Ethnic differences in erythrocyte membrane fluidity and the association with serum triacylglycerols

Michelle A. MILLER, Giuseppe A. SAGNELLA, Nirmala D. MARKANDU and Graham A. MacGREGOR
Blood Pressure Unit, Department of Medicine, Division of Physiological Medicine, St George’s Hospital Medical School, Cranmer Terrace, Tooting, London SW17 ORE, U.K.

ABSTRACT

The objectives of this study were to determine whether there are differences between black and white individuals with regard to the membrane fluidity of isolated erythrocytes, and/or in the relationships between membrane fluidity, gender and circulating lipids. Fluorescent polarization anisotropy, as an index of membrane fluidity, was determined using the fluorescent probe 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) in 52 black and 52 white individuals, of whom 39 pairs were matched for age, sex and blood pressure. In the 39 matched pairs, the TMA-DPH anisotropy was significantly higher in the black (0.262±0.007) compared with the white (0.258±0.005) subjects (P<0.005). There was also a significant difference in serum lipids. Gender differences in TMA-DPH anisotropy were observed in the white but not in the black individuals. The associations between membrane fluidity and serum lipids were examined in the total group, separated according to ethnic group. Although the associations were in the same direction in both groups, the association was only significant in the white subjects (r=−0.42; P<0.02). The ethnic difference in membrane fluidity was abolished when adjusting for serum triacylglycerols. In conclusion, ethnic differences in erythrocyte membrane fluidity, as determined by the use of TMA-DPH anisotropy, appear to be the result of ethnic differences in the level of serum triacylglycerols.

INTRODUCTION

There are racial differences in many different diseases, both in disease aetiology and in its progression [1–8]. For instance, it has been demonstrated that, although black individuals experience more strokes than white individuals [5], they have a lower prevalence of ischaemic heart disease [6,7]. Essential hypertension is a risk factor for cardiovascular disease and stroke, and there are clearly racial differences in the severity, progression and aetiology of essential hypertension, and also in the response to treatment [8].

More detailed studies have identified physiological and biochemical differences between individuals of different racial groups, and especially between black and white individuals with hypertension [9–11]. However, although the specific reasons and mechanisms for the difference in the incidence of cardiovascular disease between black and white individuals still remain unclear, recent studies have highlighted the importance of serum lipids. Indeed, racial differences in circulating levels of serum lipids have been identified [7,12,13]. By causing alterations in the structure and composition of biochemical membranes, serum lipid levels can influence the activity of various ion transporters and other biochemical processes [14–18].

The determination of membrane fluidity gives useful

Key words: ethnic, membrane fluidity, triacylglycerols.
Abbreviations: TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene.
Correspondence: Dr Michelle A. Miller (e-mail mmiller@sghms.ac.uk).
information about the physical state of biochemical membranes. Studies in white individuals have demonstrated that there is an association between membrane fluidity and serum lipids; in particular, serum triacylglycerols are positively associated with membrane fluidity in various cell types [16,18–22]. Although there are substantial differences in serum lipids between black and white individuals, it is not known whether this is also reflected in differences in membrane fluidity.

The main objectives of the present study, therefore, were to investigate the relationship between circulating serum lipids and isolated erythrocyte membrane fluidity in black subjects, and to investigate whether the lower levels of serum triacylglycerols reported previously in black individuals [12,13] are manifested in a correspondingly lower membrane fluidity.

METHODS

Subjects

Measurements of membrane fluidity were carried out in 39 white individuals matched for age, sex and blood pressure with 39 black individuals of African or Afro-Caribbean origin (see Table 1). The associations between membrane fluidity measurements and other measured variables were examined in a larger unmatched group of 52 white and 52 black individuals.

The individuals studied had a range of blood pressure of 91–225 mmHg systolic and 64–120 mmHg diastolic. All the hypertensive patients had established essential hypertension (diastolic blood pressure > 90 mmHg or systolic blood pressure > 140 mmHg [23]), with normal renal function and no clinical or biochemical evidence of secondary hypertension. None of the patients were known to suffer from diabetes mellitus. All patients either had never received treatment for hypertension or were withdrawn from treatment 3 weeks before study. All of the subjects were on their normal sodium intake. Subjects were studied between 09.00 and 10.30 hours. All subjects gave their informed consent, and local ethical committee approval was obtained.

