Epidemiological reference ranges for low-density lipoprotein cholesterol and apolipoprotein B for identification of increased risk of ischaemic heart disease

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Summary

Although there is widespread acceptance that total cholesterol (TC) value reference ranges should be based on epidemiological rather than statistical considerations, the epidemiological action limits for low-density lipoprotein cholesterol (LDL-C) are still incomplete and only statistical reference ranges for apolipoprotein B (Apo-B) levels are available. The combined use of epidemiological reference ranges for TC and incomplete or statistical reference ranges for LDL-C and Apo-B is illogical, since these parameters may fall into discordant risk categories that will hamper and complicate the management of hypercholesterolaemia.

Based on a study of lipograms obtained from ± 3 000 inhabitants of two industrialised Transvaal towns, the age-related epidemiological reference ranges for LDL-C and Apo-B were established. A comparison with published observational studies of other populations, in which comparable lipid, lipoprotein and apolipoprotein methodologies were used, reflected the severity of these lipid-related abnormalities in white South Africans, especially after the age of 30 years. In addition, the serum TC values found in this survey were not significantly different from those obtained 10 years ago.

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Atherosclerosis, the leading cause of death in the Western world, begins early in life and progresses silently for decades. By the time most individuals develop clinical manifestations of ischaemic heart disease (IHD), the atherogenic process is far advanced. Sudden death can be the first manifestation of atherosclerosis and even in non-fatal cases coronary bypass surgery may not prolong survival in patients with advanced disease.[1] The early identification and modification of risk factors are therefore essential. The use of a statistically derived reference range (population average ± 2 SD) to identify hypercholesterolaemia as one of the major modifiable risk factors, was based on the mistaken view that what is common is also good and therefore normal. Authoritative epidemiological surveys[2-4] have, in fact, shown that the relationship between cholesterol and IHD is continuous and curvilinear. This means that there is an increased risk of fatal IHD associated with serum total cholesterol (TC) levels throughout much of the range of values previously thought to be 'safe'. Consequently, the assessment of an individual patient's lipid profile should not be based on conventionally (statistically) determined reference intervals, a mistake responsible for inconsistent conclusions in early attempts to correlate serum lipid concentrations and atherogenesis. The curvilinear relationship between the incidence of IHD and TC levels suggests that cholesterol levels can be divided into three categories: desirable, moderate and high, depending on the relative risk of developing IHD. A comparison of the epidemiological data obtained from a local coronary risk factor study (CORIS)[5] in a white rural population and the Multiple Risk Factor Intervention Trial (MRFIT),[3] suggests that the 20th and 80th percentiles of TC distribution in the CORIS population should be used to distinguish between these three categories. These percentiles were recently put forward as the new cholesterol guidelines for South Africa,[6] based on the assumption that these age-related cut-off points would also apply to free-living communities with a Western lifestyle elsewhere in this country.

In most circumstances non-fasting serum TC estimations are adequate for screening and monitoring purposes. However, a lipogram consisting of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) is recommended for subjects with TC values falling into the high-risk category or subjects in the moderate-risk category who have additional risk factors.[6] A refinement of lipid risk factor analyses (LDL-C and HDL-C) has been shown to improve the prediction of the development of IHD.[7] Another reason for a more detailed lipid analysis is to exclude the occasionally elevated TC level caused by high HDL-C values but with normal LDL-C concentrations. Such subjects are not at an increased risk of IHD and therefore require no further intervention. Age-related epidemiological reference ranges are also required for LDL-C if the additional information provided by a lipogram is to be effectively utilised. A complete set of such LDL-C action limits for a South African population have not yet been determined. In many laboratories in this country, lipograms have also recently been extended to include the major apolipoproteins of LDL (Apo-B) and HDL (Apo-A1). Epidemiological surveys[8-10] have shown that these parameters

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could provide an even better indicator of risk than the currently used lipid (TC, LDL-C and HDL-C) analyses. The currently published cut-off points for Apo-B are all based on statistically determined reference ranges. However, the use of a continuum of age-related, epidemiological reference ranges in conjunction with statistically determined reference ranges may lead to situations where TC, LDL-C and/or Apo-B may fall into different risk categories. This may create practical, interpretative and management problems. The first aim of this study was therefore to test the assumption that the CORIS population, which formed the basis of South African TC epidemiological reference ranges, is representative of other free-living communities with a Western lifestyle. The second object was to provide a complete set of epidemiological action limits for LDL-C and Apo-B to facilitate interpretations of lipograms, which may lead to important decisions regarding the management of patients.

