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Original Article

Use of atherogenic index of plasma in evaluating the potential cardioprotective effects of red wine consumption: Studies in Nigerian young adult volunteers.

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ABSTRACT: There is increased interest in the biomedical basis of the "French paradox"− the epidemiological phenomenon linking co-existence of cardiovascular risk factors with moderate consumption of red wine. This study examines the predictive values and development or otherwise of atherosclerosis and cardiovascular events by using the values of atherogenic lipid of plasma (AIP), cardiac risk ratios (CRR) and atherogenic coefficient (AC) to test the hypothesis of cardio-protection of red wine consumption in young adult volunteers. It has been suggested that these indices are better than the conventional lipid profile parameters in the assessment of cardioprotective activity of acute red wine ingestion. Five ml of fasting blood was collected from each participant before they were given 300 ml of 11.5% v/v alcoholic red wine to drink within 5 minutes. One hour after ingestion of the wine, further 5 ml blood samples were collected. The plasma levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were determined, after which CRR, AC and AIP were calculated. The results show that red wine consumption induced increase in the levels of TG ($p<0.05$), HDL-c ($p<0.001$) and VLDL-c ($p>0.05$), but a decrease in the levels of TC and LDL-c ($p<0.001$). This study shows that all lipoprotein-related indices of atherogenicity (with the exception of HDL-c/LDL-c ratio) were significantly reduced ($p<0.001$) one hour after red wine ingestion in Nigerian blacks. However, the predictive value of this finding for cardiovascular events remained to be demonstrated.

KEYWORDS: Red wine; cardiac risk ratios; atherogenic lipid of plasma; atherogenic coefficient.

INTRODUCTION

Cardiovascular disease (CVD) is a major cause of mortality and morbidity in developed and developing countries and is most frequently manifested as atherosclerosis of the major arteries (Wu *et al*., 2011). Despite research efforts aimed at studying the disease, its etiology is still not completely resolved. It follows that development of cost-effective prevention and management strategies remain at the forefront of efforts aimed at combating these conditions. There is a growing body of knowledge focused on dietary agents with cardioprotective activities with the goal of understanding the "French paradox"–the epidemiological phenomenon linking co-existence of CVD risk factors with moderate consumption of red wine (Zern and Fernandez, 2005; Andrade *et al*., 2009). Studies have documented protective roles of moderate red wine consumption in both vascular and non-vascular diseases (Andrade *et al*., 2009; Wu *et al*., 2011). Following on several studies, a number of mechanisms have been suggested to explain the cardioprotective effects linked with red wine consumption. Red wine (i) inhibits oxidation of low density lipoprotein

isolated from normotensive subjects (Zou *et al*., 1999a; Zou *et al*., 2000a), (ii) reduces lipid peroxidation product (malondialdehyde) with a corresponding increase in antioxidant levels (Emokpae, 2013), (iii) suppresses platelet aggregation (Zou *et al*., 1999b; Wang *et al*., 2002; Lin *et al*., 2009), (iv) inhibits smooth muscle cell proliferation *in vitro* (Zou *et al*., 1999b; Poussier *et al*., 2005) (v) reduces endothelial cell proliferation(Bruder *et al*., 2001), attenuates intimal thickening following endothelial denudation in rabbits (Zou *et al*., 2000b), and reduces the mean area of atherosclerotic plaque (Wang *et al*., 2005). On the other hand, high alcohol intake has been associated with undesirable consequences such as elevation of blood pressure, activation of the sympathetic nervous system (Andrade *et al*., 2009) and liver damage.

The epidemiological association between triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and coronary heart disease (CHD) has been established. Specifically, raised circulating LDL-c without the need for other CHD risk factors could accelerate the development of atherosclerosis (Blaton, 1997). Reports show that half of all CVD episodes occur in subjects in whom plasma lipid levels are within 'normal' limits (Blaton, 1997) and prediction of these events is often difficult using conventional lipid profile parameters. For this reason, several lipoprotein-related indices of cardiovascular risk have been proposed and use in clinical settings in other to better identifies those at risk of cardiovascular events. This is particularly important among black African subjects because lipid levels in African population are significantly lower than that of their Caucasian counterparts (Ademuyinwa *et al*., 2008; Emokpae and Uadia, 2012). Yet, the incidence of CVD is on the increase among Africans despite lower lipid levels. In a study of some stroke patients in Nigeria, Glew *et al*. (2004) reported that both control and stroke patients had normal lipid levels in terms of TG, TC, HDL-c and LDL-c but the stroke patients were differentiated from the control subjects by the use of lipoprotein-related indices.

