Advances in Science and Technology, Vol. 2, No.2, July/August 2008, pp78-83.

# Changes in Hepatic Redox State and Serum Glucose Concentration by Co-Administration of Bonny (Nigerian) Light Crude Oil and Alcohol in the Albino Rat

Ibiba F. Oruambo* and Awolayeofori Dokubo
Department of Chemistry, Biochemistry Unit, Rivers State University of Science and Technology, Port Harcourt, Rivers State, Nigeria.
(Submitted: September 20, 2007; Accepted: June 2, 2008)

## Abstract

The effects of the administration of alcohol alone, Bonny Light Crude Oil (BLCO) alone, and alcohol plus BLCO on carbohydrate catabolism, as measured by changes in cytosolic and mitochondrial redox (NADH/$NAD^+$ ratios) states, were determined in the livers of male albino rats. The practice of co-exposure of the Niger Delta sub-population to hard liquor and spilled crude oil (BLCO) is common, hence the need for this study. Changes in the ratios of the redox pair, NADH/$NAD^+$ in rat liver cytosol and mitochondrial fractions were used to assess carbohydrate oxidation by absorption spectrophotometry at 340nm (NADH) and 260nm ($NAD^+$). The results show that alcohol alone caused a decrease in cytosolic NADH/$NAD^+$ ratio by 8.85% while increasing blood glucose level by 35.5% over the untreated controls. On the other hand, BLCO alone increased cytosolic NADH/$NAD^+$ ratio by 25.3% but increase blood glucose level slightly by 12.6%. However, alcohol and BLCO together did not change cytosolic NADH/$NAD^+$ ratio over the controls, (4.17% and 4.18%, respectively) but increased blood glucose level by 27.9% over the controls. These results suggest that in the liver cytosol, where glycolysis takes place, alcohol alone seems to depress glucose oxidation perhaps resulting in an increase in blood glucose; BLCO alone on the other hand seems to induce glucose oxidation; while alcohol and BLCO together seem not to affect glucose oxidation, while marginally elevating blood glucose level. In the mitochondrial fraction, there were no changes in redox states in alcohol alone- and BLCO alone-treated rats compared to the control except in combined alcohol and BLCO-treated rats where NADH/$NAD^+$ ratio increased by 27% over the control, a clear indication of enhanced mitochondrial oxidative process, which may have consequences for cellular energy production.

Keywords: Glycolysis, Redox state, NADH/$NAD^+$ ratio; alcohol; bonny light crude oil.

## 1.0 Introduction

The exploration and production of Bonny light crude oil (BLCO) in Nigeria takes place exclusively in the Niger-Delta region which poses health-risks to the rural population who may be exposed to spillages. In addition to the probable health risks of exposure to spilled BLCO, substantial segment of the rural Niger-Delta population engage in a socio-cultural practice of hard liquor consumption (personal experience). This combination may adversely alter metabolic energy production and possibly depress the availability of the co-enzyme, $NAD^+$ needed for continued oxidative reactions.

Metabolism of alcohol takes place primarily in the liver cytosol after absorption by the mucosal surface of the gastro-intestinal tract (see Kalent 1971. This occurs by direct diffusion into the blood and subsequent transportation to the liver for metabolism (see Kalent 1971). Here, it is oxidized in the cytosol to acetaldehyde by alcohol dehydrogenase using $NAD^+$ as co-enzyme to produce $NADH+H^+$ (see Kuston 1996). Acetaldehyde enters the mitochondria and is further oxidized to acetate by acetaldehyde dehydrogenase, again with $NAD^+$ as co-enzyme to form $NADH+H^+$; acetate is then released in the blood and transported to peripheral tissues where it is finally converted to $CO_2$, $H_20$ and fatty acid (see Kuston 1996). This process leads to increase in NADH/$NAD^+$ ratio as oxidation proceeds. Unabated increases in NADH over $NAD^+$ can affect several other metabolic reactions that use these two co-enzymes, for instance, one study reported that increase in NADH can promote the production of lactate from pyruvate, thereby deple-

* Corresponding author e-mail: ibibaforuambo@yahoo.co.uk

ting the concentration of pyruvate which can lead to lactic acidosis and inhibition of gluconeogenesis (see Abdulla and Badaway 2007). The pathway for the metabolism of crude oil in laboratory animals is not well understood (see International Agency for Research on Cancer (IARC) Monographs 1989). On the other hand, the toxic effects in laboratory animals are amply documented: increases in liver weight, hepatic proteins, RNA, glycogen, total lipids by the oral administration of Prudhoe Bay crude oil (5.0ml/kg bw daily for two days) to male Charles River CD-1 mice (see Khan, Irfan and Rahimtula, 1987); effects on reproduction and prenatal toxicity (see International Agency for Research on Cancer (IARC) Monographs 1989).

