LIPIDS IN THE HEALTH OF NIGERIANS: AN INVESTIGATIVE JOURNEY

BY

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AN INVESTIGATIVE JOURNEY

An Inaugural Lecture Delivered at University of Lagos

by

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I am grateful to the Almighty God who made the events of today possible, and Mr. Vice-Chancellor, Sir, thank you for the opportunity to deliver this lecture which in the main, is about lipids, their metabolism and the body components to which they are converted.

Lipids, a group of chemical substances, insoluble in water but soluble in organic solvents are known to the layman as fat. The greasy fat from slaughtered animals and vegetable oils sold in the market are well known forms of lipids. Lipids, however, embrace a wide range of fatty substances whose classes and chemical types are presented in Table I. The major roles of lipids are storage, metabolic and structural. In animals, lipids are stored in the adipose tissue as triacylglycerols, to supply fatty acids for energy during starvation, and strenuous exercise. They participate in metabolic control, as vitamins, anti-oxidants, membrane fluidity regulators, detergents, hormones, second messengers and cell signalling molecules (Gurr & Harwood, 1991; Alberts et al, 1999).

### Table 1: Classification of Lipids

<table>
<thead>
<tr>
<th>Names</th>
<th>Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acids</td>
<td>(a) Saturated or (b) Unsaturated. May be branched</td>
</tr>
<tr>
<td>Acylglycerols which are fatty acid esters of glycerols</td>
<td>(a) Monoacylglycerols (b) Diacylglycerols (c) Triacylglycerols (d) Glycerol Esters</td>
</tr>
<tr>
<td>Glycerolipids</td>
<td>(a) Glycero phospholipids (b) Glycosylglycerides and glycolipid (c) Sphingolipids: (i) Sphingomyelin (ii) Cerebrosides (iii) Gangliosides</td>
</tr>
<tr>
<td>Sterols and Steroids</td>
<td>Cholesterol based lipids Dihydroxy fatty acid polymers w-hydroxy and dicarboxylic fatty acid polymers Esters between long chain fatty acids and fatty alcohol.</td>
</tr>
<tr>
<td>Cutin</td>
<td>Lipid A - Rich in: (a) 3-hidromyristate (b) 12-16 carbon saturated fatty acids (c) 3-myristoxymyristate</td>
</tr>
<tr>
<td>Suberin</td>
<td>Lipopolysaccharides</td>
</tr>
</tbody>
</table>
The lipoprotein (LP) is very similar to LDL in its lipid composition and Apoprotein B content but different because it contains another glycoprotein called Apoprotein (apo) (ω APO(a)), which is linked to its APO-B by a disulfide linkage (Fig 2). APO(ω) is structurally similar to plasminogen the proenzyme of plasmin which breaks down the fibrin clot after an injury.

Lipids perform structural functions as in (i) membrane bilayer of lipids which surrounds cells; (ii) monolayer which surrounds soluble serum lipoproteins; and (iii) lipids linked to polysaccharides found in the cell wall of bacteria. (Fig. 1) Soluble lipoproteins (SL) are transported by the blood, to distribute lipids originating from dietary sources (exogenous) or synthesized in the liver (endogenous), to the rest of the peripheral tissues. The major types in the plasma are chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and the high-density lipoprotein (HDL).
atherosclerosis. Thus plasma levels are very important predictive and diagnostic tools in CHD diseases. When elevated, the LP\(^{(a)}\) is an independent inherited risk factor for a very early development of myocardial infarction and atherosclerosis.

The cell membrane

The cell plasma membrane physically separates the living cell from its surrounding environment thereby maintaining always, the essential differences between the inside (cytosol) of the cell and the outside (extracellular) water. The membrane lipids consist of phospholipids, glycolipids, sphingomyelins, and cholesterol which are amphipathic molecules. Each of the lipids moves freely and can displace its position in space. Short fatty acid tail or those with longtails but rich in cis-double bonds enhance the movement and make the membrane fluid at lower temperatures. Cholesterol decreases the bilayer permeability to small water-soluble molecules, and also inhibits phase transitions in the membrane (Albert et al., 1999).

A fluid membrane houses the integral proteins which carry out its functions, which are: selective transport of solutes, (Boszormenyi & Abaelu et al., 1977, 1986; Kareem et al., 1980), energy transduction, production and transmission of electrical signals and biologic communications between cells. In the mitochondria, the membrane proteins (enzymes) carry out the oxidation of fatty acids, generate the protomotive force leading to synthesis of Adenosine Triphosphate (ATP), are the site of the detoxification of products of respiration by catalase and numerous other reactions. This very active organelle is, therefore, easily affected by changes in the surrounding cytosol, and, therefore, very useful in monitoring the sensitivity of an organism to the changes in nutrients, toxins, drugs, etc, present in the cytosol (Weissman, 1995).

Mr Vice chancellor, Sir, in this lecture, I shall discuss our work on lipids, lipoproteins, their metabolism, medicinal plants and product development. The motivation for these studies came from previous observations, which I had made, on Nigerian foods. As a graduate student of Michigan State University, East Lansing, Professors Olaf Micklesen and Gaurth Hansen both Heads of Departments of Nutrition and Biochemistry, respectively, had directed that my research topic be on a subject relevant to solving one of the problems identified in the 1965 report of the Nigerian Nutrition Survey. Hence, I prepared and analysed Nigerian vegetable soups and two Nigerian oil seeds namely *Citrullus Vulgaris* (Melon (Egusi) seeds) and *Irvingia Gabonensis* (Apon seeds) for protein, vitamins and Amino acids. The tremendous loss of thiamin, riboflavin and ascorbic acid in Nigerian cooked sauces (Abaelu et al., 1973, 1975, 1979) prepared from composites exceedingly rich in these nutrients (Fig. 3), and the observation that Melon (Egusi) seeds had very high quality protein (28 – 32%) and lipid (55%) content, very rich in essential amino and fatty acids comparable to those of soya beans (Abaelu et al, 1979, 1980) sharpened my interest from then on to direct my research effort at Nigerian problems. I am forever grateful to these people, particularly Professor Olaf Mickelsen and his wife Mrs. Claire Mickelsen who made my sojourn in the U.S.A a memorable experience. Secondly, in the 1970's and 1980's, the Nigerian markets were flooded with imported vegetable oils labelled as “very high in polyunsaturated fatty acids”, and we wondered about the truth of these claims and the effects on the health of Nigerians and decided to embark on these studies.
Serum lipids, lipoprotein levels and membrane function:
Effect of the environment

The normal levels of serum lipids and lipoproteins are much influenced by the interactions of genetic, environmental factors and cultural practices. The degree of affluence of a population affects their types of diet and nutritional status, two strong environmental factors which influence serum and lipoprotein levels of populations. Previous reports had shown that lipids and lipoprotein levels of Nigerians, high in the socio economic ladder, were much higher than those of low economic status. The levels in the former were comparable to those reported for black Americans (Taylor & Agbodana, 1983; Onajobi, 1987; Edozien, 1965) (Fig. 4) thus showing that affluence brings the same pattern and direction of change in serum lipid levels in Nigerians as observed in the industrialised world.

EFFECT OF GENDER AND SOCIO-ECONOMIC STATUS ON AVERAGE PLASMA LIPIDS AND LIPOPROTEINS LEVEL IN NORMAL NIGERIANS

In 1993, we had measured the serum lipid and lipoprotein levels of protein-energy malnourished children attended to in LUTH (Abaelu et al, 1993) and discovered much reduced levels of all lipids and lipoproteins (50-100% reduction) with an eleven-fold increase in ketone bodies as compared to controls (Fig. 5a,b). This pattern of lipid and lipoprotein levels were very similar to that occurring in the serum of normal Nigerians and Africans of low economic status particularly in the decreased HDL value as well as TC, triglyceride, and LDL even though the subjects were free from CHD (Robinson, 1977; Knuiman & West, 1981; Taylor et al, 1982).

