Reference values for Serum Lipids in Adult Black Ugandans

Bimenya GS

Pathology Department, Faculty of Medicine, Makerere University, P. O. Box 7072 Kampala, Uganda.

ABSTRACT

To screen volunteers for reference individuals using a priori set of cut-off values obtained in urinalysis, ESR and full hemogram, blood glucose and HIV test. To determine reference values for adult Ugandans for the following serum lipids. Total cholesterol (TC), high density lipoprotein-cholesterol (HDL-cholesterol), triacylglycerol (TG), apoliporoteins A and B and lipoprotein (a). Reference values were also derived for very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterols using Friedewald algorithm. This prospective study took place at Kololo Polyclinic Laboratory for screening purposes and sampling, and Makerere University, Biochemistry Department for Lipid assays. Five hundred and forty-five healthy adult black Ugandans qualified for inclusion among the reference individuals to donate blood for the study. Lipid reference values of TC, TG and HDL were measured using enzymatic methods, from which LDL and VLDL cholesterols were calculated using Friedwald algorithm. Apolipoprotein A and B were assayed using immunoturbidimetry and ELISA respectively, against purified human apolipoprotein and Lp(a) antigens raised in goat.
Using nonparametric statistics to include the central 95 percentile of distribution, the following ranges were established. TC, 1.70 - 3.79 mmol/L; TG, 0.45 - 1.95 mmol/L; HDL, 075 - 3.21 mmol/L; VLDL; 0.14 - 0.98 mmol/L; LDL, 0.75 - 3.21 mmol/L in 545 reference individuals of which a sub sample of only 70 individuals gave the following ranges for apo lipoproteins; Apo A-I: 44 – 208 mg/L apo B: 18-158 mg/L and Lp (a): 44.5 - 423.3 mg/L. The lipid reference values of TC, TG and LDL in this work tallied with those obtained elsewhere on the African continent, but they were considerably lower than those values for European, Caucasians or American blacks. Lipoprotein (a) was wide-ranged among Ugandans and seemed higher than in Caucasians.

Keywords: Hyperlipidemia, Lipids, Lipoproteins, Reference values, Screening, Uganda.

INTRODUCTION

Coronary heart disease (CHD) is no longer rare in the indigenous populations of the developing countries[1,2]. In Uganda, autopsy reports indicate an increasing incidence of CHD as a cause of death, especially among the African middle class and business men [3].

Forty years of political independence brought changes in the dietary habits and consequent exposure to cardiovascular risk factors such as dyslipidaemia that predispose to CHD and, unfortunately, increases with monthly income[4]. This is contrary to the formerly held general belief that the average serum TC in African developing countries is as innocently low as 0.08 mmol/L (or 3.1 mg percent)[5].

Hyperlipidemia is one of the most significant biochemical risk factors associated with CHD[6]. Meaningful evaluation of CHD risk factors requires accurate data on serum lipids and lipoproteins[1], which need rigid analytical conditions and populations-based reference values.

Earlier studies showed that, reference values for plasma lipids and lipoproteins in Africans differ from those of other groups such as Caucasians due to differences in environmental and genetic factors [4,7,8]. Yet hitherto, reference values used in Ugandan medical practice are alien and textbook based. Prior to this work, of all blood lipids, only TC had been routinely analyzed in Uganda and even then without cholesterol reference values pertinent to the Ugandan population. Perhaps, indigenous Ugandan data were produced for the first time in the present author's PhD thesis [9], from which this paper is being extracted.

Thus this study was designed to establish reference values for lipid analytes among Ugandans to generate population based reference values using a priori approach in accordance with current recommendations of the International Federation of Clinical Chemistry[10]. In particular, special attention was paid to:

a) The concept of reference values[11]
b) Selection of individuals for production of reference values[12]
c) Preparation of individuals and collection of specimens for the production of reference values[13]
d) Control of analytical variation in the production, transfer and application of reference values[14]
e) Statistical treatment of collected reference values[14]
f) Determination of reference limits15 and of presentation of observed values related to reference values[16]

SUBJECTS, MATERIALS AND METHODS

A total of 849 consenting seemingly healthy, fasting individuals of mixed backgrounds from Makerere University, Mulago Paramedical school, Mulago Nurses training schools, and blood donors aged 20-50 years padded to Kololo Polyclinic laboratory on appointment and provided blood, urine and stool for screening purposes. Five hundred and forty five candidates passed by the screening tests and subsequently provided samples for lipid reference values using the a priori approach [11]. Volunteers for the study were asked to observe an overnight fast before blood was collected in a seated position from antecubital venesection without stasis between 0900 and 1200 hours. The other standard conditions for lipid analysis were followed[17].