Measurements

The subjects’ weight and height were recorded and, after 5 min of rest, blood pressure (mean of three readings) was determined using an oscillometric blood pressure monitor (Omron HEM-705CP; Omron Corp., Tokyo, Japan). After 5 min of sitting, blood for the membrane fluidity measurements was taken into acid/citrate/dextrose (85 mmol/l trisodium citrate dihydrate, 71.4 mmol/l citric acid monohydrate and 111 mmol/l dextrose in distilled water, pH 4.5; 1 part to 6 parts of blood). After centrifugation, the packed erythrocytes were washed twice with 0.9% saline (1050 g, 25 min, 4 °C) and then stored frozen in 2.5 ml aliquots at −40 °C until required. Blood was also collected into a plain blood clotting tube for the measurement of serum routine biochemistry and the enzymic determination of serum lipid levels on a Beckman LX 20 analyser (Beckman Instruments Inc.).

Measurement of erythrocyte membrane fluidity

The membrane fluidity of isolated erythrocyte membranes was measured using the fluorescent probe 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), as described previously [16]. In brief, erythrocyte membrane ghosts were prepared as described by Downes and Michell [24] and stored at −40 °C. The protein content of the membrane suspension was determined [25], and a final concentration of 100 μg/ml was used. Fluorescence polarization anisotropy, which is inversely proportional to membrane fluidity, was determined using 1.25 μmol/l TMA-DPH (Molecular Probes Inc.). Measurements were performed in a Perkin-Elmer LS-5 spectrofluorimeter with polarizers attached (excitation 350 nm; emission 430 nm; slit width 10 nm). Readings were taken at 10 min. The anisotropy was calculated, from the fluorescence intensities parallel (I II) and perpendicular (I I-) to the polarization direction of excitation light, using the modified Perrin equation:

$$r = (I I) V - G(I I-) V / (I I) V + 2G(I I-) V$$

where $r$ is fluorescence anisotropy, $V$ is vertically polarized excitation light and $G$ is a correction factor [(I II)$_H$/(I I-)$_H$, where $H$ is horizontally polarized excitation light].

Statistics

Group differences were examined using unpaired Student’s $t$-tests. A two-tailed $P$ value of $<0.05$ was taken to be significant. Correlation coefficients were determined using parametric tests (Pearson). Group results are given as means ± S. D. A general linear model was used for covariate analysis. All statistical tests were carried out using SPSS version 8.0 for Windows.

RESULTS

Ethnic differences in membrane fluidity were examined in 39 pairs of white and black individuals matched for age, sex and blood pressure. Demographic characteristics, blood pressures, selected biochemical variables and membrane fluorescence polarization anisotropy values for
Table 1  Characteristics of the 39 pairs of black and white individuals matched for age, sex and blood pressure, and TMA-DPH fluorescent polarization anisotropy results

<table>
<thead>
<tr>
<th></th>
<th>Whites</th>
<th>Blacks</th>
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<tbody>
<tr>
<td>Gender (male/female)</td>
<td>19/20</td>
<td>19/20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.1 ± 12.3</td>
<td>47.9 ± 12.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.9 ± 14.0</td>
<td>78.9 ± 12.8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.0 ± 5.0</td>
<td>28.1 ± 7.7</td>
</tr>
<tr>
<td>Supine systolic blood pressure (mmHg)</td>
<td>147.7 ± 24.2</td>
<td>147.4 ± 20.9</td>
</tr>
<tr>
<td>Supine diastolic blood pressure (mmHg)</td>
<td>90.4 ± 12.1</td>
<td>92.2 ± 11.1</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td>5.0 ± 0.55</td>
<td>5.1 ± 0.84</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>1.89 ± 1.33</td>
<td>1.03 ± 0.53</td>
</tr>
<tr>
<td>log [Serum triacylglycerols (mmol/l)]</td>
<td>0.188 ± 0.274</td>
<td>-0.037 ± 0.203**</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.92 ± 1.04</td>
<td>5.07 ± 1.02**</td>
</tr>
<tr>
<td>TMA-DPH anisotropy</td>
<td>0.258 ± 0.005</td>
<td>0.262 ± 0.007*</td>
</tr>
</tbody>
</table>

The results from the analysis of the matched pairs demonstrated that the erythrocyte membrane fluidity in the black individuals was lower than that in the white individuals, and that there were also significant differences in serum triacylglycerols and serum cholesterol between the two groups.

To investigate the possibility that the ethnic difference in membrane fluidity may be due to serum lipids and/or gender, a larger group of 52 black and 52 white individuals (26 males and 26 females in each ethnic group) was examined. As in the matched pairs, there was no significant difference in blood pressure between the groups, but there were significant differences between them in serum triacylglycerols (whites, 1.83 ± 1.20; blacks, 0.98 ± 0.5; P = 0.001) and in serum cholesterol (whites, 6.01 ± 1.1; blacks, 5.05 ± 0.93; P = 0.001). Likewise, there was a significant difference in TMA-DPH anisotropy (whites, 0.259 ± 0.005; blacks, 0.262 ± 0.007; P = 0.012) between the two groups.

