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Exposure to Gasoline Vapours: A Potential Risk Factor for Atherosclerosis in Male and Female Rats

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Abstract: A gender-dependent potential atherosclerotic risk is reported in this study to be associated with exposure to gasoline vapours in rats model. The atherosclerotic risk was assessed from the serum lipid and some electrolytes (Na^+, K^+ and Cl^-) profiles in male and female rats exposed wholly to 17.8±2.6 $cm^3/h/kg/m^3$/day of gasoline vapours (8 h daily, 6 days $week^{-1}$) for 20 weeks in exposure chambers. A significant increase ($p<0.05$) in serum total cholesterol (TC), triglycerides (TGs), Low Density Lipoprotein-Cholesterol (LDL-C), Very Low Density Lipoprotein-Cholesterol (VLDL-C), K^+ and decrease ($p<0.05$) in High Density Lipoprotein-Cholesterol (HDL-C), Na^+ and Cl^- was obtained for both male and female rats exposed to gasoline vapours. These results showed a state of hyperkalaemia, hyponatraemia, increased TG/HDL-C ratio and Atherogenic Index of Plasma (AIP) in male and female rats exposed to gasoline vapours. However, the comparative percentage increase in serum TC, TGs, LDL-C, VLDL-C, K^+, as well as percentage decrease in serum HDL-C, Na^+ and Cl^- reported to be associated with exposure to gasoline vapours, were observed to be significantly higher ($p<0.05$) in females than the male rats. Since hyperlipidaemia, hyperkalaemia and hyponatraemia are known to be implicated in atherosclerosis, the result of this study gives a clear indication that gasoline vapours is among the risk factors for atherosclerosis and that the females are more adversely affected than the males in rats model.

Key words: Gasoline vapours, hyperlipidaemia, hyperkalaemia, hyponatraemia, atherosclerosis

INTRODUCTION

Gasoline is one of the highly volatile products of petroleum fractionation. Evaporation of liquid gasoline generate gasoline vapours into the environments. These vapours are ubiquitous in the environment and constitute some components of petroleum pollutants in the atmosphere. A good percent of human populace is directly or indirectly exposed to these pollutants in the course of their day to day activities.

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However, the commonest sites of exposure to these pollutants from gasoline vapours include refineries, oil fields, refueling stations, petrochemical and petrochemical-related industries as well as traffic-congested areas. Hence, the population at greater risk of frequent exposure includes those occupationally exposed, as well as those residing in traffic-congested areas. Reports indicate that chronically exposed individuals are the petroleum drillers, refinery and petrochemical workers, refuel station attendants and automobile mechanics (Carballo *et al*., 1995; Rabble and Wong, 1996).

Potential health hazards associated with chronic or sub-chronic exposure to these ubiquitous pollutants in the environment has been the concern of the general public and scientific community in particular. It has been reported that more saturated hydrocarbons than unsaturated aromatic hydrocarbons are detected in human and animal blood after inhalation exposure to equal concentration (Zahlsen *et al*., 1993). Both the saturated and unsaturated aromatic hydrocarbons form a proportion of gasoline constituents. Unleaded gasoline is reported to contain about 300 different hydrocarbon fraction most of which are very volatile. Some of these gasoline vapours' constituents (including alkanes, benzenes, xylenes and tetraethyl lead) have been reported to be haematotoxic in humans and experimental animals (Azevedo *et al*., 1996; Rothman *et al*., 1996; Synder and Hedli, 1996). Also, our previous studies showed that inhalation and while body exposure of rats to gasoline vapours caused haematotoxicity (Uboh *et al*., 2007a), hepatotoxicity (Uboh *et al*., 2007b) and nephrotoxicity (Uboh *et al*., 2008) in the animal model. There is dearth of information on whether exposure to gasoline vapours may be risk factor to atherosclerosis or not.