(A) Screening for reference individuals

To qualify for reference values the following haematological, urinal, and faecal limits were adopted a priori as the inclusion criteria: Negative HIV screening, B-Haemoglobin (Fe) substance concentration ≥5.6 mmol/L (hemiglobincyanide method), Erythrocyte sedimentation rate (ESR) ≤30 mm in 1st hour (Westergreen), Leucocyte number concentration (WBC) ≥ $3.0x10^9$/L. Blood film had to be normocytic, normochromic euthrombocytaemic without "sickled" cells or hemoparasites. On urinalysis, a candidate was accepted for reference value if fresh mid stream urine was found without glucose, acetone, bilirubin, urobilinogen, porphobilinogen, protein, erythrocytes, leucocytes, casts, trichomonas, yeasts, bacteria, parasitic eggs and larva or human chorionic gonadotropin (hcG) using the recommended methods for intermediate hospital laboratories[18]. On faecal study, a candidate was accepted if microscopy showed no eggs, cysts or intestinal parasites. Volunteers were screened and referred when it was necessary, to the appropriate specialist health-care provider for management.

Using these criteria, 545 out of 849 adult black Ugandans: 304 men and 241 women aged 20-50 years were included in the study. There was no statistically significant difference in age between the sexes. HDL cholesterol was assayed enzymatically after precipitation of LDL and VLDL by Heparin-Manganese Chloride. VLDL and LDL were calculated from the values of TC, TG and HDL using Friedewald algorithm[19, 20].

Apolipoproteins A-1 and B were assayed immunoturbidimetrically using kits supplied by International Diagnostic Laboratory with calibrators standardized against the reference pool for serum apolipoproteins of the Centre for Disease Control USA17. Lipoprotein (a) was determined using Biopool Elisa method of goat polyclonal antibodies raised against purified human Lp (a)[21,22,23]. Details of each analysis were as follows:

(i) ***Total cholesterol Assay***

Cholesterol was assayed using the Dart® Cholesterol enzymatic method according to the following schemes:

- Cholesterol ester + H_2O $\xrightarrow{\text{Esterase}}$ Cholesterol + fatty acid
- Cholesterol + O_2 $\xrightarrow{\text{Oxidase}}$
- 4 – Cholesterone + H_2O_2
- H_2O_2 + 4 – amino antypyrine + 3, 5, dichloro – 2 – hydroxybenzenesulfonic acid $\xrightarrow{\text{peroxidase}}$ Pink chromogen + H_2O.

The absorption of the chromophore pink at 520 nm is proportional to the concentration of cholesterol[24].

(ii) ***Triacylglycerol assay***

Triacylglycerols were assayed using Dart® Triglyceride GPO reagent which quantitatively determines serum triacyglycerols according to the following scheme:

- Triacylglycerol ester + H_2O $\xrightarrow{\text{lipase}}$ glycerol + fatty acids
- Glycerol + ATP $\xrightarrow{\text{kinase}}$ glycerol – 3 – phosphate + ADP
- Glycerol – 3 – phosphate + O_2 $\xrightarrow{\text{oxidase}}$ H_20_2 + Dihydroxyacetone phosphate
- H_20_2 + leucodye complex $\xrightarrow{\text{Peroxidase}}$ Pink chromophore + H_2O_2 + HCLI

The absorbency of the pink chromophore at 520 nm is proportional to the concentration of triacylglycerols[25].

(iii) ***High Density Lipoprotein Cholesterol (HDL).***

From serum or plasma, all other cholesterol fractions (low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons) were precipitated by polyanion heparin in the presence of manganese metal ions to leave HDL in solution. The cholesterol content of the supernatant fluid was then determined using cholesterol oxidase[19].

