# THE CUMULATIVE EFFECTS OF CHRONIC CARBON MONOXIDE INHALATION ON SERUM AND VITREOUS PROTEIN AND LIPID PANELS

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## ABSTRACT

This study was designed to assess the chronic effect of CO inhalation on vitreous and serum protein and lipid panels. A total of twenty four (24) rabbits as indicated by mead's formula constituted the sample size. The study was divided into four groups including the controls. With exception of the control rabbits, others were treated daily with not more than 200 ppm of CO concentrations for thirty minutes for durations of 10 day, 20 day and 30 day respectively. Vitreous humor and cardiac blood were extracted from the rabbits using standard procedures. Data were analyzed using one-way Anova (Post Hoc-LSD) with statistical significance considered at $P < 0.05$. The serum biochemistry revealed that abumin/globulin (A/G) ratio value was significantly raised ($P < 0.05$), whereas, total protein (TP), globulin, total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) decreased significantly ($P<0.05$). Also, the vitreous TP, GLO TC, TG, HDL, LDL and VLDL exhibited significant decrease ($P < 0.05$), whereas A/G ratio increased. The findings revealed chronic CO inhalation could result to immunosuppression, hypolipidaemia and oxidative stress phenomenon.

**Keywords:** Protein profiles, lipid profiles, vitreous humor, serum, forensic science, chronic CO Intoxication.

{**Citation:** Agoro, E.S.; Akubugwo, E.I.; Chinyere, G.C.; Alabrah, P.W., Ombor, J.A. The cumulative effects of chronic carbon monoxide inhalation on serum and vitreous protein and lipid panels. American Journal of Research Communication, 2018, 6(8): 21-32} www.usa-journals.com, ISSN: 2325-4076.

## BACKGROUND STUDIES

The continuous exposure of humans and animals to moderate or high Carbon monoxide (CO) concentration is deleterious to the body. Due to preference of CO to oxygen by haemoglobin, pulsatile intoxication of CO could build up in the system leading to undesirable effect.

Carbon monoxide has 200 to 300 times more affinity for haemoglobin (Hb) than oxygen ($0_2$), forming carboxyhaemoglobin which is quite stable[1]. This renders haemoglobin incapable of carrying oxygen resulting in tissue hypoxia. As long as CO is in the atmosphere, it goes on accumulating and fixed in blood, leading to acute chemical asphysia[1]. Based on the mechanism of action of CO, it is classified as Asphyxiants. Asphyxiants are gaseous poisons which produce respiratory embarrassment, leading to asphyxia.

Carbon monoxide not only decreases the oxygen content of blood, but also decreases oxygen availability to tissues, thereby producing a greater degree of tissue hypoxia than equivalent reduction in oxyhaemoglobin caused by hypoxia[2]. It also binds to other heme proteins, such as myoglobin and mitochondrial cytochrome oxidase $a_3$, which limits oxygen use when tissue partial oxygen pressure ($PO_2$) is very low. Organs with high oxygen demand, such as the heart

and brain, are most sensitive to hypoxia and account for the major clinical sequelae of carbon monoxide poisoning.

Carbon monoxide been gaseous and lipophilic could access the circulatory system including the vitreous humor of the eye. The access by CO is the basis of the hypoxic and asphyxic mechanism of action. The blood is a major medium for the transportation of CO across the body. In the blood, CO cohabits with other components of the blood and most times even binds on some. This seamless relationship ought to be questioned as CO is known to be very toxic.

The vitreous humour is located between the lens and the retina with similar biochemical composition to that of serum[3,4]. Carbon monoxide been lipophilic could access the vitreous through the eye or blood circulatory system. The chronic effect of CO on the vitreous is another aspect of this study as it retains its composition along after dead. Hence it is of forensic importance especially in prolonged postmortem interval analysis of CO.

Due to the difficulty in using human subjects and the ethical implication, rabbits stand the choice animal for the study. The suitability of rabbit as a choice animal for this study is attributed to its anatomical and physiological similarities to human [5,6].