Subjects and methods

During 1988 a survey of the white population of two industrial towns (Vanderbijlpark and Witbank) in the Transvaal was undertaken as part of the VIGHOR study [Vanderbijlpark Information project re: Gesondheid/Health Obesity Risk factors]. A stratified random sample of 1 500 persons between the ages of 16 years and 64 years was drawn from each of the communities. The 1985 population census was used as a basis for the calculations and the Telephone Directory was the source from which individual households were drawn. A proportional number of households from each letter of the alphabet was called and 1 individual was randomly selected to participate in the survey. The survey finally included 1 407 individuals from Vanderbijlpark and 1 360 from Witbank. Blood samples were taken without prolonged venous occlusion after patients had been sitting for 5 minutes.

Laboratory analyses consisted of TC, HDL-C, LDL-C, Apo-B and Apo-A1 determinations. TC levels were measured by automated enzymatic procedures on a Technicon SMAC system.[11] To determine HDL-C, LDL and VLDL were precipitated from serum using heparin and Mg^{++} (Merck reagent kit 15007); the supernatant was used to determine HDL-C by means of the CHOD-Iodide method (Merck kit 14350). For the determination of LDL-C, LDL was precipitated from serum by heparin at its iso-electric point (Merck reagent kit 14992). After centrifugation, the cholesterol content of the supernatant was determined and this equalled the sum of HDL-C and VLDL-C. LDL-C was then calculated by: LDL-C = TC − (HDL-C + VLDL-C). Results obtained with this differential precipitation method for LDL-C in non-fasting subjects did not differ statistically significantly ($P > 0,05$; Student's t-test) from the calculated LDL-C in the same, but fasting, subjects using the Friedewald equation [LDL-C = TC − (HDL-C + Tgs/2,18)].

Commercial quality control (QC) sera were included in each batch and the results of the unknown samples were accepted only if the QC values had fallen within two standard deviations (SD) of the mean provided by the manufacturer. The coefficient of variation for TC, determined on the QC material, during the study period was 2,0%. Results from an external QC programme confirmed that there had been no biases in TC determinations and that the analytical process had been properly controlled. Apo-A1 and Apo-B were determined by immunonephelometric assays (INA) using the Behring Laser Nephelometer and methods (Behring reagent kits OSAN 14/15 and OUED for Apo-B and Apo-A1 respectively). Apolipoprotein standards, which were calibrated against the proposed International Union of Immunological Societies and the Centers for Disease Control (IUIS-CDC) reference pool for apolipoproteins,[12] were used in conjunction with commercial QC sera obtained from Behringwerke. An external QC programme for apolipoproteins verified that the analytical performance had been within 2SD of the mean that was obtained by other laboratories using comparable methods.

Data were compiled and analysed by Central Statistical Services and the Institute for Biostatistics of the South African Medical Research Council. The survey was approved by the ethical committee of the Department of Health Services and Welfare.

Results and discussion

The similarity between the actual ('unsmoothed') values of the 20th and 80th percentiles of the industrialised Transvaal population and the corresponding percentiles obtained from a smoothed graph of TC percentiles[6] from the rural western Cape population is obvious (Table I). It was also statistically confirmed with correlation coefficients of 0,92 and 0,98 for the 20th and 80th percentiles, respectively. This is the first age-related comparison with the CORIS data, which represent the largest and best-documented regional population survey in South Africa. The aforementioned two percentiles, which serve to distinguish between the three risk categories, cor-

TABLE I. A COMPARISON OF THE 20TH AND 80TH PERCENTILES OF TC (mmol/l) DISTRIBUTION IN A RURAL WHITE POPULATION (CORIS) AND IN AN INDUSTRIALISED POPULATION ACCORDING TO DIFFERENT AGE CATEGORIES IN BOTH SEXES

Age category (yrs)	Rural population		Industrialised population	
	20th percentile	80th percentile	20th percentile	80th percentile
16 - 20	**3,96**	**5,28**	**3,85**	**5,38**
21 - 25	**4,20**	**5,87**	**3,93**	**5,58**
26 - 30	**4,55**	**6,28**	**4,43**	**6,16**
31 - 35	**4,76**	**6,55**	**4,63**	**6,43**
36 - 40	**4,96**	**6,79**	**4,97**	**6,78**
41 - 45	**5,16**	**7,04**	**5,25**	**7,03**
46 - 50	**5,36**	**7,31**	**5,33**	**7,33**
51 - 55	**5,56**	**7,57**	**5,41**	**7,47**
56 - 60	**5,75**	**7,70**	**5,57**	**7,53**
61 - 65	**5,80**	**7,70**	**5,62**	**7,93**