(iv) ***Very Low Density Lipoprotein Cholesterol (VLDL)***

VLDL was estimated from triacyl glycerol value by dividing the TG value in mmo/L by 2.2 using Friedewald algorithm[19].

(v) ***Low Density Lipoprotein Cholesterol (LDL)***

Low density lipoprotein cholesterol was estimated by Friedewald algorithm wherein LDL = TC-(VLDL-HDL) mmol/L[19].

(vi) ***Chylomicrons***

Chylomicrons were detected using the standing plasma test in which the sample is left in a tube standing upright in fridge at 4°C overnight. A distinct creamy layer on the surface of the plasma indicates the presence of chylomicrons[17].

(vii) ***Apolipoproteins A and B (Apo A and Apo B)***

Apolipoproteins A-1 and B were assayed in plasma using immunoturbidimetric

goat antibodies supplied by international disgnostic laboratory[26,27].

(viii) **Lp (a) Lipoprotein**

Lp (a) was determined using a Biopool ELISA kit of polyclonal antibody raised in goat against purified human Lp (a) [22,28].

In nonparametric approach, the values were statistically analysed to set the reference values of measured TC, TG, HDL, apo A & B and lipoprotein (a). Using Friedewald algorithm derived reference values of VLDL and LDL were also calculated[19].

RESULTS

All the chylomicroninaemic cases were excluded in the screening exercise on grounds of proteinuria, glucosuria or HIV seropositivity.

The lipid reference ranges established non-parametrically among the 545 adult black Ugandans are displayed in Table 1.

Table 1: Distribution of reference lipid values among adult black ugandans

Analyte	Unit	n	Mean	SD	Reference range
TC	mmol/L	545	3.74	0.80	1.70-5.22
TG	mmol/L	545	1.16	0.39	0.45-1.95
HDL	Mmol/L	545	1.05	0.37	0.35-1.68
VLDL	mmol/L	545	0.53	0.18	0.14-1098
LDL	mg/L	545	2.15	0.72	0.75-3.21
Apo A-1	mg/L	70	1.25	0.28	0.44-2.08
Apo B	mg/L	70	0.19	0.23	0.18-1.58
Lipo (a)	mg/L	70	233.9	94.7	44.5-423.3

There were no statistically significant differences between serum mean values found in men and in women. Compared to values reported elsewhere, these values agree well with others in Africans but they are enviably lower than those reported in Caucasians before the lipid-lowering drive. Details of distribution as follows.

1. **Distribution of total cholesterol (TC)**

The distribution of TC among 545 adults Ugandans is displayed in **Figure I**.

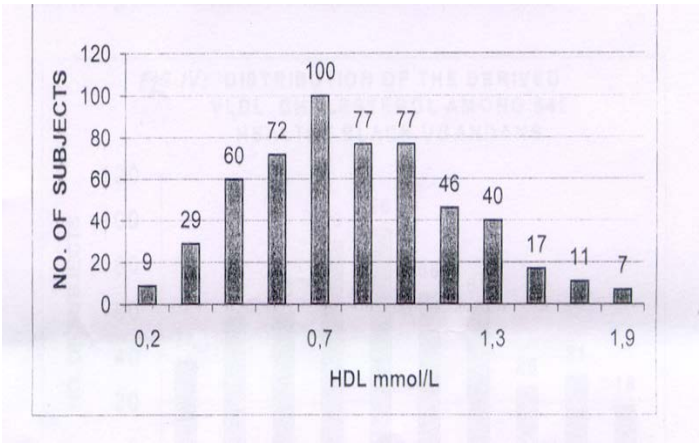


Fig. I: Distribution of serum total cholesterol among healthy black Ugandans

A previous study in Korean men and women[29], showed no statistically significant differences between serum mean values among the sexes and this corresponds with findings in Ugandan men and women. Compared to values reported elsewhere, these values agree well with others in Africans but they are enviably lower than those reported in Caucasians before the lipid-lowering drive[23, 24, 25].