Atherosclerosis and cardiovascular disease are known to be among the heading cause of deaths in the recent times (Gambhir *et al*., 2001). Atherosclerosis is known to be characterized by low level of High Density Lipoprotein-Cholesterol (HDL-C), high levels of total cholesterol (i.e., hyper cholesterolaemia), Low Density Lipoprotein Cholesterol (LDL-C) and triglycerides (TG) in the plasma (Warren and Halpert, 2004; Nasiruddin and Ahmad, 2006). Atherosclerotic condition however, may also be ascertained from such atherogenic index as the logarithm of the ratio of TG to HDL-C, i.e., Atherogenic Index of Plasma (AIP). While Gaziano *et al*. (1997) and Frohlich and Dobiáová (2003) reported that the ratio of TG to HDL-C is a strong predictor of atherosclerosis condition, hence myocardial infarction, Tan *et al*. (2004) and Dobiáová (2004) maintained that the logarithm of the ratio of TG to HDL-C in molar concentrations, i.e., log (TG/HDL), referred to as the Atherogenic Index of Plasma (AIP) is more sensitive. However, either AIP or TG/HDL-C may be used to evaluate the atherosclerotic state of a subject (Dobiáová and Frohlich, 2001), although Tan *et al*. (2004) in one of their studies reported the P values for AIP to be consistently lower than those for TG/HDL.

There are several risk factors that contribute to the development of atherosclerosis. While some of these factors can be controlled, some cannot be controlled. Among these factors is reported to include:

- Documented atheroma in any artery
- Diabetes or just upper normal blood glucose and insulin levels (Gilling *et al*., 2002)
- Dyslipidermia (cholesterol and triglyceride level disturbances) having a high blood concentration of LDL (bad cholesterol) particles, VLDL particles and low concentration of functioning HDL (good cholesterol) particles (Gambhir *et al*., 2001; Rader, 2002; Nasiruddin and Ahmad, 2006)

Several internal chemical markers indicating ongoing inflammation may also relate to relative risk (Frohlich and Dobiáová, 2003).

These risk factors usually operate synergistically to promote earlier or later consequences of atherosclerosis.

MATERIALS AND METHODS

Animals and Animal Handling

Twenty eight Wistar albino rats weighing 180-200 g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar, Nigeria and used for this study, which lasted from March, 2009 to May, 2009. The animals were allowed one week of acclimatization to laboratory conditions and handling, after which they were distributed, according to weight into three groups as outlined below. The animals were housed individually in cages with plastic bottom and wire mesh top (North Kent Co. Ltd.) and fed with normal rat chow (Guinea Feeds Product) purchased from the High Quality Livestock Feeds stores, Calabar, Nigeria. They were supplied with tap water *ad libitum* throughout the experimental period. The control groups (Mc and Fc) were maintained in the gasoline vapours-free section of the animal room adequately ventilated under standard conditions (ambient temperature, 28±2°C and relative humidity, 46%, with a light/dark cycle of 12/12 h). The test groups (Mt and Ft) were kept in the exposure chambers (vapours cupboards) previously saturated respectively with Premium Motor Spirit (PMS) blend of gasoline vapours during the exposure periods. The liquid gasoline (PMS blend) was obtained from the Mobil Refueling station, Marian Road, Calabar, Nigeria.

All animal experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

Distribution of Experimental Groups

Group	No. of rats	Treatment
Male control (Mc)	7	Vapours-free
Male test (Mt)	7	Exposed to gasoline vapour
Female control (Fc)	7	Vapours-Free
Female test (Ft)	7	Exposed to gasoline vapour

