ENHANCED LIPID PROFILE IN PLASMA AND ERYTHROCYTES OF HYPERTENSIVE TYPE-2-DIABETES MELLITUS SUBJECTS IN SOUTH-WESTERN NIGERIA

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ABSTRACT

The growing burden of hypertension and type 2 diabetes mellitus (T2DM) in Nigeria and related cardiovascular complications is becoming a public health concern. Cardiovascular risk factors were evaluated in control subjects (n=150) and patients (n=470) [hypertensive non-diabetics (n=179), normotensive diabetics (n=132), hypertensive diabetics (n=159)] attending at the Medical Out-Patient Clinic of the State Hospital, Abeokuta, Nigeria. Cholesterol, triacylglycerols and phospholipids were determined spectrophotometrically in plasma, erythrocytes and lipoproteins. The presence of either or both diseases resulted in significant (p<0.05) perturbations in blood lipids of the male and female patients. Dyslipidemia was characterised by increased concentrations of cholesterol and triacylglycerols in plasma, erythrocytes, low density lipoprotein (LDL) and very low-density lipoprotein (VLDL). The increase was more pronounced in hypertensive diabetics. High density lipoprotein (HDL) cholesterol values of the male and female patients were between 35% to 43% and 37% to 43% respectively lower than their control counterparts, while that of HDL triacylglycerols was between 8% to 10% and 6% to 23% respectively lower than their control counterparts. Plasma and erythrocyte phospholipid content increased significantly (p<0.05) in all the patients when compared with their control counterparts except in the erythrocytes of the normotensive diabetic male, where significant decrease was observed. Our findings suggest that enhanced hypercholesterolemia, hypertriacylglycerolemia and hyperphospholipidemia in plasma and erythrocytes may be responsible for increased cardiovascular complications in the comorbidity since the combined dyslipidemia are more pronounced in comorbidity of hypertension and T2DM than when either of the two conditions occurs in isolation.

Key Words: Hypercholesterolemia, Hypertriacylglyceridemia, Hyperphospholipidemia, Hypertension, Type-2-Diabetes Mellitus

Introduction

In all the continents of the world, human health is being shaped by demographic, ageing, rapid urbanization, and the globalization of unhealthy lifestyles with increased motorization, decreased physical activity, poor dietary habits, tobacco use and alcohol consumption1-3. These countries are undergoing epidemiological transition from infectious diseases to non-communicable diseases such as cardiovascular disease, cancer and chronic lung diseases as the world’s leading cause of morbidity and mortality. The major risk factors for cardiovascular disease are hypertension, type 2 diabetes mellitus and hyperlipidemia3,4.
Increasing incidence of T2DM and its commonest comorbidity, hypertension, is documented throughout the world\textsuperscript{2,4-7}. Dyslipidemia, a strong predictor of cardiovascular disease\textsuperscript{8}, causes endothelial damage\textsuperscript{9}, and the loss of physiological vasomotor activity that results from endothelial damage may become manifested as increased blood pressure (BP). Therefore, factors like dyslipidemia that cause endothelial dysfunction may lead to hypertension. Early detection and treatment of hyperlipidemia reduces the risk for cardiovascular and cerebrovascular diseases\textsuperscript{10,11}. Information on plasma lipid concentrations and prevalence of dyslipidemia among patients with T2DM and/or hypertension is, therefore, important. Few reports have confirmed that diabetes and hypertension are independently associated with dyslipidemia among Nigerians\textsuperscript{3}. Data on lipid patterns among diabetic hypertensives is, however, scanty and limited to total plasma cholesterol\textsuperscript{12}. There is also no documentation of metabolic syndrome in this population. Given the association between T2DM and hypertension and dyslipidemia, the role of lipid abnormalities as risk factors for atherosclerotic complications of diabetes and hypertension and the additive nature of these complications when both conditions occur comorbidly, it may be proposed that putative increases in plasma lipid concentrations would occur in diabetic hypertensives. The contrary may, however, be true, given the established role of hyperinsulinemia as a central link in the genesis of diabetes, hypertension, and dyslipidemia in metabolic syndrome\textsuperscript{13}. The major objective of this research was to characterize the lipid abnormality in hypertensive and T2DM subjects in Abeokuta.