2. **Distribution of Triacylglycerols**

The distribution of Triacylglycerols is displayed if Figure II**.**

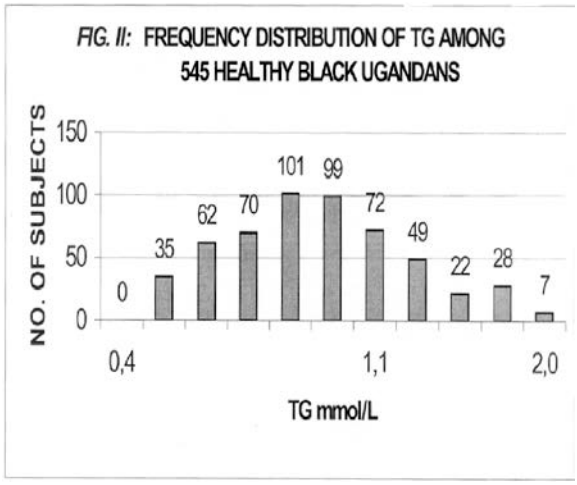


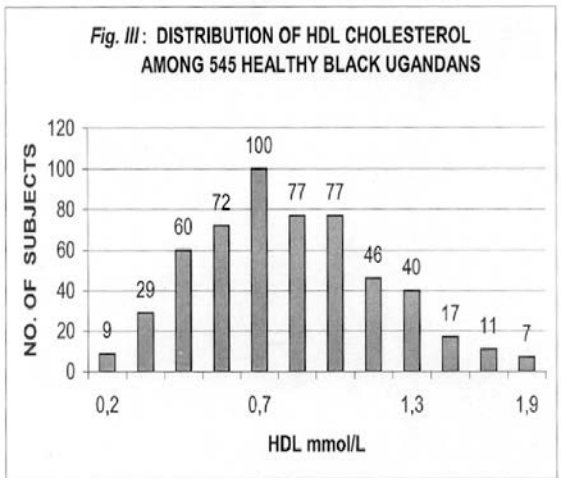
Fig. II: Distribution of TG among healthy black Ugandans

The distribution of TG substance concentration as studied in 545 reference subjects is unimodal, leptokurtic and positively skewed (Fig. II). This pattern of distribution has been observed by many researchers in different population studies[17,23,29]. As with TC, using the statistic z, at significance level of a = 0.05 there was no significant difference found between the means of TG in men and in women ($p>0.05$). Therefore, both men and women are considered one group of 545 reference individuals for plasma TG with mean (SD) of 1.116 (0.39) mmol/L. Further, following a nonparametric procedure, the reference range is read off a cumulative number fraction distribution (not shown) as 0.45 – 1.97 mmol/L of the central 95 percentile. The upper limit is enviably lower than the 3.8 mmol/L quoted by the UK National Lipid Screening Project[30,31] and by the USA National Institute of Health conference 1980[32]. It is close to the 0.15 – 2.3 mmol/L found in Nairobi adult Kenyans1, and approximates 0.20 – 1.48 mmol/L in Nigerian women[33].

3. Distribution of HDL cholesterol

The distribution of HDL reference values **(Fig. III)** was unimodal, leptokurtic and positively skewed.

The mean (SD) of HDL among men was 1.03 (0.38) and among women it was 1.07 (0.36) mmol/L apparently higher in women than in men as it has been reported by many researchers elsewhere[29,34].



As with TC and TG, using the statistic z at significance level of a = 0.05 there was no significant difference between the means of HDL among men (x = 1.03) and among women (x = 1.07). Therefore, both men and women form the same group of reference range values for HDL. Nonparametrically, and based on the central 0.95 interfractile a cumulative number fraction distribution (not shown) gives the reference range for HDL as 0.35-1.68 mmol/L. This range includes the minimum 0.91 mmol/L considered a risk factor for atherosclerosis in the Western societies[35-37]. The leptokurtic and positively skewed distribution of HDL in this work is as described by William38 in 2568 normal British. The HDL reference range is close to that of 0.25-1.80 mmol/L in Nairobi Kenyans[1].

4. Distribution of VLDL cholesterol

The distribution of 545 serum VLDL cholesterol values was polymodal, platykurtic and positively skewed. Derived from TG measurement by Friedewald algorithm, the VLDL is logically manipulated like the primary

analyte, the TG. Hence, as the mean (SD) for men and women are respectively 0.54 (0.18) and 0.52 (0.18) mmol/L, there is no statistical difference between the means of men and women. So, using the logic established for TG, TC and HDL, the reference interval is given as the central 0.95 interfractile of 0.14-0.89 mmol/L.