SERUM LIPIDS, LIPOPROTEINS AND KETONE BODY IN PRIMARY ENERGY MALNOURISHED CHILDREN

|    | PRIMARY ENERGY MALNOURISHED |   | NORMAL CONTROLS   |   |   | TOTAL CHOLESTEROL |   |   | TRACYLGlycerol |   | VLDL      |   | VERY LOW DENSITY LIPOPROTEIN |   | LDL       |   | LOW DENSITY LIPOPROTEIN |   | HDL       |   | HIGH DENSITY LIPOPROTEIN |
|----|-----------------------------|---|-------------------|---|---|-------------------|---|---|----------------|---|----------|---|-----------------------------|---|-----------|---|-----------------------------|---|-----------|
| PC | TC                          |   | PC                |   | PC | TAG               |   | PC | VLDL          |   | PC       |   | LDL                |   | HDL       |   | HDL                |

Fig. 4
Socio-economic status

Fig. 5

Fig. 5b

KB = Ketone body
Our evaluation of the nutritional status of alternate admissions of paediatric patients seen in LUTH, but for ailments unrelated to malnutrition revealed that 80% of these, were biochemically and physiologically malnourished as evident from anthropometric and hematological measurements (Renner et al., 1994). These studies showed that the levels of lipids and lipoproteins regarded as “normal” in Nigerian children and people of low economic status need re-evaluation with their nutritional status in mind.

Effects of Diets

The normal plasma lipids and lipoprotein levels are much influenced by diet, alcohol intake, physical activity, obesity and drugs, etc. In the developed world (Europe, America and Canada), normal plasma lipid levels are much higher than what obtains in the African, because their diets are high in proteins (20%) and lipids (42-43%) but moderate in carbohydrate (40%) content. The lipid content of the diet is rich in animal fat which contains high levels of saturated fatty acids which supply 17-19% of the dietary energy, (Gurr and Harwood, 1991). Excessive intake of dietary fat, rich in saturated fatty acids, leads to hypercholesteremic effect and increase in LDL particles which correlate with the very high incidence of coronary heart diseases (CHD), a condition which accounts for approximately 50% of the total number of deaths in these populations (Ahrens et al., 1957; Keys et al., 1957; Williams 1969, 1971).

Generally, among most African populations stark atherosclerosis is uncommon except in confirmed hypertensives, (Akinbue, 1976; Chuwa (1988). obese, diabetics, alcoholics or Africans who have adopted the western style of culture and diet. In these populations high levels of HDL and low LDL were implicated as the protective factors against the development of Atherosclerosis. The protection is generally ascribed to their diets which are high in carbohydrates (70%), low in fat (10-20%), but high in polyunsaturated fatty acids, obtained from vegetable oils and vegetable based diets rather than animal fat and highly processed foods (Edozien 1965).

Between 1990-1996, therefore we measured the composition of ready to eat cooked foods in the combinations purchased from bukatarias and one restaurant in five local government areas of Lagos metropolis and found that they contained between 6-11% protein, 8-21% total lipids and 62-72% carbohydrates, thus confirming (as shown in Table 2) that the composition of the diets of ordinary Nigerians has not changed.

### Table 2

<table>
<thead>
<tr>
<th>Meal Item</th>
<th>Crude Protein</th>
<th>Total Lipid</th>
<th>Total Carb</th>
<th>Ash</th>
<th>Moisture</th>
<th>Fibre</th>
<th>Iodine No</th>
<th>Saponification No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly cooked yam, beans and meat stew</td>
<td>11</td>
<td>8</td>
<td>72</td>
<td>4.1</td>
<td>4.3</td>
<td>4.0</td>
<td>69</td>
<td>196</td>
</tr>
<tr>
<td>Rice, beef stew plain or with beans or dodo</td>
<td>10</td>
<td>21</td>
<td>62</td>
<td>1.4</td>
<td>3.8</td>
<td>2.7</td>
<td>63</td>
<td>190</td>
</tr>
<tr>
<td>Eba/ Fufu with Okro or Ewedu/Soup &amp; Stew</td>
<td>7</td>
<td>19</td>
<td>70</td>
<td>3.0</td>
<td>4.4</td>
<td>2.9</td>
<td>54</td>
<td>182</td>
</tr>
<tr>
<td>Eba + Egusi soup + Meat</td>
<td>6.0</td>
<td>12.8</td>
<td>72</td>
<td>2.7</td>
<td>4.7</td>
<td>3.1</td>
<td>67</td>
<td>ND</td>
</tr>
<tr>
<td>Amala + Ewedu + Gbegi + Meat</td>
<td>11.0</td>
<td>17</td>
<td>63</td>
<td>3.0</td>
<td>4.0</td>
<td>3.0</td>
<td>69</td>
<td>ND</td>
</tr>
<tr>
<td>Poundeds Yam + Egusi or Ewedu</td>
<td>9.0</td>
<td>11</td>
<td>72</td>
<td>3.0</td>
<td>4.0</td>
<td>3.0</td>
<td>66</td>
<td>185</td>
</tr>
</tbody>
</table>

ND = Not Done
markets between 1980-1995) revealed eight of them to have a range of 35-72 iodine numbers, showing also low levels of poly unsaturation. Five of the oils derived from palm oil were found in this group (Table 3). Gas liquid chromatographic (GLC) separation of four of the oils showed them to be very low indeed in poly unsaturated fatty acids as seen in (Fig. 6a,b and Table 5) (Nwakorie et al, 1985), Osinubi (1995). None of the two oils marketed under the label of soyabean or corn oil had the expected fatty acid composition of the oils. We concluded that vegetable oils sold in Nigerian markets, though bearing labels of super unsaturation, were indeed not as described, but the exceptions were Oroyo, Golden oil of unspecified vegetable origin and Melon (Egusi) seed oil whose iodine numbers were 114, 120 and 120, respectively. GLC analysis of Egusi seed oil gave 57% 18:2 linoleic acid (di unsaturated)* 30.0% 18:1 Oleic acid (mono unsaturated), showing it to be very high in Cis unsaturated fatty acid.

Extracted lipid from these foods had iodine numbers between 54-69, showing evidence of low levels of poly unsaturation in the foods (Abaelu and Magbagbeola, 1999). A previous study of the analysis of fifteen vegetable oils (ten imported and five locally processed) sold in our local

