SERUM LIPIDS AND ANTIOXIDANTS IN GHANAIAN DIABETIC, HYPERTENSIVE AND HEALTHY SUBJECTS

A.K. NYARKO, J. ASIEDU-LARBI, M. OFOSUHENE, H. ASARE-ANANE AND MARIAN E. ADDY

Noguchi Memorial Institute for Medical Research, University of Ghana, Legon. 1Department of Biochemistry, University of Ghana, Legon and 2Department of Chemical Pathology, University of Ghana Medical School, Accra

SUMMARY

We investigated further the extent of dyslipidemia in Ghanaian hypertensive and diabetic subjects against the background of rising incidence of these conditions and worry of cardiovascular diseases becoming major killers in developing countries this millennium. Cholesterol-containing lipids, triacylglycerol, apolipoprotein B and blood antioxidant levels were measured in Ghanaian diabetic, hypertensive and apparently healthy volunteers for comparison. Total cholesterol (TC), triacylglycerol (TAG) and apolipoprotein B (apo B), and LDL-cholesterol (LDL-C) levels were significantly higher (p<0.05) in the hypertensive and diabetic subjects than in the healthy subjects. Compared to diabetics, TAG was significantly higher in the hypertensive patients (p<0.05). High Density Lipoprotein-Cholesterol (HDL-C)/LDL-C ratio was higher in the healthy subjects (p<0.05). TC, LDL-C and apo-B levels showed sex differences in the healthy and diabetic but not the hypertensive subjects. Apo-B correlated positively with LDL-C and body mass index in the diabetic and hypertensive subjects. The antioxidant Vitamin A correlated with LDL-C, HDL-C and TC for the three groups and was higher in the healthy as well as male subjects. The atherogenic lipids investigated are within Caucasian reference levels but can discriminate between healthy and diabetic or hypertensive Ghanaians. Ghanaians may have different, perhaps lower, cut points for lipids and apo-proteins that are used as risk indicators for cardiovascular diseases.

Keywords: Diabetes mellitus, hypertension, cholesterol, low density lipoprotein, apolipoprotein B, antioxidant, cardiovascular disease.

INTRODUCTION

Cardiovascular diseases (CVDs) are expected to be major causes of death in Third World countries this millennium. CVDs are caused by atherosclerosis, the accumulation of fatty deposits or plaques in artery walls of the major blood vessels and the heart. As these deposits or plaques grow, they sometimes trigger the formation of clots by causing platelets to clump together and block the flow of blood resulting in the development of CVDs.

Plaques in arterial walls consist mainly of cholesterol and cholesterol fractions associated with low-density lipoprotein (LDL) that is derived from very low-density lipoprotein (VLDL) produced by the liver from excess calories. Each LDL particle contains a molecule of apolipoprotein B (apo B), high concentrations of which confer increased risk for CVDs. Levels of LDL in blood, usually calculated is therefore, related to the total plasma apo B concentration. Unlike LDL, apo B levels hardly change with meals and can therefore, be measured using non-fasting blood.

Current evidence indicates that oxidatively modified LDL (Ox-LDL) is casually related to atherogenicity. The Ox-LDL is taken up by macrophages resulting in cholesterol accumulation and subsequent foam cell formation, however, antioxidants like vitamin A, E and β-carotenes, as well as plasma uric acid and bilirubin protect LDL from being oxidized. High antioxidants therefore, decrease LDL oxidation and reduce the risk of developing CVDs. High-density lipoprotein (HDL), another cholesterol containing lipoprotein, also protects against CVDs.

CVDs are common complications in uncontrolled diabetes mellitus and hypertension. These meta-
bolic disorders are increasingly becoming important public health problems in Ghana.

In Caucasians, these metabolic disorders are associated with elevated levels of atherogenic lipids. However, earlier reports indicate that blood cholesterol levels of apparently healthy as well as diabetic and hypertensive Ghanaians are similar and within Caucasian reference levels. We, therefore, examined the ability of cholesterol and other lipids to discriminate between healthy and diabetic or hypertensive Ghanaians and the extent of dyslipidemia in these subjects; investigating also the relationship that exist between lipid/lipoproteins and plasma antioxidants or apolipoprotein B levels in Ghanaians. To the best of our knowledge, this is the first determination of apolipoprotein B and antioxidant levels in relation to dyslipidemia in Ghanaians.

