SEX DIFFERENCES IN THE LIPIDOMICS OF SUBCHRONIC LOW LEVEL INORGANIC MERCURY EXPOSURE IN THE RAT

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ABSTRACT

Previous studies have suggested increased risk of cardiovascular diseases as a result of exposure to inorganic mercury. However, the underlying mechanisms underpinning this risk, as well as sex differences in response to mercury exposure, are not yet understood. To investigate the effects of mercury on lipid metabolism, male and female rats were exposed to mercury (as mercuric chloride) (0.5, 1.0 and 1.5 mg/kg) for 12 weeks. Control animals received distilled water also for 12 weeks after which blood, liver, kidney, brain, spleen, heart and lungs were removed from the animals and analyzed for lipid dynamics spectrophotometrically. Dyslipidemia caused by mercury in both sexes exhibited different patterns. At the highest dose, hypercholesterolemia characterized the effect of inorganic mercury in male animals as against hypocholesterolemia in the female. Hypertriglyceridemia was observed in both sexes (two-fold in male and three-fold in female). Plasma and erythrocyte free fatty acids (FFA) increased significantly in male (98% and 68%) than in female (33% and 32%) at highest dose of mercury . Reverse cholesterol transport was inhibited at highest dose of mercury in male as evidenced by decreased HDL cholesterol but increased in female respectively. Brain cholesterol and triglyceride were reduced by mercury (at 1.5mg/kg) in male as against the no effect observed in female animals. At highest dose of mercury, hepatic cholesterogenesis was observed in male whereas phospholipidosis was induced in both sexes. Pulmonary cholesterogenesis and phospholipidosis were induced in male whereas in female only phospholipidosis was observed. Pulmonary and cardiac triglyceride increased in female animals whereas hepatic triglyceride increased in both sexes. Mercury exposure caused reduction in phospholipid concentrations in brain (55% and 34%) and spleen (73% and 60%), in both sexes. Hepatic HMG-CoA reductase was up-regulated in both sexes (at 1.0 mg/kg in male but at 1.5 mg/kg in female) whereas brain HMG-CoA reductase was down-regulated in male and up-regulated in female at 1.5mg/kg. Positive associations were observed between plasma FFA and tissue mercury and HDL cholesterol and tissue mercury in females. Negative associations were observed between HDL cholesterol and tissue mercury in males. These findings indicate that inorganic mercury perturbs different pathways in lipid metabolism in both sexes and this may be responsible for its cardiovascular effects.

INTRODUCTION

Mercury is a non-essential and toxic metal in the body that is ubiquitously distributed in the environment, present in natural products and exists extensively in items encountered in daily life and therefore every human being, irrespective of age and location, is exposed to one form of mercury or another. (Park and Zheng 2012). Although mercury occurs naturally in the environment, anthropogenic activities originating from occupational or environmental sources, causes the mercury pollution of air, drinking water, food and soil environments (Clarkson 2003, Sarkar 2005).

Sub-chronic exposure to mercury is associated with a wide range of toxic effects (Hoffman et al. 2005). It results in a variety of adverse health effects including neurological, renal, respiratory, immune, dermatological, reproductive and developmental sequel (Risher & Amler 2005, Sharma et al. 2007). Recent evidences also show that mercury causes severe oxidative damages (Kim et al. 2005).

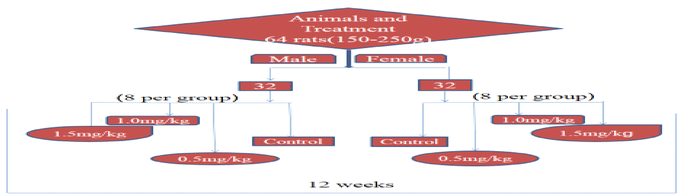
Epidemiological studies have recognized lipidomics as independent risk factors in the pathogenesis and progression of atherosclerosis and cardiovascular disease (Chrysohoou et al. 2004, Ginsberg 1994, Ademuyiwa et al. 2005). There is also increasing evidence that environmental factors/contaminants (most especially heavy metals), contribute to this dyslipidemia (Ademuyiwa et al. 2005, Aguilar-Salinas et al. 2001 , Prozialeck et al. 2008).

