Abnormalities in the Fatty-Acid Composition of the Serum Phospholipids of Stroke Patients

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The incidence of cardiovascular diseases, stroke, and myocardial infarction is increasing in sub-Saharan Africa. Since dietary polyunsaturated fatty acids (PUFA) are protective of the cardiovascular system in humans, we were interested in the question of the PUFA status of adults in northern Nigeria who had experienced a recent stroke.

We collected blood from 21 consecutive admissions for stroke (15 male patients, mean age 39.3 years and six females, mean age 40.7 years) to the Federal Medical Centre in Gombe, Nigeria and analyzed the fatty-acid composition of the serum phospholipids. Blood was collected from 30 healthy controls for comparison. The contribution palmitic acid made to the fatty-acid total was greatly decreased in the phospholipids of the stroke patients (29.2% versus 37.2 %, $p<0.001$). However, the phospholipids of the stroke patients had significantly higher percentages of 20-, 22-, and 24-carbon saturated fatty acids, as well as higher proportions of the ω-6 fatty-acid, arachidonic acid (11.4 versus 8.14%, $p<0.001$), and the ω-3 fatty-acid, docosahexaenoic acid (3.21 versus 1.80%, $p<0.001$). Using the percentages and melting points of the individual fatty acids, we estimated that the acyl chains of the serum phospholipids of the stroke patients had a lower mean melting point than the controls (27.8 versus 34.6°C, $p<0.001$). Assuming that serum phospholipids are surrogates for tissue phospholipids, we conclude that the tissue membranes of the stroke patients may be considerably more fluid than those of the controls.

Key words: polyunsaturated fatty acids ■ stroke ■ phospholipids ■ cardiovascular diseases

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INTRODUCTION

Over the past half decade, there has been a trend toward an increase in cardiovascular disease and a decrease in infectious disease in indigenous populations in sub-Saharan Africa.[1] Furthermore, it is the perception of physicians who practice medicine in this part of the world that the incidence of sudden death from stroke and myocardial infarction is increasing—particularly among younger men and women.[2-4] These trends have been attributed to a variety of factors, including the change from a more active lifestyle in a rural setting to a more sedentary way of life in an urban center; changes in dietary habits, with the consumption of less cereal and increased amounts of foods that contain more saturated fat; and the increased stress and hypertension that is often associated with economic development and urbanization.[5,6]

An inverse correlation has been shown between fish intake and risk of stroke and thrombotic infarction.[7] It is widely accepted that one of the important cardioprotective factors in fish is the ω-3 polyunsaturated fatty acids,[8-10] eicosapentaenoic acid (EPA, 20:5ω-3) in particular.[11] In the Edinburgh Artery study that involved over 1100 subjects, Leng and colleagues[12] found that the proportion of EPA in red-cell phospholipids was reduced in the group with myocardial infarction compared with a no-disease group. They also observed significantly lower levels of another ω-3 fatty acid, α-linolenic, in the red cells of the groups with stroke and lower limb disease. Fish lipids rich in EPA impair platelet aggregation and reduce thromboxane synthesis.[11] Dietary ω-3 fatty acids are thought to reduce the risk of myocardial infarction by stabilizing the myocardium electrically, thereby decreasing susceptibility to ventricular arrhythmias.[9] These fatty acids may also reduce the risk of cardiovascular disease by virtue of their anti-inflammatory effects. In a large-scale trial conducted in Italy involving 11,324 patients, daily consumption of one gram of a purified preparation of ω-3 fatty acids markedly reduced the risk of sudden car-

diac death and a reduction in all-cause mortality.[8,10] A similar study carried out in Lyon, France demonstrated that the intake of ω-3 polyunsaturated fatty acids reduced cardiovascular death and coronary heart disease by 30–35%.[13]

However, not all reports support the conclusion that moderate fish consumption lowers the risk of CVD. In the Physician's Health Study, for example, Morris and coworkers[14] reported that in the 21,185 U.S. male physicians in their study, they did not find an association between fish consumption and any cardiovascular endpoint, including myocardial infarction, stroke, or cardiovascular death.

