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Original Research Article

Time Course of Lead-Induced Dyslipidemia in Male Wistar Rats

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Abstract

Purpose: Previous studies have linked lead toxicity to dyslipidemia. However, this has not been well characterized in a time course study. This study investigates the effects of lead exposure on lipid metabolism with time, using a rat model.
Method: Seventy-two (72) male Wistar rats were exposed to lead at concentrations of 0, 200, 300, and 400 ppm in their drinking water for 4, 8 and 12 weeks, after which blood, liver, kidney, brain, heart, spleen and lungs were removed from the animals and analyzed for lead and lipid dynamics.
Results: Lead-induced inhibition of reverse cholesterol transport was both time-dependent as well as dose-dependent at 4 and 8 weeks. Plasma free fatty acids (FFAs) displayed a hormetic-like response at 4 weeks and increased dose-dependently at 12 weeks while erythrocyte FFAs increased in the 200 ppm dose at 4 weeks and in all the doses at 8 weeks. Increased hepatic, brain and renal cholesterogenesis were generally observed with highest increases occurring at 8 weeks in both organs. Hepatic, brain, renal, cardiac and pulmonary phospholipidosis were observed in all the lead doses and exposures. Cardiac cholesterol decreased while triglycerides increased at 4 weeks. Increases in hepatic and brain cholesterogenesis were neither dose nor time-dependent. Correlation studies showed both direct and inverse correlations between tissue lead and various lipid parameters.
Conclusion: Lead exposure induced significant dyslipidemia and altered cholesterol metabolism over time, underscoring potential cardiovascular and metabolic risks.

Keywords: Lead, dyslipidemia, cholesterogenesis, free fatty acids, phospholipidosis, Hydoxymethylglutaryl coenzyme A reductase.

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INTRODUCTION

Lead has continued to attract a lot of research attention despite the successful phasing out of leaded gasoline worldwide. The health hazards posed by lead to humans and animals have remained a persistent cause for concern in both high-income countries (HIC) and lower- and middle-income countries (LMIC).[1,2,3] Lead

occupies the second position on the Agency for Toxic Substances and Disease Registry's (ATSDR) "Top 20 List", appearing next to arsenic.4 It is the most common cause of heavy metal poisoning and may be dispersed by air, industrial pollution, agricultural technology and food processing.[5] Lead has many undesired effects; it has been found to induce central nervous system toxicity, especially in the developing brain,[6] inhibit δ-aminolevulinic acid dehydratase (ALAD) activity in children and adults living around smelter areas,[7] cause disruptions in humoral and cellular immune responses,[8] associated with chronic kidney disease, and affect liver function.[9,10]

Exposure to Pb results mostly from anthropogenic activities such as mining and use of industrial materials - including paints, ceramics, and piping materials.[11] The major routes of exposure include ingestion, inhalation and dermal. Once lead is absorbed, it distributes across most soft tissues and accumulates over time in bones. Children are known to be more susceptible to the deleterious effects of lead than adults because they absorb more water-soluble Pb (40-50%) than adults (3-10%).[12]

Several studies have linked lead to dyslipidemia and cardiovascular diseases,[13-15] although results from rat experiments have not always shown a consistent trend due to variations in doses administered and duration of exposure. Experiments have shown that lead induces oxidative stress and lipid peroxidation, which can lead to dyslipidemia and altered membrane structure and function.[16] Such alterations in lipid metabolism are central to the development of metabolic disorders associated with lead exposure. Results from an epidemiological study by the National Health and Nutrition Examination Survey (NHANES) between1999 to 2018 found Pb to be associated with hyperlipidemia.[14] A similar study involving 426 males in the US showed blood Pb to be positively associated with total and HDL cholesterol.[17]

Despite extensive research on the systemic toxicity of lead, its specific impact on lipid metabolism over time has not been adequately studied. Therefore, this study aims to investigate the time-course of changes in lipid profiles of blood and various organs following exposure to graded doses of Pb in rats.

MATERIALS AND METHODS

Chemicals

Analytical kits used for the determination of cholesterol and triacylglycerols were products of Linear Chemicals (Cromatest), Montgat, Barcelona, Spain, and from Sigma Aldrich, Missouri, USA. Other chemicals used were of the purest grade obtainable.

Ethical approval

Ethical approval with reference number CMUL/HREC/05/23/1043 was obtained from the Animal Ethical Committee of the Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria. The study followed the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.[18]

Experimental design

Seventy-eight (78) male albino rats were used for this study. 6 animals served as baseline animals and were sacrificed at the beginning of the study. The remaining animals were divided into twelve groups of six animals each and housed in separate cages with normal controlled temperature of 22 ± 2°C and a regular 12-hour/12-hour light-dark cycle (06:00-18:00 h). They were allowed 2 weeks to acclimatize before the commencement of lead exposure. The animals were maintained on a standard pellet diet. Four groups were exposed to lead as lead acetate in their drinking water at concentrations of 0 ppm (control), 200 ppm, 300 ppm, 400 ppm for 4 weeks; another 4 groups were also exposed in like manner for 8 weeks, and another 4 groups for 12 weeks. Table 1 below shows the summary of the experimental design.

Table 1: Experimental design

Lead dose	Duration of exposure		
	4 weeks	8 weeks	12 weeks
0 ppm (control)	6 rats	6 rats	6 rats
200 ppm	6 rats	6 rats	6 rats
300 ppm	6 rats	6 rats	6 rats
400 ppm	6 rats	6 rats	6 rats

Control animals received distilled water over the same period of time. The animals were allowed free access to food and their respective distilled water (lead-free water for control and lead acetate in distilled water for lead-exposed animals). Body weight and water consumption was recorded daily. Water consumption was measured by weighing the canister and body weight was determined using a mechanical scale with an accuracy of ± 0.1 g. These data permitted the calculation of mean lead intake per animal in terms of mg Pb/week. Lead exposure index (expressed as weekly lead intake/rat) was calculated by the formula:

Water consumption (mL) × Pb concentration (ppm)

Collection of blood samples and organs

At the end of each exposure period, the rats were sacrificed by cardiac puncture under light ether anesthesia after an overnight fast. Blood was collected into heparinized tubes. The liver, brain, kidney, spleen, heart and lungs were quickly removed, washed in ice cold 1.15% KCl solution, blotted, weighed and stored at -20°C till further analyses. Aliquots of whole blood samples were preserved separately for lead analysis while the remaining blood samples were centrifuged at 4000 rpm for 10 minutes to separate plasma and red blood cells. The erythrocytes were washed 3 times with a wash buffer containing 20 mM Tris and 0.15 M NaCl, pH 7.6. All samples were stored at -20˚C until analysed.

Determination of lead concentration in blood and organs

Lead was analyzed in nitric acid digests of whole blood and tissues using atomic absorption spectrophotometer (AAS) (Buck Scientific AAS model 200, Connecticut, USA). Results were expressed as µg Pb/mL for blood and µg Pb/g wet weight for the organs.

Determination of plasma lipid and lipoprotein profiles

Total cholesterol and triacylglycerol concentrations in plasma were determined with the aid of commercial kits (Cromatest, Linear Chemicals, Montgat, Barcelona, Spain). High-density lipoprotein (HDL) cholesterol and triglycerides were also determined in plasma with the same commercial kits for total cholesterol and triacylglycerol after very low density lipoproteins (VLDL) and low density lipoproteins (LDL) were precipitated with heparin-MnCl2 solution.[19] Total phospholipids in plasma and HDL fraction were extracted with chloroform-methanol mixture (2:1, v/v) as described by Folch et al. (1957).[20] Phospholipid content was then determined using the method of Stewart (1979).[21] Briefly, an aliquot of the phospholipid extract was evaporated to dryness at 60°C. After cooling, 2 mL of chloroform was added to the dried lipid extract and vortexed. Ammonium ferrothiocyanate (2 mL) was then added and the mixture vortexed for 1 min. They were left to stand at room temperature for 10 min for the phases to separate. The chloroform layer was taken and absorbance read at 488 nm. Phospholipid concentrations were then determined using a phospholipid standard as reference. The plasma VLDL fraction was separated using the method of Ononogbu and Lewis (1976).[22] The method involved precipitation by sodium dodecyl sulphate (SDS). Extraction of lipids from the VLDL followed the procedure of Folch et al.,[20] using chloroform-methanol mixture (2:1, v/v). For cholesterol and triacyglycerol determinations in this extract, 0.1 mL of the VLDL extract was evaporated to dryness at 60ºC. The dried extracts were re-dissolved in 20 µL of a triton-X 100/chloroform mixture (1:1, v/v) and evaporated again as before. Thereafter, 1 mL of the cholesterol or triacylglycerol reagents was added to the dried extracts and vortexed. The rest of the procedure is as outlined for plasma cholesterol and triacylglycerol. Aliquot of 0.1 mL of the VLDL extract was also evaporated to dryness and taken through the same procedure for phospholipid determination in plasma. Free fatty acids (FFA) in plasma were determined according to the method of Brunk and Swanson (1981).[23] To labelled 5 mL tubes, 100 µL of plasma, standards (palmitic acid dissolved in n-hexane) and distilled water (blank) was added. A 300 µL of copper reagent and 2 mL chloroform were added to the tubes. The tubes were corked and vortexed for 10 min. The blue aqueous layer was removed carefully. To 500 µL of each chloroform layer was added 1 mL of cuprizone reagent (bis cyclo-hexylidene hydrazide) and shaken gently. Ammonia reagent (100 µL) was added to each tube, stopped and shaken briefly by hand. Exactly 10 min after adding the ammonia reagent, absorbance was read at 620 nm against a blank. The colour was stable for about 20 - 25 min. From the standard curve of palmitic acid, FFA concentration in the samples was calculated.