TABLE II. CUT-OFF POINTS FOR LDL-C TO DISTINGUISH BETWEEN MODERATE-RISK AND HIGH-RISK CATEGORIES

Age category (yrs)	Moderate risk (mmol/l)	High risk (mmol/l)
16 - 20	**2,40 - 3,65**	**> 3,65**
21 - 25	**2,47 - 3,83**	**> 3,83**
26 - 30	**2,71 - 4,19**	**> 4,19**
31 - 35	**2,90 - 4,31**	**> 4,31**
36 - 40	**3,13 - 4,61**	**> 4,61**
41 - 45	**3,25 - 4,85**	**> 4,85**
46 - 50	**3,32 - 5,08**	**> 5,08**
51 - 55	**3,44 - 5,19**	**> 5,19**
56 - 60	**3,50 - 5,28**	**> 5,28**
61 - 65	**3,54 - 5,43**	**> 5,43**

respond with the 40th and 85th percentiles of TC distribution in the MRFIT study,[5,6] respectively. This illustrates the severity and increased relative risk in white South Africans of developing IHD. It is therefore not surprising that it has been suggested that the incidence of IHD in white South Africans may be among the highest reported for any ethnic group[13] in the Western world. As a result of the extent of this silent epidemic, increased public awareness and cholesterol screening an increased demand for lipograms may be experienced. Such age-specific epidemiological guidelines for LDL-C and Apo-B, which form an integral part of a lipogram, are as yet unavailable.

As an interim measure it was suggested[6] that the 50th and 95th percentiles of the Lipid Research Clinics (LRC) Program Prevalence Study[2] should be used for LDL-C cut-off points. Two sets of fixed reference ranges were proposed: (*i*) young adults (< 30 years) where the moderate risk category for LDL-C values lies between 2,80 mmol/l and 4,15 mmol/l; and (*ii*) middle-aged and older adults (> 30 years) where the moderate risk category lies between 3,40 mmol/l and 5,20 mmol/l. It is interesting to note from Table II that the cut-off points of LDL-C in the LRC study[2] for adults < 30 years, which distinguishes between moderate and high risk, approximate the 20th and 80th LDL-C percentiles for the age category 26 - 30 years of the South African industrialised population. The corresponding cut-off points for middle-aged and older adults of the LRC study approximate the 20th and 80th percentile of white South Africans for the age category 51 - 55 years. In other words, a gap in LDL-C epidemiological cut-off points exists between these two age categories. This complicates the interpretation of a lipogram in individuals in the third and fourth decades, which is an important age interval in terms of the assessment of premature atherosclerotic disease.

TABLE III. CUT-OFF POINTS FOR APO-B (g/l) TO DISTINGUISH BETWEEN MODERATE-RISK AND HIGH-RISK CATEGORIES BASED ON THE 20TH AND 80TH PERCENTILE

Age category (yrs)	Moderate risk	High risk
16 - 20	0,760 - 1,110	> 1,110
21 - 25	0,790 - 1,200	> 1,200
26 - 30	0,810 - 1,390	> 1,390
31 - 35	0,870 - 1,510	> 1,510
36 - 40	0,910 - 1,590	> 1,590
41 - 45	0,970 - 1,640	> 1,640
46 - 50	1,070 - 1,690	> 1,690
51 - 55	1,110 - 1,720	> 1,720
56 - 60	1,140 - 1,760	> 1,760
61 - 65	1,130 - 1,790	> 1,790

Since apolipoprotein profiling has also become common practice in many private laboratories, guidelines about such cut-off points are an important issue. The use of these investigations may become more important in future because epidemiological studies have shown that plasma levels of Apo-A1 and Apo-B discriminate better between individuals with angiographically documented IHD and normal subjects than does the cholesterol level of the corresponding lipoprotein.[8,9] There are various possible explanations for these findings,[14] e.g. it is now well recognised that the content of cholesterol ester within the core of LDL may increase, producing a more buoyant 'light' LDL particle, or decrease, yielding a denser 'heavy' LDL particle. The light or heavy particles each contains only 1 molecule of Apo-B and the measurement of LDL Apo-