In a recent study (see Oruambo and Jones 2007), we reported that BLCO caused the increased availability of crude oil hydrocarbons in the liver cells, and subsequent induction of unscheduled mitochondrial DNA synthesis, and alteration of mitochondrial/endoplasmic reticulum $Ca^{2+}$ sequestration or $Ca^{2+}$ concentration gradient, leading to between 94% to 96% decreases in liver cytosolic $Ca^{2+}$ concentration in a dose-independent manner which we suggested may inhibit $Ca^{2+}$ influx into the cytosol and thus glycogen breakdown. This we concluded may impair the ability of the liver to export glucose molecules to desiring tissues, (brain, erythrocytes, skeletal muscle) which in turn may inhibit ATP synthesis in these tissues. The probable interaction of BLCO on intermediary metabolism, specifically glycolysis, the Krebs cycle and/or the electron transport system coupled to oxidative phosphorylation has not been reported. Thus it is not clear how, if at all, crude oil (BLCO) affects cellular energy production in the liver, for sustainable metabolism, particularly, anabolic processes, while maintaining normal circulating blood glucose level; two of the liver's key functions.

In the present study, as a continuation of the work in this laboratory to understand the probable molecular pathway(s) of BLCO potential hepatotoxicity, we aimed to determine whether or not BLCO can depress or induce glycolysis in the liver cytosol, which is the initial pathway in intermediary metabolism of carbohydrates for metabolic energy synthesis; and further whether in combination with ethanol, BLCO may exert an inhibitory effect on ethanol-induced adverse effect on glycolysis. Ethanol at 0.2ml/kg bw was administered alone; BLCO at 5.0ml/kg bw was administered alone, and both were administered as single doses to adult male albino rats for six hours by intraperitoneal injection. The end-point was judged to be increases or decreases in redox state in the liver cytosol as measured by the ratios of the redox pair, $NADH/NAD^+$, with concomitant increases or decreases in blood glucose levels.

## 2.0 Materials and Methods

### 2.1 Crude Oil

Fresh Nigerian (Bonny) Light Crude Oil (BLCO) was obtained from the Nigerian National Petroleum Corporation (NNPC) in Port Harcourt, Rivers State, Nigeria, and brought to the laboratory in an amber bottle.

### 2.2 Treatment of Animals

Twenty adult male albino rats each weighing between 200 and 250gm (0.2 and 0.25 kg) were obtained from the Animal Breeding House of the University of Port Harcourt, Rivers State, Nigeria. These were separated into four groups of five animals per group. The first group each received ethanol at a single dose of 0.2ml/kg bw by intraperitoneal injection (i.p.); the second group each received a single dose of 5.0 ml/kg bw BLCO also by i.p.; the third group each received single doses of 0.2 ml/kg bw ethanol and 5.0 ml/kg bw BLCO simultaneously also by i.p.; while the fourth group was not treated and served as the control. All animals were given rodent chow and drinking water *ad libitum* prior to and following treatment, and were sacrificed six hours after treatment. Blood samples were collected in fluoride oxalate tubes just before sacrifice from each group, centrifuged at 4,000rpm for 10 minutes to obtain serum and stored at $4^oC$ for serum glucose level quantification. Liver tissues were excised, pooled by group and homogenized to 10% (w/v) in ice-cold 0.05M potassium phosphate (pH7.5) buffer.

### 2.3 Serum Glucose Quantification

Serum glucose levels were measured in all the collected samples by the glucose oxidase method

that is widely used in clinical laboratory procedures (see Bearser and Hill 1995).

To ensure reproducibility, each assay was carried-out in triplicates; and the Arithmetic Mean with their corresponding standard deviations were calculated. The results are therefore expressed as Mean $\pm$ SD. Changes over control values were calculated in percentage increase or decrease.

