2012; Fabian *et al*., 2015). This study is therefore aimed at understanding the effects of exposure to endocrine disrupting heavy metals on pituitary and gonadal hormones in normal weight auto-mechanics, with no component of MS in Ibadan.

MATERIALS AND METHODS

Participants: A total of 100 apparently healthy normal weight (BMI: 18.5-24.9 Kg/m^2) (WHO, 2000) male participants with no component of MS aged 18-64 years enrolled in this prospective cross-sectional study. Fifty automechanics, occupationally exposed to heavy metals from 5 mechanic villages in Bodija, Ibadan were matched for age, anthropometry and MS components with 50 students and staff from the University College Hospital, Ibadan with no occupational exposure to heavy metals (control). The auto-mechanics were from Bodija (11), Ring Road (14), Dandaru (9), Alalubosa (6) and Mokola (10) mechanic villages in Ibadan. Males with history of surgeries for undescended testis, varicocelectomy and orchiectomy were excluded from the study. The study protocol was approved by the University College Hospital Health Review Committee (NHREC/05/01/2008a). Informed consent was obtained from the participants before enrolment.

Gonadal Status: Participants were classified based on their reproductive hormone levels (Rey *et al*., 2012). Normal levels of LH, FSH and testosterone was classified as eugonadism, high levels of FSH, LH and normal testosterone was compensatory hypogonadism while high FSH, LH and reduced testosterone levels was hypergonadotrophic hypogonadism. Normal reference interval for hormones: testosterone=1.8-9.0 ng/mL, oestradiol $\leq$60 pg/mL, FSH=2-20 mIU/mL, prolactin=2-20ng/mL, LH=2-25mIU/mL.

Metabolic Syndrome Components: The Joint Interim Statement for MS was used to include only males with zero component of MS (blood pressure < 130/85mmHg, waist circumference < 94cm, triglyceride < 150mg/dL, fasting plasma glucose < 100mg/dL, HDL ≥ 40mg/dL) (Alberti *et al*., 2009).

Group A comprised of 49 eugonadic controls (normal FSH, LH and testosterone), group B comprised of 45 eugonadic auto-mechanics (normal FSH, LH and testosterone), group C comprised of 4 compensated hypogonadic auto-mechanics (high FSH, LH and normal testosterone) and group D comprised of 1 hypergonadotrophic hypogonadic auto-mechanic (high FSH, LH and normal testosterone). One of the controls was excluded due to elevated LH but normal testosterone levels (compensatory hypogonadism), thus reducing the number controls to 49.

Demography, Social Habits, Duration of Occupational Exposure, **Exercise, Sexual and Reproductive History**: Demographic indices (age, educational status, marital status, and parity), social habits (smoking, alcohol and drug usage), exercise, duration of occupational exposure to endocrine disrupting heavy metals, sexual and reproductive history (parity, libido, normal erection, early morning erection, and frequency of erection) were obtained from each participant using a pre-test semi-structured questionnaire.

Anthropometric Indices: Body weight, height, BMI, waist circumference, hip circumference, waist hip ratio (WHR), waist height ratio (WHR) and blood pressure (systolic and diatolic) were done according to methods described by Okoli *et al*. 2015.

Sample Collection: After an overnight fast, 15mL of venous blood was obtained aseptically by venipuncture from each participant. The blood obtained was then dispensed into tubes: fluoride oxalate tube (2mLs for fasting plasma glucose estimation), potassium ethylene diamine tetra-acetic acid tube (4mLs for plasma triglyceride and high-density lipoprotein cholesterol (HDLC) estimation), plain tube (5mLs for serum testosterone, oestradiol, FSH, LH, prolactin and total antioxidant capacity (TAC) estimations) and lithium heparin tube (4mLs for whole blood lead, mercury, arsenic and cadmium estimation). Plasma and serum were obtained by centrifuging at 500g for 5minutes and stored in small aliquots. All samples were stored frozen at -20^{0}C.

Biochemical Indices: Plasma glucose was estimated by glucose oxidase method (Dialab, Austria) (Barham and Trinder, 1972). Triglyceride was estimated using enzymatic method (Dialab, Austria) (Cole *et al*., 1997). HDL-C was estimated using enzymatic method (Dialab, Austria) (Frieldwald *et al*., 1972). FSH, LH and prolactin were determined using enzyme-linked immunosorbent assay (Bio-Inteco, UK) (Uotila *et al*., 1981). Oestradiol was determined by enzyme-linked immunosorbent assay (Bio-inteco, UK) (Tsang *et al*., 1980). Testosterone was determined by enzyme-linked immunosorbent assay (Dialab Austria) (Tsang *et al*., 1980). Lead, cadmium, mercury and arsenic were determined by atomic absorption spectrophotometer (Buck Scientific 210/211 VGP, Germany) (Koirtyoham 1980). TAC was measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999).