Exposure to Gasoline Vapours

The animals in the test groups were wholly exposed to 17.8 $cm^3/h/kg/m^3$ (target concentration) of vapourized Premium Motor Spirit (PMS) blend of unleaded gasoline (UG) vapours for 8 h day^{-1}, 6 days $week^{-1}$, for 20 weeks in a glass exposure chambers (1.5×0.9×2.1 m). Exposure conditions were chosen to reproduce those used in our previous studies (Uboh *et al*., 2007a, b, 2008). The exposures were routinely conducted from 9.00 am to 5.00 pm on week days, including holidays to mimic workplace exposure. The chamber design, exposure generation system and monitoring system were the same as those previously described (Uboh *et al*., 2007a, b, 2008), with chamber concentrations of the UG determined daily. The average daily chamber concentrations of UG during exposure periods were 17.8±2.6 $cm^3/h/kg/m^3$ (about 85.4% of target concentration). At the end of each day's exposure period, the animals were transferred to gasoline vapours-free section of the experimental animal house and maintained under the same standard conditions as the animals in control groups until the next day. During the exposure period, the initial and final volumes of liquid gasoline were respectively recorded before and after daily exposure. The daily differences in volume were used to estimate the relative concentrations of vapours used in this exposure method.

Collection and Handling of Blood Serum for Analyses

Twenty-four hours after last exposure, the animals were anaesthetized with chloroform vapour and dissected. Whole blood from each animal was collected by cardiac puncture into well-labeled non-heparinized sample tubes and allowed to clot for 3 h in iced water. The serum was separated from the clots after centrifuging at 10,000 rpm for 5 min, using bench top centrifuge (MSE Minor, England), into well-labeled plain sample bottles and used for assays.

Biochemical Assays

Serum Lipid Profile

Kits obtained from the Randox Chemical Company, UK were used for lipid profile assays. The high performance enzymatic colorimetric, CHOD-PAP method described by Richmond (1973) was used to estimate total serum cholesterol. The principle is based on the proportionate formation of hydrogen peroxide (following oxidation of free cholesterol), which is quantified when treated with chromogem, 4-aminoantipyrine. A coloured compound whose intensity is in proportion to the evolved peroxide and thus total cholesterol concentration in the sample, is formed and assayed spectrophotometrically, using HAICH, DR3000, Germany model of spectrophotometer.

Serum triglycerides were estimated by the modified enzymatic colorimeric test, according to the Glycerol Phosphate Oxidase (GPO) method. It is based on the action of L-d-glycerol phosphate oxidase on glycerol-3-phosphate and glycerol, obtained from the lipase action on triglycerides in serum. Hydrogen peroxide, a by-product of the GPO reaction is estimated as already described for total cholesterol.

HDL-cholesterol estimation employed the method of Richmond (1973). The principle entails the separation of HDL-cholesterol from chylomicrons, VLDL-C and LDL-C with a suitable precipitant and then estimation of cholesterol by the method described for total serum cholesterol.

VLDL and LDL cholesterols were obtained by calculations using the empirical relationships of Friedwald *et al*. (1972).

AIP (Atherogenic Index of Plasma) was derived from the relationship described Dobiáová and Frohlich (2001).

Serum Electrolytes

Sodium and potassium ions (Na^+ and K^+) concentrations in serum were determined by Flame Photometric method of Vogel (1960), while chloride ion (Cl^-) concentration in serum was determined by titration methods of AOAC (1990).

Statistical Analysis

All data are expressed as Mean±SEM. The mean values for the test groups were compared with the respective control group values for statistical difference using Student's t-test. Differences between groups were considered significant at $p<0.05$.

RESULTS

The effects of gasoline vapours on the serum lipid profile and some electrolytes (Na^+, K^+ and Cl^-) concentrations are summarized in Table 1 and 2. The result showed that exposure to gasoline vapours caused a significant increase ($p<0.05$) in serum TC, TG, LDL-C, VLDL-C and K^+, as well as a significant decrease ($p<0.05$) in the serum HDL-C, Na^+ and Cl^- concentrations in both male and female rats (Table 1, 2). However, the comparative

Table 1: Effect of gasoline vapours on serum lipid profile and atherogenic index in male and female rats