We were interested in the issue of 20- and 22-carbon ω-3 PUFA in the context of stroke in Gombe State in northern Nigeria for several reasons. First, since Gombe is one of the poorest regions of sub-Saharan Africa and because it is located far from the Atlantic Ocean, the general population has limited access to fish or other sources of 20- and 22-carbon polyunsaturated ω-3 fatty acids. Furthermore, the staple of the region is cereals, such as millet, sorghum, and maize, which, although they do provide adequate amounts of two essential fatty acids (linoleic acid and α-linolenic acid), are devoid of the ω-3 fatty acids, EPA, and docosahexaenoic acid (DHA) that are widely regarded as being cardioprotective. Second, internists, neurologists, and cardiologists at the two major hospitals that serve the three million inhabitants of Gombe State believe that over the past decade there has been an increase in stroke and myocardial infarction among the local population.

We, therefore, conducted a study that was aimed at determining whether the fatty-acid composition of serum phospholipids was unusual in stroke patients in Gombe, Nigeria relative to healthy controls living in the same environment. To this end, we collected blood serum from 21 consecutive admissions for stroke to the Federal Medical Centre in the city of Gombe and compared the proportions of the various fatty acids contained in the serum total phospholipid fraction with those of 30 healthy young men and women from the same region. In addition, since the fatty acids which comprise the phospholipids of tissue membranes are major determinants of the fluidity of those membranes and because serum phospholipids are widely regarded as surrogates for membrane phospholipids,[15] we were interested in comparing the calculated mean melting points (MMPs) of the composite fatty acids of the serum phospholipids of healthy controls and the patients who had experienced stroke. This study documents the many significant differences we found between the fatty-acid compositions of the serum phospholipids of the two groups.

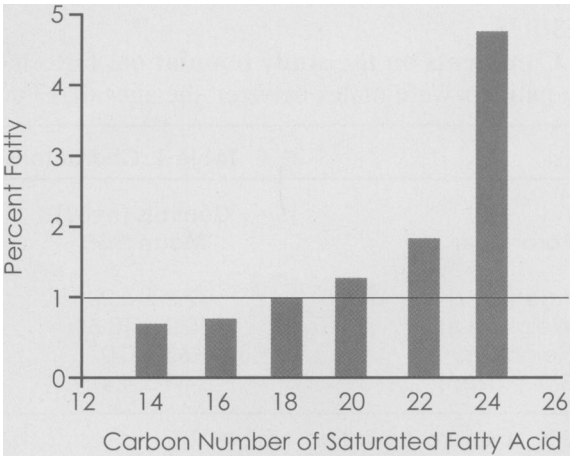
METHODS

Study population. Between April and July 2001, we enrolled 21 consecutive stroke patients who had been brought to the emergency department of the Federal Medical Centre, Gombe. For all 21 stroke patients, this was their first episode of stroke. The diagnosis of stroke was made on the basis of clinical criteria since computerized tomography was not available where this study was performed. None of the stroke patients had a history of hypertension, diabetes mellitus, liver disease, or kidney disease, and none was obese. Prior to their stroke, none of the patients had modified their normal dietary habits. The controls (six males, 24 females) were recruited from among the hospital staff and family members of the stroke patients. The patients and controls were of the same socioeconomic class based on salary level. The exclusion criteria for the controls included the following: history of transient ischemic attack, hypertension, diabetes mellitus, obesity, and drug therapy. All of the controls were in apparent good health.

Fatty-acid analysis. Ten ml of venous blood were collected into a clean, glass tube within the first hour following admission and allowed to stand at room temperature for 45 minutes before being centrifuged for eight minutes at 5,000xg. The serum fraction was withdrawn, aliquoted into 2-ml cryovials and stored for two- to six weeks at -20°C, until which time the frozen specimens were transported to Albuquerque, NM for analysis.

Preparation of fatty-acid methyl esters was carried out as previously described by Morrison and Smith.[16] Briefly, total lipids were extracted into chloroform/methanol (1:1, v/v) from 0.15 ml of serum. The extract was evaporated under a stream of nitrogen,

Figure 1. Ratio of the Percentage of Individual Saturated Fatty Acids in the Serum Phospholipids of Stroke Patients Compared to Healthy Controls



redissolved in 0.5 ml of chloroform, and loaded onto a silicic acid column which had previously been washed with chloroform. Neutral lipids were eluted with 5 ml chloroform. Total phospholipids were eluted with 6 ml chloroform/methanol (2:1, v/v). The eluate was again dried under a stream of nitrogen and redissolved in 0.5 ml 15% (w/v) BF_3 in methanol. The methylation reaction was carried out at 100°C for 10 minutes. Fatty-acid methyl esters were separated using a 0.53-mm-x-15-m fused silica Megabore DB-225 column (J&W, Folsom, CA) in a gas chromatograph (Hewlett Packard, model 5890) equipped with an integrator. Individual fatty acids were identified by the comparison of their retention times to those of fatty-acid standards.