Proteins and lipids are amongst the most common biochemical parameters found in the body's fluids and perform a lot of vital functions. They are active players in the transportation of chemicals, nutrients and other products from one part of the body to another. Also, the studied parameters are essential in the study of diseases as alterations could be diagnostic of a particular or arrays of diseases. The effects of chronic CO inhalation on protein and lipid profiles could be of clinical and forensic values. Clinical values as in knowing the causative agents of the ever

increasing idiopathic diseases. In forensics, helping in formulating a template of the cause of death or poisoning due to exposure to CO. Studies have shown that acute CO has both direct and indirect effect on lipids and proteins[7,8,9]. Replicating same in the chronic situation could be of value especially in Nigeria's environment where deliberate and non-intentional exposure to low grades of CO is rampant. The study is designed to open new vista on the effect of chronic CO inhalation on lipids and proteins in the body fluids.

## MATERIALS AND METHODS

### Study Area

The breeding and intoxication aspect of the study was conducted at Igbogene Epie in Yenagoa Local Government of Bayelsa State. The biochemical investigations were carried out at the Federal Medical Centre Yenagoa and the Niger Delta University Teaching Hospital Laboratories, Okolobiri, Bayelsa.

### Study Population

Mead's resource equation was utilized for the calculation of the sample size [10]. A total of twenty four rabbits constituted the sample size. The rabbits were exposed to not more than 200 ppm carbon monoxide concentrations daily for thirty minutes at minimum of ten days and maximum of 30 days. The rabbit where divided into four groups. The first group constituted the controls. The control group was not exposed to CO prior to mechanical sacrifice. The remaining three

groups ($10^{th}$, $20^{th}$ and $30^{th}$) were exposed to CO for 30 minutes daily for ten days, twenty day and thirty days respectively.

**Ethical Approval**

The ethical clearance and experimental protocol were approved by the Ethics Committee of the Bayelsa State Ministry of Health. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocol were stringently adhered to.

**Selection Criteria**

Only apparently healthy male albino rabbits of same age and weight were used for the study. The age range was between six to eight months. The weight brackets were 1.5-2kg. Lysed blood samples and turbid vitreous humours were rejected.

**Collection of Sample**

Vtreous humor samples were collected by the method of Coe[11] and blood samples by method postulated by Ness[12]. Both samples were collected into plain containers for the biochemical analysis. The samples were spun and the supernatants separated for the analysis. The samples were analyzed for total protein, albumin, globulin, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL).

**Laboratory Analysis**

Vitreous and serum total protein and albumin were estimated quantitatively using Biuret Method and Bromocresol Green Method as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet) respectively. The vitreous and serum globulin and albumin/golubin ratio were derived by subtracting vitreous albumin from vitreous total protein and dividing albumin by globulin respectively[13].

Vitreous and serum total cholesterol, triglyceride and HDL were estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Vitreous LDL and VLDL concentration was derived mathematically by the formula as shown by Carl and Edward [2] and Friedewald *et al.,* [14] respectively. The concentration of CO was extrapolated from the findings of Golden [15] and Struttmann *et al*. [16] in averring CO concentrations (ppm) that cannot cause to death.

**Statistical Analyses**

The data generated were analyzed using One-way ANOVA (Post Hoc-LSD) (SPSS Inc., Chicago, IL, USA; Version 18-21) by comparing the means of the various vitreous and serum biochemical parameters of the four groups of the study. A $p < 0.05$ was considered significant.

**RESULTS**

**Table 1: A Multiple Comparison of Serum Protein Profiles on the Basis of Durations of Chronic CO Intoxications**

| Parameters | Control | Duration of CO Exposure | | |
|---|---|---|---|---|
| | | Day 10 | Day 20 | Day 30 |
| **TP (g/L)** | 47 ± 6 | 37 ± 4 [a] | 34 ± 3 [a] | 34 ± 4 [a] |
| **ALB (g/L)** | 30 ± 2 | 28 ± 5 | 27 ± 4 | 29 ± 3 |
| **GLO (g/L)** | 17 ± 7 | 9 ± 2 [a] | 8 ± 5 [a] | 4 ± 1 [a] |
| **A/G Ratio** | 2.2 ± 1.2 | 3.5 ± 1.2 | 5.0 ± 4.1 | 7.1 ± 1.5 [a] |