Materials and Methods

Subjects

Patients presenting at the Medical Out-patient Clinic, State Hospital, Ijaiye, Abeokuta, Ogun State, Nigeria were used for the study. The protocol for the study was approved by the Research and Ethics Committee of the State Hospital as well as the Postgraduate Committee of the Department of Biochemistry, Federal University of Agriculture, Abeokuta. Patients (diagnosed by a Consultant Physician in the Department of Internal Medicine of the State Hospital) were made up of age- and sex-matched indigenous Nigerian normoglycemic hypertensives (n = 179); normotensive type 2 diabetes mellitus (n = 132) and patients with comorbidity of hypertension and type 2 diabetes (n = 159). Age- and sex-matched volunteers certified clinically and biochemically to be healthy, on no medication; normotensive and normoglycemic (n = 150) served as controls.

The diagnosis of diabetes mellitus was based on the WHO criteria\textsuperscript{14}. Patients on oral hypoglycemic drugs or whose diagnosis of diabetes was made at the age of 40 years and above with no record of ketosis were considered to have type 2 diabetes mellitus. Hypertensive patients were diagnosed based on WHO\textsuperscript{14,15}. Inclusion criteria included being hypertensive for > one year, use of neutral antihypertensive agents such as calcium channel blockers, angiotensin converting enzyme inhibitors, and angiotensin II receptor blockers. Excluded from the study during routine interviews, clinical investigations and laboratory tests were patients with a history of smoking, drinking alcohol, human immunodeficiency virus (HIV), systemic lupus erythematosus, systemic inflammation or systemic infection, taking oral contraceptives and lipid lowering drugs.

Biochemical Analyses

Plasma lipid profiles and fasting blood glucose

Blood samples were obtained once from the subjects by venipuncture, after an overnight fast. The blood samples were centrifuged (4000 rpm for 10 minutes) to separate plasma and red blood cells. Plasma concentrations of fasting blood
glucose, total cholesterol and triacylglycerols were determined with commercial kits (Cypress Diagnostics, Langdorpssteenweg 160.3201, Langdorp, Belgium). HDL cholesterol and triacylglycerols were determined in plasma with same commercial kits for total cholesterol and triacylglycerols after very low density lipoproteins (VLDL) and LDL were precipitated with heparin-MnCl₂ solution as described by Gidez et al.¹⁶. Total phospholipids in plasma and HDL phospholipids were extracted with chloroform-methanol mixture (2:1, v/v) as described by Folch et al.¹⁷. Phospholipids content was then determined as described by Stewart¹⁸. The concentrations of Very Low Density Lipoprotein (VLDL)-cholesterol and LDL-cholesterol were calculated by a modification of the Friedwald formula¹⁹. Atherogenic Index (AI=LDL-C/HDL-C) and Coronary Risk Index (CRI=TC/HDL-C) were then calculated for each subject.

**Erythrocytes Lipid Profile**

Because the Folch extraction¹⁷ produced lipid extracts which were highly pigmented, an improved procedure for the extraction of lipids from erythrocytes using chloroform-isopropanol (7:11, v/v) described by Rose and Oklander²⁰, was employed. For the determination of cholesterol, an aliquot of the chloroform-isopropanol extract was evaporated to dryness at 60°C. Triton X-100/chloroform mixture (1:1, v/v, 20 µL) was added to redesolve the lipids and again the solvent was evaporated. Then 1 ml of commercially available cholesterol kit reagent (Cypress Diagnostics, Langdorpssteenweg 160.3201, Langdorp, Belgium) was added and vortexed. After incubation in the dark at room temperature for 30 min, cholesterol and triacylglycerols contents were determined by colorimetry. Determination of total phospholipids in the chloroform-isopropanol extract of the erythrocyte followed the same procedure as described for plasma¹⁸.