Calculation of the MMP of the acyl chains of serum phospholipids. The MMP was determined by as described by Jensen and Patton.[17] First, the mol% of each fatty acid was calculated by dividing the mass% of each fatty acid by its respective molecular weight. Next, the mol% was multiplied by the (MP +100) of each fatty acid to obtain the melting point fractions. Finally, after the MP fractions were summed, 100 was subtracted from that sum to provide the estimate of the MMP of the acyl chains of the phospholipid preparation. The melting points used for the fatty acids are as follows: C14:0 (54°C), C15:0 (52°C), C16:0 (63°C), C16:1 (-5°C), C18:0 (69°C), C18:1ω-9 (16°C), C18:1ω-7 (44°C), C18:2ω-6 (-5°C), C18:3ω-6 (-11.3°C), C18:3ω-3 (-10°C), C20:4ω-6 (-49°C), C20:5ω-3 (-54°C), C22:5ω-3 (-54°C), C22:5ω-6 (-44.1°C), and C22:6ω-3 (-44°C).

Statistical analysis. Age-adjusted group comparisons were made using analysis of covariance (NCCS 2001, Number Cruncher Statistical Systems, Kaysville, UT). Data were tested for normality, and the Mann Whitney U test for differences in medians was used for those variables that were not normally distributed or that had unequal variances. Correlations between variables were tested using regression analysis. A p-value of 0.05 or less was considered statistically significant.

RESULTS

Comments on the study population. Fifteen of the patients were males between the ages of 17 and 50 years (mean, 39.3 ± 9.4 years) and six were women aged 18–50 years (mean, 40.7 ± 12.6 years). The average age of the six male controls was 33.5 ± 5.7 years and that of the 24 female controls was 32.0 ± 6.4 years (Table 1). The mean body mass index (BMI) values of the male patients and male controls were 23.0 ± 2.6 and 24.2 ± 5.6 kg/m², respectively. The mean BMI values of the female patients and female controls were 27.2 ± 2.9 and 24.3 ± 6.4 kg/m², respectively. None of the stroke patients died between the time of admission and the two months following admission.

Fatty-acid composition of the serum phospholipids of the patients and controls. Since a preliminary statistical analysis of the data did not reveal any significant differences between the fatty-acid composition of the phospholipids of the male and female patients or between those of the male and female controls, the data for the men and women within the control group and the patient group were pooled in order to increase statistical power. However, because the control group was significantly younger than the stroke patients and the levels of some fatty acids were found to vary with age (data not shown), comparisons of the serum phospholipids fatty-acid percentages between the stroke patients and the controls were made adjusting for age.

As shown in Table 2, there were significant differences between the proportions of more than 20 fatty acids in the serum phospholipids of the controls and the patients with stroke. Several of these differences are noteworthy, the first being the much higher proportion of palmitic acid (16:0) in the controls relative to the stroke patients (37.2 versus 29.2%, $p<0.001$). However, the proportions of stearic acid (18:0)—the second most abundant saturated fatty acid in serum phospholipids—in the serum total phospholipids of the two groups were virtually identical (17%). Eighteen-carbon stearic acid appeared to represent a crossover point since as one proceeds progressively to fatty acids of greater and greater chain length (i.e., 20:0, 22:0, 24:0), there was a progressively greater proportion of these saturated fatty acids in the serum phospholipids of the patients (Figure 1). For example, whereas the difference we observed in the

Table 1. Characteristics of the Study Populations

Parameter	Controls (n=30) Mean ± SD	Patients (n=21) Mean ± SD	p-value
Age (year)	32.3 ± 6.22	39.7 ± 10.1	0.002
Weight (kg)	60.0 ± 10.6	65.8 ± 9.0	NS
Height (m)	1.65 ± 0.07	1.64 ± 0.07	NS
BMI (kg/m²)	24.3 ± 4.4	24.4 ± 3.4	NS

SD: standard deviation; NS: p>0.05.

arachidic acid (20:0) content between the phospholipids of the patient and control groups was statistically significant (p= 0.002), the difference was small (0.20 versus 0.15%); however, for the 24-carbon saturated fatty acid (24:0), we found a nearly five-fold greater abundance of this very long-chain fatty acid in the phospholipids of the patients with stroke (0.52 versus 0.11, p<0.001).