Few studies have been carried out on the effect of inorganic mercury exposure on lipid metabolism, the dysregulation of which is an important underlying cause of CVD. In order to address this, this work evaluated the effects of sub-chronic inorganic mercury exposure on lipid and lipoprotein dynamics, as well as the potential sex difference-related health risks.

MATERIALS AND METHODS

Chemicals

Mercury chloride was products of Sigma-Aldrich, Missouri, USA.



Mercury determination

A portion of the frozen organs (≈200mg) and whole blood (0.2ml) were digested in nitric and sulphuric acid mixture. Total mercury was determined using Inductively-coupled plasma spectrometry (ICP-MS). Results are expressed as µg Hg/ml and wet weight for the organs.

Biochemical analyses

Plasma and lipoprotein lipid profiles

Plasma concentrations of total cholesterol and triglycerides were determined with commercial kits (Spin React S.A., Santa Colona, Sant Esteve De Bas, Spain). HDL cholesterol and triglycerides were determined in plasma with same commercial kits for total cholesterol and triglycerides after very low density lipoproteins (VLDL) and LDL were precipitated with heparin-MnCl2 solution as described by Gidez et al. (1982). Free fatty acids (FFA) in plasma were determined according to the method of Soloni and Sardina (1973) as modified by Brunk and Swanson (1981).

Organ lipid profiles

Lipids were extracted from the organs (liver, kidney, and brain) as described by Folch et al. (1957). After washing with 0.05M KCl solution, aliquots of the chloroform-methanol extract were then used for the determination of cholesterol and triglyceride concentrations. Cholesterol was determined in an aliquot of the chloroform-methanol extract of each organ as described by Rotimi (2011). Triglyceride concentrations in aliquots of the chloroform-methanol extracts of each organ were determined following the procedure described by Kriketos et al. (2003).

Determination of hepatic and brain HMG-CoA reductase activity

This was determined according to the method of Rao and Ramakrishnan (1975) 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

Results are expressed as mean □S.E.M. The levels of homogeneity among the groups were assessed using Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Turkey Multiple Range Test (TMRT) and paired t-test for sex variation. All analyses were done using Statistical Package for Social Science (SPSS) version 20. Correlations were calculated by Pearson's method and p values □ 0.05 were considered

RESULTS

Though exposure of the animals to inorganic mercury resulted in an accumulation of mercury in blood, kidney, liver and brain, only in the brain and liver of female animals was the accumulation found to be dose-dependent but predominantly in kidney (Table 1). Mercury-induced hypercholesterolemia in the male rat and hypocholesterolemia in female.

Dyslipidemia induced by mercury was characterised by hypercholesterolemia in male animal at all the doses while hypocholesterolemia was observed in female by 49% at 1.0mg/kg dose (Fig. 1). HDL cholesterol (reverse cholesterol transport) was reduced to 45% in male and increase by 20% in female animals (Fig. 1).

As indicated in Fig.2, mercury induced hypertriglyceridemia at all the doses and low HDL-TG in both sexes.

Mercury significantly increased the concentration of plasma free fatty acid (FFA) of both sexes but this increase are more pronounced in male animals

While mercury decreased renal and brain cholesterol concentrations (37 and 54% in male and 30% and 25% in female animal respectively) but amounted to hepatic increase of 273% cholesterol in male and no significant changes in female animals (Fig. 4).

Inorganic mercury at 0.5mg/kg dose caused a 40% and 50% reduction in renal and at highest dose (56% and 25%) reduction in brain triglyceride concentration of male and female animal respectively. On the other hand increased hepatic triglyceride (100% and 208%) was observed in both sexes (Fig. 5).