**Statistical Analysis**

Data were expressed as mean±standard error of means (SEM). Analysis of Variance (ANOVA) was carried out to test for the level of homogeneity among the groups. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). p values of <0.05 were considered to be statistically significant.

**Results**

The controls, hypertensive non-diabetics (HND), normotensive diabetic (ND) and hypertensive diabetics (HD) were similar (p>0.05) in age. The duration of diagnosis of hypertension was 5.04±2.19 years among the HND male and 4.87±1.60 years among the HD male; it was 5.26±1.66 years vs 4.94±1.62 years among the HND female and HD female respectively (p<0.05). The duration of diagnosis of diabetics was also similar (p>0.05) between the ND male, HD male, ND female and HD female; which were 4.07±1.55 years, 4.05±1.48 years, 4.22±1.19 years and 3.98±1.09 years respectively. In the diabetic patients fasting plasma glucose (FPG) was similar (p>0.05) among the ND male and ND female (196.42±3.66 mg/dl vs 188.97±4.43 mg/dl (p>0.05) but significantly (p<0.05) higher when compared with HD male and HD female patients (174.39±3.73 mg/dl vs 172.24±3.71 mg/dl). Blood pressure increased significantly (p<0.05) among the HND male, HD male, HND female and HD female (171.26±3.79/109.25±2.12 mm Hg, 177.69±3.16/111.13±2.17 mm Hg, 168.98±2.80/105.17±1.80 mm Hg and 173.54±3.08/106.01±1.97 mm Hg) respectively when compared with their corresponding controls. These demographic and clinical characteristics of subjects had earlier been published¹.
The mean±SEM values of the plasma lipid profile for male and female patients and for the control groups are depicted in Figure 1. The presence of hypertension, T2DM or comorbidity resulted in significant (p<0.05) alterations in the plasma lipid profile of the patients. In the plasma of the patients, there were significant (p<0.05) increase in the mean values of cholesterol, triacylglycerol (TAG) and phospholipids for both male and female patients when compared with their control counterparts. The increase was more marked in comorbidity. Quantitatively plasma cholesterol of the male and female patients was between 20% to 30% and 25% to 38% respectively higher than their control counterparts, while that of plasma TAG was between 38% to 57% and 34% to 42% higher than their respective control counterparts, whereas plasma phospholipid contents of the male and female patients were between 1.09 to 1.3 times and 1.12 to 1.35 times higher respectively than their control counterparts.

Figure 2 illustrates the effects of hypertension and/or type 2 diabetes mellitus on erythrocytes lipid profiles. The presence of either or both diseases resulted in significant alterations in the erythrocyte lipid profile of the subjects (p<0.05). In the erythrocyte of the patients, there were significant (p<0.05) increase in the mean values of cholesterol, triacylglycerol (TAG) and phospholipids for both male and female patients when compared with their control counterparts. Quantitatively erythrocyte cholesterol of the male and female patients was between 32% to 41% and 34% to 39% respectively higher than their control counterparts, while that of erythrocyte TAG was between 40% to 54% and 58% to 63% respectively higher than their control counterparts. Although, the erythrocyte phospholipid content of normotensive diabetics female was similar to the corresponding control female, significant (p<0.05) hyperphospho-

lipidemia was observed in other patients whereas significant (p<0.05) hypophospholipidemia was observed in normotensive diabetics male when compared with the male control counterpart.