MATERIALS AND METHODS
The proposal for this study was approved by the Scientific and Technical Committee of the Noguchi Memorial Institute for Medical Research, Legon. A total of 150 subjects, comprising 50 apparently healthy individuals (27 males and 23 females), 50 non-insulin-dependent (Type 2) diabetic patients (32 male and 18 females) and 50 hypertensive patients (20 males and 30 females) participated in the study. The apparently healthy subjects were recruited from the work force of the University of Ghana, Legon. The diabetic and hypertensive patients were outpatients recruited from the University Hospital, Legon or the Chemical Pathology Department, Korle Bu. All subjects gave their informed consent after the study and the procedures involved had been explained to them.

Inclusion Criteria
All the subjects were between the ages of 18 and 65 years, had body mass indices between 19 and 53 kg/m² and were Ghanaians who have resided in the country for a minimum of three months. Type 2 diabetic patients with fasting blood glucose (FBG) levels greater than 140 mg/dL (i.e. 7.80 mmol/L) who have had the disease for more than a year and were not on any lipid-lowering drugs were included in the study. Apparently healthy and hypertensive subjects were included in the study if their FBG levels were between 76 and 115 mg/dL (4.2 and 6.4 mmol/L). Hypertensive participants in the study have had the disease for more than a year and had a systolic and diastolic blood pressure of more than 130 and 90 mmHg, respectively.

Exclusion Criteria
Subjects were excluded if they had significant renal dysfunction (serum creatinine levels greater than 0.085 and 0.077 mmol/L for males and females respectively), or had proteinuria, hepatic dysfunction (indicated by levels of aminotransferases more than twice the upper limit of the normal values). Patients with deteriorating clinical condition or who had been severely ill within three months prior to the study and were on thyroid-stimulating drugs, corticosteroids or lipid-lowering drugs were excluded from the study. Hypertensive patients on thiazide diuretics and/or β-blockers were also excluded from the study.

Clinic Chemistry
The serum aminotransferases-ALT and AST, creatinine, total cholesterol (TC), triglycerol (TAG), and HDL-cholesterol (HDL-C) were measured with commercial kits (Randox Ltd. Antrim UK). The level of LDL-cholesterol (LDL-C) in each sample was calculated using the Friedwald formula. Apolipoprotein B (Apo B) levels in the serum samples were measured using an immunoturbidimeter assay kit (Randox Ltd., Antrim, UK).

Assay for serum anti-oxidants
Uric acid and total bilirubin levels in the serum samples were determined colorimetrically with kits (Randox Ltd., Antrim, UK). Vitamin A and E were measured simultaneously using C₁₈ reverse phase HPLC as described by Driskell et al. Retinol/retinyl acetate and tocopherol/tocopheryl acetate were added as internal standards to each serum sample prior to deproteinization. Aliquots of the supernatant were injected on to the column and eluted with a mobile phase comprising methanol/water (1:3 v/v). The concentrations of the vitamins were determined by comparing peak areas of the vitamins with the respective standards.

Statistics
Two-way parametric and non-parametric analysis of variance (ANOVA) and Student Neuman-Keuls post analysis were performed to determine statistical significance. Significance was set at 5% for all analysis. All statistical tests were performed with Jandel SigmaStat Statistical Software version 2.0 (1992-1995).

RESULTS
Characteristics of the Subjects
The physical and chemical parameters, which were used to include or exclude the subjects in the various categories, are shown in Table 1. Both systolic and diastolic blood pressures of the hypertensive
Lipid and lipoprotein levels
Table 2 shows the levels of the various lipids and lipoprotein in the serum, measured or calculated for the different categories of subjects. Total cholesterol (TC) levels for the apparently healthy subjects were significantly lower (p<0.05) than those for the hypertensive and diabetic subjects, which
Whereas the apparently healthy female subjects had lower levels of TC compared to their male counterparts, diabetic female subjects had significantly higher total cholesterol levels than their male counterparts (p<0.05). A similar pattern of variation was also seen for apo B levels and the computed LDL-cholesterol.

