Effects of a Mixture of Methylated Spirit and Carbonated Cola Drink (A Novel Substance Abused by Nigerian Youths) On the Lipid Profile of Wistar Rats

[1]*, Ikimi, C.G. and [2]* Addy P. S.
[1]Department of Biochemistry, Faculty of Science,
Federal University Otuoke, Bayelsa State, Nigeria
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, Bayelsa Medical University, Imgbi Road, Yenagoa, Bayelsa State, Nigeria.
peremobowei.addy@bmu.edu.ng
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Abstract

Absence of information on the health effects of the intake of a mixture of methylated spirit and carbonated cola drink affects decisions on its use. The campaign for the disuse of the substances recently being abused by young people across Nigeria shall be baseless without empirical evidence to validate such clamours. This study is an attempt to provide primary data on how some of these novel mixtures impact the body. It aims to determine the effects of a mixture of methylated spirit and a carbonated cola drink on the lipid profile of male wistar rats as a means of assessing its health impact. Male wistar rats were obtained from the animal house of the Department of Biochemistry, University of Benin, Edo state, Nigeria. Methylated spirit and carbonated cola drinks were purchased from Otuoke Pharmacy, Otuoke, Bayelsa state and used for the research. Methylated spirit (50ml) was added to 50ml of cola drink in a beaker to obtain 100ml of the mixture. The animals were grouped into 3: A, B and C with seven animals in each group. A single dose of 0.1ml of the mixture were orally administered to those in group A and 0.2ml to those in group B using plastic oral intubation and gavage tube. Those in group C where orally administered 1 ml of distilled water and served as the control. The concentrations of total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride and very low density lipoprotein were analyzed using semi-automated biochemistry analyzer with standard reagent kits. The result of the investigation reveals that intake of the mixture increased the mean levels of total cholesterol $(3.774 \pm 0.82 mmol/L)$*, triglyceride* $(2.026 \pm 0.42 mmol/L)$*, high density lipoprotein* $(1.468 \pm 0.25 mmol/L)$*, low density lipoprotein* $(1.385 \pm 0.38 mmol/L)$*, very low density lipoprotein* $(0.9205 \pm 0.19 mmol/L)$ *for group A and total cholesterol* $(2.782 \pm 0.03 mmol/L)$*, triglyceride* $(1.450 \pm 0.68 mmol/L)$*, high density lipoprotein* $(1.043 \pm 0.17 mmol/L)$*, low density lipoprotein* $(1.080 \pm 0.16 mmol/L)$*, very low density lipoprotein* $(0.659 \pm 0.31 mmol/L)$ *for group B when compared to the control total cholesterol* $(4.671 \pm 0.13 mmol/L)$*, triglyceride* $(0.6160 \pm 0.10 mmol/L)$*, high density lipoprotein* $(1.084 \pm 0.01 mmol/L)$*, low density lipoprotein* $(3.321 \pm 0.05 mmol/L)$*, very low density lipoprotein* $(0.2910 \pm 0.03 mmol/L)$*. The observed altered the lipid profiles in the experimental subjects may initiate further metabolic changes as high lipid levels have been associated with weight gain, obesity and atherosclerosis. Although alcohol is a substance of complex physiological effects that vary according to gender, age, race, body weight and consumption patterns,*

methanol as used in the mixture is not advisable, because its continual consumption will result in the development of cardiovascular diseases in abusers.

Key Words: *Methanol, Lipid Profile, Substance Abuse, Mixture and Cardiovascular Disease*

INTRODUCTION

The use of psychoactive substances among youths for non-conventional purposes has become a concern at different spheres of the society. Globally, it has been estimated that 90% of the population aged 12 years and above, are associated with dependency on psychoactive substances (Oluremi, 2012). In Nigeria, the illicit consumption of drugs and the harmful use of psychoactive substances such as alcohol, pharmaceutical drugs, inhalants and solvents have increased at an alarming rate over the years. Available reports indicate that Nigeria is currently the highest consumer of cannabis and amphetamine in Africa (Soyibo and Lee, 2003). It is estimated that 60% of children in Nigeria had abused one or more substances. 1.3% had used substances such as cocaine and marijuana. Most of the studies in Nigeria reported that alcohol is the most abused substance among students. Studies indicate that children began abusing certain household substances at the age of 12 to 13 (Soyibo and Lee, 2003).