Figure 3 is the summary of effects of hypertension and/or T2DM on HDL lipid profile. HDL responded to the presence of hypertension and/or T2DM with a significant (p<0.05) decrease in HDL-C and TAG levels. For HDL-C, the decrease was more marked in normotensive diabetics male when compared with control and other patients (29.93±0.96 mg/dl vs 52.16±1.44 mg/dl), meanwhile for HDL TAG, the decrease was more pronounced in hypertensive non-diabetes female when compared with control and other patients (27.79±0.90 mg/d vs 36.12±2.05 mg/d). Quantitatively, HDL-C values of the male and female patients were between 35% to 43% and 37% to 43% respectively lower than their control counterparts, while that of HDL TAG was between 8% to 10% and 6% to 23% respectively lower than their control counterparts. HDL phospholipid content of hypertensive and/or diabetics male and female patients was similar to their corresponding controls, however significant (p<0.05) hyperphospholipidemia was observed in HDL of hypertensive non-diabetics female.

Figures 4 depicts the LDL-C, VLDL-C, coronary risk index (CRI) and atherogenic index (AI) in the controls and patients. The presence of either or both diseases resulted in significant (p<0.05) alterations in the LDL-C and VLDL-C of the subjects. In the lipoprotein of the patients, there were statistically significant (p<0.05) increase in the mean values of LDL-C and VLDL-C for both male and female patients when compared to their control counterparts. Quantitatively LDL-C of the male and female patients was between 70% to 94% and 92% to 106% higher respectively than their control counterparts. Quantitatively LDL-C of the male and female patients was between 70% to 94% and 92% to 106% higher respectively than their control counterparts, while that of VLDL-C was between 38% to 43% and 34% to 42% higher respectively than their control counterparts.
Enhanced Lipid Profile in Plasma and Erythrocytes respectively than their control counterparts. Atherogenic and coronary risk indexes of hypertensive and/or diabetics patients increased significantly (p<0.05) when compared to their corresponding control counterparts. Normotensive diabetics male has the highest of the two indexes when compared with other patients, (3.5 fold increase for atherogenic index and 2.4 fold increase for coronary risk index). Quantitatively atherogenic index of the male and female patients was between 207% to 249% and 217% to 255% higher respectively than their control counterparts, while the coronary risk index of the male and female patients was between 112% to 136% and 112% to 134% higher respectively than their control counterparts.

Table I shows the effects of hypertension and/or diabetes on cholesterol to phospholipid ratio in different compartments (plasma, erythrocyte, HDL and LDL+VLDL) of the subjects. The presence of either or both diseases resulted in significant (p<0.05) alterations in the cholesterol to phospholipids in different compartments of the subjects. Plasma cholesterol to phospholipids ratio of hypertensive non-diabetic female and normotensive diabetic female increased significantly when compared to their corresponding controls, however the ratio was similar in other patients when compared with the control counterparts. In the erythrocyte and LDL+VLDL of the patients, there was statistically significant increase in the mean values of cholesterol to phospholipid ratio for both male and female patients when compared with their control counterparts. Quantitatively erythrocyte cholesterol to phospholipid ratio of the male and female patients was between 11% to 42% and 12% to 28% higher respectively than their control counterparts, while that of LDL+VLDL cholesterol to phospholipid ratio was between 9% to 138% and 110% to 163% higher respectively than their control counterparts. In contrast to erythrocyte and LDL+VLDL, HDL-cholesterol to phospholipid ratio decreased significantly for both male and female patients when compared with their control counterparts. Quantitatively HDL-cholesterol to phospholipid ratio of the male and female patients was between 21% to 31% and 26% to 43% lower respectively than their control counterparts.

Figure 1: Effects of hypertension and/or type 2 diabetes mellitus on levels of plasma lipids. Each bar represents the mean ± SEM. Bars with different alphabets are significantly different at p<0.05.
Figure 2: Effects of hypertension and/or type 2 diabetes mellitus on levels of erythrocytes lipids. Each bar represents the mean±SEM. Bars with different alphabets are significantly different at p<0.05.