The results of the correlation analysis demonstrated that there was a significant negative correlation between log serum triacylglycerols and TMA-DPH anisotropy in the white individuals (r = -0.42; P = 0.002; n = 52). Although a similar association was seen in the black individuals, this was not statistically significant (r = -0.23; P = 0.096) (Figure 1; Table 2). There were no significant associations between TMA-DPH anisotropy and serum cholesterol in the white (r = -0.27) or black (r = 0.02) groups.

There were insufficient subjects in this study to perform a detailed analysis of the associations in the normotensive white and black groups separately. However, analysis of the hypertensive subjects alone showed that, as in the total group, there was a stronger association between TMA-DPH anisotropy and log serum triacylglycerols in the white (r = -0.49; P = 0.001; n = 40) compared with the black (r = -0.25; P = 0.11; n = 40) hypertensive group.

The association between serum triacylglycerols and TMA-DPH anisotropy was investigated further within the gender subgroups (see Table 2). The subgroup analysis demonstrated that there were significant gender differences in TMA-DPH anisotropy in the white subjects (P < 0.02), but not in the black subjects (see Table 2 for values). However, there were also significant differences in serum triacylglycerols between the different gender groups (P = 0.002 in the white subjects and P = 0.005 in the black subjects; see Table 2 for values). The association between serum triacylglycerols and membrane fluidity was in the same direction in each of the
groups, although the highest coefficient was seen in the white female individuals ($r = -0.47; P < 0.02$).

Analysis of variance demonstrated that, even when gender was taken into account, there was still a significant difference in TMA-DPH anisotropy between the black and white individuals ($P = 0.016$), as there was no interaction between race and sex. Also, after adjustment for the significant difference in serum triacylglycerols (between the two ethnic groups), the difference in TMA-DPH anisotropy between the black and white individuals was abolished ($P = 0.398$). The estimated marginal means were 0.260 and 0.261 (at a log serum triacylglycerol value of 0.065) for the white and black individuals respectively.

### DISCUSSION

There is evidence to suggest that the observed differences in the incidence of cardiovascular disease between black and white individuals may be a result of the differences in circulating lipids in these ethnic groups [8]. It is postulated that differences in circulating lipids may cause changes in the cellular membrane environment, which in turn will alter the transport processes occurring within the cells [20]. These changes may lead to metabolic abnormalities and the development of disease [22]. The purpose of the present study was to investigate possible differences in membrane structure between black and white individuals by measuring membrane fluidity, and to investigate the relationship between serum lipid levels and membrane fluidity in black individuals.

Previous studies have demonstrated differences in membrane fluidity related to gender [16,26]. Also, differences between white normotensive and hypertensive individuals have been demonstrated using different methods or different dyes [16,20,21]. Therefore possible ethnic differences in membrane fluidity between black and white individuals were examined in 39 pairs of black and white individuals matched for age and blood pressure (11 normotensive and 28 hypertensive pairs). It was found that there was a significantly higher anisotropy, and hence lower membrane fluidity, in the black compared with the white group. Serum triacylglycerols and serum cholesterol were also significantly lower in the black group.

An association between serum triacylglycerols and membrane fluidity in white individuals has been demonstrated previously, both in erythrocytes and in platelets [16,22,27–30]. Also, a recent study demonstrated that the fluidity of low-density lipoprotein is related to its triacylglycerol content [31].

In the present study, the association between serum lipids and membrane fluidity was examined in 52 white and in 52 black individuals. It was found that, as in our previous studies [16,27], there was a strong negative association between serum triacylglycerols and the fluorescent polarization anisotropy values ($r = -0.42$; $P = 0.002$) in the white individuals. However, although there was a similar association in the black individuals (slope $-0.0081$ and $-0.0075$; constant 0.261 and 0.260 for black and white subjects respectively), the significance of the relationship was reduced ($r = -0.23; P = 0.096$), even though the number of subjects was the same in each group. The decreased strength of the association in the black individuals may be due partially to the smaller range of triacylglycerol values in the black individuals and to the greater spread of TMA-DPH anisotropy values.

When analysed separately, it was found that the strength of the association between serum triacylglycerols and TMA-DPH anisotropy in the white subjects was gender-dependent. Nethertheless, it was demonstrated that, while adjustment for gender attenuated slightly the ethnic difference in membrane fluidity, adjustment for the ethnic differences in serum triacylglycerols abolished the difference completely. This suggests, therefore, that the observed difference in membrane fluidity between black and white individuals is a result of the differences in serum triacylglycerols between these two different ethnic groups.