Assay for erythrocyte membrane lipid profile

Extraction of lipids from erythrocytes membrane was done using chloroform-isopropanol (7:11, v/v) mixture as described by Rose and Oklander (1965).[24] For the determination of cholesterol, an aliquot of the extract was evaporated to dryness at 60°C. Triton X-100/chloroform mixture (1:1, v/v, 20 µL) was added to resolve the lipids and again the solvent was evaporated. Then 1 mL of commercially available cholesterol kit reagent (Cromatest, Linear Chemicals, Montgat, Barcelona, Spain) was added and vortexed. After incubation in the dark at room temperature for 30 min, cholesterol content was determined.[25] For triacylglycerol determination, an aliquot of the extract was evaporated to dryness as in cholesterol determination and the rest of the procedure was as described for VLDL triacylglycerol. Determination of total phospholipids and free fatty acids in the chloroform-isopropanol extract of the

erythrocyte followed the same procedure as described for plasma.[21,23]

Isolation of erythrocyte ghost and determination of its lipid profile

Erythrocyte membranes were prepared within 24 hours of collection of blood using the method of Gratzer (1982).[26] Washed red blood cells (0.5 mL) were used in preparation of erythrocyte membranes. A 19.5 mL volume of cold lysis buffer (5 mM Tris-HCl buffer, pH 7.6) was added to the cells, vortexed and allowed to stand for 10 min before dividing into four 5 mL centrifuge tubes. The tubes were centrifuged at 20,000 rpm for 20 min and gently removed from the rotor and placed in an ice-bucket. The supernatant, which was dark-red at this stage, was gently removed with a Pasteur pipette. More ice-cold lysis buffer was added to the precipitate up to the 5 mL mark on the centrifuge tubes to re-dissolve the pellet, and the centrifugation was repeated. The supernatant was again removed as before. The washing procedure with the lysis buffer was repeated once more. At this stage the erythrocyte membranes were creamy white in colour. Finally, the resultant pellets were weighed and dissolved in 500 µL of cold Tris–HCl buffer, stored in Eppendorf tubes, and kept frozen at −20°C. Lipids were extracted from the membrane suspensions and determined as described for erythrocytes.

Determination of organ lipid profiles

Lipids were extracted from the organs (liver, kidney, brain, heart and lungs) of the rats by the method of Folch et al.[20] After washing of the homogenate with 0.05 M KCl solution, aliquots of the extract were used for the determination of cholesterol, using the procedure described for erythrocytes. Phospholipids were determined as described for plasma. Triacylglycerol concentrations in aliquots of the extracts were determined following the procedure described by Kriketos et al. (2003).[27]

Assay for hepatic and brain 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase activity

Assay for HMG-CoA reductase was done according to the method of Rao and Ramakrishnan (1975)[28] by measuring the hepatic and brain concentrations of HMG-CoA and mevalonate. The ratio of HMG-CoA to mevalonate is taken as an index of the activity of HMG-CoA reductase. An increase in this ratio indicates inhibition of cholesterogenesis while a decrease indicates enhanced cholesterogenesis.

Statistical analysis

Data were expressed as mean ± SD. Data were analyzed by One way analysis of variance (ANOVA) followed by Tukey's post hoc test, with $p < 0.05$ considered significant. Relationships among the parameters were assessed by Spearman's correlations. All analyses were done using Statistical Package for Social Sciences (SPSS) version 20.

RESULT AND DISCUSSION

Lead exposure indices

Table 2 depict the indices of lead (Pb) exposure. Total Pb ingested by each animal increased dose-dependently across all the exposures (4, 8 and 12 weeks).

Table 2: Total Pb intake (mg) of each animal after 4, 8 and 12 weeks of Pb exposure

	4-week Pb Exposure Lead dose			**8-week Pb Exposure Lead dose**			**12-week Pb Exposure Lead dose**		
Parameter	200 ppm	300 ppm	400 ppm	200 ppm	300 ppm	400 ppm	200 ppm	300 ppm	400 ppm
Total Pb intake (mg)	107.19	152.80	249.26	269.23	427.95	540.87	397.85	633.36	809.71
Mean Pb intake /week ± SD (mg)	26.80 ± 5.87	38.20 ± 4.68	62.32 ± 2.29	37.40 ± 2.44	53.49 ± 1.83	67.61 ± 3.27	33.15 ± 3.75	52.78 ± 4.56	67.48 ± 5.27

Tissue lead (Pb) concentration

Tissue lead (Pb) concentration is depicted in Table 3. After four weeks of lead exposure, rats accumulated Pb in a dose-dependent manner (30.77 ± 10.00, 33.97 ± 12.08, and 43.52 ± 13.02 µg/dL at 200, 300, and 400 ppm, respectively). After 8 weeks of lead exposure, mean blood Pb concentration was no longer dose-dependent, rather the 300 ppm dose group accumulated the highest blood Pb, while at 12 weeks, the 200 ppm dose group accumulated the highest blood Pb. Blood Pb concentration tended towards dose-dependence only at 4 weeks of exposure.

Table 3: Lead concentrations in the tissues of the animals

Group	Control	200 ppm	300 ppm	400 ppm
		Blood (µg/dL)		
0 weeks	1.93 ± 0.05^{a}	1.93 ± 0.05^{a}	1.93 ± 0.05^{a}	1.93 ± 0.05^{a}
4 weeks	0.81 ± 0.49^{b}	30.77 ± 10.00^{d}	33.97 ± 12.08^{e}	43.52 ± 13.02^{g}
8 weeks	3.68 ± 0.08^{a}	21.28 ± 14.66^{c}	69.19 ± 21.33^{i}	54.14 ± 9.63^{h}
12 weeks	1.74 ± 0.52^{a}	56.64 ± 14.45^{h}	39.61 ± 1.10^{f}	42.09 ± 3.37^{f}
		Liver (µg/g wet weight)		
0 weeks	0.43 ± 0.07^{a}	0.43 ± 0.07^{a}	0.43 ± 0.07^{a}	0.43 ± 0.07^{a}
4 weeks	0.37 ± 0.05^{a}	1.67 ± 0.48^{d}	2.37 ± 0.37^{e}	3.61 ± 1.11^{f}
8 weeks	0.96 ± 0.04^{b}	0.83 ± 0.03^{b}	3.54 ± 1.10^{f}	5.73 ± 2.01^{g}
12 weeks	1.25 ± 0.38^{c}	3.56 ± 1.44^{f}	5.92 ± 1.30^{g}	0.53 ± 0.33^{a}
		Kidney (µg/g wet weight)		
0 weeks	0.78 ± 0.08^{a}	0.78 ± 0.08^{a}	0.78 ± 0.08^{a}	0.78 ± 0.08^{a}
4 weeks	1.36 ± 0.06^{a}	17.26 ± 3.80^{f}	24.83 ± 6.89^{g}	31.04 ± 6.72^{h}
8 weeks	1.56 ± 0.88^{a}	5.81 ± 2.65^{b}	9.30 ± 2.55^{d}	10.56 ± 3.28^{e}
12 weeks	0.98 ± 0.31^{a}	5.80 ± 2.84^{b}	5.14 ± 1.66^{b}	7.05 ± 2.11^{c}
		Brain (µg/g wet weight)		
0 weeks	BDL	BDL	BDL	BDL
4 weeks	BDL	3.07 ± 0.94^{a}	3.93 ± 1.64^{b}	2.89 ± 0.50^{c}
8 weeks	BDL	2.33 ± 0.80^{d}	2.53 ± 0.58^{e}	0.45 ± 0.01^{f}
12 weeks	BDL	1.24 ± 0.29^{g}	1.40 ± 0.21^{h}	1.03 ± 0.02^{i}
		Heart (µg/g wet weight)		
0 weeks	BDL	BDL	BDL	BDL
4 weeks	BDL	BDL	BDL	BDL
8 weeks	BDL	BDL	BDL	BDL
12 weeks	BDL	3.97 ± 1.96^{a}	4.02 ± 0.99^{a}	3.11 ± 0.92^{b}
		Lungs (µg/g wet weight)		
0 weeks	BDL	BDL	BDL	BDL
4 weeks	BDL	BDL	BDL	BDL
8 weeks	BDL	BDL	BDL	BDL
12 weeks	BDL	0.36 ± 0.01^{a}	3.22 ± 1.14^{c}	2.57 ± 0.49^{b}
		Spleen (µg/g wet weight)		
0 weeks	BDL	BDL	BDL	BDL
4 weeks	BDL	BDL	BDL	BDL
8 weeks	BDL	BDL	BDL	BDL
12 weeks	BDL	BDL	BDL	BDL

BDL = Below Detection Limit
Value represents the mean ± standard deviation (SD), n = 6. Values within the same organ with different alphabets are significantly different at $p < 0.05$.

Liver lead increased dose-dependently at 4 and 8 weeks, while at 12 weeks, 400 ppm lead dose accumulated the lowest lead concentration (0.53 ± 0.33 µg/dL) which was about 40% of control (1.25 ± 0.38 µg/dL). There was a dose-dependent accumulation of lead in the kidney of the animals at 4 and 8 weeks of lead exposure, while lead decreased with time from 4 to 12 weeks in the 300 and 400 ppm doses. Brain lead concentration was below detection limit (BDL) in baseline animals (0 week) and in all control animals. Afterwards all the doses quickly accumulated brain lead at 4 weeks which then decreased with time from 4 to 12 weeks in the 200 and 300 ppm doses while 400 ppm dose accumulated the lowest lead concentration (0.45 ± 0.01 µg/g) at 8 weeks of exposure. Heart lead was BDL at 4 and 8 weeks and in all controls. Lead was detected in the heart only after 12 weeks of exposure and it was not dose-dependent, with the 300 ppm dose group accumulating the highest Pb (4.02 ± 0.99 µg/g). The same pattern of lead accumulation in the heart was observed in the lungs where lead only accumulated after 12 weeks of exposure, with the 300 ppm dose group accumulating the highest Pb (3.22 ± 1.14 µg/g). Spleen lead was BDL in all the doses and exposure time.