**Table 2: A Multiple Comparison of Vitreous Protein on the Basis of Durations of Chronic CO Intoxications**

| Parameters | Control | Duration of CO Exposure | | | F-Value | P-Value |
|---|---|---|---|---|---|---|
| | | Day 10 | Day 20 | Day 30 | | |
| **TP (g/L)** | 4.3 ± 2.5 | 2.8 ± 1.1 | 2.3 ± 1.3 [a] | 1.8 ± 0.4 [a] | 3.048 | 0.070 |
| **ALB (g/L)** | 1.0 ± 0.8 | 1.3 ± 0.5 | 0.6 ± 0.5 | 0.8 ± 0.5 | 0.929 | 0.457 |
| **GLO (g/L)** | 3.5 ± 2.4 | 1.6 ± 0.6 [a] | 1.7 ± 0.9 [a] | 1.1 ± 0.7 [a] | 5.199 | 0.016 |
| **A/G Ratio** | 0.17 ± 0.16 | 0.85 ± 0.24 [a] | 0.36 ± 0.33 | 0.64 ± 0.55 | 2.754 | 0.098 |

Data are mean ± SD; Significant at 0.05 Confidence ($P < 0.05$)

**Symbols-** *a*: $P < 0.05$ vs control, *b*: $P < 0.05$ vs Day 10, *c*: $P < 0.05$ vs Day 20

**Legend**: TP- Total Protein; ALB-Albumin; GLO- Globulin; A/G- Albumin/Globulin.

**Table 3: A Multiple Comparisons of Serum Lipid Profile Exposed on the Basis of Durations of Chronic CO Intoxications**

| Parameters | Control | Duration of CO Exposure | | |
|---|---|---|---|---|
| | | Day 10 | Day 20 | Day 30 |
| TC (mmol/L) | 3.2 ± 0.2 | 3.1 ± 0.29 | 2.0 ± 0.3 $^{ab}$ | 1.9 ± 0.2 $^{ab}$ |
| TG (mmol/L) | 1.12± 0.09 | 1.62± 0.23 $^{a}$ | 1.54 ±0.03 $^{a}$ | 1.53 ±0.31 $^{a}$ |
| HDL (mmol/L) | 0.91± 0.18 | 0.59± 0.18 $^{a}$ | 0.31± 0.01 $^{ab}$ | 0.22± 0.18 $^{ab}$ |
| LDL (mmol/L) | 0.39± 0.16 | 0.77± 0.75 $^{a}$ | 0.28 ± 0.24 $^{b}$ | 0.20± 0.16 $^{b}$ |
| VLDL (mmol/L) | 1.99± 0.18 | 1.15± 0.44 $^{a}$ | 1.45±0.29 $^{ab}$ | 1.48± 0.30 $^{a}$ |

**Table 4: A Multiple Comparison of Vitreous Lipid Profiles on the Basis of Durations of Chronic CO Intoxications**

| Parameters | Control | Duration of CO Exposure | | |
|---|---|---|---|---|
| | | Day 10 | Day 20 | Day 30 |
| TC (mmol/L) | 1.44± 0.14 | 0.85± 0.13 $^{a}$ | 0.67± 0.17 $^{a}$ | 0.55± 0.13 $^{ab}$ |
| TG (mmol/L) | 0.76± 0.06 | 0.23± 0.04 $^{a}$ | 0.19± 0.03 $^{a}$ | 0.16 ± 0.05 $^{ab}$ |
| HDL (mmol/L) | 0.39 ± 0.04 | 0.12 ± 0.02 $^{a}$ | 0.10 ± 0.01 $^{a}$ | 0.13 ± 0.01 $^{a}$ |
| LDL (mmol/L) | 0.71 ± 0.05 | 0.63 ± 0.10 $^{a}$ | 0.49 ± 0.15 $^{a}$ | 0.38 ±0.10 $^{ab}$ |
| VLDL (mmol/L) | 0.345±0.026 | 0.105±0.017 $^{a}$ | 0.090±0.014 $^{a}$ | 0.078±0.022 $^{a}$ |