Statistical analysis

The statistical package for the social sciences (SPSS version 22.0) was used for all calculations and statistical analyses. Individual parameters were expressed as mean ± SD. Student's t-test and analysis of variance were used for comparison of quantitative variables while Chi Square test was used to find associations. Post hoc analysis (Fisher's least significant difference) was used for comparison of means within subgroups while multiple regression was used to find relationships. Data obtained were significant at $p < 0.05$.

RESULTS

Gonadal Status: Among the 50 males in the control group, 49 (98%) were eugonadic (Group A) while 1(2%) had compensatory hypogonadism (elevated LH but normal testosterone) and was excluded from the control group. Among the 50 auto-mechanics, 45 (90%) were eugonadic (Group B), 4 (8%) had compensatory hypogonadism (group C) while 1 (2%) had hypergonadotrophic hypogonadism (group D).

Demographic Characteristics and Social Habits between Auto-Mechanics and Controls: Table 1 shows comparisons of demographic characteristics and social habits in auto-mechanics and controls. There was a significant difference in the association lower educational status but increased parity of auto-mechanics than controls (p<0.001). Parity was significantly higher in automechanics compared with controls (p<0.001).

Duration of Occupational Exposure of Auto-mechanics, History and Frequency of Physical Exercise among Auto-Mechanics and Controls: Table 2 shows duration of occupational exposure of automechanics, associations/comparisons of number and hours at work, physical exercise, history and frequency between auto-mechanics and controls. All auto-mechanics (100%) were occupationally exposed to endocrine disrupting heavy metals for >6 years, out of which 47 (94%) were exposed for >10years.

Sexual History of Auto-Mechanics and Control: Table 3 shows an association of sexual history of auto-mechanics and controls. There were significant differences in the associations of frequency of early morning erection and libido, which were lower in auto-mechanics than controls (**P**<0.05).

Anthropometric Measures, Components of Metabolic Syndrome, Indicators of Obesity, Heavy Metals, Hormones and Oxidative Stress Indicator of Auto-Mechanics and Controls: Table.4 shows comparisons of anthropometric measures, components of MS, indicators of obesity, heavy metals, hormones and oxidative stress indicator of auto-mechanics and controls.

Table 1.
Comparison of Demographic Characteristics and Social Habits of Auto-Mechanics and Controls

Category	Indices	Auto-mechanics n (%)=50	Control n (%)=49	X^2	t	P
+Age (years)		40.78 ± 8.3	39.08 ± 8.0		1.034	0.300
Marital Status	Single	3 (6.0)	5 (8.2)	0.68		0.715
	Married	47 (94.0)	45 (91.8)			
Educational Status	No Formal Education	4 (8.0)	0 (0.0)			
	Primary School	13 (26.0)	1 (2.0)			
	JSC	5 (10.0)	0 (0.0)	76.88		<0.001*
	SSC	25 (50.0)	2 (4.1)			
	Graduate	3 (6.0)	33 (67.3)			
	Post Graduate	0 (0.0)	13 (26.5)			
Ethnic Group	Yoruba	46 (92.0)	41 (83.7)	9.28		0.319
	Others	4 (8.0)	8 (16.3)			
+Parity		2.98 ± 1.6	1.67 ± 0.8		4.9	<0.001*
Smoking	Yes	1 (2.0)	1 (2)	0.00		0.988
	No	49 (98.0)	48 (98)			
Alcohol	Yes	15 (30.0)	10 (20.4)	1.21		0.272
	No	35 (70.0)	39 (79.6)			

+ = values in mean ± SD, p<0.05 is considered statistically significant, * = significance, X^2 = chi-square, t = student t test, ***P*** = probability, JSC = junior school certificate, SSC = senior school certificate, controls = males without occupational exposure to heavy metals

Table 2.
Comparison of Duration of Occupational Exposure and Exercise History of Auto mechanics and Controls