Environmental and occupational pollution is often associated with the accumulation of heavy metals such as lead which are resistant to biodegradation. The results of the present study showed lead

accumulation in the organs in the following order: kidney > liver > brain > heart and lungs. The presence of Pb in these organs correlates with the presence of lead-binding proteins in several organs.[29,30] The role of these low molecular weight proteins is to sequester Pb in a non-toxic form in these organs. A previous study detected the presence of these proteins in the kidneys of lead-exposed rats but not in control animals,[31] implying that these proteins were inducible. Further work by Egle and Shelton (1986)[32] discovered these proteins termed p32/6.3 constitutively present in the brain of adult rats. Lead binding proteins therefore play a crucial role in determining the bioavailability of lead and explains the differences in the ability of organs to accumulate lead and display toxic effects of lead. An explanation for the decrease in blood and kidney lead concentration with time observed in this study could be due to increased sequestering of Pb by the bone and uptake by other organs such as brain, heart and lungs. Bone lead is known to be the best index for long term exposure[33] although bone lead was not assayed in this work.

Effect of lead on plasma lipid and lipoprotein profiles

A crucial finding of this study was that lead induced perturbation in the metabolism of lipids in different compartments of the rats. Figure 1 (A-I) shows the effect of lead (Pb) on plasma lipid and lipoprotein (HDL and VLDL) profiles of rats. Plasma total cholesterol (TC) was marginally increased above control in all the doses at 4 weeks (Figure 1A). This increase was highest in the 300 ppm dose (8%) followed by the 400 ppm dose (5%) and finally the 200 ppm dose (4.5%). At 8 weeks, an increase in plasma TC was observed in the 200 ppm (10%) but no significant difference ($p < 0.05$) in plasma TC in the 300 and 400 ppm doses compared to the control. After 12 weeks of lead exposure, plasma TC decreased in all the doses compared to control. Hypertriglyceridemia heralded lead exposure in all the durations except in the 200 ppm 8 - and 12 - week exposures, however these increases were not dose-dependent (Figure 2B). The highest accumulation of triglycerides was observed in the 400 ppm dose in all the durations of exposure. Significant dose-dependent decreases ($p < 0.05$) in mean plasma phospholipids in all the doses were observed at 4 and 8 weeks (Figures 1C). At 12 weeks, decreases in phospholipids in the 200 and 300 ppm doses amounted to 20 and 13%, respectively however there was no significant difference ($p > 0.05$) in the 400 ppm dose compared to control.

Plasma HDL cholesterol decreased in a time-dependent manner from 4 to 12 weeks in the 200 and 300 ppm doses but in the 400 ppm dose, the decrease was only time-dependent from 4 to 8 weeks (Figure 1D). Decreases were dose-dependent at 4 and 8 weeks while at 12 weeks, the concentration of plasma HDL cholesterol in all three doses stood at about 55% of control. The result of plasma HDL triglycerides in the rats is presented in Figure 1E. At 4 weeks, there was no significant difference ($p > 0.05$) in HDL triglycerides in 300 and 400 ppm doses compared to control, however, a 10% increase in the 200 ppm dose was observed. At 8 and 12 weeks, fluctuations in HDL triglycerides were observed in the doses compared to control. At 4 weeks, HDL phospholipids concentrations in the 300 and 400 ppm doses were not significantly different from control (Figure 1F), however, a marginal increase was observed in the 200 ppm dose. After 8 weeks of lead exposure, all three doses showed significant ($p < 0.05$) decreases in HDL phospholipids compared to control, with lowest value occurring in the 300 ppm dose (55% of control) and highest value in the 400 ppm dose (77% of control). This trend was sustained at 12 weeks, with the lowest value of HDL phospholipids occurring in the 200 ppm dose (82% of control) and the highest value in the 400 dose group. At 4 weeks of lead exposure, there was no significant difference in VLDL cholesterol concentration between 300 ppm and control but 200 and 400 ppm doses increased by 31 and 13%, respectively (Figure 1G).

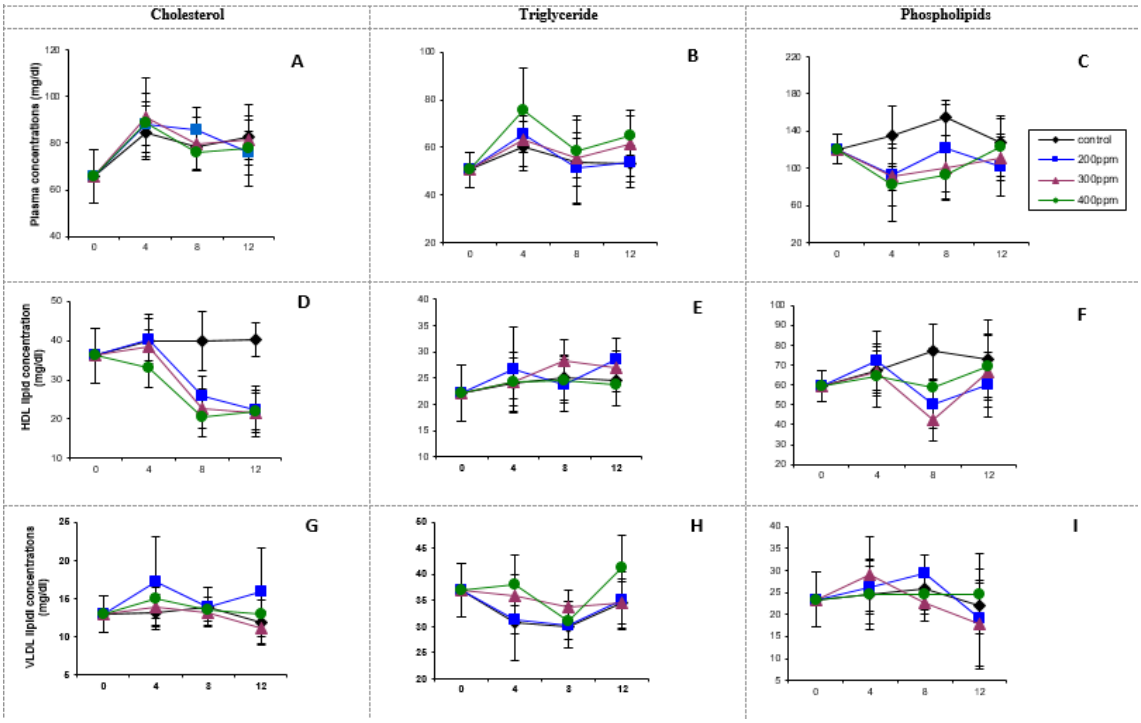

Figure 1: Effect of Pb on Plasma lipids (cholesterol, triglyceride, and phospholipid) and lipoprotein (HDL, and VLDL) concentrations in rats plotted against time course in weeks (0, 4, 8 and 12 weeks). Each point in the graphs represents mean ± standard deviation (SD), n = 6.

A trend reversal at 8 weeks resulted in no significant difference ($p > 0.05$) between all three doses and control. After 12 weeks of exposure, significant increases of 34 and 10% were again observed in the 200 and 400 ppm doses, respectively with no significant difference in VLDL cholesterol concentration in the 300 ppm dose. Mean VLDL triglyceride concentration of the 300 and 400 ppm doses increased significantly at 4 and 8 weeks compared to the controls (Figure 1H). At 12 weeks however, there was no significant difference between 200 and 300 ppm doses and control while 400 ppm dose increased by 20%. At 4 weeks, VLDL phospholipids of the 200 and 300 ppm Pb doses increased by 7 and 19%, respectively while there was no significant difference ($p > 0.05$) in the 400 ppm dose (Figure 1I). At 8 weeks, the observed increase in VLDL in the 200 ppm dose was sustained with time and then decreased at 12 weeks. VLDL phospholipids in the 300 and 400 ppm doses likewise exhibited fluctuations between 8 and 12 weeks of exposure.

Effect of lead on erythrocyte and erythrocyte membrane lipid profiles

The effect of lead (Pb) on erythrocyte and erythrocyte membrane lipid profiles (cholesterol, triglyceride and phospholipid concentrations) are shown in Figure 2(A-F). At 4 weeks of lead exposure, erythrocyte cholesterol concentrations of the 200 and 400 ppm doses increased by 16 and 18%, respectively while there was no significant change in the 300 ppm dose compared to the control (Figure 2A). At 8 weeks of lead exposure, a reversal of trend resulted in 33 and 13% decrease in erythrocyte cholesterol concentration in the 200 and 300 ppm doses, respectively while no significant difference was observed in the 400 ppm dose.At 12 weeks of exposure, erythrocyte cholesterol concentration increased in the 200 ppm dose and decreased in the 400 ppm dose. Mean erythrocyte triacylglycerol concentration of the 200 ppm dose was consistently higher than that of the control at 4 weeks (32%), 8 weeks (6%) and 12 weeks (21%) of lead exposure (Figure 2B). This increase was also sustained in the 300 and 400 ppm doses at 4 and 12 weeks while at 8 weeks, a decrease of 9% was observed in the 300 and 400 ppm doses. Erythrocyte phospholipids exhibited up-down fluctuations in all the doses across the exposures (Figure 2C).

Erythrocyte membrane cholesterol concentration in the 200 ppm dose group was consistently lower than control at 4, 8 and 12 weeks (15, 15 and 20%, respectively) (Figure 2D). However, in the 300 ppm dose group, there was 39 and 23% increase in erythrocyte membrane cholesterol concentration above that of the control at 4 and 12 weeks while there was no significant difference in erythrocyte membrane cholesterol concentration at 8 weeks.

On the other hand, the 400 ppm dose group displayed a 49 and 36% decrease in erythrocyte membrane cholesterol concentration compared to the control at 4 and 8 weeks of lead exposure, while there was no significant difference compared to control value at 12 weeks. Mean erythrocyte membrane triglyceride concentrations at 4 and 8 weeks were significantly increased ($p < 0.05$) in all three doses, but the increases were not dose-dependent (Figure 2E). After 12 weeks of lead exposure, there was no significant difference in erythrocyte membrane triacylglycerol concentration in the 200 ppm dose, while 300 and 400 ppm doses increased by 31 and 79%, respectively.