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**TABLE 3**

**PHYSICO-CHEMICAL PROPERTIES OF NIGERIAN VEGETABLE OILS**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SOURCE</th>
<th>IODINE NUMBER</th>
<th>SAPONIFICATION NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported Oils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finesse*</td>
<td>NS</td>
<td>55</td>
<td>210</td>
</tr>
<tr>
<td>Golden Cup*</td>
<td>NS</td>
<td>60</td>
<td>186</td>
</tr>
<tr>
<td>Angel Fish*</td>
<td>NS</td>
<td>58</td>
<td>200</td>
</tr>
<tr>
<td>Kings*</td>
<td>NS</td>
<td>65</td>
<td>194</td>
</tr>
<tr>
<td>Turkey Brand*</td>
<td>NS</td>
<td>58</td>
<td>205</td>
</tr>
<tr>
<td>Golden Oil*</td>
<td>NS</td>
<td>124</td>
<td>186</td>
</tr>
<tr>
<td>Kraft Oil*</td>
<td>NS</td>
<td>72</td>
<td>182</td>
</tr>
<tr>
<td>Caroli*</td>
<td>Corn Oil</td>
<td>88</td>
<td>190</td>
</tr>
<tr>
<td>Vegola*</td>
<td>Soya Bean</td>
<td>96</td>
<td>210</td>
</tr>
<tr>
<td>Corn Oil*</td>
<td>NS</td>
<td>124</td>
<td></td>
</tr>
</tbody>
</table>

| Nigerian Processed Oils       |                 |               |                       |
| Oils (Industrial)             |                 |               |                       |
| Groundnut Oil                 |                 | 97            | 189                   |
| Soya bean Oil                 |                 | 131           | 197                   |
| AVOP*                         |                 | 35            | 201                   |
| Oroyo                         |                 | 114           |                       |

| Locally Processed and Unrefined|                 |               |                       |
| Red Palm Oil                  | Iddo            | 53            | 199                   |
| Melon Seed Oil                | CMUL Lab        | 120           | 205                   |
| Serewe/Ibo variety            |                 |               |                       |
| Oil Bean Seed                 |                 | 76            | 196                   |
| Breadfruit Oil                |                 | 102           | 198                   |

* N/S = Not Specified
* = Trade Mark Name

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* Foot note – Other reports show linoleic content of freshly extracred melon (Egusi) seed oil to range between 70-75% and oleic between 7.8 – 10.7% (Ogugie, 1988)
EFFECT OF NIGERIAN DIET ON RAT PLASMA LIPID AND LIPOPROTEIN

PF = PFIZER FEED
P = PALM OIL (RED)
RP = REFINED PALM OIL (BLEACHED)
S = SOYABEAN OIL
G = GROUND NUT OIL
A = AVOP VEG. OIL

Fig. 7a

Fig. 7b
**TABLE 4.**

LIPID COMPOSITION OF LIVER TISSUE

<table>
<thead>
<tr>
<th>GROUP INDEX</th>
<th>TYPE OF OIL</th>
<th>% LIPID OF LIVER TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td>CONTROL COD-LIVER</td>
<td>3.10 ± 0.11</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>MELON SEED</td>
<td>4.83 ± 0.02</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>SOYA-BEAN SEED</td>
<td>4.73 ± 0.06</td>
</tr>
<tr>
<td>GROUP 4</td>
<td>FINESSE</td>
<td>7.00 ± 1.2</td>
</tr>
<tr>
<td>GROUP 5</td>
<td>CAROLI CORN</td>
<td>4.15 ± 0.01</td>
</tr>
</tbody>
</table>

Average for three determinations

**TABLE 5.**

FATTY ACID COMPOSITION OF OILS USED IN THE FEEDING EXPERIMENT

<table>
<thead>
<tr>
<th>Type of oil</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18.1</th>
<th>C18.2</th>
<th>C18.3</th>
<th>C20.4</th>
<th>C22.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melon seed oil (egusi oil)</td>
<td>0.8</td>
<td>4</td>
<td>0.8</td>
<td>8.3</td>
<td>30</td>
<td>57</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Soya-bean oil (vegola)</td>
<td>0.1</td>
<td>2</td>
<td>18</td>
<td>14</td>
<td>63</td>
<td>2</td>
<td>2</td>
<td>ND</td>
</tr>
<tr>
<td>Corn oil (caroli)</td>
<td>L</td>
<td>3</td>
<td>25</td>
<td>57</td>
<td>1</td>
<td>25</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Finesse oil</td>
<td>41</td>
<td>43</td>
<td>0.4</td>
<td>6</td>
<td>10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = NOT DETECTED

We fed these oils between 1982-1996 to rats at the level of 10-18% of the diet and between 10-20% protein and 65.5-70% carbohydrate, simulating Nigerian dietary composition, and observed that generally all the vegetable oil groups had normal serum lipids and lipoprotein levels in the short term of 28 days, i.e., in the total cholesterol (TC), triacylglycerols (TAG) and LDL cholesterol content (Fig. 7a,b). However, TC was generally lower (65-89mg/dl) and HDL (anti atherogenic factor) significantly highest for the palm oil/olein groups (60-73mg/dl) while for AVOP vegetable oil (Iodine No 35), the HDL was 27mg/dl and very low (Osinubi, 1995). When dietary protein was borderline (10-11%) refined palm oil and Finesse oil of unspecified vegetable origin, gave a condition known as fatty liver (liver total lipids was above 7%) (Table 4) while Finesse gave increased levels of all lipids measured in the serum, heart and liver of treated rats at the end of eight weeks (Nwakorie, 1982). Gas liquid chromatographic analysis of Finesse oil revealed that it contained 41% myristic, 43% palmitic 6% stearate and only 10% oleic acids (Table 5). Our samples of Finesse and AVOP vegetable oils were of very poor quality (Osinubi, 1995; Nwakorie, 1985).

We studied the metabolism of the oils further in rats fed 10% vegetable oil, 15% protein and 65.5% carbohydrate, by measuring the liver and red blood cells activity of Glucose-6-phosphate dehydrogenase (G6PD), a cytosolic enzyme known to be highly sensitive and adaptable to the degree of saturation of fatty acids in the dietary fat. We observed that vegetable oils (red and bleached palm oil) rich in saturated fatty acids increased the liver G6PD activity 5-7 fold above those of pure melon, and soyabean oils which were rich in poly-unsaturated fatty acids of linoleic acid, and 2-3 times above that of controls consuming the commercial Pfizer rat stock feed (Fig. 8a). Furthermore, a 50:50 mixture of red palm oil and melon seed oil gave 3 fold increase in G6PD activity above the soyabean oil group (Abaelu et al, 1992).

On the other hand, the liver inner mitochondrial membrane suuccinic dehydrogenase (SDH) and oligomycin sensitive ATPase were significantly lower in animals fed the saturated fatty acid rich palm oil/olein at 65% SDH and between 36-66% of the ATPase activities found in the sunflower and soyabean groups, respectively (Fig. 8b). We noted, however, that, the mitochondrial SDH and ATPase activities of the palm oil groups were approximately the same as those of the control animals consuming the commercial rat stock feed (Fig. 8b). Intestinal membrane transport of L-leucine, an essential amino acid also revealed approximately same rate of transport by palm oil groups and commercial stock feed groups but a significant 25% increase in melon oil groups thus showing that normal membrane functions were retained in the intestines of rats.
feder palm oil diets, but that the melon groups had an enhanced performance (Abaelu et al, 1996).

The literature on dietary lipids composition and their metabolism in health and disease is voluminous. Referring to the existing knowledge from results of previous studies (Sinclair, 1964; Gurr and Harwood, 1991), we were able to interpret the results of our experiments thus:

1. Most of the imported vegetable oils sold in the Nigerian local markets between 1980-1995 ranged between very low to moderate in their content of polyunsaturated fatty acids, many of them were bleached palm oil even when not specified.

2. Normal Nigerian dietary composition (1990-1996) is still very high in carbohydrate (62-72%), moderate in lipids (8-21%) and deficient to borderline in protein (6-11%). When protein is deficient, such a diet would result in accumulation of lipids in the liver as fatty liver syndrome and reduced membrane (intestinal) transport of nutrients, e.g., amino acids, etc. (Abaelu, A.M. et al, 1976; Boszormeny and Abaelu, 1976; Nwakorie, 1982).