As demonstrated previously, both in our and in other studies [16,28,30], there was no association between membrane fluidity and serum cholesterol in the white subjects. In addition, the results of the present study

<table>
<thead>
<tr>
<th>Group</th>
<th>TMA-DPH anisotropy</th>
<th>Serum triacylglycerols (mmol/l)</th>
<th>log [Serum triacylglycerols (mmol/l)]</th>
<th>$r$</th>
<th>Slope</th>
<th>Constant</th>
<th>$n$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whites (all)</td>
<td>0.259 ± 0.005</td>
<td>1.83 ± 1.20</td>
<td>0.186 ± 0.255</td>
<td>−0.42</td>
<td>−0.0075</td>
<td>0.260</td>
<td>52</td>
<td>0.002</td>
</tr>
<tr>
<td>Blacks (all)</td>
<td>0.262 ± 0.007</td>
<td>0.98 ± 0.50</td>
<td>−0.056 ± 0.186</td>
<td>−0.23</td>
<td>−0.0081</td>
<td>0.261</td>
<td>52</td>
<td>0.096</td>
</tr>
<tr>
<td>White females</td>
<td>0.261 ± 0.004</td>
<td>1.50 ± 1.23</td>
<td>0.11 ± 0.265</td>
<td>−0.47</td>
<td>−0.0075</td>
<td>0.261</td>
<td>26</td>
<td>0.016</td>
</tr>
<tr>
<td>White males</td>
<td>0.257 ± 0.004</td>
<td>2.07 ± 1.13</td>
<td>0.262 ± 0.224</td>
<td>−0.18</td>
<td>−0.0031</td>
<td>0.257</td>
<td>26</td>
<td>0.390</td>
</tr>
<tr>
<td>Black females</td>
<td>0.262 ± 0.007</td>
<td>0.82 ± 0.50</td>
<td>−0.122 ± 0.184</td>
<td>−0.12</td>
<td>−0.0046</td>
<td>0.262</td>
<td>26</td>
<td>0.558</td>
</tr>
<tr>
<td>Black males</td>
<td>0.261 ± 0.007</td>
<td>1.14 ± 0.46</td>
<td>0.019 ± 0.187</td>
<td>−0.29</td>
<td>−0.0107</td>
<td>0.261</td>
<td>26</td>
<td>0.147</td>
</tr>
</tbody>
</table>
show that there was no such association in the black subjects either.

There is increasing evidence to suggest that the aetiology of cardiovascular diseases is different in black compared with white individuals. However, the possible mechanisms remain unclear. The present study demonstrates that there is a difference in erythrocyte membrane fluidity between black and white individuals. However, as the individuals in the present study were matched for blood pressure, these differences are likely to be due to a blood pressure-independent effect. Notwithstanding this, the results demonstrate that, for any given blood pressure level, there is a tendency for membrane fluidity to be lower in black individuals.

Although the difference in membrane fluidity observed in the present study is relatively small, previous studies have demonstrated that changes in membrane fluidity can modulate the activity of ion transport proteins, signal transduction mechanisms, Ca$^{2+}$ handling and intracellular pH regulation [22]. It is therefore feasible that this small but significant difference in membrane fluidity between black and white individuals could have an effect on these cellular mechanisms. In the long term, these cellular alterations could contribute to the processes responsible for the difference in disease progression (e.g. those associated with hypertension [8]) between individuals of different ethnic origin.

As the use of both dietary and drug-induced modification of serum lipids become increasingly advocated, so the need to understand the possible consequences of such intervention in individuals of different ethnic origin becomes paramount. In the present and other studies performed using both erythrocytes [16,30] and platelets [28,30], there was no correlation between membrane fluidity and cholesterol. However, it has been demonstrated that augmentation of the cholesterol content of endothelial cells by incubating them with low-density lipoprotein is accompanied by a decrease in membrane fluidity [32]. Interestingly, in Japanese individuals, low blood cholesterol is associated with an increased risk of intracerebral haemorrhage [33]. Therefore differences in the relationships between serum lipids and cell membrane lipids in different cell types also merit further investigation.

In conclusion, the present study demonstrates that there are differences in erythrocyte membrane fluidity between black and white individuals. It also demonstrates that serum triacylglycerol levels are an important determinant of membrane fluidity in black as well as white individuals, and that serum triacylglycerols can account for the observed differences in membrane fluidity between these two ethnic groups. However, further studies need to be performed in order to determine the possible significance of these differences in membrane fluidity in relation to the differences in disease prevalence and progression between these two ethnic groups.

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**REFERENCES**


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