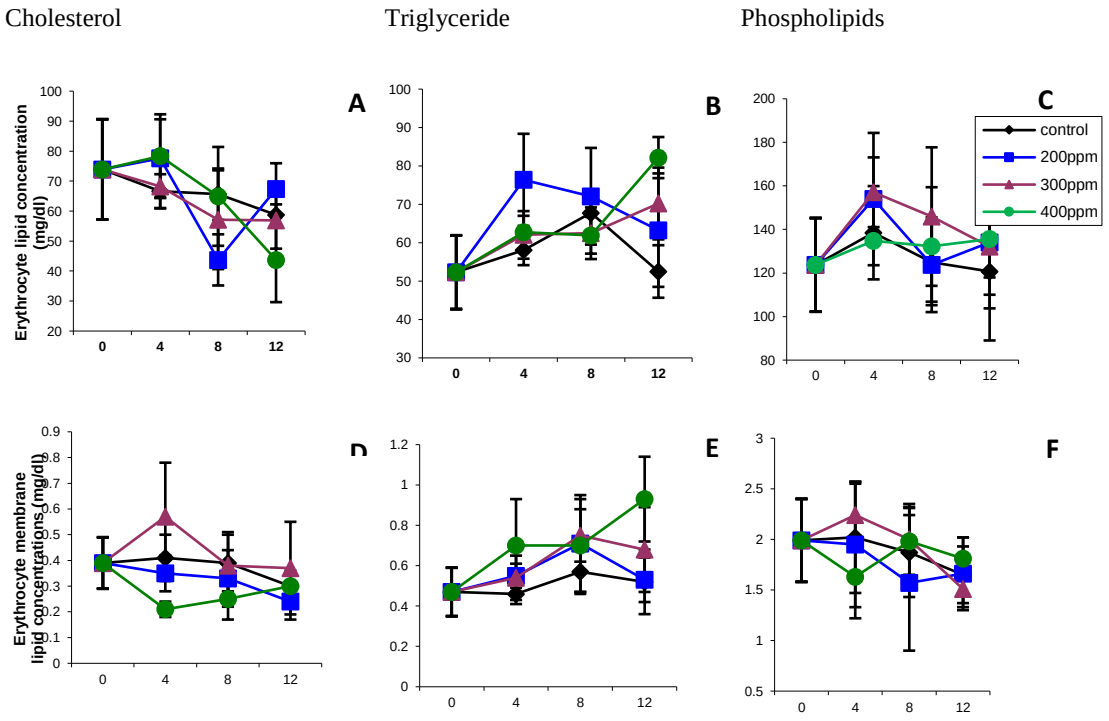

Figure 2: Effect of Pb on erythrocyte and erythrocyte membrane lipids (cholesterol, triglyceride, and phospholipid) concentrations in rats plotted against time course in weeks (0, 4, 8 and 12 weeks). Each point in the graphs represents mean ± standard deviation (SD), n = 6.

There was no significant difference in erythrocyte membrane phospholipids concentration between the 200 ppm dose group and the control group but there was an increase of 11% in the 300 ppm dose group and a decrease of 19% in the 400 ppm dose group (Figure 2F). At 8 weeks, a decrease of 16% was observed in the 200 ppm dose group, while the 300 and 400 ppm dose groups displayed only marginal increases. At 12 weeks, there was no significant difference in erythrocyte membrane phospholipid concentration in the 200 ppm dose group and the control group, while the erythrocyte membrane phospholipid concentration in the 300 ppm dose group decreased by 9%, and increased by 10% in the 400 ppm dose group.

The erythrocytes are non-excitable cells responsible for the distribution of lead throughout the body.[34] Alterations in chemical and physical characteristics of erythrocyte membrane in lead exposure have been reported.[35,36] Investigators have attributed these effects to a modification in membrane lipid composition36 while others have associated them with interference of Pb with the activity of membrane-bound enzymes such as Ca2+-Mg2+ ATPase.3,[37] In the present study, erythrocyte and erythrocyte membrane cholesterol/phospholipid ratios were distorted in the lead-dosed animals with the ratio favouring increased membrane fluidity in most of the lead-exposed groups. In the study of Quintanar-Escorza and his team,[38] microscopic images of erythrocytes obtained from lead-exposed workers and control revealed dramatic changes in erythrocyte shapes in lead-exposed workers.

Effect of lead on plasma and erythrocyte free fatty acid (FFA) concentrations

The effects of lead (Pb) on plasma and erythrocyte FFAs are presented in Figure 3. At 4 weeks, there was a decrease of 51% in plasma FFA in the 200 ppm dose group while a 2-fold and a 1.5-fold increase were observed in the 300 and 400 ppm doses, respectively (Figure 3A). Conversely, decreases were observed across board at 8 weeks, amounting to 20, 54 and 37% in the 200, 300 and 400 ppm doses, respectively. At 12 weeks, the trend was reversed and dose-dependent increases in plasma FFAs concentrations were observed across board amounting to 11, 14 and 30% in the 200, 300 and 400 ppm doses, respectively. An increase of 9% and a decrease of 6% in mean erythrocyte FFA was observed in the 200 and 300 ppm doses, respectively at 4 weeks while there was no significant difference in the 400 ppm dose (Figure 3B). At 8 weeks, increases were observed in all the doses (7% in the 200 and 300 ppm doses and 18% in the 400 ppm dose). At 12 weeks, there was no significant difference in erythrocyte FFAs in the 200 and 400 ppm doses while 300 ppm decreased by 16%.

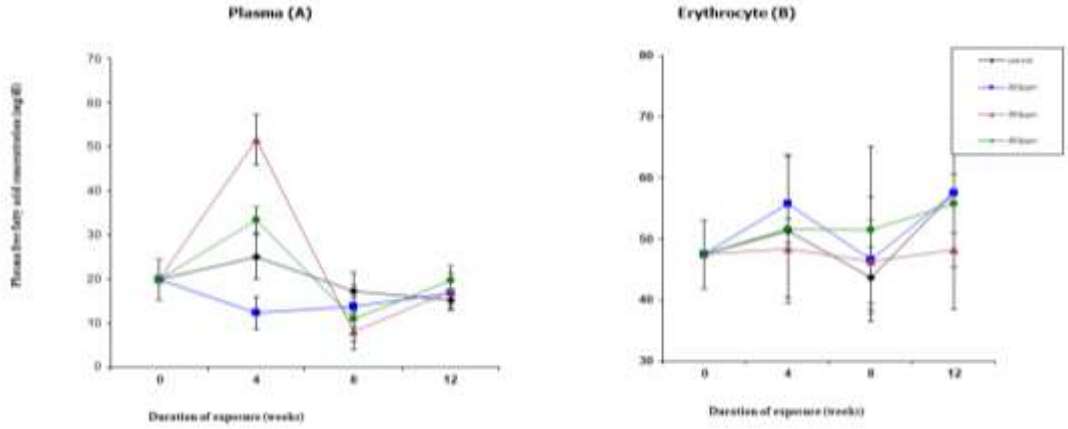

Figure 3: Effect of Pb on free fatty acid concentrations in plasma (A) and erythrocyte (B) of rats plotted against time course in weeks (0, 4, 8 and 12 weeks). Each point in the graphs represents mean ± standard deviation (SD), n = 6.

Lead-induced lipid alterations primarily occur through the mechanism of oxidative stress. Lead exposure can trigger the generation of reactive oxygen species (ROS); superoxide radicals (O2•−), singlet oxygen (1O2), lipid peroxides, hydroxyl radicals (•OH), and hydrogen peroxide (H2O2), which disrupt cellular redox homeostasis, overwhelming the cell's antioxidant defenses, leading to lipid peroxidation. This process damages cell membranes and disrupts lipid metabolism, contributing to the toxic effects of Pb.[39,40] Generation of ROS, a byproduct of normal cellular metabolism also goes on in the cell unrelated to Pb exposure and this further undermines the antioxidant defences of the cell, leading to heightened oxidative stress and all its attendant pathological outcomes such as mitochondrial dysfunction, DNA damage, telomere shortening, lipid peroxidation, and protein oxidative modification, all of which trigger apoptosis and senescence.[41,42] Hence, establishing the time-dependent factor in the outcome of lead toxicity and dyslipidemia.

In this study, the high plasma level of FFAs observed in the animals after 4 and 12 weeks of lead exposure may be an offshoot of hydrolysis of triglycerides and subsequent release of FFAs into the plasma. Much of this hydrolysis occurs in the adipose tissue and is catalyzed by a triglyceride lipase distinct from lipoprotein lipase which occurs outside the adipose tissue cell.[43] Increased plasma FFA concentration has also been observed in other conditions such as obesity, insulin resistance, non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, and related comorbidities such as cardiovascular disease,[44] confirming previously established correlations between exposure to lead and some of these diseases.[33,40] FFA is also known to be modulated with age as observed in this study. Corvilain et al. (1961)[45] first noted a similar high FFA in children up to the age of 10 years. This finding has long been confirmed by other workers.[46] From their data, Corvilain et al.

suggested that the abrupt fall in the FFA level occurred between the 10th and 18th year in humans. The reason for the fall in FFA during adolescence has been attributed to endocrine changes in adolescence, particularly as fat metabolism is to a great extent under hormonal control. Hence the decrease in plasma FFAs with time in controls in this study may be as a result of sexual maturation. Also the spike in FFA concentration by the 200 ppm dose at 4 weeks in this study shows a biphasic response which may suggest a possible hormetic effect, although this was not statistically validated in the present study. Hormesis is a response characterized by a low dose stimulation and a high dose inhibitory effect of an environmental agent.[47] The response of the cell or organism to the low dose of the toxin is considered an adaptive compensatory process following an initial disruption in homeostasis.