Group	TC	TG	LDL-C	VLDL-C	HDL-C	TG/HDL	AIP
Mc	4.47±0.28	2.86±0.18	1.74±0.38	1.86±0.13	4.05±0.35	0.71±0.31	0.85±0.12
Mt	7.26±2.45*	5.02±1.24*	3.05±1.41*	2.97±0.19*	2.20±0.26*	2.28±1.01*	1.36±0.23*
Fc	3.85±0.21	2.58±0.34	1.65±0.27	1.68±0.11	4.10±1.32	0.63±0.38	0.80±0.15
Ft	7.63±1.10*	4.98±1.31*	3.12±2.10*	3.00±0.63*	1.83±0.10*	2.72±0.93*	1.43±0.28*

All values are expressed as Mean±SEM, n = 7, *p<0.05 compared with controls. Mc: Male control, Mt: Male test, Fc: Female control, Ft: Female test, TC: Total cholesterol, TG: Triglycerides, LDL-C: Low density lipoprotein-cholesterol, VLDL-C: Very low density lipoprotein-cholesterol, HDL-C: High density lipoprotein-cholesterol, AIP: Atherogenic index of plasma

Table 2: Effect of gasoline vapours on some serum electrolytes profile in male and female rats

Group electrolyte	Mc	Mt	Fc	Ft
Na^+	119.64±6.71	90.25±5.65*	118.45±5.86	85.54±4.72*
K^+	4.81±1.06	7.87±2.10*	4.68±1.11	8.85±2.21*
Cl^-	101.25±3.75	98.31±4.06*	100.58±3.56	95.86±3.89*

All values are expressed as Mean±SEM, n = 7, *p<0.05 compared with controls. Mc: Male control, Mt: Male test, Ec: Female control, Ft: Female test

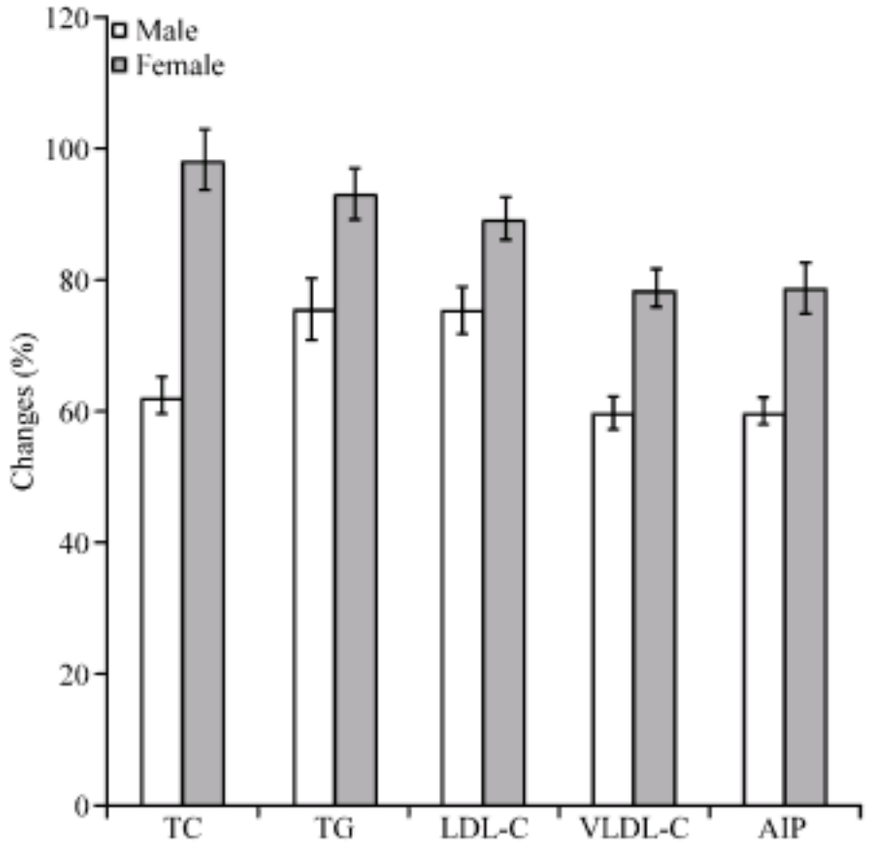

Fig. 1: Comparative percentage increase in serum lipid profile and artherogenic index in male and female rats exposed to gasoline vapours