5. Distribution of LDL cholesterol

The distribution of LDL cholesterol values for the 545 reference subjects polymodal, mesokurtic and positively skewed, with mean (SD) of 2.15 (0.72) mmol/L. The cumulative number fraction distribution was within the desirable limits for LDL in the Western Society [39,40] and is close to, but lower than that of 1.68-4.90 mmol/L found in Kenyan adults in Nairobi[1] and lower than the 2.56 (0.90) of Korean men and the 2.55 (0.75) of Korean women[29].

6. Distribution of Apo A and Apo B

The distribution of apolipoprotein A and apoliprotein B are given in Fig. IV(A) and Fig. IV(B).

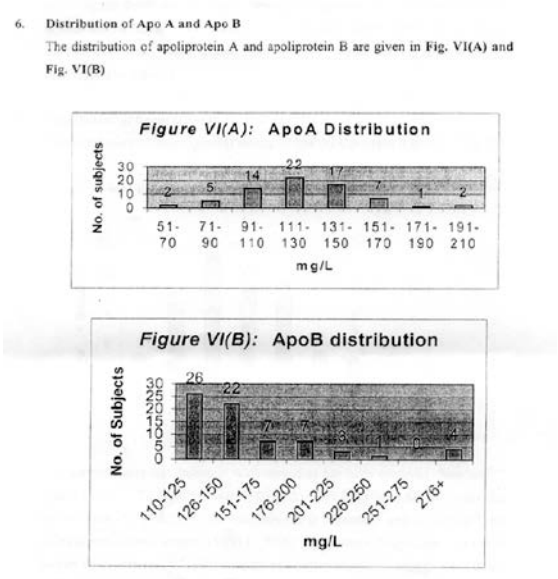


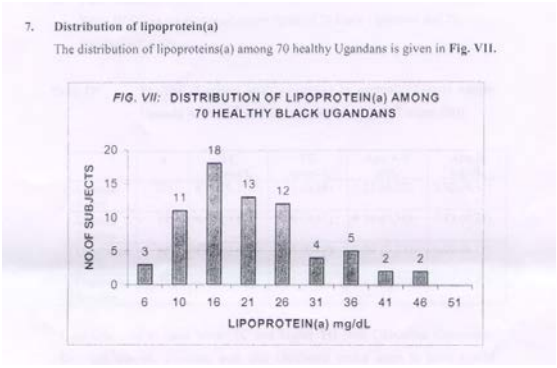
Fig IV (A & B) Distribution of ApoA [Figure VI(A)] and ApoB [Figure VI(B)] among healthy black Ugandans

The distribution of Apo A-1 among 70 Ugandans, mean (SD) of 1.25 (0.28) g/L is close to 1.33 (0.25) in Korean men and 1.38 (0.22) in Korean women and seemingly higher in women than in men but the difference is not statistically significant 29.

Similarly the distribution of Apo B among 70 Ugandans mean (SD) of 0.91 (0.23) was like that of Korean men 1.00 (0.33) and Korean women 0.97 (0.26) where the difference between sexes is not significant[29].

7. Distribution of Lipoprotein (a)

The distribution of lipoproteins (a) among 70 healthy Ugandans is given in Fig. V.



The lipoprotein (a) distribution was wide-ranged as has been observed elsewhere[41,42] with a tendency for females mean (SD) of 335 (101.5) to have higher values than males, mean (SD) 232 (88.9) as was recorded in Japanese43 and in Korean[29] men 130 (22) and Korean women 155[19]. While the Japanese distribution was similar to that of Caucasians[23,43], this study gives a wider empirical range of 90-500 mg/L seemingly agreeing with Dahler[44] that blacks have higher values of Lp (a) than whites even of the same locality[41,44,45].

8. **Comparison of Ugandans with others in literature**

There are hardly any apolipoprotein studies reported for indigenous Africans. Consequently, comparisons are made with Caucasians and Orientals.

(a) Ugandans compared with Oklahoma Caucasians.

Table II Gives the profile of serum lipids of 70 black Ugandans and 70 Oklahoma Caucasians.