Indices	Auto-mechanics n (%)=50	Control n (%)=49	X^2	T	P
Duration of Occupational Exposure					
<2 years	0 (0.0)				
2-5 years	0 (0.0)				
6-9 years	3 (6.0)				
≥10 years	47 (94.0)				
+Hours at Work/day	9.18 ± 0.3	7.82 ± 0.2		3.794	0.010*
+Days at Work/week	6.00 ± 0.1	5.22 ± 0.1		6.98	<0.001*
Physical Exercise					
Yes	32 (64.0)	33 (67.3)	0.12		0.735
No	18 (36.0)	16 (32.7)			
Exercise Frequency					
Daily	18 (56.3)	13 (39.3)			
Weekly	8 (25.0)	15 (45.5)	3.01		0.223
Occasionally	6 (18.7)	5 (15.2)			

*+ = values in mean ± SD, p<0.05 is considered statistically significant, * = significance, t = student t test, **P**= probability, duration of occupational exposure (years of exposure + hours of work/day + days of work/week) = duration of occupational exposure to endocrine disrupting heavy metals.*

Heavy metals (lead, cadmium and mercury) and hormones (testosterone, oestradiol, and FSH) levels were significantly higher in auto-mechanics compared with controls (**P**<0.05). There was a significant decrease in TAC levels in auto-mechanics when compared with controls (**P**<0.01). Prolactin, arsenic, LH, anthropometric measures (height, weight and hip circumference), components of MS (waist circumference, blood pressure, triglyceride, glucose and HDLC) and indices of adiposity (BMI, WHR and WHtR ratio) were not significantly different in auto-mechanics and controls (*p*>0.05).

Hormones, Heavy Metals and Total Antioxidant Capacity Based on Gonadal Status: Table 5 shows comparisons of hormones, heavy metals and TAC of auto-mechanics and controls based on gonadal status. Among the auto-mechanics, 45 (90%) were eugonadic, 4 (8%) had compensatory hypogonadism and 1 (2%) auto-mechanic was hypergonadotrophic hypogonadic. There were significant differences in levels of testosterone, oestradiol, FSH, LH, lead, cadmium, mercury and TAC within all groups (**P**<0.01). There was no significant difference in prolactin and arsenic levels within all groups (**P**>0.05)

Table 3.
Comparison of Sexual History of Auto-Mechanics and Controls

	Indices	Auto-mechanics; n =50 (%)	Control; n =49 (%)	X^2	P
Normal Erection	Yes	49 (98)	49 (100)	0.99	0.320
	No	1 (2)	0 (0)		
Early Morning Erection	Yes	46 (92)	49 (100)	4.09	0.043*
	No	4 (8)	0 (0)		
Erection Frequency	Daily	34 (68)	40 (81.6)		
	Weekly	7 (14)	5 (10.2)	2.73	0.255
	Occasionally	9 (18)	4 (8.2)		
Libido	Daily	12 (24)	23 (46.9)	8.733	
	Weekly	21 (42)	20 (40.8)		0.013*
	Occasionally	17 (34)	6 (12.2)		

***P**<0.05 is considered statistically significant, * = significance, X^2 = chi-square, **P** = Probability*

Table 4. Comparison of Anthropometric Measures, Components of Metabolic syndrome, Indicators of Obesity, Heavy Metals, Hormones and Oxidative Stress Indicator of Auto-Mechanics and Controls