A second major distinction between the fatty-acid compositions of the two groups was in the proportions of two important polyunsaturated fatty acids, namely arachidonic acid (20:4ω-6), which is a critical-membrane fatty acid and precursor to the prostaglandins and leukotrienes, and DHA, which is a major fatty-acid component of the membrane phospholipids of the retina and central nervous system. The phospholipids of the stroke patients contained a greater proportion of both of these critical polyunsaturated fatty acids. Furthermore, compared to the controls, the serum phospholipids of the

Table 2. Comparison of the Fatty-Acid Composition of the Serum Phospholipids of Patients and Controls

Fatty Acid	Patients (n=21) Weight % Mean ± SD	Controls (n=30) Weight % Mean ± SD	p-Value
Saturated Fatty Acids			
12:0	0.005 ± 0.01	ND	–
14:0	0.37 ± 0.10	0.52 ± 0.15	<0.001
15:0	0.15 ± 0.05	ND	–
16:0	29.2 ± 2.57	37.2 ± 2.13	<0.001
17:0	0.22 ± 0.08	ND	–
18:0	16.9 ± 1.88	17.0 ± 2.10	NS
20:0	0.20 ± 0.05	0.15 ± 0.04	0.002
22:0	0.40 ± 0.17	0.23 ± 0.08	<0.001
24:0	0.52 ± 0.32	0.11 ± 0.05	<0.001
Monounsaturated Fatty Acids			
14:1ω-9	0.05 ± 0.03	0.08 ± 0.07	0.02
16:1ω-9	0.60 ± 0.33	0.53 ± 0.19	NS
18:1ω-7	0.42 ± 0.16	1.21 ± 0.25	<0.001
18:1ω-9	11.8 ± 2.57	10.3 ± 1.31	0.02
20:1ω-7	ND	0.04 ± 0.05	–
20:1ω-9	0.28 ± 0.08	0.17 ± 0.05	<0.001
22:1ω-9	1.13 ± 0.37	0.08 ± 0.03	<0.001
24:1ω-9	0.33 ± 0.21	0.08 ± 0.04	<0.001
ω-6 Fatty Acids			
18:2ω-6	16.5 ± 2.47	17.6 ± 2.59	NS
18:3ω-6	0.13 ± 0.10	0.08 ± 0.04	0.02
20:2ω-6	0.48 ± 0.50	0.27 ± 0.05	<0.001
20:3ω-6	3.74 ± 1.16	2.60 ± 0.53	<0.001
20:4ω-6	11.4 ± 2.93	8.14 ± 1.53	<0.001
22:4ω-6	0.69 ± 0.23	0.23 ± 0.08	<0.001
22:5ω-6	0.17 ± 0.06	0.25 ± 0.11	0.003
ω-3 Fatty Acids			
18:3ω-3	0.13 ± 0.06	0.10 ± 0.04	0.02
18:4ω-3	ND	0.07 ± 0.03	–
20:5ω-3	0.32 ± 0.17	0.41 ± 0.38	NS
22:5ω-3	0.64 ± 0.24	0.34 ± 0.11	<0.001
22:6ω-3	3.21 ± 1.52	1.80 ± 0.70	<0.001
Σ Saturated *Fatty Acids*	48.0 ± 2.59	55.1 ± 3.18	<0.001
Σ ω-3 fatty acids	4.30 ± 1.77	2.73 ± 1.09	<0.001
Σ ω-6 fatty acids	33.2 ± 3.49	29.2 ± 3.46	<0.001
Σ ω-3/Σ ω-6 fatty acids	0.13	0.093	
MMP (°C)	27.8	34.6	<0.001

SD: standard deviation; ND: not detected (<0.005 mg/g); NS: nonsignificant (P≥0.05); MMP: mean melting point

patient group contained significantly less total saturated fatty acids (48.0 versus 55.1%, $p<0.001$) and more total ω-3 polyunsaturated fatty acids (4.30 versus 2.73%, $p<0.001$) and ω-6 polyunsaturated fatty acids (33.2 versus 29.2%, $p<0.001$).