Table 3 Levels of endogenous and exogenous (dietary) anti-oxidants in the sera of the three categories of subjects. The numbers are means and standard errors of the mean.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=27)</th>
<th>Female (n=23)</th>
<th>Male (n=20)</th>
<th>Female (n=30)</th>
<th>Male (n=32)</th>
<th>Female (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin</td>
<td>0.52±0.07</td>
<td>0.42±0.07d</td>
<td>0.37±0.08</td>
<td>0.36±0.08</td>
<td>0.40±0.06</td>
<td>0.24±0.08d</td>
</tr>
<tr>
<td>Urate (mg/dL)</td>
<td>7.28±0.65</td>
<td>4.64±0.71</td>
<td>5.41±0.76</td>
<td>5.41±0.76</td>
<td>5.48±0.60</td>
<td>5.27±0.68</td>
</tr>
<tr>
<td>Vitamin A (mg/L)</td>
<td>456.00±20.00</td>
<td>421.00±22.00</td>
<td>419.00±24.0</td>
<td>419.00±19.00</td>
<td>463.00±19.00</td>
<td>384.00±25.00</td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>9.15±1.11</td>
<td>8.26±1.21</td>
<td>7.41±1.29</td>
<td>9.66±1.10</td>
<td>9.23±1.02</td>
<td>9.00±1.36</td>
</tr>
</tbody>
</table>

Significant difference (p<0.05) between: (a) apparently healthy and hypertensives; (b) hypertensive and diabetics; (c) apparently healthy and diabetics; (d) diabetic males and females; (e) apparently healthy males and females; (f) between hypertensive males and females.

Table 4 Correlation between levels of antioxidants, cholesterol, apolipoprotein B and the ratio of HDL-cholesterol to LDL cholesterol in the three categories of subjects which showed relatively high correlation coefficient. Figures are given as the square of the correlation coefficient ($r^2$)

<table>
<thead>
<tr>
<th>Variables Compared</th>
<th>Total Population</th>
<th>App. Healthy</th>
<th>Categories of subjects</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A vrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.10</td>
<td>0.12</td>
<td>0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.07</td>
<td>0.12</td>
<td>0.25</td>
<td>0.16</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.30</td>
<td>0.27</td>
<td>0.34</td>
<td>0.29</td>
</tr>
<tr>
<td>HDL-C/LDL-C</td>
<td>0.11</td>
<td>0.12</td>
<td>0.25</td>
<td>0.16</td>
</tr>
<tr>
<td>Apo B vrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.17</td>
<td>0.12</td>
<td>0.59</td>
<td>0.21</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.33</td>
<td>0.09</td>
<td>0.71</td>
<td>0.16</td>
</tr>
<tr>
<td>HDL-C/LDL-C</td>
<td>0.04</td>
<td>0.01</td>
<td>0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>0.11</td>
<td>0.01</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL-Cholesterol vrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.10</td>
<td>0.12</td>
<td>0.27</td>
<td>0.16</td>
</tr>
</tbody>
</table>

did not show any significantly differences between them (p>0.05). There were significant sex differences among the diabetic and apparently healthy subjects (0.05) with respect to total cholesterol (TC) levels.
The apparently healthy subjects had the lowest levels of triacylglycerol (TAG) (p<0.05), followed by the diabetic subjects. There were significant sex differences in each category of subjects (p<0.05).
However, whereas in the hypertensive subjects the males had higher levels of TAG compared to the females, the opposite was true for the diabetic as well as the apparently healthy subjects. The hypertensive males had the highest levels of TAGs.

There were no significant differences in the HDL-cholesterol levels among the three different categories of subjects. However, within the groups, the female subjects had significantly higher levels of HDL-cholesterol compared to their male counterparts (p<0.05).