Recently, non-medical consumption of cough syrups in Northern Nigeria has become a subject of public interests, largely due to its potential to cause serious health problems for its abusers (Rhems *et al*, 2003). Substance abuse is generally associated with a range of social, physiological, behavioral and health risk including increased risk of accidents, violence and suicide (Rhems *et al*, 2003). The use of illicit substances usually begins with tobacco and alcohol, thereafter, it advances to the abuse of inhalants, marijuana and drugs such as sleeping pills. If the abuse persists until late adolescence, abusers typically become more involved with other non-drug substances such as mixtures of various kinds of substances and other natural biochemical processes in the environment. Factors that bring about addiction to substances abuse include environmental factors; genetic factors; social factors, mainly peer pressure; and health factors, majorly mental illness (NIDA, 2003).

The abuse of several mixtures of substances are made possible because they are common, easily accessible, available in large quantities, cheap and very portable. This makes them the most abused substances in recent times.

The aim of this research is to determine the effects of a mixture of methylated spirit and a carbonated cola drinks on selected lipid profile following oral administration of sub-lethal doses in wistar rats, as a means of assessing its health impact.

METHODOLOGY

Study Area

This study was conducted in department of Biochemistry laboratory, Faculty of Science, Federal University Otuoke, Bayelsa state, Nigeria. Laboratory analysis of blood sample obtained from experimental animals was done at Enis biomedical laboratory, Igbogene, Bayelsa state. (Agoro *et al*, 2017).

Preparation of Substance for Administration

50ml of methylated spirit was added to 50ml of cola drink in a beaker. The mouth of the beaker was immediately sealed with aluminum foil to prevent evaporation.

Determination of LD_{50}

12 male wistar albino rats were used for determination of LD_{50} (median lethal dosage) using Lorke method (Lorke, 1983). This method is divided into two phases. In the first phase, nine (9) animals were divided into 3 groups of three animals each. The animals were administered graded doses of 10mg, 100mg and 1000mg of methylated spirit and cola drink mixture. 3 deaths were recorded after administration of the mixture within a period of 10 minutes.
In phase two, three (3) animals were grouped into three (3) groups of one (1) animal each and were administered graded doses of 1600mg, 2700mg and 5000mg. Within a period of 5 minutes all the three (3) animals died. The mixture was administered orally using syringes but without needles.

Selection criteria

Male wistar rats were obtained from the Animal house of Department of Biochemistry, University of Benin, Edo State, Nigeria. The wistar rats were allowed to acclimatize for one week with free access to diet (commercial pellet) and water adlibitum. The rats were housed in plastic cages under conditions of room temperature. The weights of the rats varied between 100-170grams. The experimental period lasted for 21 days (3 weeks).

EXPERIMENTAL DESIGN

After one week of acclimatization, 21 male wistar albino rats weighing 110kg and 120kg were grouped into three for the experiment. Each group had seven animals which were categorized in the format stated below:

Group A: This group consist of seven animals each animal received 0.1ml of the mixture once a day throughout the experimental period.

Group B: This group consist of seven animals each animal received 0.2ml of the mixture once a day throughout the experimental period.

Group C (Control): This group is the control group and consist of seven animals each animal received 1ml of distilled water once a day.

The experimental period lasted for three weeks. Blood samples were collected between experimental intervals of 7 days of the three weeks of the experimental period. The rats were sacrificed under mild anesthesia with chloroform in line with recommended animal right laws of the European treaty for the protection of vertebrate animals 2013. Blood samples were collected from the heart via cardio puncture before the death of the rats using a 2ml sterile syringe and needle. Blood samples were collected into EDTA bottles for haematology and plain bottles to obtain serum.

Preparation of Blood Samples

Whole blood collected into plain bottles was spun in the centrifuge at 3500cpm for 5 minutes to obtain serum for the biochemical assays.

ANALYSIS OF SAMPLE

Determination of TC content of samples

The enzymatic method was used for the determination of total cholesterol using standard kits produced by Agappe.

Assay Principle

Enzymatic colorimetric determination of total cholesterol according to the following reactions:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{4- cholesten-3- one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinine} + 4H_2O$$

3.2.6.2 Determination of TG content of samples

The endpoint colorimetric method was used for the determination of triglyceride using standard kit (Agappe).