percentage increases in serum TC, TG, LDL-C, VLDL-C and K^+ concentrations obtained from female rats were observed to be significantly higher ($p<0.05$) compared to the values obtained for male gender following exposure to gasoline vapours (Fig. 1). Also, the comparative percentage decrease in serum HDL-C, Na^+ and Cl^- concentrations obtained from female rats (55.4±2.7, 27.8±3.8 and 4.7±1.8%, respectively) were as well observed to be significantly higher ($p<0.05$) compared to the comparative percentage decreases obtained for the male rats (45.7±2.3, 24.6±3.5 and 2.9±1.2%, respectively) exposed to gasoline vapours.

The result of this study also showed that the TG/HDL-C ratio and Atherogenic Index of Plasma (AIP) were significantly increased ($p<0.05$) in both male and female rats following exposure to the vapours (Table 1). From these results, it is evidenced that exposure to gasoline vapours may cause hyperlipidaemia, hyperkalaemia, hyponatraemia and hypochloraemia in rats model. These adverse conditions are also observed to be

sex-dependent, with the female being more vulnerable. The hyperlipidaemia, as seen in increased TG/HDL-C ratio and AIP, hyperkalaemia reported in this study to be associated with exposure to gasoline vapours, suggests that gasoline vapours is one of the risk factors for atherosclerosis.

DISCUSSION

Hyperlipidaemia is well known to be one of the major risk factor for atherosclerosis and cardiovascular disease. Hence, any condition of life that causes hyperlipidermia may therefore be considered to be among the causative or risk factor for atherosclerosis. The present study was designed to assess the comparative atherosclerotic risk associated with inhalation and whole body exposure to gasoline vapours in male and female rats. The results of this study showed that exposure to gasoline vapours caused increase in serum total cholesterol (hypercholesterolaemia), LDL-C, VLDL-C, triglycerides, Atherogenic Index of Plasma (AIP) and K^+, as well as decrease in serum HDL-C, Na^+ and Cl^- levels in male and female rats. Increase in AIP value have been reported to be associated with different cases of cardiovascular diseases (Dobiáová and Frohlich, 2001; Dobiáová, 2004; Tan *et al*., 2004). This observation indicated that exposure to gasoline vapours is associated with hyperlipidaemia, hyponatraemia, hypochloraemia and hyperkalaemia; thereby describing its atherogenic potential. However, the female gender is observed to be more adversely affected than the males.

In this study, exposure to gasoline vapour is considered a crucial factor responsible for hyperlipidaemia in the atherosclerotic risk factor profile and consequently for the increase in atherosclerotic risk. It has been reported that hypercholesterolaemia and smoking are two major risk factors for the development of atherosclerosis and both have been shown to be associated with impaired endothelial function (Zeiher *et al*., 1995; Heitzer *et al*., 1996, 1999, 2001). The observations made in this study agree with our previous report that exposure to gasoline vapours alters the normal serum lipid profile and causes oxidative stress in hepatocyte of male and female rats (Uboh *et al*., 2007b). The results reported in this study also indicated that exposure to gasoline vapours have a similar atherosclerotic effect as that reported for smoking (Zeiher *et al*., 1995; Heitzer *et al*., 1996, 1999, 2001). Oxidative modification of LDL-C has also been shown to be an important step in promoting atherosclerosis (Witzum, 1994; Stocker and Keaney, 2004). Also increased vascular production of super-oxide anion has been implicated as contributing to impaired endothelium-dependent vascular relaxation in animal models of hypercholesterolaemia (Ohara *et al*., 1993). In our previous study, a high extent of lipid peroxidation, evidenced in elevated level of malonyl dialdehyde (MDA) and reduced superoxide dismutase (SOD) activity in rats exposed to gasoline vapours was reported (Uboh *et al*., 2007b). Hence, the atherosclerotic condition observed in this study may be attributed to oxidative modification of lipoproteins which promotes endothelial dysfunction.