Data are mean ± SD; Significant at 0.05 Confidence ($P < 0.05$)

**Symbols-** *a*: $P < 0.05$ vs control, *b*: $P < 0.05$ vs Day 10, *c*: $P < 0.05$ vs Day 20

**Legend**: Group I- 10th Day; Group II- 20th Day; Group III- 30th Day CO ; TC- Total Cholesterol**;** TG-Triacylglycerol**;** HDL-High Density Lipoprotein**;** LDL-Low Density Lipoprotein**;** VLDL-Very Low Density Lipoprotein

## DISCUSSION

Chronic carbon monoxide poisoning is the inhalation of low quantity of CO over a long duration. The rampancy of CO in the environment and the increasing chronic health challenges in Nigeria and the world at large need an inquisitive research. This will in no measure open up a new vista of seamless relationships between CO and some idiopathic diseases conditions.

This study revealed a significant decrease ($P < 0.05$) in concentrations of serum and vitreous total protein and globulin and an increase in A/G ratio across the chronic CO intoxication period (Table 1 & 2). The reduction in serum and vitreous total protein and globubin reported in this study could be attributed to immune-suppression. Globulins are immune proteins produced in response to infection and inflammation. The consistent exposure to CO resulted to depression of the immune system with consequent reduction in globulin as observed in this study. Moreover, the reduction in total protein is due to the fall in globulin as seen in the increased A/G ratio. An increase in A/G is attributable to immunodepression and compromise [17]. Chronic CO intoxication has the capacity of compromising the immune system, hence exposing the body vulnerable to arrays of diseases.

The plasma and vitreous lipid profiles analyzed include; total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein

(VLDL). The findings of this study exhibited a pan significant decrease ($P < 0.05$) in concentration of serum and vitreous lipid profiles except for triacylglycerol (Table 3 & 4).

The scientific basis of the alterations observed in this study is multifaceted. The plasma and vitreous lipid profile which were lower (except serum TG) could be attributed to massive infiltration of lipids into cells and tissues occasioned by the consistent hypoxia generated by CO. The movement results to decrease in lipid concentration in body fluids and increase in cells and tissues. The research is of the view that CO enhances the permeability of cell membranes, which effortlessly enhances the infiltration of lipids. The main mechanism by which CO causes heart disease is basically due to hypoxia. The infiltration mechanism as postulated in this research is in accordance with arrays of findings that observed same in terms of increased cholesterol accumulation in cells. Studies in rabbits and monkeys have reported that exposure to CO produced accumulation of cholesterol in the aorta and coronary arteries and endothelial damage[18]. Widlansky *et al*.[19] also showed an endothelial dysfunction occasioned by cholesterol infiltration.

Oxidative stress is a condition characterized by elevated levels of intracellular reactive oxygen species (ROS) which are the progenitors of free radicals. Free radicals are highly reactive, and capable of damaging almost all types of biomolecules (proteins, lipids, carbohydrate, and nucleic acids). The harmful effect also extends to cells and tissues [2, 20, 21]. Hence, the free radicals generated during CO effect have the propensity of distorting and degrading lipids in the systems. It is this effect that cascade into lipid peroxidation, which in turn reflected the reduction in concentration of studied lipid profiles.

The increase in serum TG is a product of the capacity of CO to causing diabetes mellitus. Due to the insufficiency and inefficiency of insulin and glucagon, the body usually derives energy from another source especially TG. Increase in serum TG is a medical fact of the pathophysiology of diabetes mellitus [22].

## CONCLUSIONS

The aim of the study is to assess the chronic implication of moderate inhalation CO on serum and vitreous proteins and lipids profiles. This study revealed a significant decrease in concentrations of serum and vitreous total protein and globulin across the chronic CO intoxication period. Furthermore, the study showed that a pan decline in concentrations of lipid profiles in both the vitreous and serum. These findings have clearly shown that chronic CO intoxication could produce immune suppression and hypolidaemia which are basis of a lot of diseases.

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