Assay Principle

Enzymatic determination of triglyceride is based on the following reactions:

$$\text{Triglyceride} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty acid}$$

$$\text{Glycerol} + ATP \xrightarrow[Mg^{++}]{\text{Glycerol kinase}} \text{Glycerol-3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol-3-phosphate oxidase}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{TOPS} \xrightarrow{\text{POD}} \text{Violet colored complex}$$

Determination of HDL content of samples

The phosphotungstate method was used for the determination of HDL using standard kits (Agappe).

Assay principle

The chylomicrons, very low density lipoproteins (VLDL) and low density lipoproteins (LDL) of serum are precipitated by phosphotungstic acid and magnesium ions. After centrifugation, high density lipoproteins are in the supernatant. HDL content of supernatant is measured by an enzymatic method.

Determination of LDL content of samples

The Friedewald's formula was used for the determination of LDL.

Assay Principle

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

LDL-Cholesterol = TC – ($\frac{TG}{2.2}$ +HDL)

LDL carries most of the circulating cholesterol in man and when elevated contributes to the development of coronary atherosclerosis. LDL-Cholesterol is measured to assess risk for CHD and to follow the progress of patients being to lower LDL-Cholesterol concentrations.

Determination of VLDL content of samples

Assay principle

VLDL-cholesterol is calculated from measured values of total cholesterol, HDL-cholesterol and LDL-Cholesterol according to the relationship:

VLDL-Cholesterol = TC – (HDL + LDL)

VLDL-C is similar to LDL-C in the sense that it contains mostly fat and not much protein. VLDL-Cholesterol is the lipoproteins that carry cholesterol from the liver to organs and tissues in the body (Sundaram and Yao, 2010).They are formed by a combination of cholesterol and triglycerides. Moreover, VLDLs are heavier than LDL, and are also associated with atherosclerosis and coronary heart disease.

STATISTICAL ANALYSIS

The data obtained from the experiments were analyzed using statistical package for service solution (SPSS) software for Windows version 21 (SPSS Inc. Chicago, Illinois, USA). All the data were expressed as mean$\pm$standard deviation (SD). The limit of significance were set as $P<0.05$. Data obtained were subjected to test of significance using Student's T-test to determine if significant differences exist between the mean of test and control groups.

RESULT

In figure 1 below, total cholesterol level was significantly decreased in group A and B when compared to the control for day 1. Group A and B were significantly elevated compared to the control in day 7. Group A was decreased and group B was increased compared to the control in day 14.

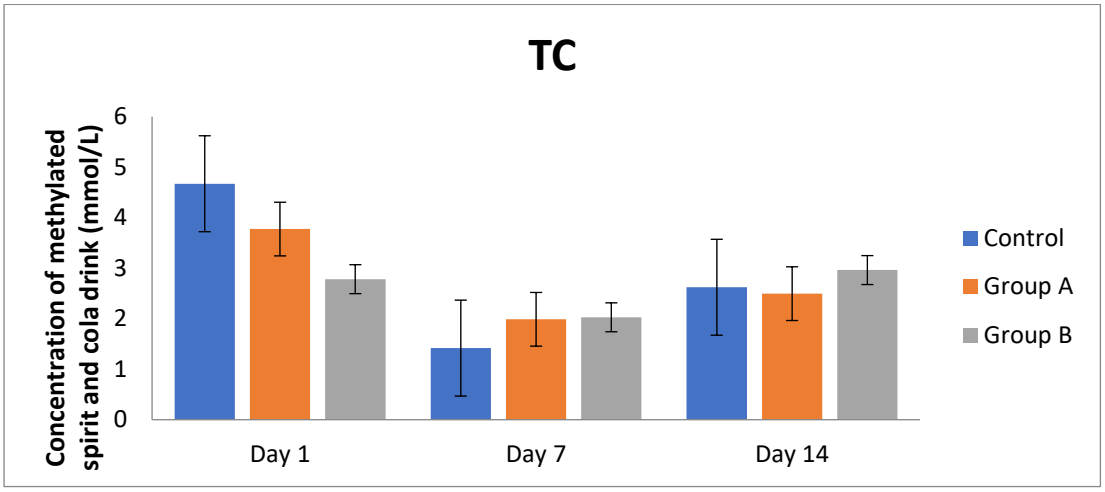

Fig 1: Levels of serum Total cholesterol (TC) in animals from different groups.