### 2.4 Determination of NADH/NAD$^+$ ratios in Cytosolic and Mitochondrial Fractions

Following homogenization, the homogenate was centrifuged at 1000 x g for 10mins to sediment the nuclei which was discarded (see Oruambo and Jones 2007); the resultant supernatant was collected and re-centrifuged at 3,000 x g for 10min. to sediment the mitochondria (see Oruambo and Jones 2007) which was collected and re-suspended in potassium phosphate buffer solution; this represented the mitochondrial fraction. The resultant supernatant was decanted and re-centrifuged at 10,000 x g for 10min. This time, the sediment was discarded while the supernatant was collected and represented the cytosolic fraction (see Oruambo and Jones 2007).

The NADH/NAD$^+$ ratios were determined in the liver cytosolic and mitochondrial fractions by near-visible and ultraviolet absorption spectrophotometry of NADH and NAD$^+$ respectively (see Nelson and Cox 2000). NADH, the reduced form of the co-enzyme absorbs maximally at 340nm, while NAD$^+$, the oxidized form of the co-enzyme has no absorbance here. Conversely, NAD$^+$, the oxidized form of the co-enzyme absorbs maximally at 260nm, while NADH has no absorbance here at this wavelength (personal experience). Consequently, absorbances of each sample of each group were obtained at 340nm and 260nm almost simultaneously in triplicates in both the cytosolic and mitochondrial fractions (see Oruambo and Jones 2007). Results are expressed as mean $\pm$ SD; and changes over control, as percent change, as described above.

## 3.0 Results

In Table 1, the effects of treatment regimen i.e. alcohol alone, BLCO alone or combination of alcohol and BLCO, on blood glucose concentration are shown. Treatment was by i.p. at single doses of ethanol (0.2ml/kg bw) and BLCO (5.0ml/kg bw) for six hours. All three regimens showed increases in blood glucose concentrations: there was a sizeable increase of 35.5% in ethanol-treated rats over the untreated controls; a marginal increase of 12.6% in BLCO-treated rats; and a moderate increase of 27.9% in ethanol and BLCO-treated rats. Clearly, ethanol induced the largest increase in blood glucose when compared to BLCO and the combination of ethanol and BLCO. Indeed, it seems that BLCO, in this result, may have acted antagonistically in counteracting what may have been the induction effect of ethanol.

Table 2 shows the effects of the treatment regimes on the levels of the redox pair, NADH and NAD$^+$ in the liver cytosol. Ethanol caused a decrease in NADH while increasing NAD$^+$ levels, thus resulting in slight decrease of 8.85% in NADH/NAD$^+$ ratio over the control; which suggests that glucose oxidation via glycolysis in the cytosol may have been depressed by ethanol. If this is true, blood glucose level may be higher in the presence of ethanol; in Table 1, it was higher by 35.5%.On the other hand comparatively, BLCO caused an increase in NADH and a decrease in NAD$^+$ levels over the control, thus resulting in a moderate increase of 25.3% in NADH/NAD$^+$ ratio over the control. This suggests a BLCO-induced acceleration of glucose oxidation by glycolysis in the cytosol. Again, if true, blood glucose level should either decrease or remain unchanged as the animals were fed throughout the experiment: In Table 1, blood glucose level actually increased but slightly by 12.6%.

However, combination treatment of ethanol and BLCO yielded an interesting result for the NADH/NAD$^+$ ratio: there was no change over the control (4.17 and 4.18, respectively), which suggests what we postulated in Table 1, that is, BLCO may have counteracted the ethanol-induced depression of glycolysis in the cytosol, although blood glucose level did not reflect this absolutely, but only marginally as it increased by 27.9% over the control.

In Table 3, we show the NADH/NAD$^+$ ratios in mitochondrial fraction of all three treatment regimes. Compared to the untreated control, ethanol alone and BLCO alone showed little change in NADH/

$NAD^+$ ratios (4.8% and 5.9% decreases, respectively), while combination of ethanol and BLCO produced a 27% increase in NADH/$NAD^+$ ratio. This may mean that Krebs cycle reactions, in particular, may have been induced by this treatment regimen.