Table II: Profiles of serum apolipoproteins in normolipidemic adults: Uganda Africans [9] and Oklahoma Caucasian [39]. Values are given as mean (SD)

	n	**TC mmol/L**	**TG mmol/L**	**Apo A-1 g/dL**	**Apo B mg/dL**
Uganda males	35	4..05 (1.17)*	1.05 (0.38)	1.21 (0.22)	0.92 (0.15)
Uganda females	35	4.32 (0.83)	1.26 (0.47)	1.26 (0.34)	0.81 (0.28)
Oklahoma males	35	4.76 (0.93)	0.98 (0.37)	1.27 (0.23)	1.00 (0.27)
Oklahoma females	35	4.91 (0.98)	0.95 (0.44)	1.47 (0.26)	0.92 (0.23)

Ugandans tended to have lower TC and higher TG than Oklahoma Caucasians. Ugandan women, Ugandan men and Oklahoma males seemed to have similar apolipoprotein A-1 levels which are summarily lower than in Oklahoma females. Oklahoma males seemed to have higher apolipoprotein B than Oklahoma females, Ugandan males and Ugandan females.

Table III: Lipoprotein values of Ugandans [9] compared with those of Koreans[29]. Values are given as mean (SD)

Analyte	**Korean men**	**Korean women**	**Ugandan men**	**Ugandan Women**
Apo A-1 mg/L	133 (25)	1.33 (22)	1.22 (22)	1.26 (34)
Apo B mg/L	100 (33)	97 (26)	92 (15)	81 (28)
Lp(a) mg/L	130 (0.22)	155 (18)	232.6 (88.9)	235.1 (101.5)
TC mmol/L	4.50 (0.95)	4.44 (0.75)	4.05 (1.85)	4.32 (0.83)
TG mmol/L	1.59 (0.82)	1.50 (0.95)	1.05 (0.38)	1.26 (0.4)
HDL mmol/L	1.21 (0.29)	1.38 (0.28)	1.92 (26)	1.06 (0.29)
LDL mmol/L	2.56 (0.90)	2.55 (0.72)	2.12 (0.71)	2.16(0.69)

DISCUSSION

As found out in this work and as reported elsewhere among Africans [1,9] and among Koreans [29] there was no statistical difference between the levels of serum lipids found in men and women [44] carefully matched for age, ontology and activity. This contrasts with the analogous reports in Caucasians[45] before the lipid lowering intervention. Further, compared to reports in Caucasians and as reported in the Nairobi study group[1] the African has lower serum lipids particularly TC and LDL than Caucasians. Though this sounds like good news, in a clinical study among Ugandans however, it has been shown[9] that though Ugandans have lower serum lipids than Caucasians, terminal expression of CHD (sudden deaths) tend to occur in the Ugandan at a lower concentration of plasma lipids than in the Caucasians and at a lower age (45-55 years) than Caucasians. This has been generally observed in African blacks[46,47]. As CHD seems on the increase in Africans there is no room for complacency: more research is needed to elucidate lipid metabolism in the middle class post-independence Africans. The apparent high range of lipoprotein (a) among Ugandans in particular and in blacks in Houston[44] with twice as much in blacks as in whites in Zimbabwe is a cause for further investigations as lipoprotein (a) is currently considered the major culprit for atherosclerosis[42] possibly through its interaction with plasminogen[48].