As designated in Fig. 6, mercury did not affect HMG-CoA/mevalonate ratio of the brain and liver of female animal but, the 1.0 and 1.5mg/kg mercury doses resulted in 72% and 25% increase in brain and liver HMG-CoA/mevalonate ratio of the male animals respectively.

The associations between tissue mercury levels and some lipid parameters are depicted in Table 2. While tissue mercury levels correlated positively with plasma FFA in both sexes (with the exception of kidney and blood mercury in male and brain mercury in female animals where no correlation was observed), reverse cholesterol transport was negatively correlated (in male) but positively (in female) with tissue mercury levels. Hepatic mercury correlated positively with brain HMG CoA reductase and blood mercury correlated with hepatic HMG CoA reductase.

Table 1: Mercury concentrations in the tissues of the animals

	Mercury dose(mg/kg body weight)							
	Control		0.5		1.0		1.5	
	Male	Female	Male	Female	Male	Female	Male	Female
Brain Mercury µg/g	0.050±0.000[a]	0.055±0.002[a]	0.055±0.004[a]	0.058±0.004[a]	0.060±0.007[a]	0.061±0.007[a]	0.120±0.016[b]	0.090±0.012[b]
Liver Mercury µg/g	0.080±0.000[a]	0.055±0.003[a*]	0.173±0.010[b]	0.273±0.049[b]	0.429±0.031[c]	0.689±0.055[c*]	1.365±0.186[d]	0.886±0.064[d*]
Kidney Mercury µg/g	0.143±0.009[a]	0.322±0.029[a*]	29.207±0.881[b]	22.582±1.754[b]	17.470±0.214[c]	43.834±4.913[c*]	23.782±1.587[c]	77.893±7.768[d*]
Heart mercury µg/g	0.162±0.043[a]	0.750±0.000[a*]	0.750±0.000[b]	0.750±0.000[a]	0.750±0.000[b]	0.750±0.000[a]	0.663±0.026[b]	0.420±0.025[b*]
Spleen mercury µg/g	0.076±0.006[a]	0.032±0.002[a*]	0.071±0.003[a]	0.078±0.007[b]	0.063±0.007[b]	0.169±0.014[c*]	0.089±0.003[c]	0.097±0.013[b]
Lung mercury µg/g	0.031±0.005[a]	0.031±0.006[a]	4.330±1.768[b]	0.100±0.001[a]	0.169±0.005[a]	0.335±0.035[b*]	0.272±0.011[a]	0.826±0.170[c*]
Whole mercury µg/ml	0.004±0.000[a]	0.005±0.001[a]	0.035±0.010[c]	0.020±0.004[b]	0.022±0.005[b]	0.037±0.010[c]	0.041±0.007[c]	0.067±0.016[d]

Table 2: Association between tissue mercury levels and some lipid parameters

	Plasma FFA		HDL cholesterol		Hepatic HMG CoA reductase		Brain HMG CoA reductase	
	Correlation coefficient (r)		Correlation coefficient (r)		Correlation coefficient (r)		Correlation coefficient (r)	
	Male	Female	Male	Female	Male	Female	Male	Female
Brain mercury	0. 664**	0.261	-0.373*	0.097	0.233	-0.2	0.228	-0.285
Kidney mercury	0.334	0.502**	- 0.638**	0.229	0.124	-0.138	0.192	-0.158
Liver mercury	0.453**	0.374*	-0.425*	0.063	0.244	-0.177	0.400*	-0.083
Heart mercury	0.339	-.457**	-.659**	-.624**	-0.116	0.199	0.318	0.183
Spleen mercury	0.167	0.301	0.025	-.368*	0.234	0.05	0.156	0.012
Lung mercury	-0.009	.383*	-0.115	.426*	0.169	-.366*	-0.314	-0.175
Blood mercury	0.155	0.435*	-0.419*	0.164	0. 458**	-0.121	0.116	-0.176