Table 1. Glucose Concentrations in Serum of Male Albino Rats Treated with Ethanol alone, (0.2ml/kg bw), BLCO alone (5.0 ml/kg bw) or Ethanol + BLCO (same single doses) by intraperitoneal injection for six hours.

| Treatment | Serum Glucose Concentration (mmol/L) | Percent Increase over control |
|---|---|---|
| Control (untreated) | 6.03. ± 0.21 | Baseline |
| Ethanol (alone) | 8.17 ± 0.50 | 35.5 |
| BLCO (alone) - | 6.79 ± 0.52 | 12.6 |
| Ethanol +BLCO - | 7.71 ± 0.41 | 27.9 |

Values are expressed as Means ± S.D. of 4 determinations

Table 2: NADH/ $NAD^+$ Ratios in Liver Cytosolic Fractions of Male Albino Rats Treated With Ethanol alone, (0.2ml/kg bw), BLCO alone (5.0 ml/kg bw) or Ethanol +BLCO (same single doses) by intraperitoneal injection for six hours

| Treatment | NADH (340nm) | $NAD^+$ (260nm) | NADH/ $NAD^+$ Ratio | Percent Change over control |
|---|---|---|---|---|
| Control (untreated | 0.557±0.002 | 0.138±0.0040 | 4.18 | Baseline |
| Ethanol (alone) | 0.712±0.002 | 0.187±0.0001 | 3.81 | -8.85 |
| BLCO (alone) | 0.686±0.001 | 0.131±0.0010 | 0.131±0.001 | + 25.3 |
| Ethanol + BLCO | 0.771±0.001 | 0.185±0.0020 | 4.17 | Nil. |

Values are expressed as Means ± S.D. of 4 determinations

Table 3: NADH/$NAD^+$ Ratios in Liver Mitochondrial Fraction of Male Albino Rats Treated with Ethanol alone, (0.2ml/kg bw), BLCO alone (5.0 ml/kg bw) or Ethanol + BLCO (same single doses) by intraperitoneal injection for six hours

| Treatment | NADH (340nm) | $NAD^+$ (260nm) | NADH/$NAD^+$ Ratio | Percent change over control |
|---|---|---|---|---|
| Control (untreated) | 0.959±0.023 | 0.519±0.0210 | 1.85 | Baseline |
| Ethanol (alone) | 1.443±0.005 | 0.818±0.0005 | 1.76 | -4.8 |
| BLCO (alone) | 1.205±0.001 | 0.693±0.0009 | 1.74 | -5.9 |
| Ethanol + BLCO | 0.995±0.021 | 0.423±0.0030 | 2.35 | +27 |

Values are expressed as Means ± S.D. of 4 determinations

## 4.0 Discussion

The results obtained in Tables 1,2, and 3 showed that ethanol alone seemed to depress glucose breakdown (oxidation) via glycolysis as the ratio of the redox pair NADH/$NAD^+$ decreased and blood glucose level increased significantly; BLCO alone seemed to induce glucose oxidation as the NADH/$NAD^+$ ratio increased by a moderate margin, though a slight increase was observed in the blood glucose level, while the combination of ethanol and BLCO did not change NADH/$NAD^+$ ratio, but also increased blood glucose level by a moderate margin. Clearly, the state of the redox pair, NADH/$NAD^+$ seems to correlate with blood glucose levels: when NADH level rises, it signals the predominance of oxidation (i.e. glycolysis), consequently, a depletion of blood glucose until replenished (see Depre Rider and Hue 1998), on the other hand, when $NAD^+$ level rises at the expense of NADH, it signals the predominance of anabolism or the depression of glycolysis; a catabolic process (see Depre Rider and Hue 1998). Blood glucose level is then expected to rise. Hence, when ethanol alone, BLCO alone or ethanol + BLCO alter the state of the ratio of the redox pair, NADH/$NAD^+$, it directly or indirectly affect blood glucose levels either concomitantly or correlatively.

The relationship is borne out from the biochemical reaction pathway of carbohydrate metabolism because it is as glucose that the bulk of dietary carbohydrate is absorbed and transported to the

bloodstream and to the liver where it undergoes oxidation via the reactions of glycolysis in the cytosol. This pathway is primarily cellular/metabolic energy-producing in the form of Adenosine tri-phasphate (ATP) when linked to the Krebs cycle, electron transport chain and oxidative phosphorylation in the mitochondria (see Depre Rider and Hue 1998).