The need to identify risk factors and serum markers of atherosclerosis in the process of early detection and prediction of risk for CVD is gaining increasing attention. Mathematical models are often used to estimate how much atherogenic LDL-c is driving progression of atherosclerosis (Tariq and Ali, 2012). Cardiac risk ratio (CRR) assessment is the ratio of total cholesterol value to HDL-c value (John and Brunzell, 2008), atherogenic coefficient (AC) is calculated by subtracting the value of HDL-c from TC, all divided by HDL-c value. More recently, Dobiasova and Frohlich (2001), proposed a term atherogenic index of plasma (AIP), defined as log(TG/HDL-c), that indicated plasma atherogenicity was also a significant independent predictor of CHD. However, these lipoprotein-related indices have not been extensively used to evaluate the effects of red wine on atherogenic lipid levels. This study therefore examines the predictive values to the development or otherwise of atherosclerosis and cardiovascular events by using the values of AIP, CRR and AC to test existing hypothesis of the mechanism of cardioprotective effects of red wine consumption in Nigerian young adult volunteers.

MATERIALS AND METHODS

Subjects and study design

All the participants were Nigerians drawn from a university undergraduate population and comprised of 20 volunteers; 10 males and 10 females, mean age 23±2 years. They reported no obvious health problems and all displayed normal serum lipid levels. They were non-smokers, non-athletes (did not participate in any team sport or engage in rigorous aerobic activity beyond 30 minutes three times a week), and were not on any medication likely to interfere with lipid metabolism. In addition, they consume typically Nigerian low-fat, high carbohydrate and protein diet. All participants gave informed consent to participate in the study. On the day the blood specimens were collected, all subjects were on 8–10 hours fast.

Administration of Treatment

Treatments were administered in the fasting state to avoid changes in plasma constituents associated with the ingestion of meal. This approach was taken because the levels of plasma constituents including lipids can be affected by the ingestion of meal. Fasting state decreases the variability in the concentrations of plasma constituents and to standardize blood collection. Previous studies have shown that alcohol is rapidly absorbed in an empty stomach, attaining the highest concentration in the blood within 30–45 minutes (Wilkinson *et al*., 1977). Boban and Modun (2010) showed that significant serum antioxidant capacity and indeed metabolic changes take place within one hour after ingestion of 300 ml of red wine.

Collection of samples and assays of biochemical parameters

Fasting blood (5 ml) was collected from the volunteers before they were given 300 ml alcoholic red wine (Carlos Rossi, 11.5% v/v alcohol) to drink within 5 minutes in the fasting state. One hour after the ingestion of the red wine, 5 ml of blood was aseptically collected and dispensed into lithium heparin plastic containers. The specimens were centrifuged at 2000 rpm for 10 minutes and plasma was stored at -20 ^{o}C until they were analyzed. TG and TC were assayed by enzyme-catalyzed reactions using reagents supplied by Randox Laboratories, UK. The triglycerides were determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from peroxidase-catalyzed reactions of hydrogen peroxide, 4-aminophenazone and 4-chlorophenol. Cholesteryl ester is hydrolyzed by cholesteryl ester hydrolase to cholesterol and fatty acid. The 3-OH group of cholesterol is

then oxidized to a ketone in an oxygen requiring reaction catalyzed by cholesterol oxidase to produce cholest-4-en-3-one with the simultaneous production of hydrogen peroxide. The hydrogen peroxide produced is coupled with the oxidation of 4-aminoantipyrine by peroxidase to form a colour dye (Tietz, 1990). High density lipoprotein cholesterol (HDLC) was assayed in the supernatant after selective precipitation of VLDL-c and LDL-c using phosphotungstate-Magnesium chloride reagent while LDL-c and VLDL-c were calculated using Friedewald's equation (Friedewald *et al.*, 1972). CRR and AC were calculated as shown above in the *Introduction* section. AIP was calculated as log (TG/HDL-c) with TG and HDL-c expressed in molar concentrations (Dobiasova and Frohlich, 2001).