Figure 3: Effects of hypertension and/or type 2 diabetes mellitus on levels of HDL lipids. Each bar represents the mean±SEM. Bars with different alphabets are significantly different at p<0.05.

Figure 4: Effects of hypertension and/or type 2 diabetes mellitus on levels of LDL-cholesterol, VLDL-cholesterol, Arterogenic index (AI), Coronary risk index (CRI). Each bar represents the mean±SEM. Bars with different alphabets are significantly different at p<0.05.
Table I: Cholesterol: phospholipid ratio in the plasma, erythrocyte and lipoprotein of the subjects.

<table>
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<tr>
<th></th>
<th>CM (n=74)</th>
<th>HNDM (n=76)</th>
<th>NDM (n=64)</th>
<th>HDM (n=68)</th>
<th>CF (n=76)</th>
<th>HNDF (n=103)</th>
<th>NDF (n=68)</th>
<th>HDF (n=91)</th>
</tr>
</thead>
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<tr>
<td>Plasma</td>
<td>0.91±0.06bc</td>
<td>0.84±0.02ab</td>
<td>1.00±0.15c</td>
<td>0.83±0.02ab</td>
<td>0.86±0.02ab</td>
<td>0.98±0.03c</td>
<td>0.97±0.02c</td>
<td>0.81±0.02a</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>0.45±0.01a</td>
<td>0.50±0.01bc</td>
<td>0.64±0.01c</td>
<td>0.53±0.01bd</td>
<td>0.49±0.01ab</td>
<td>0.57±0.03d</td>
<td>0.63±0.02c</td>
<td>0.55±0.01cd</td>
</tr>
<tr>
<td>HDL</td>
<td>0.98±0.06c</td>
<td>0.77±0.04b</td>
<td>0.68±0.04bc</td>
<td>0.74±0.05b</td>
<td>1.05±0.04c</td>
<td>0.60±0.05a</td>
<td>0.76±0.05b</td>
<td>0.78±0.04b</td>
</tr>
<tr>
<td>LDL + VLDL</td>
<td>0.45±0.01a</td>
<td>1.05±0.03c</td>
<td>0.87±0.03b</td>
<td>1.07±0.03c</td>
<td>0.41±0.01a</td>
<td>1.05±0.03c</td>
<td>0.86±0.02b</td>
<td>1.08±0.04c</td>
</tr>
</tbody>
</table>

CM = Control male, HNDM = Hypertensive non-diabetic male, NDM = Normotensive diabetic male, HDM = Hypertensive diabetic male, CF = Control female, HNDF = Hypertensive non-diabetic female, NDF = Normotensive diabetics female, HDF = Hypertensive diabetics female. Each value represents the mean±SEM. Values within the same row with different superscripts are significantly different at p<0.05.

Discussion

Cardiovascular disease (CVD) is the leading cause of death in developing countries and accounts for 35% of all deaths in the United States and 49% of deaths in Europe. It has been estimated that by 2020, CVD will account for approximately 40% of all global deaths. The growing burden of hypertension and type 2 diabetes mellitus (T2DM) in Nigeria and related cardiovascular complications is presently becoming a public health concern and calls for integrated intervention. Cardiovascular risk was thus evaluated in controls and patients using lipidomics as metric.

The major finding of this study was that the presence of hypertension and/or T2DM perturbs the metabolism of lipids in different compartments (plasma, erythrocyte and lipoproteins) of the patients. These perturbations were reflected as up-/down-regulation of the concentrations of the major lipids (cholesterol, triacylglycerols and phospholipids).

The presence of hypertension, T2DM or comorbidity resulted in significant alterations in the plasma and erythrocyte lipid profile of the patients. The major characteristics of these alterations were increased cholesterol in plasma, erythrocyte, low density lipoprotein (LDL) and very low density lipoprotein (VLDL). However, cholesterol in high density lipoprotein (HDL) of the patients was significantly decreased relative to their respective control groups.