The physiological consequences of this elevated plasma FFAs could be diverse and could be viewed from the metabolic roles of FFAs. The major source of energy in many tissues including the liver, heart, kidney, muscle, lung, testis, brain and adipose tissue are FFAs, although the brain cannot extract them from the blood.[48] They are also the substrates for triglyceride synthesis in the liver.[49] Thus, the increased plasma FFAs in the lead-exposed animals indicates that hepatic uptake of these lipids may have been inhibited by Pb, hence compromising energy metabolism (production) in this organ.

Effect of lead (Pb) on organ lipid profiles

The effect of lead (Pb) on organ cholesterol, triglycerides, and phospholipid concentrations in the organs are presented in Figures 4 and 5. At 4 weeks of Pb exposure, liver cholesterol concentration in the 200 and 400 ppm doses increased by 12 and 11%, respectively, however there was no significant difference in cholesterol concentration in the 300 ppm dose (Figure 4A). At 8 weeks, all three doses increased significantly ($p < 0.05$) with the highest increase recorded in the 400 ppm dose (94%), followed by 200 ppm (41%) and 300 ppm dose (39%). After 12 weeks of lead exposure, liver cholesterol in the 200 and 300 ppm doses increased by 28 and 32%, respectively, while 400 ppm dose decreased to 7.5% below control. At 4 weeks of lead exposure, kidney cholesterol concentration increased in all three doses but not dose-dependently with the highest increase observed in 300 ppm dose (18%), followed by 200 ppm dose (16%) and the 400 ppm dose (11%) (Figure 4B). At 8 weeks, the increase was maintained only in the 200 ppm dose (10%), while 400 ppm dose decreased by 18%. There was no significant difference in kidney cholesterol concentration in the 300 ppm dose at 8 and 12 weeks but 200 and 400 ppm doses increased by 16 and 18%, respectively at 12 weeks. After 4 weeks of lead exposure, brain cholesterol concentration increased by 21% in the 200 ppm dose, 33% in the 300 ppm dose and 16% in the 400 ppm dose (Figure 4C). This increase was sustained through 8 weeks although it was not dose-dependent (57, 54 and 25% in the 200, 300, and 400 ppm doses, respectively). At 12 weeks, there was no significant difference in brain cholesterol concentration and control in the 400 ppm dose, while the brain cholesterol concentration in the 200 and 300 ppm doses increased by 49 and 26%, respectively.

Mean liver triglyceride concentration in the 300 ppm dose group decreased by 12% but there was no significant difference in the 200 and 400 ppm doses and control at 4 weeks (Figure 4D). After 8 weeks of lead exposure, liver triglyceride concentration decreased by 8 and 15% in the 200 and 300 ppm doses, respectively, while 400 ppm dose increased by 15%. After 12 weeks of lead exposure, liver triglyceride concentration in all three doses decreased but not dose-dependently (16, 14 and 8% in the 200, 300 and 400 ppm doses, respectively). Mean kidney triglyceride concentration increased in the 200 and 300 ppm doses at 4 weeks (Figure 4E). This increase amounted to 32 and 18%, respectively while in the 400 ppm dose group, kidney triglyceride concentration decreased by 6%. At 8 weeks of lead exposure, there was no significant difference in kidney triglyceride concentrations in the 200 and 400 ppm doses, while in the 300 ppm dose group, kidney triglyceride concentration increased by 17%. After 12 weeks of lead exposure, kidney triglyceride concentration in the 200 ppm dose increased by 9% while there was no significant difference in kidney triglyceride concentration between the 300 and 400 ppm doses and control. At 4 weeks of lead exposure, brain triglyceride concentration increased by 66% in the 200 ppm dose but decreased by 17% in the 300 ppm dose (Figure 4F). However, there was no significant difference in brain triglyceride concentration between the 400 ppm dose and control. At 8 weeks, there were decreases of 23 and 26% in the 200 and 300 ppm doses, respectively while there was no significant difference between 400 ppm dose and control. After 12 weeks of lead exposure, there was significant decrease of 33% in the 200 ppm dose while a reversal of trend led to a 22 and 12% increase in the 300 and 400 ppm doses, respectively.

The effect of lead (Pb) on liver, kidney, and brain phospholipid concentrations are shown in Figure 4(G-I). Liver phospholipids increased in all three doses by 22, 30 and 6% in the 200, 300, and 400 ppm doses, respectively at 4 weeks (Figure 4G). This trend was sustained at 8 weeks with highest increase observed in the 300 ppm dose (27%), followed by 200 ppm dose (23%), and 400 ppm dose (1.23%). At 12 weeks of lead exposure, there was no significant difference in liver phospholipids concentration between control and 400 ppm doses, while liver phospholipids concentration in both 200 and 300 ppm doses increased by 24 and 25%, respectively. Mean kidney phospholipids concentration increased in the 200 and 300 ppm doses by 38 and 32%, respectively at 4 weeks of lead exposure (Figure 4H), however there was no significant difference in kidney phospholipids concentration between the 400 ppm dose group and the control. After 8 weeks of lead exposure, kidney phospholipids concentration in the 200 and 300 ppm doses also increased by 17 and 26%, respectively while in the 400 ppm dose group, it increased marginally by 3.41%. After 12 weeks of exposure, kidney phospholipids concentration in all three doses increased (32, 31, and 23% in the 200, 300, and 400 ppm doses, respectively) although the increases were not dose-dependent. At 4 weeks of lead exposure, increases of 42, 48 and 21% in brain phospholipids were observed in the 200, 300 and 400 ppm doses, respectively (Figure 4I). This increase was sustained through 8 and 12 weeks, amounting to 27%, in 200 and 300 ppm doses and 8% in 400 ppm dose at 8 weeks and 31, 35, and 8% in 200, 300 and 400 ppm doses, respectively at 12 weeks.

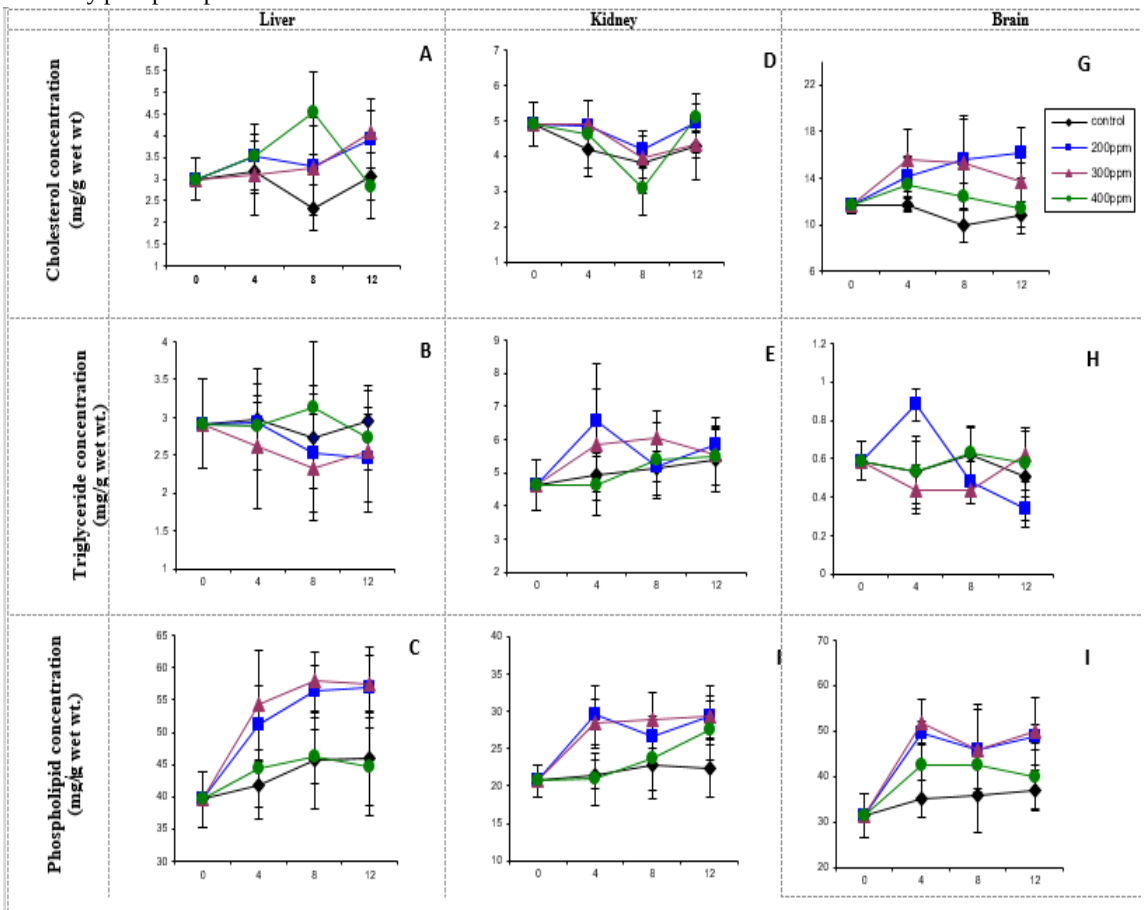

Figure 4: Effect of Pb on liver, kidney, and brain lipid concentrations in rats plotted against time course in weeks (0, 4, 8 and 12 weeks). Each point in the graphs represents mean ± standard deviation (SD), n = 6.

Spleen cholesterol concentration in 300 and 400 ppm doses decreased by 24 and 28%, respectively at 4 weeks of Pb exposure but no significant difference was observed in the 200 ppm dose (Figure 5A). After 8 weeks of lead exposure, spleen cholesterol concentrations in all three doses decreased significantly ($p < 0.05$) but not dose-dependently (29, 20, and 18% in the 200, 300, and 400 ppm doses, respectively). These decreases became minimal at 12 weeks (5, 15 and 2% for 200, 300 and 400 ppm doses, respectively). At 4

weeks of lead exposure, significant decreases in heart cholesterol were observed in all the doses (29, 29, and 37% in the 200, 300, and 400 ppm doses, respectively) (Figure 5B). At 8 weeks of lead exposure, heart cholesterol concentration in the 200 ppm dose increased by 23%, while there were no significant differences in the heart cholesterol concentrations between the control and the 300 and 400 ppm doses. After 12 weeks of lead exposure, the trend remained sustained in the 200 ppm dose with increase of 29% observed, and a marginal increase of 8% in the 400 ppm dose and decrease of 8% in the 300 ppm dose. After 4 weeks of lead exposure, an increase of 13% in lung cholesterol concentration was observed in the 300 ppm dose, while 200 and 400 ppm doses decreased by 5 and 14%, respectively (Figure 5C). After 8 weeks of lead exposure, significant increases of 33 and 15% were observed in the 200 and 300 ppm doses, respectively, while there was no significant difference in lung cholesterol concentration in the 400 ppm dose. At 12 weeks of lead exposure, increases in lung cholesterol concentration were again observed across board (11, 27 and 26% in the 200, 300 and 400 ppm doses, respectively).