Table 1: Plasma lipid profile levels in subjects pre- and post-ingestion of red wine.

Measured variable	Pre	Post	p-value
	(n=20)	(n=20)	-
Age (Years)	23±2	23±2	-
Triglyceride (mmol/L)	0.63±0.09	0.83±0.09	0.001
Total cholesterol (mmol/L)	3.82±0.19	3.18±0.16	0.001
HDL-c (mmol/L)	0.74±0.03	0.90±0.03	0.001
LDL-c (mmol/L)	2.93±0.22	2.04±0.18	0.001
VLDL-c (mmol/L)	0.27±0.12	0.36±0.10	>0.05

Values are expressed as mean ± S.E.M.

Statistical Analysis

The results were expressed as mean ± SEM. A $p \leq 0.05$ was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS-16, Chicago, USA). Student t-test was used to compare the mean value of each lipid fraction, CRR, AC and AIP levels between pre- and post-red wine consumption.

RESULTS AND DISCUSSION

This study was conducted to ascertain the ability of lipid profile parameters and lipoprotein-related indices to assess the cardioprotective activity of acute red wine consumption in apparently healthy young adults. The results of this study showed that acute red wine ingestion caused modulation of lipoprotein levels. Table 1 indicates that post red wine consumption induced increase levels of TG ($p<0.05$), HDL-c ($p<0.001$), and VLDL-c($p>0.05$), while on the other hand caused decrease levels ($p<0.001$) of TC and LDL-c. As shown in Table 2, all the indices of atherogenicity except (HDL-c/LDL-c) were significantly reduced ($p<0.001$). There was a significant increase in the HDL-c/LDL-c ratio when post-red wine consumption was compared with pre-red wine consumption.

There were increases in the levels of TG, HDL-c and VLDL-c, but decreased levels of TC and LDL-c one hour after red wine consumption. This observation is consistent with findings reported in previous studies (Wu *et al.*, 2011; Zern and Fernandez, 2005; Andrade *et al.*, 2009). Likewise the atherogenic indices were found to be significantly reduced ($p<0.001$) except for HDL-c/LDL-c ratio that was significantly increased ($p<0.001$). High plasma level of TG is both an independent and synergistic risk factor for CVD. In this study, we found that red wine consumption produced a significant increase in TG level. In a study of the effects of alcohol consumption in patients with Diabetes mellitus, Sushith *et al.* (2012) observed that alcohol consumption significantly increased the levels of cholesterol, TG, and LDL-c, but caused a decrease in HDL-c levels in diabetic alcoholics compared to diabetic non-alcoholics. The mechanisms that may contribute to alcohol-induced raised TG levels in blood include increased generation of VLDL particles degradation and increase in TG levels in the blood that usually occurs after meal (Sushith *et al.*, 2012). Increase in TC level is a well-recognized risk factor for developing atherosclerosis.

Table 2: Comparison of the indices of lipid atherogenicity in subjects pre- and post-ingestion of red wine.

Biomarkers of cardiovascular risk	**Pre**	**Post**	**p-value**
	(n=20)	(n=20)	
AIP	0.21±0.03	0.17±0.01	0.001
Cardiac risk ratios (CRR)			
TC/HDL-c	5.24±0.56	3.52±0.26	0.001
LDL-c/HDL-c	3.94±0.52	2.25±0.25	0.001
HDL-c/LDL-c	0.25±0.06	0.44±0.05	0.001
Atherogenic coefficient (AC)	4.14±0.08	2.54±0.02	0.001