The levels of endogenous as well as exogenous (dietary) antioxidants measured in the sera from the different categories of subjects are presented in Table 3. Total bilirubin was lower in female subjects who were either diabetic or apparently healthy. However, no significant differences were observed among the three categories (p>0.05). Urate level was higher in apparently healthy male subjects compared to their female counterparts (p<0.05). Vitamin A levels were significantly higher in the apparently healthy subjects compared to the other two groups (p<0.05). Within each category, the female subjects had lower levels of vitamin A compared to their male counterparts (p<0.05). No significant differences were observed in the levels of vitamin E for the various groups (p>0.05).

The highest correlation observed was between apo B and LDL-cholesterol followed by that between apo B and total cholesterol for the hypertensive patients (Table 4). The correlations observed for vitamin A and other parameters were also high. Hypertensive subjects appeared to have the highest correlation coefficient values. There was no correlation between age and total cholesterol.

**DISCUSSION**

Cholesterol in some lipoprotein and increased oxidation of low-density lipoprotein (LDL) predispose humans to CVDs. Although evidence points to a rising incidence of diabetes mellitus and hypertension in Ghana, indications are that unlike Caucasians, these metabolic disorders, in Ghanaians, are associated with lipid and lipoprotein levels that are within currently accepted reference values. Risk levels of these parameters, however, differ for different populations. Levels of cholesterol-containing lipoproteins, triglycerides, apolipoprotein B and antioxidants were therefore, determined in apparently healthy, hypertensive and diabetic Ghanaians to evaluate whether they can discriminate between patients and healthy subjects.

The mean total cholesterol (TC) obtained for apparently healthy subjects in this study is lower than those previously reported for adult Ghanaians. Seasonal variations in total cholesterol level demonstrated elsewhere might explain this observation. Furthermore, the significantly lower TC levels (p<0.05) of our healthy female subjects compared to their male counterparts may be explained by the fact that their mean age is pre-menopausal, which has been shown to have a lowering effect on TC levels.

The TC levels for the hypertensive and diabetic subjects are similar to those reported previously. The TC and TAG levels reported here are however, below reference limits that suggest risk for CVDs in Caucasians. This could mean that Ghanaians hypertensive and diabetic subjects might be at a lower risk of developing CVDs. It is worth noting, however, that compared to the apparently healthy group TC and TAG values for the hypertensive and diabetic subjects are significantly higher (p<0.05) and there are reports indicating that uncontrolled hypertension and diabetes mellitus are associated with abnormalities in lipid levels. Thus, the upper limit of blood TC that may point to increased risk for CVDs in Ghanaians might be lower than the generally accepted upper limit of 5.2 mmol/L based on Caucasian reference values. This has implications for the management of dyslipidemias associated with diabetes mellitus and hypertension in Ghanaians.

The hypertensive subjects in this study had normal fasting blood glucose levels (Table 1) suggesting that this group of subjects did not develop hypertension secondary to diabetes. The elevated diastolic pressure may be due to the high blood TC recorded for the hypertensive group. The lower levels of TC (p<0.05) in the apparently healthy female subjects compared to their male counterpart was not observed in the diabetic and hypertensive subjects, suggesting that the dyslipidemia associated with these pathological states may have a sex component. This effect was more marked with the diabetic subjects among whom female TC was significantly higher (p<0.05) compared to the male subjects.

The mean LDL-C values for all the three categories of Ghanaians in the present study were below the upper limit of 3.90 mmol/L reported for Caucasians. High levels of this lipoprotein are un-
desirable because of its strong association with atherogenesis. Therefore, compared to Caucasian reference values, the relatively lower LDL-C level could point to decreased risk for CVDs among Ghanaians. However, the significantly higher (p<0.05) levels of LDL-C among the hypertensive and diabetic subjects compared to the healthy group suggest increased pre-disposition of these patients to pathogenesis of CVDs in spite of the LDL-C levels of these patients falling below the cut-off indicating CVD risk for Caucasians. This emphasizes the need to establish the upper limits of LDL-C for this population.