Comparison of the fluidity of the serum phospholipids of the stroke patients and controls. Having observed marked differences in the proportions of saturated and polyunsaturated fatty acids in the serum phospholipids of the patient and control groups, we were interested in objectively assessing the impact these differences might have had on the relative fluidity of the total phospholipids in their sera. One way to accomplish such a comparative assessment was by using the percentages and melting points of the individual fatty acids that comprise these phospholipids to calculate the MMP of each of the phospholipid preparations. Table 2 summarizes the results of our MMP estimations. The MMP of the acyl chains of the serum total phospholipids of the stroke patients was significantly lower than those of the phospholipids of the controls (27.8 versus 34.6°C, $p<0.001$), indicating that the acyl chains of the serum phospholipids of the patients are more fluid compared to those of the controls.

DISCUSSION

The main result of our study of 21 young stroke patients in northern Nigeria was the finding of statistically significant differences in the proportions of more than 20 fatty acids that comprise their serum phospholipids relative to those of a control group comprised of healthy men and women of similar socioeconomic status. These differences were not restricted to any one particular fatty-acid class but instead were seen with the saturated fatty-acid series (14:0-24:0), one of the essential fatty acids (α-linolenic acid), and both the ω-3 and ω-6 polyunsaturated fatty-acid classes. In a search of the literature, we were unsuccessful in finding any reports in which the fatty-acid composition of serum or tissue phospholipids was compared between stroke patients and controls. Neither are we aware of any reports that have addressed the question of the levels of fatty-acid desaturases or chain-elongation enzymes in stroke patients.

The finding of higher proportions of 22:5ω-3 and 22:6ω-3 and lower percentages of 22:5ω-6 in the stroke patients relative to the controls suggests that the controls in the present study may have had a subclinical ω-3 fatty-acid deficiency. The higher percentage of 22:5ω-6 in the phospholipids of the controls relative to the patients is an indication of a relatively low ω-3 fatty-acid status, since with less ω-3 fatty acids to compete for the Δ-4 desaturase, the proportion of 22:5ω-6 would be increased.[15] It is noteworthy in this regard that the percentage of α-linolenic acid (18:3ω-3) was significantly lower in the controls compared to the stroke patients. This observation is cause for speculating that the nutrition of the controls may have been suboptimal with regard to the ω-3 fatty acids. However, Mead acid (20:3-9) was not detected in the control subjects. Mead acid is normally not seen unless the individual whose blood serum was analyzed was deficient to a significant degree in α-linolenic acid.[15]

Since it is widely accepted that the fatty-acid composition of an individual's serum phospholipids is reflective of the fatty acids of their tissue phospholipids,[18] these differences in fatty-acid composition that we have found between stroke patients and healthy controls suggest that the lipid composition and structure/function properties of the tissue membranes of the stroke patients in our study may have differed substantially from those of the controls. The extent to which the membranes of the stroke patients and healthy controls might have differed was indicated by our comparative analysis of the MMPs of the acyl chains of the serum total phospholipids of the two groups. The MMP of the acyl chains of the phospholipids of the patients was about 6°C lower than that of the controls (Table 2). Even this 6°C difference in MMP values could have untoward consequences on one or more of the functions of tissue membranes of the stroke patients, such as solute transport (e.g., amino acids, glucose) across the plasma membrane, membrane potential, ligand: receptor interactions, and membrane trafficking. In this regard, for example, it has been shown that alterations in the fatty-acid composition of the muscle membrane phospholipids of patients with diabetes correlate with insulin sensitivity.[19-21] It is conceivable that in the stroke patients we studied the differences in the fatty-acid composition and fluid property of their membrane phospholipids relative to the controls might have had some adverse effect on platelet-platelet or platelet-endothelium interactions that predisposed the stroke patients to blood clots, which in turn contributed to the cerebrovascular accident each experienced. The altered fluidity of the tissue membranes of the stroke patients in the present might also have compromised other critical cellular functions mentioned above.