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REFERENCES

1. Ojwang P. J. Ogada T., Maina F.W., Ssekadde-Kigonvu and Mati J.K.G. *Reference values for serum Lipids and Lipoprotein Cholesterol in Adults and Cord Blood of Kenyan Africans*. East African Medical Journal 1984; 61 (5): 367-71.
2. Bertrand E. *Coronary Heart Disease in Black Africans*: An overview east African Medical Journal 1995; 72 (1): 37-41.
3. Kalungi Sam. *Autopsy Report 2003* unpublished. Makerere University, Pathology Department Report.
4. Report of Natural education Program: *Expert Panel on detection Evaluation and Treatment of High Blood Cholesterol in Adults.* Arch. Intern. Med. 1988: 148; 36-69.
5. Read A.E., Barrit D.W., Hawa R.L. *Modern Medicine*. A textbook for Students, 2nd Edition, Pitsman, 1982.
6. Lewis B., Chait A., Wooten I.D.P., Krikler D.M., Onitiri A., Sigurdsson G., and February A. *Serum Lipoprotein abnormalities in Patients with Ischaemic Heart Disease:* Comparison with a Control Population. BMJ 1974; 3:489-493.
7. Sharper A.G., Leonard P.J., Jones K.W., Jones. *Environmental effects on Body Build, Blood Pressures and Body Chemistry of Nomadic Warriors serving in the Army in Kenya.* E. Afri. Med. J. 1969; 46:282-289.
8. Ononogbu J.C. *Reference Values for Serum Lipoprotein Cholesterol in Nsukka area*. Clinical chemistry News Letter 1:982.
9. Bimenya G.S. *Lipidaemia studies among Ugandans.* Ph.D thesis, Makerere University, 1990.
10. International Federation of Clinical Chemistry 1987/9. *Approved Recommendation (1987) on the theory of Reference Values.* Determination of reference limits. J.clin. Chem. Clin. Biochem. vol. 25: 645-656.
11. Expert Panel on Theory of Reference Values: *The Concept of Reference Values Clin. Chem.* Acta 1987; 165:111-118.
12. Expert Panel on Theory of Reference Values: *Approved Recommendation of Production of Reference Values.* J. Clin. Chem. Clin. Biochem 1987; 25: 639-644.

13. Expert Panel on theory of Reference Values: *Preparation of Individuals and Collection of Specimens for the Productaion of Reference Values Clin. Chem.* Acta 1988; 177:51-52.
14. Expert Panel on Theory of Reference values: Expert Panel on Theory of Reference values*: Control of Analytical Variation in the Production, Transfer and Application of reference values.* Ann Biol Clin. 1991; 49:487-490.
15. Expert Panel on theory of reference Values: *Statistical treatment of Collection reference values.* J. Clin Chem. Clin. Biochem. 1987; 25:645-656.
16. Expert Panel on theory of Reference Values: *Presentation of observed values Related to Reference Values*. Lab Media 1988; 5: 27-30.
17. Bachonik P.S. Collection and Processing of Specimens: *In lipid research Clinics Program Manual of Labouratory Operations* pg. 1-23. US Department of Health and Human Resources National Institute of Health, 1983.
18. *Methods Recommended for essential Clinical and Haematological Tests for Intermediate Hospital Labouratories*. WHO working Group on essessment of Clinical Technologies, 1986; 86/3:3-15.
19. Friedewald W.T., Levy R.I., Friedewickson D.S. *Estimation of the Concentration of Low Density Lipoprotein Cholesterol without the use of Preparative Ultracentrifuge*. Clin. Chem. 1972, 18:499-502.
20. Wilson and Spiger. *A dual Precipitation Method for Quantitative Plasma Lipoprotein Incasement without Ultracentrifugation*. J. Lab. Clin. Med. 1973; 82:473-482.
21. Cooper G.R., Smith S.J., Wieba D.A., Kuckmak M., and Hannon W.H. *International Survey of Apolipoproteins* A1 and B measurements 1983-1983 (review). Clin. Chem. 1985; 31:223-28.
22. *Biopol ELISA purified assay* Insert. Biopool A B. P.O. Box 1454 S-901 24 Umea Sweden.
23. Roengren A., Willelimenson, Erikson E., Risberg B., Wedel H., Lipoprotein (a) and Coronary Heart Disease. *A prospective Case Control Study in a General Population Sample of Middle aged men*. B.M.J 1990; 301:1248-1252.
24. Coulter Electonics: *Technical Brochure on Total Cholesterol Enzymatic assay*. Hialeah Florida.
25. Coulter electronics: *Technical Brochure on Triglyceride enzymatic assay.* Hialeah Florida.
26. International Diagnostic Laboratory Ltd. Apo A-1 *Immunoturburbidimetric Assay Kit* Insert. P.O Box 5577, Jerusalem 91054, Israel, Nov. 1987.
27. International Diagnostic Laboratory Ltd. Apo A-1 *Immunoturbidimetric Assay Kit* Insert. Missouri 63017 USA, Jan 1987.
28. Biopool Tint elize Lp(a) Kit. Porton Products Ltd. Maidenhead(UK).
29. Min. W.K., Lee J.O., Lee J.O., Kim c.H., Song H., and Kim J. *Identification of Apo(a) phemotypes in a Korean population using standardized nomeclature system based on the number of Kringle IV repeats* Ann clin. Biochem., 1997; 34: 681-687.
30. Margoli S. *Physician Strategies for the Prevention of Coronary Heart Disease*: John Hpkine Med. J., 1977; 14(3): 170-176.
31. Sgerphad J., Betteridge D.J., Durrignton P., et al. *Strategies for Reducing Coronary Heart Disease and Desirable Limits for Blood Lipid Concentration:* guidelines of the British Hyperlipidaemia Association. B.M.J., 1987; 295:12406.
32. The Lipid research Clinis Population Studies Book Vol. 1: Prevalence Study, NIH Publications No. 80.1507, July 1980.
33. Oyedola O.O., Kashope D.T., Olusi S.O., *Plasma Lipids and Lipoproatein Cholesterol Fractions in Nigerian Women on Steroidal Contraceptive Therapy* Biochemica Clinica 1990; 14(14): 1585-1589.
34. Shaefer et al. *Apoliprotein and Lipoprotein in Human Plasma*: an Overview Clin. Chem. 1988; 34/B:B4-B8.
35. Bush, fried and Conor. *Cholesterol, Lipoproteins, and Coronary Heart Disease in Women.* Cli. Chem. 1988; 24/8(B): B60-B70.
36. Castelli et al. *Incidence of Coronary Heart Disease and Lipoprotein Cholesterol Levels*: The Framming ham Study. JAMA 1986; 256:2835-2838.
37. Lang and Schettler in: *Arteriosklerose: Schettler* and Gross (Eds). Deutscher arzer-Verlag, Koln, 1985.
38. William P. Robinson D., Bailey A. *High Density Lipoprotein and Coronary Risk factors in Normal Men.* The Lancet 1979; (1) 72-75.