Values are expressed as mean ± S.E.M

This study indicates that red wine consumption produced significantly lower concentration of TC. On the other hand, increase level of HDL-c is cardioprotective, and we observe in this study that red wine consumption caused a significant increase in HDL-c level. The cardioprotective effects of HDL-c are linked with decreasing the rate of entry of cholesterol into the cell via LDL and increasing the rate of cholesterol release from the cell by enhancing reverse cholesterol

transport to the liver for excretion through bile (Barter, 2005). HDL-c also acts as cholesterol scavenger from peripheral tissues followed by esterification through Lecithin:cholesterol acyl transferase (LCAT) and delivering it to the liver and steroidogenic organs for eventual synthesis of bile acids as well as elimination from the body (reviewed in Ademuyinwa *et al.*,2005 and Ikewuchi and Ikewuchi, 2009). Unfortunately, these observed changes in lipid profile parameters cannot effectively explain the cardioprotective activity of red wine consumption because the changes in lipid levels are within the reference limits observed in healthy subjects. Because of the increasing cardiovascular events in Africans despite the lipid profile parameters being within 'normal' limits in the general population, the association between lipid and cardiovascular events would be better predicted using lipoprotein-related indices rather than the conventional lipid profile parameters. Hence, the analysis and description of the cardioprotective activity of red wine and other dietary elements could be demonstrated in a more informative and useful ways by using these atherogenic indices.

In this study, we observed that red wine consumption produced significantly reduced atherogenic indices of CRR, AC and AIP. Low atherogenic indices are protective against cardiovascular events (Usoro *et al.*, 2006). Ademuyinwa *et al.* (2008) used the lipid ratios TC/HDL-c and LDL-c/HDL-c to evaluating cardiovascular risk in postmenopausal Nigerian women, and demonstrated that the study population seemed to have unfavourable risk profile for CVD which was not obvious using simple lipid profile parameters. These lipid ratios were described as more effective indices of assessing risk for CVD (Brochu *et al.*, 2000). The recommended ratios by the American Heart Association for TC/HDL-c and LDL-c/HDL-c is <3.5 (Van der Sande *et al.*, 2001). In this study we observed that TC/HDL-c changed from 5.24 to 3.52 one hour after ingestion of red wine, while LDL-c/HDL-c ratio decreased from 3.94 to 2.25 (Table 2). Glew *et al.* (2004) distinguished stroke patients from control subjects using these ratios in a study in which all participants had 'normolipid' levels in terms of TG, TC, HDL-c and LDL-c. It was suggested that no single level of lipid profile parameters could separate individuals who are at high risk from those who are not (Ademuyinwa *et al.*, 2008). In a related approach, Gaziano *et al.* (1997) observed that TG/HDL-c ratio was a strong predictor for myocardial infarction. The isolated increase in TG as observed post red wine ingestion may increase CVD risk, but its effect may be counteracted by increased levels of HDL-c (Stensvold *et al.*, 1993).

The use of AIP as an additional index when assessing cardiovascular risk was proposed and used as a very good predictor of cardiovascular risk (Dobiasove and Frohlich, 2001). The various levels at which an individual is at risk of cardiovascular events have been proposed using AIP. It has been reported that AIP values of -0.3–0.1 are associated with low, 0.1–0.24 with medium, and >0.24 with high cardiovascular risk (Dobiasova *et al.*, 2005). In this study, we found that red wine consumption reduced AIP from 0.21±0.03 to 0.17±0.01 ($p<0.001$). It was demonstrated that the development of cardiovascular event is a function of particle size of LDL-c and HDL-c, with the small particle size exhibiting great atherogenic potential (Khazaal, 2013). Fractional cholesterol esterification rate of HDL-c (FER_{HDL}) has a strong correlation with lipoprotein particle size and thus can be considered as a functional risk marker for coronary heart disease (Dobiasova *et al.*, 2005). Logarithmic transformation of TG/HDL-c ratio as proposed is the best determinant for FER_{HDL-c} and hence a better predictor of cardiovascular risks than lipid profile parameters that were previously used (Dobiasova *et al.*, 2005).

We have shown in this study that all lipoprotein-related indices, with the exception of HDL-c/LDL-c ratio, were reduced one hour after red wine ingestion and are better than lipid profile parameters in the assessment of cardioprotective activity of acute red wine ingestion. Our findings support previous reports that lipoprotein-related indices are potentially better tools in assessing cardiovascular risks especially when lipid profile parameters were within normal reference levels.

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