Hyperkalaemia, hyponatraemia and hypochloraemia are also reported in this study to be associated with exposure to gasoline vapours in the rat model. These disorders in electrolytes balance are also known to be implicated in atherosclerotic condition. Eteng *et al*. (2006) demonstrated the relationship between serum electrolytes (Na^+, K^+ and HCO_3^-) and serum lipid profile. Na+ and Cl^- excretion from the body is reported to be a function of arterial blood pressure (Guyton, 2006).

Moreover, Na^+ depletion is reported to stimulate rennin release and subsequent production of Angiotensin II, which is a potent vasoconstrictor (Guyton, 2006). Angioensin

II and small increase in plasma K^+ is known to stimulate the production/release of aldosterone. The Rennin-Angiotensin System (RAS) and particularly the Angiotensin II is reported to play a key role in blood pressure homeostasis and atherogenesis through its hypertensive effect (Mazzolai *et al*., 2004; Nussberger *et al*., 2008). Also, primary hyperaldosteronism is considered to be associated with residual hypertension. It is interesting to note that several of the proposed mechanism for atherogenesis are similar to those associated with Angiotensin II mediated events (Su *et al*., 1998; Keidar *et al*., 2001; Mazzolai *et al*., 2000). More so, animal experiments and human studies both indirectly and directly demonstrated that pharmacological blockade of the RAS has been of beneficial effect on atherosclerosis (Strawn *et al*., 2000; Yusuf *et al*., 2000). From these reports, it may be suggested that the atherosclerotic condition reported in this study is likely to be caused by the RAS stimulating effect of hyponatraemia and hyperkalaemia associated with exposure to gasoline vapour.

The result of this study also showed that the atherosclerotic risk of gasoline vapours is sex-dependent in rats. This observation supported our earlier reports that haematotoxicity and hepatotoxicity effects associated with exposure to gasoline vapours are sex-dependent, with females being more adversely affected (Uboh *et al*., 2007a, b). The specific mechanism of this sex-dependent effect is not very clear. However, Lambrinoudaki *et al*. (2006) reported that increased androgenicity is associated with unfavourable cardiovascular risk profile, while high endogenous estradiol is related to a pro-atherogenic lipid profile in healthy postmenopausal women. It has also been reported that pre-menopausal women have a lower incidence of cardiovascular disease (CVD) compared with men of the same age (Matthews *et al*., 1989). According to Connor and Bush (1991), Liu (2001) and Warren and Halpert (2004), female sex hormones deficiency, as in menopausal state, is associated with a pro-atherogenic lipid profile characterized principally by lower HDL-C, higher total cholesterol, triglycerides, LDl-C and VLDL-C levels. In our previous study, we reported that exposure to gasoline vapours caused significant reduction in steroidal sex hormones in female rats and increase in sex hormones in male rats models (Uboh *et al*., 2007c). Hence, the sex-dependent atherosclerotic risk of gasoline vapour reported in this study may be attributed to increase adverse effect of the vapours' constituents on sex hormonal system in female rats than the males.

From the observations made in this study it may apparently be concluded that exposure to gasoline vapours is also among the risk factors for atherosclerosis and hence cardiovascular disease. This study also make it clear that the atherosclerotic risk associated with exposure to gasoline vapours is sex-dependent in rats models and that he female gender is at greater risk.

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