	Indices	Auto-mechanics; n = 50	Controls; n = 49	t	*P*
Anthropometric measures	Height (m)	1.73 ± 0.1	1.73 ± 0.1	-0.025	0.980
	Weight (cm)	62.87 ± 6.0	64.33 ± 6.5	-1.164	0.247
	HC (cm)	91.9 ± 6.2	89.49 ± 7.7	1.708	0.091
Components of MS	SBP (mmHg)	115.38 ± 9.1	114.73 ± 6.1	0.412	0.681
	DBP (mmHg)	76.66 ± 11.3	76.69 ± 10.9	-0.015	0.988
	WC (cm)	79.05 ± 5.8	76.81 ± 6.7	1.799	0.078
	Glucose (mg/dL)	88.08 ± 13.7	90.1 ± 14.3	-0.706	0.482
	Triglyceride(mg/dL)	118.62 ± 10.5	118.12 ± 9.9	0.243	0.809
	HDL-C (mg/dL)	40.98 ± 8.9	42.59 ± 8.5	-0.923	0.358
Indicators of adiposity	BMI (kg/m^2)	21.14 ± 1.8	21.59 ± 1.7	-1.261	0.210
	WHR	0.86 ± 0.0	0.85 ± 0.0	1.093	0.277
	WHtR	0.46 ± 0.0	0.44 ± 0.0	1.853	0.067
Heavy metals	Lead (ug/dL)	8.48 ± 2.2	6.04 ± 0.7	5.201	< 0.001*
	Cadmium (ug/dL)	0.28 ± 0.1	0.24 ± 0.0	3.219	0.002*
	Mercury (ug/dL)	0.25 ± 0.1	0.19 ± 0.0	4.124	< 0.001*
	Arsenic (ug/dL)	0.05 ± 0.0	0.06 ± 0.0	-1.682	0.096
Hormones	Testosterone (ng/mL)	8.79 ± 2.5	6.89 ± 2.4	3.791	< 0.001*
	Oestradiol (pg/mL)	32.75 ± 11.5	28.84 ± 6.9	2.043	0.044*
	Prolactin (ng/mL)	19.77 ± 9.2	20.14 ± 7.7	-0.219	0.827
	FSH (mIU/mL)	10.81 ± 13.4	6.89 ± 2.4	3.791	0.045*
	LH (mIU/mL)	7.73 ± 3.9	6.59 ± 2.0	1.806	0.074
Oxidative stress indicator	TAC (umol/L)	866.56 ± 30.1	1086.11 ± 53.6	2	< 0.001*

*Values are in mean ± SD, p<0.05 is considered statistically significant, * = significance, t = student t test, p = p value, WC = waist circumference, HC = hip circumference, WHC = waist to hip ratio, WHtR = waist to height ratio, FSH = follicle stimulating hormone, LH = luteinizing hormone, MS = metabolic syndrome, TAC = total antioxidant capacity*

Hormones among the Groups Classified Based on Gonadal Status: Table 6 shows comparisons of hormones among the groups classified based on their gonadal status using post hoc test. Group B had significantly increased levels of testosterone and oestradiol compared with group A (**P**<0.001) while group D had significantly lower levels of testosterone and oestradiol compared with groups A, B and C (**P**<0.05). FSH and LH levels were significantly lower in group A compared with group C (**P**<0.01). FSH and LH levels were significantly higher in groups D compared with groups A, B and C (p<0.001). Testosterone levels in all groups except D were within normal reference interval while FSH levels in groups C and D were within the normal reference interval.

Comparison of Heavy Metals and Total Antioxidant Capacity among the Groups Classified Based on Gonadal Status: Table 7 shows comparisons of heavy metals between the different groups classified based on gonadal status using post hoc test. Auto-mechanic groups B, C and D had higher levels of heavy metals (lead, cadmium and mercury) compared with control group A (**P**<0.05). Group D had higher lead and mercury levels compared with groups A, B and C (**P**<0.001). TAC level was significantly lower in groups B, C, and D compared with group A (**P**<0.001). However, there was no significant difference in arsenic level among all groups (**P**>0.05).

Table 5. Comparison of Hormones, Heavy Metals and Total Antioxidant Capacity Based on Gonadal Status

Indices	A (n = 49)	B (n = 45)	C (n = 4)	D (n = 1)	*P*
Testosterone (ng/mL)	6.89 ± 2.4	8.94 ± 2.4	9.04 ± 0.8	0.56	< 0.001*
Oestradiol (pg/mL)	28.84 ± 6.9	33.23 ± 10.7	32.51 ± 17.4	9.40	0.002*
FSH (mIU/mL)	6.85 ± 2.4	7.10 ± 2.8	38.86 ± 24.1	65.60	< 0.001*
Prolactin (ng/mL)	20.14 ± 7.8	20.10 ± 9.3	18.67 ± 9.2	10.91	0.496
LH (mIU/mL)	6.59 ± 2.0	7.01 ± 2.3	11.85 ± 7.4	23.95	< 0.001*
Lead (ug/dL)	6.04 ± 0.7	8.18 ± 1.6	9.20 ± 1.2	19.42	< 0.001*
Mercury (ug/dL)	0.19 ± 0.0	0.24 ± 0.1	0.22 ± 0.0	0.76	< 0.001*
Cadmium (ug/dL)	0.24 ± 0.0	0.28 ± 0.1	0.31 ± 0.0	0.34	0.006*
Arsenic (ug/dL)	0.06 ± 0.0	0.05 ± 0.0	0.06 ± 0.0	0.04	0.262
TAC (umol/L)	1084.69 ± 53.2	867.31 ± 30.7	862.01 ± 26.3	858.06	< 0.001*