After 4 weeks of lead exposure, spleen triglyceride concentration increased by 14% in 200 ppm dose but decreased by 24 and 5% in the 300 and 400 ppm doses, respectively (Figure 5D). After 8 weeks of lead exposure, increases of 30, 8, and 12% in spleen triglyceride concentrations were observed across the 200, 300, and 400 ppm doses, respectively. This trend was sustained at 12 weeks, with decreases of 19, 21 and 20% in the 200, 300, and 400 ppm doses, respectively. On the other hand, there was significant ($p < 0.05$) increase in heart triglyceride concentration in all the three doses after 4 weeks of lead exposure (Figure 5E). This increase amounted to 54, 36, and 22% in the 200, 300, and 400 ppm doses, respectively, and was sustained through 8 weeks (39 and 17% in the 200 and 400 ppm doses, respectively) while there was no significant difference in heart triglycerides between the 300 ppm dose and the control. At 12 weeks of lead exposure, there was no significant difference ($p > 0.05$) in heart triglycerides concentration between 400 ppm dose group and the control, while 200 and 300 ppm doses increased by 20 and 15%, respectively. Triglycerides concentrations in the lungs increased in all the doses after 4 weeks of lead exposure, although these increases were not dose-dependent (Figure 5F). After 8 weeks of lead exposure, no significant difference ($p < 0.05$) was observed between the triglycerides concentration in the lungs of the 400 ppm dose group and that of the control group. However, there were significant increases in lung triglycerides concentration in the 200 ppm and 300 ppm doses. groups (33 and 9%, respectively). After 12 weeks of lead exposure, the trend was reversed with all the doses decreasing significantly ($p < 0.05$). Phospholipids concentration in the spleen as shown in Figure 5G was decreased by 14% in the 200 ppm dose after 4 weeks of lead exposure, while an increase of 20% was observed in the 400 ppm dose (Figure 5G). After 8 weeks of lead exposure, there was an increase of 10% in the 200 ppm dose group and decreases of 14 and 11% in the 300 and 400 ppm doses, respectively. At 12 weeks of lead exposure, there were decreases of 8, 12, and 8% in the 200, 300 and 400 ppm doses respectively. Significant increases in heart phospholipids concentrations were observed in all the doses at 4 weeks of lead exposure (Figure 5H). This trend was sustained at through 8 and 12 weeks. The increases were not dose-dependent, however dose-dependent increases were observed in lung phospholipid concentrations; (25, 28, and 36% in the 200, 300 and 400 ppm doses respectively) at 4 weeks (Figure 5I). At 8 and 12 weeks, phospholipids in the lungs increased across all the doses but not dose-dependently.

Findings from the present study have shown a reduced concentration of triglycerides in the liver of some of the lead-exposed animals. On the other hand, renal triglycerides increased across most of the doses studied except in the 400 ppm dose at 4 weeks and the 200 ppm dose a 12 weeks which is consistent with the work of Wirthensohn et al. (1980)[50] and Ademuyiwa et al. (2009).[51] Although the triglyceride content of the brain compared to other lipids in this organ is very small, lead exposure resulted in a significant increase in this lipid in the brain of some of the doses especially in the 200 ppm dose at 4 weeks that exhibited the highest increase in triglyceride content which may imply that the 200 ppm dose is most detrimental to the brain. Since the brain cannot take up FFA from the plasma, a lead-induced damage to the blood-brain barrier and subsequent uptake of the plasma FFA may lead to the hypertriglyceridemia observed in the brain at this dose. Several studies have linked Pb exposure to blood-brain-barrier dysfunction.[52]

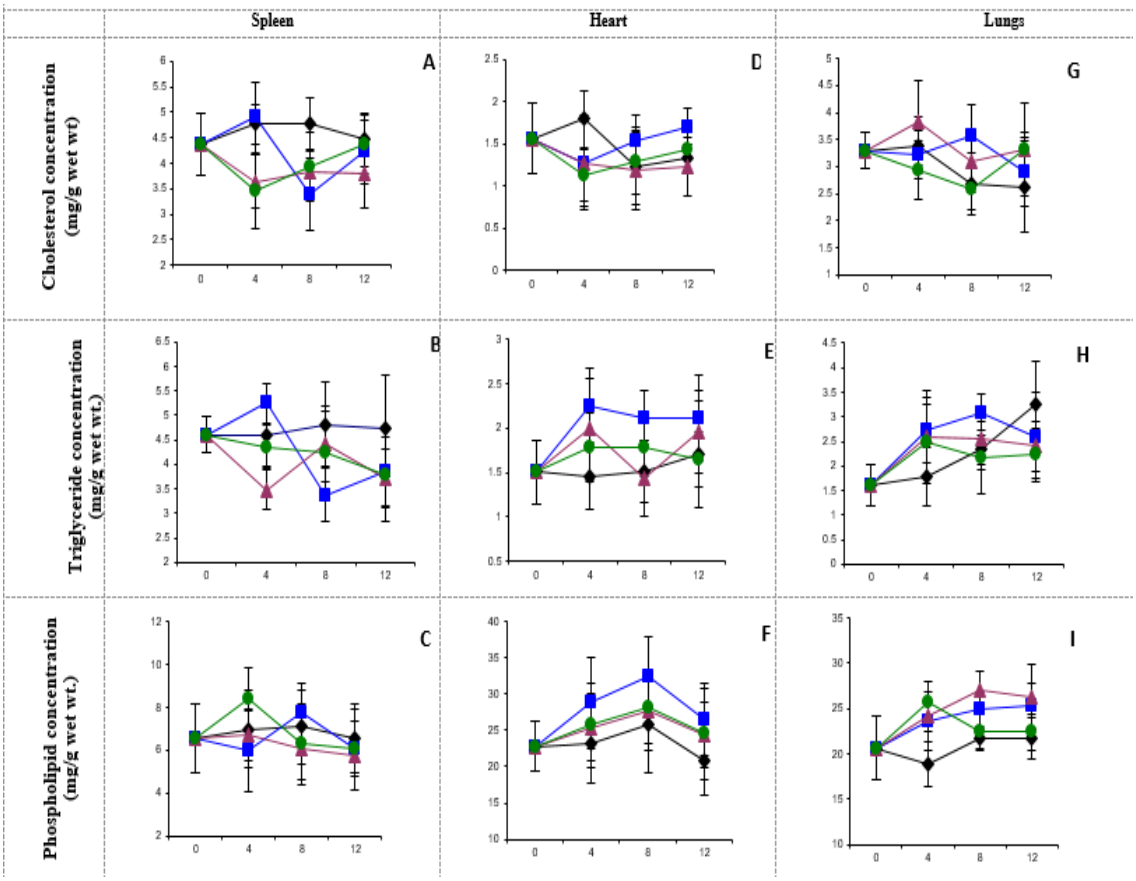

Figure 5: Effect of Pb on spleen, heart and lung lipid concentrations in rats plotted against time course in weeks (0, 4, 8 and 12 weeks). Each point in the graphs represents mean ± standard deviation (SD), n = 6.

Cholesterol and phospholipids are components of the plasma membranes of living organisms. While cholesterol also functions as the precursor of steroid hormones, phospholipids function as emulsifying agents to maintain the proper colloidal state of the cytoplasm. They are also involved in the transport of hydrophobic constituents into and out of the cell. Cholesterol and triglycerides have been widely studied in plasma during various metabolic malfunctions as a result of their involvement in vascular disorders; however, very little attention has been given to plasma phospholipids, as well as lipid profiles of tissues in various pathologies.[53] The observation of elevated plasma cholesterol in the lead-exposed animals at 4 weeks together with a concomitant dose-dependent increase in LDL cholesterol and decreases in HDL cholesterol in many of the lead-exposed groups are factors that have been shown to predispose to cardiovascular disease. This relationship between lead and plasma cholesterol suggests an altered cholesterol metabolism related to lead exposure. A similar work but using male rabbits also reported significantly higher mean levels of total cholesterol, HDL cholesterol and LDL cholesterol with decreased VLDL cholesterol and triglyceride level.[54]

Effect of lead (Pb) on liver and brain HMG CoA reductase activity

The effect of lead (Pb) on liver and brain HMG CoA reductase activity, measured as HMG CoA/mevalonate ratios is summarized in Figure 6. After 4 weeks of lead exposure, liver HMG CoA/mevalonate ratio was significantly ($p < 0.05$) decreased across all the doses, indicating an increase in HMG CoA reductase activity (Figure 6A). Decreases in the ratio were 28.5, 35 and 16.5 % in the 200, 300 and 400 ppm doses, respectively. After 8 and 12 weeks of lead exposure, there were no significant differences in HMG CoA reductase activity between the control and 400 ppm dose group, while 200 and 300 ppm doses decreased significantly ($p < 0.05$). The mean brain HMG CoA/mevalonate ratio of the animals decreased across all three doses and exposure times with highest decreases (highest enzyme activity) occurring in the 200 ppm doses (Figure 6B). The enhanced cholesterogenesis observed especially in the liver and brain of the animals in this study may

be attributed to lead-induced activation of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis.[55] The activity of this enzyme was shown to be increased in the liver and brain of most of the lead-exposed animals. To maintain cholesterol homeostasis, HMG-CoA reductase is subject to regulation by multiple mechanisms such as transcription and translation,[56] rate of enzyme degradation,[57] reversible phosphorylation and dephosphorylation,[58] and feedback inhibition.[59] The enzyme is also subject to hormonal regulation by different hormones; glucocorticoids acting at the posttranslational level and insulin acting at both the transcriptional and posttranslational levels.[60,61]