Apo B, a component of LDL-C and an independent risk factor with respect to the onset of CVDs, correlated positively with LDL-C in this study (r² = 0.33 for the total population and as high as 0.71 for the hypertensive subjects). Apo B levels were not significantly different (p>0.05) among the diabetic and hypertensive patients, but were significantly higher (p<0.05) compared to values for the healthy subjects. The positive correlation between the derived LDL-C and apo B obtained by measurement, indicates that either parameter could be a risk indicator for CVDs among Ghanaians.

For the hypertensive subjects, apo B correlation positively with TC (r² = 0.59). Furthermore, the apo B levels of the diabetic and hypertensive subjects in this study were close to, or exceeded the upper limit for Caucasians. Defects in apo B gene affect LDL-C levels and apo B is reported to be the most important risk indicator for CVDs. It is thus, reasonable to infer that despite the apparently normal atherogenic lipid levels, they can discriminate between healthy persons and diabetic or hypertensive patients, and may also predict risk for CVDs in Ghanaians.

Elevated HDL-C, unlike LDL-C levels, is antiatherogenic. It protects against CVDs by removing cholesterol from the tissues and returning it to the liver to be metabolized. Hence, a high level of HDL-C is beneficial. As previously reported, HDL-C levels for the various categories of subjects investigated are comparable to values reported for Caucasians. Compared to their male counterparts, the lower LDL-C levels reported for the apparently healthy females and the elevated HDL-C in hypertensive and diabetic females are consistent with the effects that female hormones have on these lipoproteins. Diabetic females, however, had higher LDL-C (p<0.05) that did not correlation negatively with HDL-C.

In this study, vitamin A levels were significantly higher (p<0.05) among the apparently healthy subjects compared to the hypertensive and diabetic subjects. Furthermore, significant sex differences were observed for this antioxidant, which also correlated positively with HDL-C but negatively with LDL-C. The vitamin A levels of our subjects fall within the 95% confidence interval of 343.72 – 830.66 μg/L reported recently for a population of similar age. Vitamin E, the often referred to antioxidant that has been shown to relate inversely with ischemic heart diseases, however, did not vary among the groups. Levels of this vitamin were, however, similar to levels reported for Caucasian community. These may suggest that compared to vitamin E, vitamin A may be a more important dietary antioxidant that could reduce the risk for CVDs among Ghanaians. It would be worthwhile to investigate further, the total antioxidant status of Ghanaians, including the factors that affect it. Antioxidants, which prevent LDL-C oxidation and lower LDL-C levels, are beneficial in the management of atherosclerosis. This is in view of the role played by post-secretory oxidation of LDL in atherogenesis.

Changes in ratio of cholesterol content of lipoproteins are also predictors of risk for pathogenesis of CVDs. For example, the higher the HDL-C/LDL-C ratio, the better one is protected from the onset of CVDs. In this study, the HDL-C/LDL-C ratios for all the categories of subjects are comparable to those reported previously. Although the hypertensive and diabetic subjects in this study had apo B levels suggestive of risk for atherogenesis, their HDL-C/LDL-C ratios appeared to be within desirable levels. However, the ratios for hypertensive and diabetic subjects are lower than those for the apparently healthy subjects, an indication that the hypertensive and diabetic subjects could be more predisposed to CVDs.

In conclusion, TC, LDL-C, HDL-C and HDL-C/LDL-C ratio as well as plasma apo B levels showed significant differences between the apparently healthy and the other subjects. These parameters therefore, discriminate between healthy and diabetic or hypertensive Ghanaians. The data further shows that like Caucasians, diabetes mellitus and hypertension, in Ghanaians, are associated with abnormalities in lipids that may predispose these patients to CVDs. The study also shows that vitamin A is likely to be an important dietary antioxidant for Ghanaians. These have implications for the diagnosis, management and prognosis of atherogenic complications of diabetes mellitus and
hypertension among Ghanaians. It is therefore, important to ascertain levels of lipid and lipoproteins that should be regarded as the upper limit for Ghanaians.

REFERENCE