In figure 2 below, triglyceride level was significantly elevated in group A throughout the experiment when compared to the control. Group B was significantly elevated in day 1 and 14 when compared with the control.

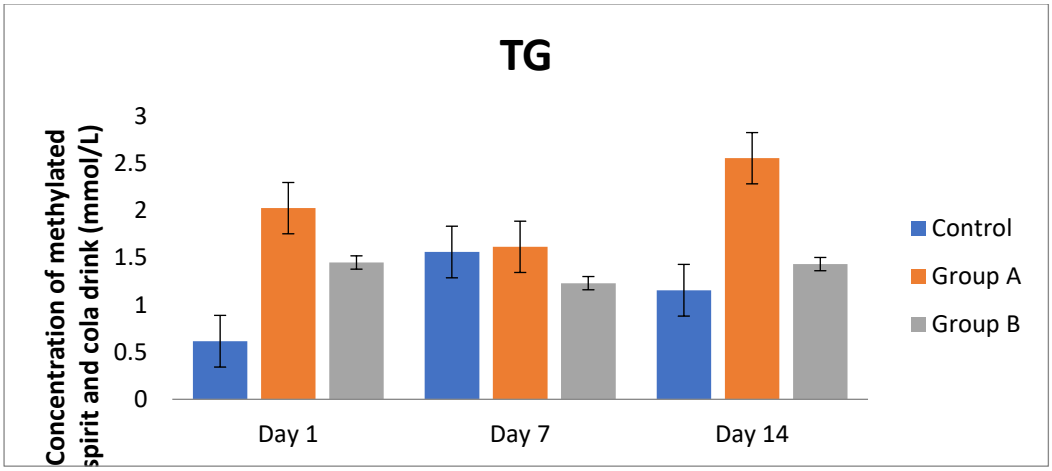

Fig 2: Levels of Triglycerides (TG) in animals from different groups.

In figure 3 below, high density lipoprotein cholesterol was significantly elevated in group A and group B was decreased in day 1 when compared to the control. Group B was significantly elevated in day 7 and 14 when compared to the control.

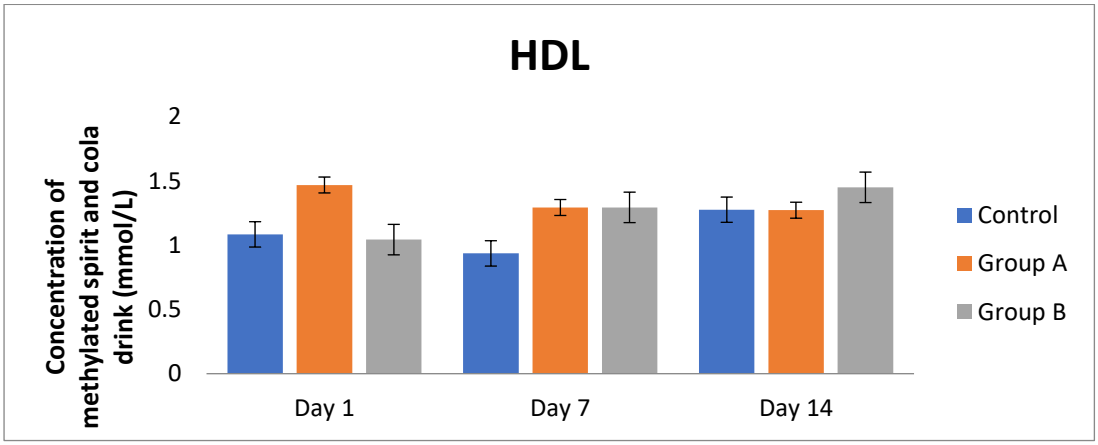

Fig 3: Levels of High density lipoprotein (HDL) in animals from different groups.

In figure 4 below, low density lipoprotein cholesterol was significantly decreased in group A throughout the days compared to the control. Group B was significantly decreased in day 1 and 7 compared to the control. Group B increased in day 14 compared to the control.