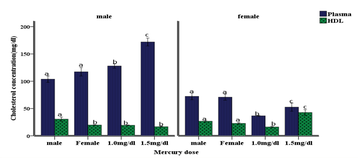


Fig.1: Effects of inorganic mercury on plasma and HDL cholesterol concentrations. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

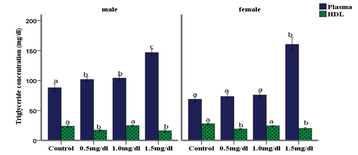


Fig.2: Effects of inorganic mercury on plasma and HDL triglyceride concentrations. Each bar represents the means±S.E.M. of 8 rats. Bars with different alphabets are significantly different from each other. *p<0.05, compared to male.

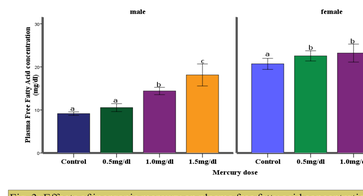


Fig. 3: Effects of inorganic mercury on plasma free fatty acid concentrations of male and female rats. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05

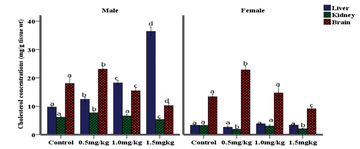


Fig. 4: Effects of inorganic mercury on hepatic, renal and brain cholesterol concentrations. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05.

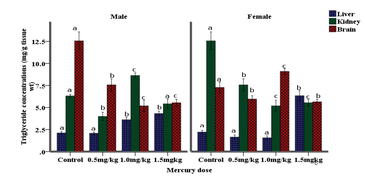


Fig. 5: Effects of inorganic mercury on hepatic, renal and brain triglyceride concentrations. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05.

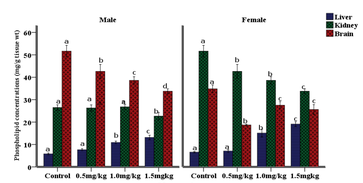


Fig. 6: Effects of inorganic mercury on hepatic, renal and brain phospholipid concentrations. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05.

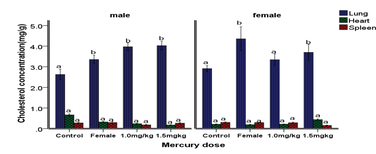


Fig. 7: Effects of inorganic mercury on pulmonary, cardiac and splenic cholesterol concentrations. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05

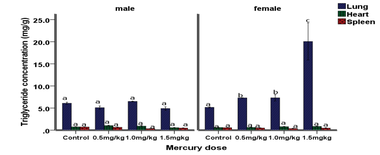


Fig. 8: Effects of inorganic mercury on pulmonary, cardiac and splenic triglyceride concentrations. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05.

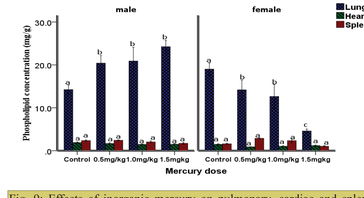


Fig. 9: Effects of inorganic mercury on pulmonary, cardiac and splenic phospholipid concentrations. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05.

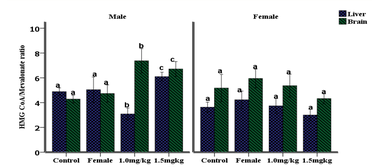


Fig. 10: Effects of inorganic mercury on hepatic and brain HMG CoA/Mevalonate ratios as an Index of HMG CoA reductase activity. Each bar represents the mean±S.E.M. of 8 rats. Bars with different alphabets are significantly different at p < 0.05.

CONCLUSIONS

The findings of this study indicate that sub-chronic exposure to inorganic mercury produced two common denominators of dyslipidemia namely: inhibition of reverse cholesterol transport and increase in plasma FFA. These two denominators which are sex oriented (in addition to other individual perturbations of lipid metabolism induced by mercury), might mediate the observed cardiovascular effects of inorganic mercury exposure.

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