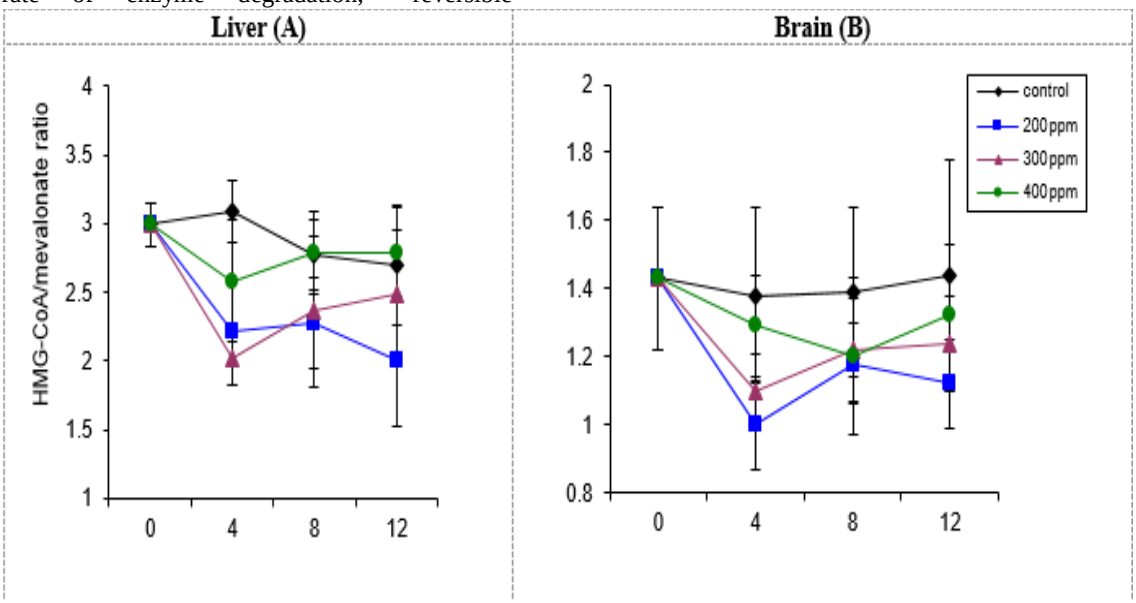

Figure 6: Effect of Pb on HMG-CoA/mevalonate ratio in liver (A) and brain (B) of rats plotted against time course in weeks (0, 4, 8 and 12 weeks). Each point in the graphs represents mean ± standard deviation (SD), n = 6.

It has been discovered that phosphorylation/dephosphorylation of the enzyme plays an important role in regulation of the hepatic cholesterol biosynthesis process.[62] Serine871 in rodents[63] and serine872 in humans[64] have been identified as the sites of phosphorylation on HMG-CoA reductase. Investigation at the molecular level may be necessary to establish the exact mechanism of lead induced up-regulation of HMG-CoA activity. The accumulation of cholesterol observed by other extrahepatic tissues of the lead-exposed animals in this study may be mediated by an enhanced cholesterol efflux from the liver since most extrahepatic tissues obtain their cholesterol from the liver. This suggests the inhibition of the enzyme cholesterol-7α-hydroxylase, the rate-limiting enzyme in the conversion of cholesterol to bile acids (a major route for the excretion of cholesterol from the body).[65] Another mechanism of cholesterol removal from extra-hepatic tissues is the apolipoprotein-mediated cholesterol removal. An impairment of this process is usually indicated by decreased HDL cholesterol level as observed in this study. HDL and apolipoprotein (apo) A-I have been reported to be receptors of cellular cholesterol.[66] In addition, phospholipid transfer protein mediates a transfer of phospholipids and cholesterol between triglyceride-rich lipoproteins and HDL, and a reduction in phospholipid transfer protein activity results in lower HDL levels.[67] Therefore, a reduction in phospholipid transfer protein activity could presumably decrease cellular cholesterol removal as well. Other factors that influence the level of tissue cholesterol are the activities of plasma lecithin: cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP).[68] While LCAT is required for the conversion of free cholesterol into cholesterol ester so that it can then move into the core of HDL, CETP mediates the exchange of cholesterol ester in HDL for triglycerides in triglyceride-rich lipoproteins, an important step in the delivery of cholesterol to the liver from extrahepatic tissues.[69] Although the activities of the two enzymes were not determined in this study, reduction in their activities may be responsible for inhibition in reverse cholesterol transport as observed in this study.

Index of membrane fluidity

The molar ratio of cholesterol to phospholipids is one of the indices of membrane fluidity. An increase in this ratio indicates decreased fluidity while a decrease signifies increased fluidity.[70,71] While lead exposure decreased this ratio in the kidney, an increase was observed in the brain after 8 and 12 weeks of lead exposure. Neither increase nor decrease is desirable for the cell. Reports have associated changes in cholesterol/phospholipid ratios with impaired signal and general dyslipidemia in heavy metal exposure.[72] A massive accumulation of phospholipids in the cell leads to the formation of numerous multi-lamellar inclusion bodies in cell cytoplasm resulting in loss of cellular function and viability.[70] Numerous studies in humans and animals indicate that lead exposure can promote brain dysfunction and this may present as behavioural changes and defective cognitive functions.[73,74] Since lead is both neurotoxic and nephrotoxic, induction of cholesterogenesis and phospholipidosis may be mechanisms underlying the cellular effects of lead.

Cholesterol/phospholipid ratios of the different compartments

Cholesterol/phospholipid ratios in different compartments of the animals on exposure to lead are depicted in Table 4. Four different patterns were observed: In the first pattern, this ratio generally increased in some of the doses compared to control. This was exemplified in the plasma, liver, kidney and brain. In the second pattern, the ratio generally decreased in some doses compared to control and this was exemplified in the erythrocytes and spleen. In the third pattern, the ratio fluctuated, both decreasing and increasing between doses. This pattern was observed in the VLDL, erythrocyte membrane, heart and lungs. In the last pattern, the ratio was generally constant across the doses when compared with the control and this was depicted in the HDL.

Ta**ble 4:** Cholesterol: phospholipid ratios in the different compartments

Plasma				
Group	**Control**	**200 ppm**	**300 ppm**	**400 ppm**
0 weeks	0.52 ± 0.09^{a}	0.52 ± 0.09^{a}	0.52 ± 0.09^{a}	0.52 ± 0.09^{a}
4 weeks	0.66 ± 0.14^{b}	1.07 ± 0.38^{d}	1.02 ± 0.24^{d}	1.26 ± 0.47^{e}
8 weeks	0.51 ± 0.07^{a}	0.67 ± 0.16^{b}	1.00 ± 0.27^{a}	0.80 ± 0.22^{c}
12 weeks	0.70 ± 0.18^{b}	0.82 ± 0.27^{c}	0.76 ± 0.18^{c}	0.66 ± 0.12^{b}
HDL				
0 weeks	0.56 ± 0.10^{a}	0.56 ± 0.10^{a}	0.56 ± 0.10^{a}	0.56 ± 0.10^{a}
4 weeks	0.60 ± 0.13^{c}	0.58 ± 0.14^{a}	0.58 ± 0.06^{a}	0.53 ± 0.12^{a}
8 weeks	0.50 ± 0.15^{d}	0.56 ± 0.19^{a}	0.55 ± 0.11^{a}	0.73 ± 0.10^{c}
12 weeks	0.49 ± 0.06^{b}	0.52 ± 0.09^{a}	0.50 ± 0.07^{a}	0.46 ± 0.11^{b}
VLDL				
0 weeks	0.48 ± 0.05^{a}	0.48 ± 0.05^{a}	0.48 ± 0.05^{a}	0.48 ± 0.05^{a}
4 weeks	0.46 ± 0.07^{c}	0.57 ± 0.08^{b}	0.47 ± 0.11^{a}	0.83 ± 0.16^{c}
8 weeks	0.47 ± 0.09^{d}	0.39 ± 0.05^{a}	0.74 ± 0.30^{a}	0.48 ± 0.06^{c}
12 weeks	0.46 ± 0.12^{b}	0.58 ± 0.35^{b}	0.85 ± 0.32^{a}	0.80 ± 0.32^{b}
Erythrocyte				
0 weeks	0.61 ± 0.16^{a}	0.61 ± 0.16^{a}	0.61 ± 0.16^{a}	0.61 ± 0.16^{a}
4 weeks	0.53 ± 0.09^{c}	0.52 ± 0.11^{b}	0.43 ± 0.05^{a}	0.65 ± 0.22^{c}
8 weeks	0.60 ± 0.13^{d}	0.36 ± 0.08^{a}	0.41 ± 0.12^{a}	0.57 ± 0.14^{c}
12 weeks	0.57 ± 0.23^{b}	0.32 ± 0.11^{b}	0.44 ± 0.08^{a}	0.33 ± 0.08^{b}
Erythrocyte membrane				
0 weeks	0.21 ± 0.06^{a}	0.21 ± 0.06^{a}	0.21 ± 0.06^{a}	0.21 ± 0.06^{a}
4 weeks	0.23 ± 0.08^{c}	0.26 ± 0.10^{b}	0.25 ± 0.04^{a}	0.14 ± 0.02^{c}
8 weeks	0.25 ± 0.08^{d}	0.23 ± 0.07^{a}	0.19 ± 0.05^{a}	0.16 ± 0.03^{c}
12 weeks	0.26 ± 0.04^{b}	0.16 ± 0.09^{b}	0.37 ± 0.09^{a}	0.15 ± 0.03^{b}
Liver				
0 weeks	2.99 ± 0.48^{a}	2.99 ± 0.48^{a}	2.99 ± 0.48^{a}	2.99 ± 0.48^{a}
4 weeks	3.16 ± 0.47^{b}	3.54 ± 0.33^{c}	3.10 ± 0.93^{b}	3.51 ± 0.75^{c}
8 weeks	2.34 ± 0.53^{d}	3.29 ± 1.12^{e}	3.26 ± 0.98^{e}	4.53 ± 0.96^{f}
12 weeks	3.07 ± 0.54^{b}	3.93 ± 0.66^{g}	4.06 ± 0.79^{g}	2.84 ± 0.75^{a}
Kidney				
0 weeks	4.90 ± 0.62^{a}	4.90 ± 0.62^{a}	4.90 ± 0.62^{a}	4.90 ± 0.62^{a}
4 weeks	4.17 ± 0.73^{b}	4.85 ± 0.73^{c}	4.90 ± 0.69^{a}	4.61 ± 0.97^{d}