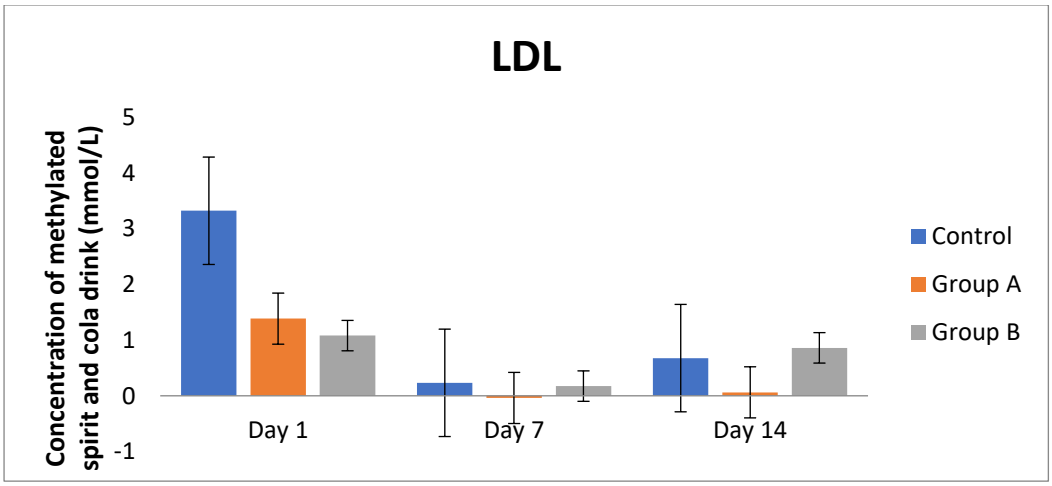

Fig 4: Levels of low density lipoprotein (LDL) in animals from different test groups.

In figure 5 below, very low density lipoprotein was significantly elevated in group A and B compared to the control but decreases in day 7 compared to the control. Group B decreased compared to the control.

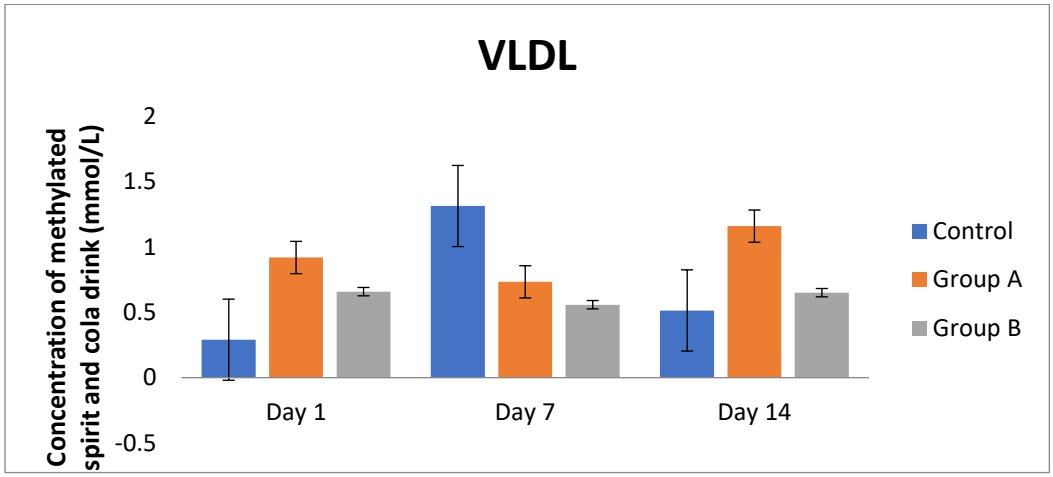

Fig 5: Levels of very low density lipoprotein (VLDL) in animals from different test groups.

DISCUSSION

It was observed that from the result of figure1, the levels of total cholesterol in group A were higher than from group B when compared to the control. In a previous study (Millán *et al*, 2009) for instance, moderate alcohol consumption was also associated with the smallest rise in the total-cholesterol:HDL-cholesterol ratio. An increase in the total cholesterol concentration is an atherogenic marker, whereas lower HDL-cholesterol concentrations are related to risk factors such as metabolic syndrome, the total-cholesterol: HDL-cholesterol ratio is considered to be a sensitive index of CVD risk. Increased levels of total cholesterol are found in hypercholesterolaemia, nephritic syndrome and uncontrolled diabetes, while decreased levels are found in malabsorption, malnutrition, anemia and liver diseases.