*values were in mean ±SD, **P**<0.05 is considered statistically significant, F = F statistics, FSH = follicle stimulating hormone, LH = luteinizing hormone, TAC = total anti-oxidant capacity, normal reference interval for Hormones; Testosterone=1.8-9.0ng/mL, oestradiol = <60pg/mL, FSH=2-20mIU/mL, Prolactin=2-20ng/mL, LH=2-25mIU/mL; eugonadic= normal testosterone, LH, FSH; hypergonadotrophic hypogonadism=high FSH, LH, low testosterone; compensated hypogonadism = high LH, FSH, normal testosterone; group A= Eugonadic controls, group B = eugonadic auto-mechanics, group C = compensated hypogonadic auto-mechanics, group D = hypergonadotrophic hypogonadic auto-mechanics.*

Table 6. Comparison of Hormones among the Groups Classified Based on Gonadal Status

Hormones	Group	Mean ± SD	Group	Mean ± SD	*P*
	A	6.89 ± 2.4	B	8.94 ± 2.4	< 0.001*
Testosterone	A	6.89 ± 2.4	C	9.04 ± 0.8	0.082
(ng/mL)	A	6.89 ± 2.4	D	0.56	< 0.001*
	B	8.94 ± 2.4	C	9.04 ± 0.8	0.937
	B	8.94 ± 2.4	D	0.56	< 0.001*
	C	9.04 ± 0.8	D	0.56	< 0.001*
Oestradiol	A	28.84 ± 6.9	B	33.23 ± 10.7	< 0.022*
(pg/mL)	A	28.84 ± 6.9	C	32.51 ± 17.4	0.447
	A	28.84 ± 6.9	D	9.4	0.005*
	B	33.23 ± 10.7	C	32.51 ± 17.4	0.873
	B	33.23 ± 10.7	D	9.4	0.001*
	C	32.51 ± 17.4	D	9.4	0.005*
FSH	A	6.85 ± 2.4	B	7.10 ± 2.8	0.811
(mIU/mL)	A	6.85 ± 2.4	C	38.86 ± 24.1	< 0.001*
	A	6.85 ± 2.4	D	65.60	< 0.001*
	B	7.10 ± 2.8	C	38.86 ± 24.1	< 0.001*
	B	7.10 ± 2.8	D	65.60	< 0.001*
	C	38.86 ± 24.1	D	65.60	< 0.001*
Prolactin	A	20.14 ± 7.8	B	20.13 ± 9.3	0.965
(ng/mL)	A	20.14 ± 7.8	C	18.67 ± 9.2	0.738
	A	20.14 ± 7.8	D	10.91	0.133
	B	20.10 ± 9.3	C	18.67 ± 9.3	0.752
	B	20.10 ± 9.3	D	10.91	0.137
	C	18.67 ± 9.2	D	10.91	0.292
LH	A	6.59 ± 2.0	B	7.01 ± 2.3	0.425
(mIU/mL)	A	6.59 ± 2.0	C	11.85 ± 7.4	< 0.001*
	A	6.59 ± 2.0	D	23.95	< 0.001*
	B	7.01 ± 2.3	C	11.85 ± 7.4	< 0.001*
	B	7.01 ± 2.3	D	23.95	< 0.001*
	C	11.85 ± 7.4	D	23.95	< 0.001*

P= significance, * = significance, FSH = follicle stimulating hormone, LH = luteinizing hormone, group A = eugonadic controls, group B = eugonadic auto-mechanics, group C = compensated hypogonadic auto-mechanics, group D = hypergonadotrophic hypogonadic auto-mechanics.

Multiple Regression of Hormones with Endocrine Disrupting Heavy Metals, Duration of Occupational Exposure to Endocrine Disrupting Heavy Metals and TAC in Auto Mechanics: Table 8 shows regression of hormones with endocrine disrupting heavy metals, duration of occupational exposure to endocrine disrupting heavy metals and TAC in auto-mechanics. There was an inverse relationship between lead and testosterone (**P**= 0.001) and a direct relationship between lead and FSH (**P**= 0.013) in the auto-mechanics. There was also a direct relationship between mercury and LH in the auto-mechanics (**P**= 0.031). TAC had an inverse relationship with LH (P<0.01). There was an inverse relationship between the hours at work per day and FSH (**P**= 0.033). However there was no relationship between the hormones and arsenic, number of years of occupational exposure and days spent at work in a week.