We also compared our data with the guidelines of risk factors for cardiovascular disease given by the American Heart Association. According to these guidelines, blood pressure <130/85 mmHg; total cholesterol <200 mg/dl; triacylglycerols <200 mg/dl; HDL >40 mg/dl and LDL <130 mg/dl, are favorable risk factors. In addition, certain lipid ratios like total cholesterol/HDL-C (coronary risk index) and the LDL-C/HDL-C (atherogenic index) also correlate with cardiovascular disease. The recommended ratios for the two are ≤3.523. Indications from this comparison are that while the total cholesterol, LDL-C and triacylglycerol concentrations of both the controls and patients were within the acceptable range prescribed by the American Heart Association, hypertension and/or T2DM patients with mean HDL-C ranging from 29.93 mg/d to 34.19 mg/dl seem to have unfavorable risk profiles for cardiovascular disease when compared with HDL-C acceptable range. Furthermore, the LDL-C/HDL-C ratios of the patients indicate that normotensive diabetic male have a greater
risk of cardiovascular disease when compared with other patients and controls. As regards total cholesterol/HDL-C ratio, all the patients groups seem to be at risk of cardiovascular disease.

The significantly higher plasma total cholesterol in the hypertensive than in the control in the present study is in agreement with earlier studies\textsuperscript{25}. Again, studies in non-blacks have demonstrated similar trends of hypercholesterolemia in hypertensive to normotensive controls\textsuperscript{26}. Increase in plasma total cholesterol levels, especially in the presence of hypertension has been associated with coronary heart disease (CHD). Studies have shown that atherosclerosis risk factor such as high plasma cholesterol levels is less in blacks compared to the European white population. In a study in South Africa, adult white males had higher mean serum cholesterol than blacks (5.27 mmol/l vs. 4.29 mmol/l)\textsuperscript{27}. Despite the lower concentration of total cholesterol, LDL-C and triacylglycerol in the patients when compared to the commonly accepted limit as prescribed by the American Heart Association. Some researchers however have argued that the desired range of plasma total cholesterol concentrations as advocated for developed countries may have to be reviewed for developing countries based on the suspicion that subjects in developing countries could be prone to developing CHD at lower plasma cholesterol level\textsuperscript{28}. Racial variations in plasma lipid concentrations are also largely attributable to differences in the fiber component of diet. Traditional African diet is high in plant fiber and low in fats. In Zimbabwe, for example, fats made up 17.8\% and 42.7\% of diet among Africans and whites, respectively. High fiber diet reduces plasma lipids through reduction of total fat intake, reduction of fat absorption, and increased bile secretion. The non-intake of cigarettes and alcohol among our patients are additional factors contributing to the comparatively lower lipid concentrations obtained in the current study.

Timothy \textit{et al.}\textsuperscript{29} in an earlier prospective study of CHD showed that adult blacks have higher mean levels of HDL-C than whites. The racial differences in HDL-C have been suggested to be either due to genetic or environmental factors, Genetic factor, more than environmental factor is adjudged to be responsible for the higher level of HDL-C in blacks. However, cord blood HDL-C in neonate revealed no difference between races. Similarly, cord blood from infants of subjects from high and low-income groups did not have any differences in the levels of total and HDL-C in a Nigerian study. The above findings in cord blood across racial and socio-economic groups support the fact that environment plays an important role in HDL-C metabolism. Findings on the preponderance of hyper HDL-C in women are varied. Some studies showed increased HDL-C levels in pre-menopausal women who enjoy relative immunity from CHD while others showed a significant increase in HDL-C even in elderly women\textsuperscript{25}, however our findings show no significant difference in gender HDL-C concentration in both controls and patients.