3. Such diets when metabolised would yield predominantly acetyl CoA to provide energy and be an endogenous source of raw materials for the synthesis of long chain saturated fatty acids, Triacylglycerols (TAG) and cholesterol, (Beaton and Mc'Henry, 1964) which are packaged into LDL and LP(a) lipoproteins, possibly much more LP(a) than LDL because its synthesis depends completely on endogenously produced TAGs and cholesterol. As shall be seen later in this lecture, LP(a) levels in Nigerians/blacks in general are very high and might have arisen as an adaptation to use up excess acetyl CoA from high carbohydrate diets for synthesis of lipids (e.g. TAG and cholesterol) in the liver for distribution to the peripheral tissues. Kostner (1990) had thought otherwise by hypothesizing that:

"in the civilized human population of the industrialized world LP(a) may have lost its importance phylogenetically, because"
cholesterol rich lipoproteins, notably, LDL are highly abundant. Whereas in the black African, for which the food supply is not as regulated as that of other ethnic groups plasma LPₐ levels are more than twice that in the Caucasians”.

Phylogenetically, therefore, he felt that LPₐ has remained exceedingly important in the black because supply of cholesterol to the kidneys, brain, endocrine and genital organs cannot be left to chance. But I think the difference in LPₐ concentrations in the two groups may lie in the content of the diet rather than the frequency or regularity of it, i.e., high carbohydrate but moderate quantity of saturated fatty acids in the African diet on one hand and large quantities of saturated fatty acid with moderate levels of carbohydrate on the other, as found in the Caucasian diet. This argument is further supported by the fact that:

4. Generally, high carbohydrate and saturated lipid diets but adequate protein stimulate the activity of G6PD, (Nace et al; 1970; Teppenman et al, 1958; Niemeryer et al, 1962). In our studies, all the diets (adequate in protein) containing red/bleached palm oil (42% palmitate, 3% myristate, and 4% stearate) induced 5-7 fold the activity of G6PD in a pathway where, as a result, the production of NADPH would be tremendously increased. NADPH is the reducing power needed in the synthesis of the following metabolites: long chain saturated fatty acids and cholesterol from acetyl CoA and the utilisation of folic acid for RNA and DNA synthesis. These are ingredients for increasing cell size (hypertrophy) and coupled with increased ribose synthesis in that pathway, cell proliferation (hyperplasia) of adipose tissue. Hence, regular and excessive intake of such diets would lead to diabetes and obesity. However, because long chain saturated fatty acids (C16:0 carbon chain and above) have very little effect on blood cholesterol, (Beaton & M’cHenry, 1964), this may give a false picture of well being, i.e., a normal to reduced levels of total serum cholesterol and LDL, a picture normally seen in the Nigerian subjects and in the laboratory animals studied (LDL was not measured in rats). But it appears that Nigerians eat much palm oil in their diet as evident from the results of the physical constants from analyses of vegetable oils and food purchased from the bukaterias. Lipids, which are high in mono-unsaturated fatty acids are known to depress LDL and slightly elevate HDL. Results of our experiments confirmed those of previous workers who identified the 42% oleic acid (18:1 cis-mono-unsaturated fatty acid) and 9% linoleic present in palm oil to be responsible for its effects in not elevating serum LDL as well as being a HDL booster (Grundig, 1986; Pereira and Sinniah, 1990; Sundram et al, 1990; Matson et al, 1985; Mata, 1992; Adelotan, Usuanlele and Aabelu, 1992; Osinubi et al, 1995). In addition its failure to elevate blood total cholesterol even though it contains 50% long chain saturated palmitic acid is because the latter occupies the alpha position in the palm oil triglycerides rather than the beta position which causes elevated blood LDL-cholesterol (Bajust, 1965; Elson, 1992). Moreover, palm oil is very rich in carotenoids and tocotrienols, vitamins A and E active substances which are potent antioxidants and suppressors of cholesterol synthesis, hence its anticarcinogenic and antithrombotic activities (Hornstra, 1988). Consequently, palm oil which is regularly used in the Nigerian diet appears to be a HDL (antiatherogenic) booster, a total cholesterol and LDL non-elevating vegetable oil but excessive consumption will result in obesity and diabetes.

5. With moderate consumption, increased nicotinamide adenine dinucleotide hydrogen phosphate (NADPH) production (Abaelu et al, 1992) should be a positive adaptation in providing an important reducing agent in the body, e.g. in the protection of red blood cells against lysis particularly in G6PD deficient individuals. The utilisation of judicious mixtures of palm oil and other oils, rich in poly unsaturation levels as demonstrated in our experimental use of mixture of melon/palm oil, will be valuable in providing the
protection as well as furnishing adequate essential fatty acids.

6. Saturated fatty acid rich oils fed in protein deficient diet, however, cannot be recommended as sole sources of vegetable oils in the Nigerian diet because signs of essential fatty acid deficiency (alopecia) were observed in the young growing rats fed these oils and this was accompanied by raised serum levels of triacylglycerol, liver total lipids and heart cholesterol at the end of eight weeks feeding.

COMPARISON OF PLASMA LIPIDS AND LIPOPROTEINS IN NIGERIANS AND OTHER POPULATIONS

Hypertension, but not atherosclerosis, is quite common in the Nigerian adult population (Akinkugbe, 1976). By 1995, 11% of the population above age 15 with a BP 160/90 was the national hypertension average: but today, with the pronouncement by WHO that an individual with a BP of 140/90 is deemed to be at risk, the figure has doubled (Ajuluchukwu, 2004). How well then do the experimental data described above support the values of serum lipids in Nigerian?

In the course of our research, we studied the serum lipids and lipoprotein composition of Nigerians in health and disease as compared to other racial populations. Our experimental subjects consisted of a population of normal, unrelated healthy Nigerian volunteers, employed in LUTH, College of Medicine, Idi-Araba and from Lagos metropolis in general, Coronary Heart Disease (CHD) patients attending the cardiology clinic of LUTH, and a normal UK (caucasians) population associated with Charring Cross and West Minister Medical School of the University of London. We compared our results with the values obtained for other racial groups as shown in Fig. 9a, b: (i) all the lipids and lipoprotein values obtained fell within normal limits; (ii) We confirmed that in Nigerians and other Africans there were significantly decreased levels of plasma total cholesterol, triacylglycerol, LDL, and increased levels of HDL (the anti-atherogenic lipoprotein) when compared with other racial groups as previously reported by others. Hence from these results, Nigerians should be much less predisposed to CHD while, clearly, genetic make up and the environment have influence on serum lipids and lipoprotein values (Bhatnagnar et al, 1993; Rhoads et al, 1986; Nig. Nut. Sur., 1965; Chuwa, 1988; Osinubi et al, 1993; Taylor & Agbedana, 1983; Robinson et al, 1979; Knuiman and West 1981).
NORMAL PLASMA LIPIDS AND LIPOPROTEIN AVERAGE VALUES IN SOME POPULATIONS

C - CAUCASIAN
J - JAPANESE
I - INDIAN
N - NIGERIAN

Fig. 9a

HDL APO A-I AND LDL APO B AVERAGE VALUES IN SOME POPULATIONS

C - CAUCASIAN
J - JAPANESE
I - INDIAN
N - NIGERIAN

Fig. 9b

EFFECT OF GENDER ON AVERAGE PLASMA LIPID LEVELS IN NIGERIANS AND TWO TANZANIAN RURAL POPULATIONS

■ = MALE
■ = FEMALE
TC = TOTAL CHOLESTEROL
TAG = TRIACYLGLYCEROL

Fig. 10a NORMAL NIGERIANS 1965 TO 1996

Fig. 10b TANZANIAN RURAL POPULATIONS
Comparing the effects of gender and age, we found that in general and by contrast with Europe and America, the Nigerian and African females consistently tended to have slightly higher lipids and lipoprotein values than their male counterparts with the exception of the triglyceride value. The higher values in the female are maintained in spite of differences in the socio economic status of the people (Figs. 10a, 10b and 4). Overall, within the sex, lipid levels, particularly total cholesterol (TC) triglycerides and LDL, gradually increase until 49 years of age after which a decrease occurs in the males, while marked increases which coincide with menopause are observed in the females (Onajobi, 1987; Taylor et al., 1983; Osinubi et al., 1993; Abaelu, 2002; Swai and Mtngao, 1988).