Table 7.
Comparison of Heavy Metals and Total Antioxidant Capacity among the Groups Classified Based on Gonadal Status

Heavy Metals	Group	Mean ± SD	Group	Mean ± SD	P
Lead	A	6.04 ± 0.7	B	8.18 ± 1.6	< 0.001*
(ug/dL)	A	6.04 ± 0.7	C	9.20 ± 1.2	< 0.001*
	A	6.04 ± 0.7	D	19.42	< 0.001*
	B	8.18 ± 1.6	C	9.20 ± 1.2	< 0.120
	B	8.18 ± 1.6	D	19.42	< 0.001*
	C	9.20 ± 1.2	D	19.42	< 0.001*
Mercury	A	0.19 ± 0.0	B	0.24 ± 0.1	< 0.001*
(ug/dL)	A	0.19 ± 0.0	C	0.22 ± 0.1	0.274
	A	0.19 ± 0.0	D	0.76	< 0.001*
	B	0.24 ± 0.1	C	0.22 ± 0.1	0.423
	B	0.24 ± 0.1	D	0.76	< 0.001*
	C	0.22 ± 0.0	D	0.76	< 0.001*
Cadmium	A	0.24 ± 0.0	B	0.28 ± 0.1	0.005*
(ug/dL)	A	0.24 ± 0.0	C	0.31 ± 0.0	0.055
	A	0.24 ± 0.0	D	0.34	0.033
	B	0.28 ± 0.1	C	0.31 ± 0.0	0.429
	B	0.28 ± 0.1	D	0.34	0.184
	C	0.31 ± 0.0	D	0.34	0.526
Arsenic	A	0.06 ± 0.0	B	0.05 ± 0.0	0.089
(ug/dL)	A	0.06 ± 0.0	C	0.06 ± 0.0	0.979
	A	0.06 ± 0.0	D	0.04	0.224
	B	0.05 ± 0.0	C	0.06 ± 0.0	0.515
	B	0.05 ± 0.0	D	0.04	0.466
	C	0.06 ± 0.0	D	0.04	0.318
TAC	A	1084.69 ± 53.2	B	867.31 ± 30.7	< 0.001*
(umol/L)	A	1084.69 ± 53.2	C	862.01 ± 26.3	< 0.001*
	A	1084.69 ± 53.2	D	858.06	< 0.001*
	B	867.31 ± 30.7	C	862.01 ± 26.3	0.818
	B	867.31 ± 30.7	D	858.06	0.768
	C	862.01 ± 26.3	D	858.06	0.914

***P**= Probability, * = significance, group A = eugonadic controls, group B = eugonadic auto-mechanics, group C= compensated hypogonadic auto-mechanics, group D = hypergonadotrophic hypogonadic auto-mechanics.*

Multiple Regression of Hormones with Endocrine Disrupting Heavy Metals, and TAC in controls: Table 9 shows regression of hormones with endocrine disrupting heavy metals in controls. There was no relationship between the hormones, the endocrine disrupting heavy metals and TAC.

DISCUSSION

Infertility is a problem of public health importance in Nigeria and many other developing nations. This is due to its high prevalence and its serious social implications on affected couples and families (Owolabi *et al*., 2013). Although little is known about the aetiology of the decline in male fertility, significant associations have been reported between impaired fertility and exposure to heavy metals (Abarikwu, 2013).

Significant increases in heavy metals (lead, cadmium and mercury) were observed when auto-mechanics were compared with controls (***P***<0.05). This is in tandem with other studies in Nigeria that reported significant increases in lead, cadmium, mercury and arsenic in auto-mechanics (Arinola and Akiibinu 2006; Utang *et al*., 2013). However, there was no significant increase in arsenic level in auto-mechanics compared with controls in this present study. Auto-mechanics engage in activities (including repairs of brakes and steering, repair of engines, spray painting, recharging of batteries, welding, soldering, electrical wiring) which could lead to the release of heavy metals to their environment (Aloysius *et al*., 2013).

Chemical toxicity ensuing from poor safety measures in handling and transporting chemical wastes had been reported (Anetor *et al*., 2009). This may be as a result of poor education and poor awareness of safety measures. In this present study, auto-mechanics had lower educational status than controls (***P***<0.01). Only 3 (6%) of the auto-mechanics were graduates while 46 (93.8%) of the controls were graduates. Moreover, auto-mechanics had more children than the controls (***P***<0.01). These findings are in consonance with a survey in Nigeria which reported low family planning practice in poorly educated couples resulting in increased parity (Gizaw and Regassa, 2011).