Although we report a higher prevalence of hypercholesterolemia in hypertensive diabetics than hypertension or T2DM alone in plasma and erythrocyte, this difference was not significant in erythrocyte of female. Interestingly, hypertensive diabetics (both sexes) have more HDL-C when compared with hypertensive or diabetics. The presence of hypertension may however be contributory to the greater prevalence of dyslipidemia in hypertensive diabetics than T2DM. The relationship between insulin resistance or compensatory hyperinsulinemia may partly explain the above scenario\textsuperscript{30}. Insulin resistance often leads to increased intracellular hydrolysis of TAG and release of fatty acids into the circulation and the resultant inability of fat cells to store TAG is the initial step in the development of dyslipidemia. Consistent with this
was our finding of increased concentration of triacylglycerol in the plasma, erythrocyte and lipoproteins.

The significantly higher plasma TAG in patient groups than the control in the present study is in agreement with earlier studies\textsuperscript{25}. Some studies have looked prospectively at the relationship between plasma lipids and the future development of hypertension. A 7-year follow-up of 1039 initially non-diabetic, non-hypertensive subjects from the San Antonio Heart Study suggested that risk factors for atherosclerosis, including triacylglycerols, also predicted hypertension\textsuperscript{31}. A prospective study of 1482 adults in Utah, followed for 7 years with 40 cases of incident hypertension reported a significant age-adjusted relative risk (RR) of 1.42 for a 1 standard deviation (SD) increase in triacylglycerols (110 mg/dl) and a non-significant RR of 0.82 for HDL-C (11 mg/c)\textsuperscript{31}. We would expect that if dyslipidemia played a role in the development of hypertension, then treating dyslipidemia would have some effect on BP. Elevation of plasma triacylglycerol level could be the result of either increased VLDL production or decreased VLDL clearance\textsuperscript{32} as observed in this study. Significant increase in patients' TAG compared with their respective controls in the present work is of particular importance since some workers are of the opinion that serum TAG is an independent risk factor for CHD. Also, plasma TAG level has been found to be more predictive of heart disease in women than men, though, our HND, ND and HD female did not show significant increase in plasma TAG when compared with their male counterpart. On the other hand, report has shown that hypertriglyceridemia and not hypercholesterolemia was associated with myocardial infarction\textsuperscript{33}.

Hypertriglyceridemia, the most common form of dyslipidemia in insulin-resistant states and non-insulin dependent diabetes mellitus (NIDDM), is closely associated with a number of metabolic and coagulation abnormalities which have been shown \textit{in vitro} and in clinical studies to be atherogenic. While there is undoubtedly a close genetic link between various aspects of this plurometabolic syndrome, there is also good evidence that hypertriglyceridemia aggravates many of these associated metabolic abnormalities. Treatment of hypertriglyceridemia with diet, exercise, weight loss and pharmacological agents can effectively reverse not only fasting and postprandial TAG levels but also many of the associated metabolic and prothrombotic abnormalities\textsuperscript{34}.

Erythrocytes lipid composition is currently becoming a matter of great interest because the etiology of many diseases has been shown to have their root in erythrocyte lipid abnormalities\textsuperscript{35,36}. It has been reported that the erythrocyte lipid is composed almost entirely of un-esterified cholesterol and phospholipids\textsuperscript{37}. The small quantity of cholesterol esters and phospholipids imprint a pattern of erythrocyte lipid clearly different from that of the plasma environment\textsuperscript{38}. Despite this rather divergent lipid composition of the erythrocyte and its plasma environment, circulating mature erythrocytes from mammals are limited in their lipid metabolism in a number of ways. Firstly, there is little evidence of de novo synthesis of lipid by the erythrocyte\textsuperscript{39,40}. Secondly, \textit{in vivo} and \textit{in vitro} evidences suggest that a major pathway for replacement of red cell lipids is through exchange with plasma lipids\textsuperscript{37,41,42}. Compounds such as dimethylsulfoxide, acetone, urea and alcohols have been shown to promote this exchange\textsuperscript{37}. Since the presence of hypertension and/or T2DM resulted in increased plasma concentrations of the major lipids, the increase observed in erythrocyte lipids might be attributed to exchange of these lipids between plasma (lipoproteins) and erythrocytes. However, since the concentrations of the erythrocyte and plasma lipids did not attain equal values, it is possible that the lipid levels of
erythrocytes may be composed of several pools with more and less readily exchangeable molecules and that hypertension and/or T2DM might promote transfer of these lipids from these other pools into the erythrocyte.