**LDL, HDL, and the genetic make up**

In the Western Countries, apart from life style, genetic factors also predispose most sufferers to early myocardial infarction because CHD gene aggregate in families. The conditions, familial hyperlipidemia and hypercholesterolaemia in which fasting plasma remains turbid, or production of functional LDL receptors is impaired have been implicated as frequent causes of ischaemic heart diseases (Beaton & M’c Henry, 1964; Brown and Goldstein, 1984).

The LDL is a heterogeneous molecule and consists of three subclasses: LDL-1, LDL-2, and LDL-3. The LDL-3 subfraction in the caucasian has been found to increase significantly in CHD subjects. In Nigerians, we observed two major subclasses, the LDL-1 and LDL-2 (Fig.11a,b). The LDL-3 associated with a three fold increased risk of myocardial infarction was not seen in the subjects studied (Osinubi, 1995; Abaelu, 2002). A lot more research is, needed in this area to establish the number of LDL subclasses in Nigerians and the relationship between the LDL subclass pattern and CHD in the population. We however observed that though in Nigerians the LDL value is lower and HDL higher than his Caucasian counterpart, his APOB level (99mg/dl) of LDL is significantly higher while the corresponding APOA value (135mg/dl) of HDL is significantly lower than the Caucasian values of APOB (81mg/dl) and APOA1 167mg/dl, respectively (Osinubi et al., 2000). This new findings should be confirmed by further research because it shows that Nigerians either have a higher turn - over rate for LDL and a lower rate for HDL or a higher rate of APOB and lower rate of APOA synthesis. Another possibility is the presence of a high protein to lipid ratio in serum lipoproteins of Nigerians. We did not measure this and it should be an interesting future study. Thus genetically, the Nigerian LDL appears to possess more APOB proteins for binding the LDL receptors but less HDL-APOA, for binding HDL receptors, and so would appear to distribute more LDL-cholesterol molecules to the peripheral tissues faster than would the Caucasian subjects.

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**Fig. 11a** LDL subclass patterns by density gradient ultracentrifugation; primary peak LDL 1.

**Fig. 11b** LDL subclass patterns by density ultracentrifugation; primary peak LDL 11.
LP(\(_a\)) is an LDL-like particle whose APO(\(_A\)) is a genetic marker. Numerous clinical studies in Whites and the Japanese have established a very strong association between elevated LP(a) levels and susceptibility to develop CHD (Rhoades et al., 1986; Armstrong et al., 1986, etc.). In general, LP(\(_a\)) levels greater than 30mg/dl is evident of a risk of CHD. Comparing the LP(\(_a\)) values in, Figure 12, normal Nigerian LP(\(_a\)) average value of 22.1 mg/dl is 2.4 times that of the average for normal Caucasian subjects, almost 2 times of the Japanese and 1.12 times of the Asian value. Indeed, 35% of the normal Nigerians studied had values higher than 30mg/dl (Osinubi, 1995; Abaelu, 2002). Thus confirming all previous studies, which have been undertaken in the African and Black Americans.

The LP(\(_a\)) levels of Nigerians suffering from CHD rose non-significantly from 22.1 to 24 mg/dl, by only 9% and moreover, only 40% of CHD Nigerians had LP(\(_a\)) levels > 30mg/dl (Osinubi et al, 1999). Whereas in the Caucasian the mean LP(\(_a\)) value for CHD subjects was 15.2mg/dl that is 180% or approximately twice that of the normal subjects. Thus, while it is confirmed that increased LP(\(_a\)) levels is predictive of CHD in the Caucasian, it cannot be used to differentiate between normal and CHD Nigerians, thus having no predictive value in them (Osinubi et al, 2000). High normal LP(\(_a\)) levels are found also in American blacks and Congolese blacks of French extraction (Guyton, 1985; Para et al, 1987).
The LP Molecule Phenotypes

The Apoprotein $\alpha$ (APO$\alpha$) glycoprotein is a heterogeneous molecule whose determined isoforms (by ultracentrifugation, SDS–polyacrylamide electrophoretic separation followed by immunoblotting), gave six phenotypes classified as: F, B, S$S_2S_3$ and S$S_4$. In our study, all the high molecular weight isoforms S$S_2S_3$ and S$S_4$ were identified in both the Nigerian and Caucasian subjects. We, however, observed that the frequencies and distribution of the APO$\alpha$ isoforms were different in Nigerians because S$S_2$ and S$S_3$ were found more frequently among the UK (Caucasian) normal subjects, whereas there was a tendency towards the S4 isoform in Nigerians (Fig 13a,b). The B and S1 phenotypes associated with the highest LP$\alpha$ levels among the Caucasians with CHD and are thought to be better predictors of CHD (Amstrong 1986, Boerwrinkle 1989) were rare in both normal and CHD Nigerians. Moreover, APO$\alpha$ locus variation was explained by only 13% in Nigerians, in Asians 70% and in Caucasians 41%, thus LP$\alpha$ level is affected minimally by APO$\alpha$ locus in Nigerians, therefore, other factors must be involved. The high normal LP$\alpha$ levels in Nigerians possibly evolved as a combination of increased synthesis coupled with reduced rate of degradation and therefore would appear to serve other important functions.

Though LP$\alpha$ levels cannot be used to discriminate the normal from CHD subjects in Nigerians, other serum lipids and lipoproteins remain valuable predictors of CHD in them. For instance, only the serum triglyceride and LDL-lipoprotein levels in the normals were statistically, significantly different from those of the CHD and these seem to be the best discriminators of individuals at risk of coronary events in the Nigerian population (Osinubi et al. 1993). In the female, serum triglyceride alone is enough to discriminate between CHD and normal subjects. The mean concentrations of total cholesterol, triglyceride and LDL were mildly elevated while HDL was lower in the CHD but the changes were not statistically significant. Moreover, there was no significant difference between total HDL – cholesterol levels of the CHD and normal subjects nor in the levels of APO-A1 and APO B, which are better discriminators of CHD from normal subjects.