Table 8. Multiple Regression of Hormones with Endocrine Disrupting Heavy Metals, Occupational Exposure to Heavy Metals and TAC in Auto-Mechanics

Variable		Predictor constant	Beta	t	*P*
Testosterone	R^2 adj.=0.266, F=3.224, P=0.006	Lead	-0.623	-3.673	0.001*
		Mercury	0.050	0.311	0.757
		Cadmium	0.059	0.370	0.713
		Arsenic	0.120	0.885	0.382
		TAC	-0.085	-0.654	0.516
		Years of work	-0.070	-0.538	0.593
		Hours at work	-0.026	-0.191	0.850
		Days at work	-0.005	-0.035	0.972
Oestradiol	R^2 adj.=0.059, F=1.386, P=0.231	Lead	-0.208	-1.083	0.285
		Mercury	-0.064	-0.353	0.726
		Cadmium	0.028	0.154	0.878
		TAC	-0.156	-1.062	0.295
		Arsenic	0.069	0.451	0.655
		Years of work	0.039	0.265	0.792
		Hours at work	-0.270	-1.784	0.082
		Days at work	0.066	0.398	0.692
FSH	R^2 adj.=0.272, F=3.292, P=0.005	Lead	0.431	2.543	0.015*
		Mercury	0.218	1.357	0.182
		Cadmium	-0.108	-0.683	0.498
		Arsenic	-0.021	-0.155	0.878
		TAC	-0.123	-0.954	0.346
		Years of work	0.165	1.265	0.213
		Hours at work	-0.282	-2.117	0.040*
		Days at work	0.221	1.515	0.138
Prolactin	R^2 adj.=0.008, F=1.050, P=0.416	Lead	-0.022	-0.110	0.913
		Mercury	-0.108	-0.580	0.565
		Cadmium	0.181	0.981	0.332
		Arsenic	-0.016	-0.101	0.920
		TAC	-0.047	-0.313	0.756
		Years of work	-0.137	-0.897	0.375
		Hours at work	0.294	1.888	0.066
		Days at work	0.122	0.716	0.478
LH	R^2 adj.=0.335, F=4.086, P=0.001	Lead	0.200	1.236	0.224
		Mercury	0.361	2.356	0.023*
		Cadmium	0.155	1.029	0.310
		Arsenic	-0.170	-1.317	0.195
		TAC	-0.371	-3.001	0.005*
		Years of work	0.246	1.970	0.056
		Hours at work	0.097	0.765	0.449
		Days at work	-0.138	-0.987	0.329

*$p<0.05$ is considered statistically significantly, * = significance, t = student t test, p = p value, Beta = regression coefficient, LH=luteinizing hormone, FSH= follicle stimulating hormone*

A significant decrease in TAC in auto-mechanics when compared with controls was observed in this study ($p<0.01$). Okoli *et al*. (2015) showed significant decrease in TAC in auto-mechanics thus, implicating oxidative stress. This could be as a result of the induction of oxidative stress caused by endocrine disrupting heavy metals. Heavy metals have been reported to increase the production of ROS which can overwhelm the cells' intrinsic antioxidants (Ercal *et al*., 2001). It is possible that the observed low TAC in these auto-mechanics was due to the increased production of ROS resulting from the heavy metals they were exposed to in this study.

Oestradiol, testosterone and FSH levels were significantly increased in auto-mechanics when compared with controls in this study ($P<0.05$). However, these levels were within the normal reference interval. Positive correlations were observed between oestrogen and heavy metals (lead and mercury) (Agusa *et al*., 2007; Georgescu *et al*., 2011). It was postulated that increased oestrogen concentration could be due to oestrogen mimicry, displacement or competition with oestrogen binding; or interaction with oestrogen receptors by endocrine disrupting heavy metals-lead, cadmium, arsenic and mercury (Takiguchi and Yoshishara, 2006; Georgescu *et al*., 2011; Dyer 2007). Though oestradiol is involved in the development and maintenance of male fertility, it increases the concentration of SHBG (Kalme *et al*., 1999). This high level of SHBG is associated with arbitrary high level of total testosterone (Ronde *et al*., 2013).