Plasma lipid dynamics have been widely studied during various metabolic malfunctions due to their involvement in vascular disorders. Cholesterol and triacylglycerols have been the components of major interest with very little attention being given to plasma phospholipids.

Phospholipids, as well as cholesterol, are components of the plasma membrane of living organisms. While cholesterol also functions as the precursor for the biosynthesis of steroid hormones, phospholipids function as emulsifying agents to maintain the proper colloidal state of the cytoplasm. They are also involved in the transport of hydrophobic constituent into and out of cells. Cholesterol and triacylglycerol have been widely studied in the plasma during various metabolic malfunctions due to their involvement in vascular disorders. However, compared with plasma cholesterol measurement, very little attention has been given to plasma, erythrocyte and lipoprotein phospholipids in various pathologies. The data from this research indicate that the presence of hypertension, T2DM or comorbidity was associated with phospholipidosis in plasma and erythrocyte. In the lipoproteins, hypophospholipidemia was observed, whereas phospholipidosis was observed in HDL of hypertensive non-diabetic (HND) female. A massive accumulation of phospholipids in the cell leads to the formation of numerous multi-lamellar inclusion bodies in cell cytoplasm resulting in loss of cellular function and viability. The induction of phospholipidosis is characterized by: (1) inhibition of lysosomal phospholipase activity is regarded as the primary mechanism of induction, (2) inhibition of lysosomal enzyme transport as a result of down-regulation of genes involved in lysosomal enzyme transport, (3) enhanced phospholipid biosynthesis due to enhanced free fatty acid availability and (4) enhanced cholesterogenesis.

The molar ratio of cholesterol to phospholipids is one of the indices of membrane fluidity. An increase in this ratio indicates decreased fluidity. The data from this study indicate that the presence of hypertension, T2DM or comorbidity was associated with increased molar ratio of cholesterol to phospholipids in plasma, erythrocyte and lipoprotein. Several reports have associated changes in lipid cholesterol/phospholipids profiles with impaired signal as well as energy trasduction.

Since the male and female controls in the present study were of approximately the same age as the patients who had experienced hypertension and/or T2DM, we did not have to be concerned that our conclusions regarding lipid profiles levels in the patient and control groups may have been confounded by age considerations. Furthermore, the possible confounding variable of age appears not to have been a factor in our study because when we tested for possible correlations between LDL-C, total cholesterol, HDL-C or triacylglycerols versus age in both female and male pairs, none was found. However, sex was found to be a possible determinant of the pattern of lipid profile levels in the patients. We have reported earlier other variables such as body mass index (BMI), waist circumference and other anthropometric parameters and their correlation with hypertension comorbidly occurring with diabetes in some residents of Abeokuta, Nigeria.

In conclusion, data from this research revealed enhanced cholesterogenesis, hypertriacylglycerol-
Erythrocytes and phospholipidosis in plasma and erythrocytes may be responsible for increased cardiovascular complications (heart disease, stroke, kidney failure, premature mortality and disability) in the comorbidity since the combined dyslipidemia are more pronounced in comorbidity of hypertension and T2DM than when either of the two conditions occurs in isolation. Therefore, lipid profiling for all persons with T2DM and/or hypertension should be a routine test. It might be appropriate at this time in Nigeria for T2DM and/or hypertension patients to consider physical activity and/or pharmacological interventions in their lowering blood lipids.

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Enhanced Lipid Profile in Plasma and Erythrocytes