8 weeks	3.81 ± 0.88^{e}	4.20 ± 0.52^{b}	3.95 ± 0.60^{e}	3.11 ± 0.81^{f}
12 weeks	4.26 ± 0.93^{b}	4.93 ± 0.84^{a}	4.34 ± 0.38^{b}	5.08 ± 0.41^{a}
		Brain		
0 weeks	11.62 ± 0.61^{a}	11.62 ± 0.61^{a}	11.62 ± 0.61^{a}	11.62 ± 0.61^{a}
4 weeks	11.62 ± 0.61^{a}	14.13 ± 1.74^{d}	15.57 ± 2.69^{e}	13.48 ± 2.25^{b}
8 weeks	9.92 ± 1.44^{g}	15.55 ± 3.49^{e}	15.30 ± 4.07^{e}	12.43 ± 1.14^{b}
12 weeks	10.86 ± 1.08^{h}	16.18 ± 2.13^{f}	13.63 ± 1.61^{c}	11.35 ± 2.08^{a}
		Spleen		
0 weeks	4.37 ± 0.62^{a}	4.37 ± 0.62^{a}	4.37 ± 0.62^{a}	4.37 ± 0.62^{a}
4 weeks	4.77 ± 0.39^{b}	4.90 ± 0.69^{b}	3.64 ± 0.53^{c}	3.45 ± 0.73^{d}
8 weeks	4.78 ± 0.50^{b}	3.41 ± 0.71^{d}	3.82 ± 0.43^{e}	3.93 ± 0.68^{e}
12 weeks	4.46 ± 0.52^{f}	4.23 ± 0.63^{a}	3.81 ± 0.67^{e}	4.38 ± 0.57^{a}
		Heart		
0 weeks	1.56 ± 0.42^{a}	1.56 ± 0.42^{a}	1.56 ± 0.42^{a}	1.56 ± 0.42^{a}
4 weeks	1.80 ± 0.34^{b}	1.27 ± 0.50^{c}	1.28 ± 0.56^{c}	1.13 ± 0.31^{d}
8 weeks	1.24 ± 0.46^{c}	1.53 ± 0.31^{a}	1.18 ± 0.46^{d}	1.29 ± 0.38^{c}
12 weeks	1.33 ± 0.16^{c}	1.71 ± 0.21^{b}	1.23 ± 0.35^{c}	1.44 ± 0.26^{e}
		Lungs		
0 weeks	3.29 ± 0.33^{a}	3.29 ± 0.33^{a}	3.29 ± 0.33^{a}	3.29 ± 0.33^{a}
4 weeks	3.39 ± 0.53^{a}	3.23 ± 0.44^{b}	3.82 ± 0.78^{c}	2.93 ± 0.55^{d}
8 weeks	2.68 ± 0.57^{e}	3.57 ± 0.56^{f}	3.09 ± 0.56^{b}	2.58 ± 0.37^{e}
12 weeks	2.63 ± 0.83^{e}	2.91 ± 0.63^{d}	3.33 ± 0.32^{a}	3.30 ± 0.86^{a}

Each value represents the mean ± standard deviation (SD), n = 6. Values within the same organ with different alphabets (a-i) are significantly different at $p < 0.05$.

Result of correlation study

The intensities of association among some parameters are depicted in Table 5. Significant positive and negative correlations were observed among the parameters. A typical pattern of correlation was observed between lead accumulation and tissue lipid profiles. While positive correlation was observed between tissue cholesterol and phospholipids versus lead concentration in the liver, kidney, brain, heart, and lungs, negative correlations were observed between liver and brain HMG-CoA/mevalonate ratios versus brain lead concentration, HDL cholesterol versus blood and liver lead concentration.

Table 5: Intensity of association among parameters

Parameter	Correlation coefficient	p-Value
Blood lead vs HDL cholesterol	-0.523	0.000
Blood lead vs Liver cholesterol	0.336	0.000
Blood lead vs Lung phospholipids	0.485	0.000
Liver lead vs HDL cholesterol	-0.416	0.000
Liver lead vs Liver cholesterol	0.476	0.000
Liver lead vs Lung phospholipids	0.400	0.000
Kidney lead vs Plasma cholesterol	0.340	0.000
Kidney lead vs plasma FFA	0.498	0.000
Brain lead vs Plasma cholesterol	0.352	0.000
Brain lead vs Brain HMG CoA/mevalonate	-0.335	0.000
Brain lead vs Liver HMG CoA/mevalonate	-0.494	0.000
Brain lead vs Lung phospholipids	0.472	0.000
Heart lead vs HDL cholesterol	-0.413	0.000
Lung lead vs HDL cholesterol	-0.423	0.000
Plasma cholesterol vs plasma cholesterol/phospholipids	0.347	0.000
Brain cholesterol vs Liver HMG CoA/mevalonate	-0.419	0.000
HDL cholesterol vs liver phospholipids	-0.342	0.000
Liver HMG CoA/mevalonate vs kidney phospholipids	-0.421	0.000
Liver HMG CoA/mevalonate vs brain phospholipids	-0.463	0.000

Summary of dyslipidemia

A summary of dyslipidemia in all the compartments is presented in Table 6. In this study, confounding factors such as nutritional status, hormonal fluctuations, or stress- induced metabolic changes were reduced to minimum by striving for consistency in animal care, experimental procedures, and sample collection.

Tables 6: Summary table of dyslipidemia in rats through 4, 8 and 12 weeks

	Cholesterol									Triglyceride									Phospholipids								
Wk	4			8			12			4			8			12			4			8			12		
ppm	200	300	400	200	300	400	200	300	400	200	300	400	200	300	400	200	300	400	200	300	400	200	300	400	200	300	400
Pl	↑	↑	↑	↑	↑	—	↓	—	↓	↑	↑	↑	—	↑	↑	—	↑	↑	↓	↓	↓	↓	↓	↓	↓	↓	—
HDL	—	—	↓	↓	↓	↓	↓	↓	↓	↑	—	—	↑	↓	—	↑	↑	—	↑	—	—	↓	↓	↓	↓	↓	↓
VLDL	↑	—	↑	—	—	—	↑	—	↑	—	↑	↑	—	↑	↑	—	—	↑	↑	↑	—	↑	↓	↓	↓	↓	↑
Er	↑	—	↑	↓	↓	—	↓	—	↓	↑	↑	↑	↑	↓	↓	↑	↑	↑	↑	↑	—	—	↑	↑	↑	↑	↑
ErM	↓	↑	↓	↓	—	↓	↓	↑	—	↑	↑	↑	↑	↑	↑	—	↑	↑	—	↑	↓	↓	↑	↑	—	↓	↑
L	↑	—	↑	↑	↑	↑	↑	↑	↓	—	↓	—	↓	↓	↑	↓	↓	↓	↑	↑	↑	↑	↑	↑	↑	↑	—
K	↑	↑	↑	↑	—	↓	↑	—	↑	↑	↑	↓	—	↑	↑	↑	—	—	↑	↑	↑	↑	↑	↑	↑	↑	—
B	↑	↑	↑	↑	↑	↑	↑	↑	—	↑	↓	—	↓	↓	—	↓	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
S	—	↓	↓	↓	↓	↓	↓	↓	↓	↑	↓	↓	↓	↓	↓	↓	↓	↓	↓	—	↑	↑	↓	↓	↓	↓	↓
H	↓	↓	↓	↑	↓	—	↑	↑	—	↑	↓	—	↓	↓	—	↓	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑	↑
Lg	↓	↑	↓	↑	↑	—	↑	↑	↑	↑	↑	↑	↑	↑	—	↓	↓	↓	↑	↑	↑	↑	↑	↑	↑	↑	↑

Legend: ↑ = increase; ↓ decrease; Wks = Exposure in weeks; ppm = doses in parts per million; Pl = plasma; HDL = High-density lipoprotein; VLDL = very low-density lipoprotein; Er = Erythrocytes. ErM = Erythrocyte membrane; L = Liver; K = Kidney; B = Brain, S = Spleen; H = Heart; Lg = Lung

A major limitation of this study is its applicability. Animal models, although useful, cannot accurately simulate human environments, which could result in inaccurate predictions of human outcomes and the application of research findings in clinical settings. Significant species differences in anatomy, physiology, metabolism, and genetics are major restrictive factors.

CONCLUSION

The antioxidant capacity of the cell, which encompasses the enzymatic and non-enzymatic antioxidant system, determines the generation and progression of oxidative stress and this system is dynamic. It mounts a continuous fight against ROS. An initial large dose of a toxicant may temporarily overwhelm it causing cell injury while a chronic low dose exposure to the same chemical may elicit subclinical effects. The interplay of these factors with time may be responsible for the observed fluctuations in the major lipids in this study. These findings have implication in managing exposure to lead. The fluctuations in dyslipidemia with time and dose observed in this study correlate with up-regulation of HMG-CoA reductase activity, inhibition of reverse cholesterol transport and enhanced phospholipidosis resulting from increased availability of FFAs. Further studies at the molecular level may further corroborate these findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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