Thus, in conclusion, we suggest that levels of serum triglyceride and LDL-cholesterol are the most important factors with respect to
predicting the risk of CHD in the Nigerian population, and that research is needed to determine the number and usefulness of LDL-1,2 and 3 subfractions, in predicting CHD in Nigerians. Also confirm or otherwise the higher APOB and lower APO-A levels in Nigerians. The probable need for the high levels of LP\textsubscript{a} and APO-B proteins for distributing cholesterol to the peripheral tissues:

Metabolic studies have shown that LDL and LP\textsubscript{a} share common route of metabolism as well as have certain pathways that proceed via different routes for LP\textsubscript{a}. In “in vivo” experiments using radiolabelled LP\textsubscript{a} and LDL\textsubscript{l}, LP\textsubscript{a} was preferentially taken up in rabbits by the spleen > ovaries > kidney. Indeed the spleen took up 2-3 times more LP\textsubscript{a} while in the kidney and ovaries it was 30-50% more LP\textsubscript{a} than LDL\textsubscript{l}. Also kidney patients have grossly elevated plasma LP\textsubscript{a} levels and in pregnancy, plasma levels rose in the first trimester and fell to basal levels at the time of delivery. Thus, Kostner (1990) suggested that the function of LP\textsubscript{a} is the supply of cholesterol derived from the liver to the periphery, independently of dietary influences, to the endocrine organs with high steroid hormone production. It is possible, that in Nigerians/Africans in addition to hormone production, phylogenetically, very high level of LP\textsubscript{a} is needed and might have evolved to supply cholesterol to those organs whose cells continually synthesise more membranes during cell proliferation processes such as in the testis and the uterine cycle but particularly in the Reticulo endothelial systems such as the spleen, which plays a significant role in the immune system, contains platelets as well as being a very important blood filter. The spleen is an exceptionally effective means of phagocytic removal of bacteria and deformed cells, e.g., sickled red blood cells, or those which contain malarial parasite as obtained in tropical areas of the world where such diseases and infections are endemic. New cell membrane (whose composition consists of phospholipids glycolipids and cholesterol) is needed to replace the portions continually removed by phagocytosis in order to restore the cell size to normal or replace shed tissues in the uterine cycle (Ganong, 1995).

In support of this is the enlargement of the spleen and livers commonly observed in people living under tropical conditions. The bulk of this cholesterol need appears to be better supplied by LP\textsubscript{a}, a lipoprotein whose endogenous synthesis in the liver is assured from raw materials, such as acetyl CoA which a high carbohydrate and saturated lipid diets supply. In other words, apart from hormone synthesis, LP\textsubscript{a} is needed much more by those tissues where new membranes are needed very urgently.

Thrombotic risks associated with elevate LP\textsubscript{a}

A very strong association exists between the elevations of LP\textsubscript{a} values and susceptibility to develop cardiovascular diseases (CHD). Increased LP\textsubscript{a} levels linked with CHD diseases is thought to be related to interference with thrombogenesis and thrombolysis processes, This is because the APO\textsubscript{a} of LP\textsubscript{a} is structurally similar to plasminogen, but cannot be converted into an active protease for the dissolution of the fibrin clot. However, LP\textsubscript{a} competes with plasminogen and tissue plasminogen activator (tP\textsubscript{a}) in binding fibrinogen and thereby slows down plasminogen activation, and even doubles the time for the lysis of the clot. Hence, clinically, individuals with elevated levels of LP\textsubscript{a} do not lyse clots as effectively as those with normal levels (Kostner et al, 1990; Lascalzo et al, 1990).

In Nigeria, hypertension and diabetes are common in most communities and they predispose to cardiovascular diseases (Okuwobi, 1968; Akinkugbe, 1976; Chuma, 1988), and eventually to stroke. The occurrence of a very high LP\textsubscript{a} value in the African makes it tempting to suggest from the above account that after thrombi forrmation unng a stroke, the inhibition of fibrinolysis of the blood clot is assured. However, it is known that normally, fibrinolytic activity is greater in the black African compared with Americans and Indians, age and sex, matched and remains active in them, throughout life, while it decreases in Indians and Europeans (Williams, 1988). Even though research is yet to unravel why fibrinolysis remains high in the African it can be suggested here that a high LP\textsubscript{a} may necessarily not affect the fibrinolytic activity adversely because as mentioned earlier, we observed from our experiments that the B and S1 APO\textsubscript{a} Phenotypes frequently found among Caucasians with cardiovascular disease (CHD) are very rare in both normal and CHD Nigerians. Research is very much needed to find out any differences in the degree of competitive binding of the fibrin clot between plasminogen,
As pointed out earlier, our oil seeds are very rich in proteins, lipids, essential amino acids and fatty acids. In Nigeria, many of our highly prized fermented foods (Ogi, Gari, Fufu, Lafun, etc.) beverages (burukutu and pito and condiments, (Dawadawa, iru, ogiri) are obtained from cereals, roots, tubers and protein rich oil seeds using chance fermentation processes. As an example, iru the flavouring agent made from African locust beans (Parkia biglobosa) is very rich in protein (Campbell-Platt 1980) and also serves as meat supplement among poor families. But production is laborious, time consuming, and the product has very short shelf life. On the other hand, ogiri traditionally produced from melon and castor oil beans has very strong objectionable ammoniacal smell (Eka, 1980; Odunfa, 1983; Ogundana 1978).

In our Product Developmental effort we tapped from the knowledge that Bacteria and fungi could be used to cause desirable changes in the texture, flavour and taste of foods because, genetically, they possess adaptive mechanisms, for inducing the synthesis (derepression) of the various hydrolases needed for the utilisation of substrates which may be novel to them. Mutations occur frequently due to changes in the DNA structural genes coding, for proteins and/or changes at the transcriptional levels between DNA and RNA; and because their life cycles are very short the changes are fast. We had previously isolated and characterised the mixed flora responsible for the fermentation of African locust beans into the food flavouring agent, iru. We obtained four pure cultures of the wild types of Bacillus: namely, B. licheniformis, B. subtilis, B. Pumillis and another strain of B.licheniformis (Abaelu et al. 1990). Melon seeds, are ubiquitous in occurrence, and unlike African locust beans, are available in commercial quantities. Therefore, it was highly desirable that these organisms should ferment melon seeds into acceptable products. To test this hypothesis, we proceeded to study the ‘in vitro’ fermentation of melon seeds with the pure culture of each bacillus specie and were pleased to discover products that were defined by the biological characteristics of each singular organism (Table 6). Thus we obtained three melospice products; one, from B.licheniformis which tasted exactly like ogiri but with highly refined pleasing odour and flavour, a second from B.subtilis and all the S S S S S 1 and B. APO (a) isoforms.
which was similar to *iru* normally obtained from fermented African locust beans. The third product was from *B. Pumulis* which had a cheeselike, sharp sweet and sour taste, with an odour equally mild and sweet in aroma.

There were increases in free amino acids, free fatty acids and reducing sugars (Fig 14a,b) indicative of induced production of the various proteases, lipases and amylases by the fermenting organisms. We observed that the unsaturated fatty acids were converted into saturated ones, thus resulting in decreased rancidity of the products (Abaelu *et al*, 1990; Bencheat *et al*, 1974; Kushi *et al*, 1976). But fermentation time was quite long (7 days) before products that were consistent with the desired condiments began to form.
ACTIVITY OF AMYLASE IN WILD TYPE AND MUTANT STRAINS OF B. Licheniformis

![Graph showing specific activity of amylase in wild type and mutant strains.](image)

Fig. 14c

ACTIVITY OF PROTEASE IN WILD TYPE AND MUTANT STRAINS OF B. Licheniformis

![Graph showing specific activity of protease in wild type and mutant strains.](image)

Fig. 14d

Fig. 15a  pH changes during fermentation of melon seeds by mutants of B. licheniforms.
So, we began work on the genetic improvement of the fermenting organism obtained from the above work in order to increase the overall efficiency in growth rate, tolerance to acidity and temperature, enhanced flavour and retardation of spoilage. Using N-methyl-N-nitrosoguanidine mutagenesis to cause mutation in *B. licheniformis* I, (the specie which gave ogiri-like products) we succeeded in isolating 18 mutants four of which were true hyperproducers of extracellular alpha amylase and 23 other mutants three of which were true proteases hyperproducers (Adeniran et al., 1991). The mutants secreted up to 2-3 times more alpha-amylase and proteases (Figs 14a, b) above those of the wild type when grown in Mueller-Hinton (MH) medium but 3-16 fold alpha amylase and 6-144% protease in Bouillon yeast medium than the wild type. Two of the mutants, 173 and 213 whose partially purified α-amylase and protease showed the most desirable properties (in terms of high activity, pH effect, thermostability, substrate affinity, and temperature stability) (Figs.15a, b, c, d.) were selected and studied for their fermentation of melon seeds. The mutants reduced the fermentation time to half of that of the wild type, from 92 hours to 48 hours. For both wild type and mutants there were increases in the reducing sugar and amino acid content ranging from 1.5-3.5 times the starting values as shown in (Figs 16a,b) (Adeniran et al., 2002). These are desirable characteristics previously noticed in the production of the Japanese Miso (Shibasaki and Hesseltine, 1962) soy sauce (Wang and Hesseltine, 1970) and in the Indonesian mould fermented food product (Wang, 1965).