39. Naito H.K. *The Cholesterol Challenge*: from Laboratory to Clinic. Clin. Chem. 1988;34:444-449.
40. Assman. *Lipiddiagnostik heute*. Page 29ff. In greten H. et al. Lipoprotein and Herzinjackt. Witzrok-Verlag Baden, W. Germany/Inermist 1979; 20:559.
41. Dube N. Voordbij R., Leus F., Gomo Z.A.R. *Lipoprotein(a) concentrations and apolipoprotein(a) isoform distribution in a Zimbabwean population* Cent Afri. J. Med 2002; 48(7)(8), 83-84.
42. Bkita H., Matsubara M., Shibuya H., Fuda H., Chiba H. *Effect of Ageing on plasma(a) levels*. Ann Clin. Biochem 2002; 39:237-240.
43. Nago N., Kayaba K., Hiraoka J., Matsuo H., Goto T., Kario K., Tsutusmi A., Nakamura Y., and Igarashi M. *Lipoprotein(a) levels in the Japanese population: Influence of Age and Sex and Relation to Atherosclerotic risk factors. American Journal of Epidemiology* 1995; 1141 (6): 815-821.
44. Dahlen G.H., Guton J.R., attar M., Farmer J.A., Kautz J.A., Gotto A.M. *Association of Levels of Lipoprotein Lp(a), plasma lipids and other lipoproteins with* Coronary Artery Disease Documented by Angiography. Circulation 1986, 74 (4): 758-765.
45. Ononogbu I.C. *Comparison of High Density Lipoprotein and Serum Cholesterol Levels in a European and an African Community Atherosclerosis* 1979; 34:49-52.
46. Anabwami G.M. *Heart Disease in Africa. What is Know?* East African Medical Journal 1996; 73(4): 213-214.
47. Ahaneku J.E., Nwosu M.C., Ahaneku G.I., and Okugba P.C. *Utilization of Clinical Chemistry Tests, with Special reference to Lipid Profile in Disease* Management in Nigerian Setting. East African Medical Journal 1999; 76(3): 72-75.
48. Annika Roengren, Lars Willelimenson, Elsa Erikson Bo Risbery, Hans Wedel. *Lipoprotein(a) and Coronary Heart Disease*: A Prospective Case Control Study in a General Population Sample of Middle Aged Men B.M.J. 1990; 301: 1248-1253.