Table 9
Multiple Regression of Hormones with Endocrine Disrupting Heavy Metals, and TAC in Controls

Dependent variable		Predictor Constant	Beta	T	P
Testosterone	R^2 adj.=0.010, F=1.692, P=0.378	Lead	0.208	1.357	0.182
		Mercury	0.090	0.603	0.550
		Cadmium	0.178	1.156	0.254
		Arsenic	-0.092	-0.617	0.540
		TAC	-0.010	-0.072	0.943
Oestradiol	R^2 adj.=0.064, F=0.421, P=0.831	Lead	-0.063	-0.398	0.693
		Mercury	0.108	0.697	0.489
		Cadmium	-0.056	-0.348	0.729
		Arsenic	0.016	0.106	0.916
		TAC	-0.143	-0.956	0.344
FSH	R^2 adj.=0.021, F=0.806, P=0.552	Lead	0.003	0.019	0.985
		Mercury	0.070	0.463	0.646
		Cadmium	-0.250	-1.597	0.118
		Arsenic	0.046	0.303	0.763
		TAC	0.130	0.885	0.381
Prolactin	R^2 adj.=0.069, F=0.376, P=0.862	Lead	-0.091	-0.569	0.572
		Mercury	0.075	0.482	0.632
		Cadmium	-0.086	-0.536	0.595
		Arsenic	0.099	0.641	0.525
		TAC	0.039	0.262	0.795
LH	R^2 adj.=0.068, F=1.704, P=0.154	Lead	-0.271	-1.827	0.075
		Mercury	0.205	1.408	0.166
		Cadmium	0.133	0.891	0.378
		Arsenic	-0.029	-0.201	0.842
		TAC	0.260	1.856	0.070

***P*<0.05 is considered statistically significantly, * = significance, t = Student t test, *P*= probability, Beta = regression coefficient, LH=luteinizing hormone, FSH= follicle stimulating hormone**

Total testosterone and not free testosterone was estimated in this study. There was an inverse relationship between hours spent at work in a week and FSH (p= 0.033). The reason for this is not clear. However, this relationship may be subtle as endocrinopathies were observed in the auto-mechanics. Future studies may identify these relationships in the different endocrinopathies.

Among the auto-mechanics, 90% were eugonadic, 8% had compensatory hypogonadism and 2% were hypergonadotrophic hypogonadic. Hypergonadotrophic hypogonadic auto-mechanic and compensatory hypogonadic auto-mechanics have testicular dysfunction. Queiroz and Waissmann (2006) reported that heavy metals have an adverse effect on spermatogenesis and testicular function. This report corroborates this present study as the levels of lead and mercury were significantly increased in these groups with testicular dysfunction compared with eugonadic auto-mechanics (p<0.05). Hypergonadotrophic hypogonadic auto-mechanic had significantly elevated levels of lead and mercury compared with eugonadic and compensatory hypogonadic auto-mechanic. This corroborates a report by Georgescu *et al*. (2011) that lead has inhibitory effects on testosterone at high level exposure.

Lead had an inverse relationship with testosterone (***P***= 0.001) but direct relationship with FSH (***P***= 0.013) in the auto-mechanics. LH had a direct relationship with mercury (β= 0.343, p= 0.031) but indirect relationship with TAC (p<0.001). These findings suggest that lead and mercury acting as endocrine disruptors may directly disrupt testicular function. It is probable that lead may affect Sertoli cells and disrupt spermatogenesis while mercury may affect Leydig cells leading to disruption of testosterone synthesis. Antioxidants in auto-mechanics may be overwhelmed by the increased oxidative stress leading to reduced TAC. High level of lipid peroxidation, DNA damage and apoptosis appear to play a role in testicular dysfunction (Agarwal *et al*., 2008).

In conclusion, increasing industrialization has been associated with a decline in fertility. The aetiology of the decline in male fertility is not well known but significant associations have been reported between impaired fertility and exposure to heavy metals. Observations from this study show that the auto-mechanics were occupationally exposed to endocrine disrupting heavy metals- lead, cadmium and mercury and not arsenic, which could lead to an alteration in the pituitary and gonadal hormones levels. Lead and mercury acting as endocrine disruptors may directly disrupt testicular function. It is probable that lead may affect sertoli cells leading to disruption of spermatogenesis while mercury may affect Leydig cells leading to disruption of testosterone synthesis. Antioxidants in auto-mechanics may be overwhelmed by the increased oxidative stress indicated by reduced TAC levels. Education of auto-mechanics on safety measures to reduce the exposure to endocrine disrupting heavy metals may be necessary. The use of protective clothing, proper handling and disposal of their wastes may be helpful in reducing the exposure to endocrine disrupting heavy metals.

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