The α-amylase produced by our mutagenised strains of *B. licheniformis* were found to be more thermostable than those of the wild type at 58°C and had optimal temperature for activity between 55-65°C (Fig. 15b) and thus could be useful in producing amylases for industrial use locally rather than depend on importation. With this in mind, we proceeded and cloned the α-amylase gene as a 3.0 kilobase PstI
fragment from chromosomal DNA of *B. licheniformis* mutant 213 in to purified plasmid PAT 153 to obtain a 6.6Kb recombinant plasmid. This was transferred into *E. coli* HB 101 and amylase productivity was expressed in the presence of starch and tetracycline (Adeniran et al., 1991a,b) as shown in (Figs 17 and 18a,b).

**Fig. 18a** Changes in Reducing Sugars during fermentation of melon seeds by mutants of *B. licheniformis*

**Fig. 18b** Amylase positive *E. coli* HB 101 (pSAA 1) on blood agar base plate containing tetracycline and 1% starch.

**Medicinal Plant Lipids**

Lipids and Organic solvent soluble substances in medicinal plants are important in the maintenance of Nigerian health. We screened two plants, namely *Bridelia ferruginea* (*Epo ira*) stem bark extracts (BF) (family Euphorbiaceae) and leaf extracts of *Solanum nodiflorum* (SN) (*Odu*) (family solanacea) for efficacy as anti-microbial and anti-viral
ABLE 7: Screening of *B. ferruginea* Extracts and fractions for anti-bacterial activity.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Ethanolic Extract</th>
<th>Acetone fraction</th>
<th>Butanol fraction</th>
<th>Chloroform fraction</th>
<th>FII fraction</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>SS</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>SS</td>
<td>S</td>
<td>S</td>
<td>SS</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Std. E. coli</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>SS</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Y. enterolitica</td>
<td>SS</td>
<td>S</td>
<td>S</td>
<td>SS</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td>Std. Staphylococcus aureus</td>
<td>SS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>SS</td>
</tr>
<tr>
<td><em>N. gonorrhoea</em></td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>-</td>
<td>-</td>
<td>S</td>
</tr>
<tr>
<td><em>Streptococcus Strain A, C. and G</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S = Sensitive</td>
<td>SS = Slightly sensitive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R = Resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FII = Fraction eluted from aqueous extract (WE) chromatographed on Sephadex %50 column.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- = Not done because of shortage of specimen.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 8:** Effects of *B. ferruginea* extracts on microbial growth

<table>
<thead>
<tr>
<th>Extract</th>
<th>Amount of extract in ug</th>
<th>Zone of Inhibition of microorganism measured in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Yersinia enterolitica</em> S. d2</td>
</tr>
<tr>
<td>Ethanol (EE)</td>
<td>200</td>
<td>11</td>
</tr>
<tr>
<td>Ethylacetate</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Butanol (BF)</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>Aqueous (WE)</td>
<td>200</td>
<td>12</td>
</tr>
<tr>
<td>Coumarin</td>
<td>100</td>
<td>23</td>
</tr>
<tr>
<td>Std. Ampicillin</td>
<td>1.51U</td>
<td>-</td>
</tr>
<tr>
<td>Or Penicillin</td>
<td>2001U</td>
<td>-</td>
</tr>
</tbody>
</table>
Extracts from both plants, exhibited broadspectrum bactericidal activities towards both gram positive and negative organisms in the thirteen different bacterial species studied, while SN showed anti-viral activity in addition (Tables 7 & 8, Figs 19a,b) (Abaelu et al, 1993, 1997). Susceptible viruses included potiskum, and other viruses, thereby justifying the use of these medicinal plants for the various infections, which the people take them for. When tested the BF extracts showed no anti-fungal activity.

Fig. 19a: Salmoneilla & Shigella spp.
Salmoneilla and Shigella species were tested for sensitivity against A, C, D and E extracts on dextrose sensitivity test Agar. Picture shows Salmoneilla and Shigella to be resistant to A Extract. A, B and E remain as dark brownish spots while C extract remain as centres of clear zones around the filter paper indicates sensitivity whereas a flake indicates resistance.

Fig. 19b: E. coli and Staphylococcus aureus were tested for sensitivity against A, B, C and E extracts on dextrose sensitivity test Agar. Picture shows E. coli and Staphylococcus aureus to be sensitive to all the extracts.
The extracts of BF stem back after phytochemical screening (by means of thin layer chromatography, on silica gels, uv/vis spectrophotometry and infra-red spectroscopy (Figs. 20a,b; 21a,b; 22) contained predominantly mixtures of secondary metabolites (Phenolic compounds) such as flavonoids, coumarins some lipids (saponins) and tannins (Owumi et al, 1993). Rutin, umbelliferone and saponin (hecogenin) were quantified by means of HPLC (High performance liquid chromatography) (Fig. 23).

*Solanum nodiflorum* leaves contained 27% lipid, 14% protein and 36% carbohydrate on dry weight basis and, when phytochemically screened and quantified by HPLC, contained high levels of lipids such as sterols, saponin, (e.g., stigmasterol, dehydrocholesterol, sitosterol, hecogenin), mono and poly unsaturated fatty acids, e.g., oleic and linoleic acids and flavonoids (e.g., quercetin and umbeliferone) while alkaloids, tannins and phlobatans were absent. Carotenoids and vitamin E occurred in microquantities only (Sotomi, 1995; Abeta et al, 1997; Dissertations 1998, 2001, 2002).

Flavonoids were discovered by the Biochemist Albert Szent Gyorgyi (1937 Nobel prize winner) which he labelled Vitamin P. and are needed to maintain capillary wall integrity. They are potent anti-oxidants capable of stopping tissue damage from free radicals which are implicated in numerous diseases, e.g., inflammatory and cardiovascular diseases, diabetes, etc., (Fotsis et al, 1997; Gabor, 1988; Hertog, 1993; Furhman, 1997).

Saponins and sterols (sitosterol) are also known to be potent anticancer/anti-viral agents, immune boosters, anti-inflammatory agents and the source of hormones, DHEA as well as being, natural anti-biotics.
because they are cholesterol lowering agents, they are used in the
treatment of hypercholesterolemia. Hecogenin is the usual starting material
for industrial production of corticosteroids, anti-inflammatory agents

The richness of Bridelia ferruginea and Solanum nodiflorum
extracts in Lipids – (sterols, saponins) and phenolic compounds (Flavonoids)
may be the reason for the efficacy of these extracts as anti-bacterial and
anti-viral agents observed in our work. Thus it appears that these two
plants should be very important local sources of Flavonoids, saponins and
sterols.