Normally, since glycolysis is catabolic, it is oxidative in nature and therefore has a net requirement for the oxidized form of the co-enzyme, $NAD^+$. As the reaction sequence proceeds, $NAD^+$ is reduced to its redox partner, $NADH(H^+)$, $NAD^+$ level decreases while NADH level increases; thus for every molecule of glucose that is oxidized by aerobic glycolysis in the liver, when not linked to skeletal glycolysis, there is a net gain of a pair of electrons in the form of $NADH (H^+)$. Consequently, the equilibrium state of the ratio of this redox pair, $NADH/NAD^+$, serves as indicator of on-going oxidation of glucose via glycolysis (see Depre Rider and Hue 1998). Furthermore, in addition to this role of the liver in producing metabolic/cellular energy via glycolysis, the liver is also an exporter of glucose to such tissues as the brain, testes, erythrocytes, and skeletal muscle, and maintains the normal concentration of circulating blood glucose concentration (see Depre Rider and Hue 1998) . It is from this function of the liver, that we observe the correlation between active glycolysis ($NADH/NAD^+$ ratio) and blood glucose concentration.

When ethanol seemed to depress glycolysis by decreasing the ratio of the equilibrium constant of the redox pair $NADH/NAD^+$, it is plausible to suggest that blood glucose level would rise. In our results, it did just that by 35. 5%. As shown, when BLCO induced glycolysis by increasing the $NADH/NAD^+$ ratio, we suggested that blood glucose level should decrease or remain unchanged; it however increased but only marginally by 12.6%. We note that this represents a 23% differential (decrease) from the alcohol- included 35.5%.

However, when the combination of ethanol and BLCO did not change the ratio of $NADH/NAD^+$, we expected that the blood glucose level would also not change, that is remain at equilibrium; not so, blood glucose level increased by a moderate 27.9%. It is not totally clear why this is so, but we observe that this increase is 7% lower than the increase caused by ethanol alone and about 15% higher than BLCO-induced marginal increase of 12.6%; perhaps together, BLCO may be acting to modulate the hyperglycemic tendency of ethanol.

As this study-approach has not been reported before, we were unable to compare our results; however, because the practice of hard liquor consumption in the Niger Delta region of Nigeria may exacerbate the health risks from chronic exposures to crude oil spillages, the potential for exacerbation or modulation of the potential hepatotoxicity of BLCO inspired this study. We conclude that BLCO may not starve tissues of metabolic energy in form of ATP from glucose oxidation, but may slow down the rate of availability of glucose to these tissues for ATP- produced glycolysis; this therefore may be one of the molecular pathways of BLCO- induced hepatotoxicity.

## References

Abdulla, A.B., Badaway. B. A review of the effect of alcohol, on carbohydrate metabolism, in: *Medical Council on Alcohol Oxford Journals, Oxford University Press,* 2007.

Bearser, R.S. and Hill, Joan 1995, In: The Joslin Guide to Diabetes. New York: Simon and Schuster Publishers, p.158.

Depre, C., Rider, M.H., and Hue, L. 1998, Mechanisms of Control of Heart and Skeletal muscle glycolysis, Enr. J. Biochem, ***258***, 277-290.

International Agency for Research on Cancer (IARC) Monographs, Occupational Exposures in Petroleum Refining Crude Oil and Major Petroleum Fuels, **1989** ***45***,144.

Kalent, H. 1971, "Absorption, distribution and elimination of ethanol: effect on biological membranes" in :*The Biology of Alcoholism, B. Kissin and H. Begleiter (Eds), Plenum Press, New York,* ***1***, 1-62.

Khan, S, Irfan, M. and Rahimtula, A. D. 1987**, "**The hapatotoxic potential of a Prudhoe Bay crude oil: effect on mouse liver weight and composition", *Toxicology,* ***46***, 95-105.

Kuston, K.E. 1996, "Ethanol and acetaldehyde metabolism: past, present and future", Alcohol Clin. Exp. Res., *20*: 662-674.

Nelson, D.L. and Cox, M.M.: Bioenergetics and

Metabolism; In: Lehninger Principles of Biochemistry, Worth Publishers, New York. 2000, p. 519.

Oruambo, I.F. and Jones, A.B. 2007, Alterations in the concentrations of liver mitochondrial DNA, Cytoplasmic Total Hydrocarbon and Calcium in Guinea pigs After Treatment with Nigerian light crude oil. *Int.J.Environ. Res. Public Health, **4** (1),* 23-27.