The levels of triglyceride in group A and B from the result of figure 2 were significantly higher than from the control. Alcohol is known to enhance concentrations of hepatic triglycerides. This increase is due to an increased synthesis of triglycerides in the liver (Chanda *et al*, 2013). The actions of alcohol on plasma triglycerides are more variable and depend upon the dose of alcohol administered, the underlying diet, the genetic predisposition to hypertriglyceridemia, and the duration of administration (Toshikuni *et al*, 2014). In a previous study (Deushi *et al*, 2007) for instance, the increase in plasma levels of triglycerides following the administration of very large amounts of alcohol was only transitory. Elevated plasma triglycerides and very low density lipoproteins are strongly and related to a greater incidence of coronary heart disease. Increased levels of triglyceride are found in hyperlipidemia, diabetes, and hypothyroidism. Increased levels are risk factor for arterioclerotic coronary disease, peripheral vascular disease, acute pancreatitis. Decreased levels are found in malnutrition and hyperthyroidism.

It was observed from the result of figure 3 the concentration of HDL in group A and B were significantly higher at $p<0.05$ than from the control. Several epidemiological studies suggest that alcohol raises plasma HDL-CH (Castelli *et al*, 1977). This effect also has been demonstrated in experimental animals (Rudel *et al*, 1981). To our knowledge, however, it has not been shown that controlled administration of alcohol raises HDL concentrations in man under metabolic ward conditions. Thus, despite the fact that alcohol raised triglycerides, which usually is associated with decrease in HDL-CH, there was instead a definite and paradoxical

increase in HDL-CH. The mechanism for this rise in HDL-CH induced by alcohol remains to be determined. High levels of high density lipoprotein have been found to be a protective factor for the development of that disease, so that decreased levels constitute a risk factor.

A previous epidemiologic report noted an inconsistent reciprocal relationship between alcohol intake and plasma LDL-CH (Castelli *et al*, 1977). This might imply that alcohol per se lowers LDL. In the present study, however, no such effect was seen. For the group as a whole, no changes in levels of LDL-CH were observed. Increased levels of LDL indicate a risk of atherogenic heart and peripheral vascular disease. The levels of VLDL in group A and B were significantly higher than from the control. In a study by Crouse and Grundy (Crouse *et al*, 1984), normal subjects did not have any differences in VLDL-TG production rates when alcohol was included in fat-free meals; alcohol induced a 45% increase in several obese subjects, however. Wolfe *et al*. (1976) reported that alcohol increased VLDL-TG production above pre-alcohol levels when normal subjects had fasted 69 h but not after a 15-h fast. The increased VLDL-TG production in the 69-h fasted subjects was apparently linked with increased peripheral release of FFA, as both FFA flux and FFA concentrations were increased over baseline levels following alcohol infusion in the 69-h fasted group but not the 15-h fasted group. It is possible that the stress induced by the induction of hypoglycemia stimulated a catecholamine response and subsequently overcame the usual antilipolytic effect of acetate (Abramson *et al*, 1968; Crouse *et al*, 1968; Nilsson *et al*, 1978) and increased peripheral lipolysis (Galster *et al*, 1981) in the 69-h fasted subjects. The increased VLDL-TG production after alcohol observed by Wolfe et al. (Wolfe *et al*, 1976) in 69-h fasted individuals and by Crouse and Grundy (1984) in obese subjects may have been driven by increased lipolytic rates.

CONCLUSION AND RECOMMENDATION

The effects of moderate alcohol consumption on the lipid profile are well-documented, showing an association between alcohol-induced increases in HDL-C levels and cardio protection (though there remains some debate). The mechanism of this potential cardio protective effect of alcohol is fertile ground for research. Whereas prior research was focused on alcohol-induced changes in cholesterol and triglycerides levels, the paradigm has shifted to the composition of lipoproteins, with emphasis on smaller lipid molecules such as sphingolipids. These molecules may impact the endothelium by their interaction with cell membranes and ultimately be responsible for the potential atheroprotection afforded by alcohol. Finally, direct evidence to recommend drinking alcohol in moderation for decreasing cardiovascular risk is still lacking, and presents another avenue for clinical research.

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