![Graph showing oxygen consumption rates](image)

**Fig. 24b**

**PLOTS OF RATE OF OXYGEN CONSUMPTION BY MITOCHONDRIA
RESPIRING ON SUCCINATE**

**GROUP A**

**GROUP B**

**GROUP E**

**Succinate + Rotenone**

**ADP**

**CCCP**

**EAF** = Ethylacetate Extract in 95% ethanol.
Further work revealed that extracts of BF and SN were acutely toxic when administered in gram quantities per kilogram body weight, for BF histochemically, deleterious effects were observed in mice in the following organs: the lungs, kidneys, brain and the heart, all effects pointing to disturbances in ion fluxes and organ damage (Ofogba et al., 1998; Abaelu et al., 1993; Senior (Hons) projects 1991).

Biochemical effects showed up for BF at much lower levels of intake (a quarter - one gram/kilogram body weight) when all groups showed a decrease in blood glucose (hypoglycemic effect), thus confirming previous observation that the leaf extract is efficacious in the traditional treatment of diabetes. The serum ions and metabolites which increased or decreased were the same for all dosage levels.

Mitochondria electron transport (MET) was inhibited both "in vitro" and "in vivo" with little stimulation of catalase at low dosages (Fig. 24a,b,c), and so energy (ATP) synthesis was minimally affected (Owumi et al., 1990; Abaelu et al., 1993 and 1998).

At high dosages, however, the extract caused large inhibition of MET, induced a two fold increase in catalase activity (Fig. 25) caused the reversion of three mutant strains of amino acid dependent bacteria - (E. Coli and Salmonella typhimurium) (Abaelu et al., 1998) and increased the levels of four serum metabolites, the transaminase and phosphatase enzymes, thus indicating potentials for carcinogenesis and organ damage at high concentrations (Ofogba et al., 1998).

CONCLUSIONS

- In the plasma, LDL lipoprotein turns over faster, and together with total cholesterol and triacylglycerol levels, are much lower while HDL is higher in Nigerians than in other racial groups.

- In spite of the above, the normal Nigerian diet which is protein deficient, very high in carbohydrate, moderate in lipids rich in saturated fatty acids, may predispose Nigerians to obesity, diabetes, liver and cardiovascular diseases.
• LP(α) level is two and a half times higher in Nigerians than in the Caucasian but it has no predictive value for CHD in Nigerians as it does in the Caucasians and Japanese.

• Elevated levels of triacylglycerols and LDL cholesterol predict coronary heart disease, specifically in Nigerians.

• Nigerian females tend to have higher plasma lipid levels than their male counterparts.

• In the Nigerian diet, palm oil acts as an HDL booster and contains carotenoids and tocotrienols which have anti-oxidant properties.

• Two Nigerian medicinal plants studied were discovered to be efficacious as Anti-bacteria, antiviral and anti-diabetic agents, but, if taken in high concentrations and for prolonged periods, become toxic.

• The plants are rich in Bioflavonoids and lipids, which are also anti- many metabolic diseases possibly due to their anti-oxidant properties.

• Biotechnological methods are valuable tools in the enhanced production and improved quality of Nigerian Fermented products.

RECOMMENDATIONS

(1) Previous studies (Irvine E., 1988), and our work have shown, that Plasma lipid and lipoproteins should be established for populations living under similar environmental conditions and cultural practices, because the mean blood levels of these bimolecules differ from one environment to another. This example and many others show that biochemical differences occur among the races and yet there are very few data on the values of biomolecules, their concentrations, and activities specific to Africans. In health care delivery, therefore, Nigerian/African doctors generally use the available data in the literature mostly relevant to non- Africans. In recognition of the weakness of this practice, Hudson and Stern (2002) in a recent book detailed all the parameters, based on research findings, predisposing the black American to cardiovascular diseases. Some of the information in this book may not necessarily apply to Africans who, though, are genetically similar, live in a different environment with different nutrition and cultural practices.

I therefore recommend research to collect, document, and collate existing data where available on normal levels of biomolecules in Nigerians /Africans in order to reduce the applications of inappropriate “normal levels” of medical indices which were originally determined for other races. This will go a long way to improving the health care delivery system.

While still on this topic, I will like to plead with clinical and basic scientists to come together to pursue collaborative work in all areas of medical research. To me, basic and clinical scientists in Nigerian universities appear to work as separate and independent entities, which leads to the duplication of effort, they should come together to discuss the health problems and work together to find solutions to them. I have no doubt they will be more productive and effective that way.

(2) The storage of food products in ill ventilated warehouses and their display in the hot sun, are ideal conditions for the biodegradation of organic matter. This may well have occurred to the imported vegetable oils in our study. I therefore recommend that food marketing be completely re-organised in Nigeria especially to create awareness among the participants, of the need for proper storage and handling of foods in order to preserve the nutrient content as is done in all industrialised countries of the world.

Concerning quality control of food and drug, National Agency for Food and Drug Administration and Control (NAFDAC) has been doing an excellent job in the area of fake and expired drugs, but it appears that a lot of work is needed in the area of food. Much of the food sold in our markets even from casual inspection appear to be unfit for human
consumption. NAFDAC will need to establish laboratories for the routine analysis of samples of food products (both imported and locally produced), and the surrounding waters (Sea Lagoon and fresh water) in every state for nutrient content, microbiological contamination and for residual poisonous chemicals, e.g., chlorinated organo pesticides, mercury, lead, etc. Also, state governments should consider establishing their own independent laboratories to perform the same function should NAFDAC be unable to do so.

(3) In this lecture we have been reminded that protein deficient diet would predispose Nigerians who consume poor quality lipid to the development of liver diseases, e.g., fatty liver.

Protein sufficiency is easily met by eating both animal and plant proteins in a mixed diet. Nigeria is blessed with numerous inexpensive plant seeds containing very high levels of protein both in quality and quantity. Apart from cow peas, many other examples abound e.g. Cucumeropsis edulis Vulgaris*, Melon (Egusi) seeds (30-32% protein) *Parkia clappertoniana, (Dawadawa/ iru) seeds (39% protein) and Cucumus melo (Musk melon) (29% protein), etc. (Osagie, 1988) These seeds are very rich in essential amino and poly unsaturated fatty acids. Nigerian scientists have attempted the production of milk and yoghurt substitutes from melon seeds (Sanni et al, 1999; Abaelu et al, 1991, 1993) which were displayed at the Federal Ministry of Science and Technology Fair in 1989.

I therefore, recommend that efforts be devoted to push the increased production of these indigenous plant oil seeds, especially melon (Egusi) which is needed in commercial quantity. This should be supported with research to develop improved varieties for both domestic consumption and export.

There is need to create the environment for acquiring proper food and nutritional knowledge, by establishing departments of food and nutrition such as those at the University of Ibadan and the University of Nigeria, Nsukka, in more universities and polytechnics.

(4) Our medicinal plants appear to be efficacious for the ailments that the people use them for. Continued research is recommended to establish dosage levels, the exact active principles (molecular species) probable toxicological effects, especially "in vivo" and the mechanism of action of the active principle of Nigerian medicinal plants. Meanwhile, the two plants – B. Ferruginea and S. nodiflorum - cited above appear to be good sources of anti-oxidants (Bio flavonoids) and anti viral (saponins and steroids) agents and should be exploited for that purpose.

(5) Biotechnological methods such as those used in the production of melospices should be exploited by industries to give improved food products, or to produce enzymes and other biomolecules needed in our industries. To this end, Mr. Vice-Chancellor, Sir, I wish to note that the University will be of invaluable assistance to us, in the Department of Biochemistry, by setting up a Unit for Proteomics and Molecular Biology along with the required equipment, materials and personnel.

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* These were referred to as Citrullus vulgaris and Parkia biglobosa, respectively in the experiment.
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