If the lipid composition and fluid property of the membranes of stroke patients were indeed different from the corresponding parameters of healthy individuals, then it may be possible to detect this difference using bioelectrical impedance analysis. Two parameters measured in impedance analysis are resistance and reactance. Resistance is a measure of total body water, whereas reactance is a measure of the phase shift between current and voltage due to

the capacitance of tissue membranes. From the resistance and reactance data, one can calculate the phase angle as the arc-tangent of the resistance/reactance ratio. A number of studies involving human diseases that have a nutritional component[22-25] have shown that the phase angle is generally directly proportional to the overall well-being of an individual and the quality and integrity of their tissue membranes. We, ourselves, have shown that the proportions of certain fatty acids in the membrane phospholipids of children with sickle cell disease correlate with their phase angle.[26] We plan to extend the present study to encompass a larger number of stroke patients and controls; in that larger study, we would compare the phase angle of the two groups of subjects and inquire to determine to what extent differences in phase angle might be correlated with differences between the fatty-acid composition of their red-cell phospholipids and those of the controls. Such an experiment would allow us to test the hypothesis that the 6°C difference we observed in the MMPs of the acyl chains of the serum phospholipids between the controls and stroke patients could influence membrane function. Of course, membrane structure and function are not the only biological issues to be considered when the proportions of ω-3 and ω-6 fatty acids change in a particular subset of a population. Fatty acids also exert their effects by influencing the nature of the eicosanoids cells and tissues synthesize.

What might account for the differences we observed between the fatty-acid compositions of the serum phospholipids of the stroke patients and controls? One obvious consideration is diet. It is conceivable that the dietary habits of the 21 individuals who experienced stroke differed significantly from those of the 30 healthy individuals who served as controls in the present study. Andersson and associates[27] demonstrated recently that the fatty-acid profiles of the skeletal-muscle phospholipids of healthy men and women differ in groups whose dietary lipids have different fatty-acid compositions. They found that subjects whose diets had been supplemented with 2.4 g of EPA and DHA acids per day (equivalent to about 100–200 g of fatty fish, such as salmon) had proportions of long-chain ω-3 polyunsaturated fatty acids in skeletal muscle that were 2.5 times higher than those in control subjects. Having already had experience conducting comprehensive analyses of the diets of populations in northern Nigeria,[28] it should be possible for us to obtain additional information regarding the relation between diet and stroke in that region of Africa.

Alternatively, the fatty-acid abnormalities we documented in the stroke patients could be the result of differences between the patients and the controls in the activity levels of the various enzymes that comprise the pathways that elongate and desaturate long-chain fatty acids. The trend towards progressively increasing proportions of saturated fatty acids in the serum phospholipids of the stroke patients (Figure 1) is consistent with the hypothesis that the acyl-chain elongating enzymes were more active in the patients relative to the controls. Similarly, the finding of larger percentages of ω-3 and ω-6 polyunsaturated fatty acids in the stroke patients suggests that one or more of the fatty-acid desaturases may also be more active in the stroke patients.

There were several limitations to our study. First, since there were only 21 stroke patients, it is important to repeat the study with larger numbers of subjects and to do so at several other geographic sites in rural sub-Saharan Africa. Second, a lack of sophisticated diagnostic imaging facilities at the Federal Medical Centre–Gombe prevented us from differentiating the kinds of stroke and the anatomic site of the injury in the patients. Third, dietary information to assess the possible difference in intake of various fatty acids between patients and controls was not collected. Fourth, since culture, including dietary practices, may influence the risk of stroke, it would have been beneficial to have obtained ethnic data for the subjects.

It would be useful to determine if our findings are reproducible in other populations, especially in ethnic groups other than those who are indigenous to Africa. It would also be worthwhile mounting a study to determine if the kinds of fatty-acid abnormalities we observed in the serum phospholipids of stroke patients in northern Nigeria might have predictive value vis-à-vis stroke and cardiovascular disease in general (e.g., myocardial infraction, blood clots). A remarkable aspect of the stroke patients in the present study was their relatively young age (mean 39.7 years). We have no explanation for why stroke occurred at such a young age in these individuals. Furthermore, it seems worthwhile to explore the possibility that modification of the dietary habits of the population in the region where the present study was conducted might lead to the normalization of the fatty-acid composition of the serum phospholipids of the stroke patients who survived their cerebrovascular event. A considerable literature supports the recommendation that the intake of moderate doses of long-chain ω-3 fatty acids to prevent sudden cardiac death.[29] Our finding of increased levels of ω-3 polyunsaturated fatty acids in the serum phospholipids of stroke patients in Nigeria should caution against applying this generalization to all populations, especially adults in northern Nigeria and perhaps other regions in the western Sahel of Africa as well. In light of the findings reported herein, perhaps the conclusion should not be that the fatty-acid status of the stroke patients was unusual but rather that it was

the fatty-acid nutrition of the controls that was abnormal, and that the unique fatty-acid pattern of the phospholipids of the controls somehow conferred upon them a reduced risk of stroke.

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