TABLE IV. LIPID AND LIPOPROTEIN LEVELS (MEAN ± SD) IN HIGH- AND LOW-RISK WHITE POPULATIONS

Age (yrs)	Total cholesterol			LDL-cholesterol			HDL-cholesterol		Apo-AI		Apo-B	
	SA (mmol/l)	UK (mmol/l)	Italy (mmol/l)	SA (mmol/l)	UK (mmol/l)	Italy (mmol/l)	SA (mmol/l)	Italy (mmol/l)	SA (g/l)	Italy (g/l)	SA (g/l)	Italy (g/l)
20 - 29	5,14 ± 1,1	5,15 ± 1,1	4,81 ± 0,54	3,30 ± 0,99	3,10 ± 1,0	2,89 ± 0,57	1,08 ± 0,26	1,51 ± 0,13	1,348 ± 0,221	1,218 ± 0,168	1,050 ± 0,315	0,863 ± 0,155
30 - 39	5,72 ± 1,2	5,45 ± 1,1	5,06 ± 0,70	3,80 ± 1,08	3,30 ± 1,05	3,08 ± 0,73	1,13 ± 0,28	1,51 ± 0,14	1,415 ± 0,220	1,341 ± 0,1625	1,233 ± 0,364	0,920 ± 0,1435
40 - 49	6,30 ± 1,26	5,9 ± 1,17	5,28 ± 0,64	4,21 ± 1,20	3,68 ± 1,05	3,30 ± 0,67	1,11 ± 0,29	1,52 ± 0,16	1,438 ± 0,233	1,326 ± 0,140	1,413 ± 0,381	0,909 ± 0,1525
50 - 59	6,52 ± 1,23	6,33 ± 1,18	5,57 ± 0,69	4,32 ± 1,15	4,03 ± 1,15	3,57 ± 0,71	1,12 ± 0,3	1,46 ± 0,16	1,467 ± 0,239	1,337 ± 0,194	1,460 ± 0,380	0,967 ± 0,181
+ 60	6,59 ± 1,37	ND	5,75 ± 0,63	4,38 ± 1,20	ND	3,66 ± 0,70	1,17 ± 0,35	1,53 ± 0,25	1,488 ± 0,252	1,377 ± 0,182	1,448 ± 0,385	1,065 ± 0,169

Only TC and LDL-C values were available for the UK study.[20]

TABLE V. PERCENTAGES OF VARIOUS POPULATIONS IN SA RISK CATEGORIES

	Desirable TC (≤ 20th percentile)			Moderate TC (> 20th & < 80th percentile)			High TC (≥ 80th percentile)		
Age (yrs)	SA	UK	Italy	SA	UK	Italy	SA	UK	Italy
20 - 29	**20**	**17,36**	**9,85**	**60**	**59,67**	**88,45**	**20**	**22,97**	**1,70**
30 - 39	**20**	**25,75**	**32,28**	**60**	**61,51**	**65,50**	**20**	**12,71**	**2,22**
40 - 49	**20**	**28,77**	**48,01**	**60**	**60,48**	**51,93**	**20**	**10,75**	**0,06**
50 - 59	**20**	**23,89**	**45,62**	**60**	**60,96**	**54,18**	**20**	**15,15**	**0,20**
> 60	**20**	**—**	**31,21**	**60**	**—**	**68,71**	**20**	**—**	**0,08**

B can thus provide a more accurate assessment of the number of LDL particles than measurement of LDL-C alone. Sniderman *et al.*[15] found that the majority of the patients they studied with angiographically documented IHD had elevated levels of Apo-B and normal levels of LDL-C. They introduced the term 'hyperapobeta-lipoproteinaemia' or 'hyperapo B' to distinguish this condition from hyperbeta-lipoproteinaemia (type II hypercholesterolaemia), in which both LDL-C and Apo-B are elevated. Since the LDL particle is depleted in cholesterol esters, the patient is usually 'normocholesterolaemic' or has only a borderline high cholesterol level. Because of the altered composition of LDL, the elevated LDL levels in hyperapo-B can be detected more accurately by measuring Apo-B than by measuring LDL-C. It has been shown that heavy (dense) LDL particles were removed at a slower rate than the light LDL particles.[14] Thus, while there is no apparent defect in the LDL receptor in hyperapo-B, the shift to the denser LDL particles is not advantageous, because dense LDLs have a longer residence time in plasma than light LDL. This observation suggests that dense LDL might bind less well to LDL receptors than light LDL. For this reason, it is perhaps not surprising that Apo-B is a better risk predictor than LDL-C; the latter does not accurately reflect the increased LDL particles in hyperapo-B patients.

Based on the new epidemiological definition of 'normal cholesterol value', it is reasonable to expect that optimum/desirable ranges should be similarly set for apolipoproteins. Regression analysis on approximately 3 000 individuals in this study has given a correlation coefficient of $r = 0{,}82$; $P < 0{,}001$ between Apo-B and LDL-C values. Since the 20th and 80th percentiles of TC and LDL-C in the South African adult population seem to correlate well with suggested action limits derived from large epidemiological studies abroad,[2] and because of the good correlation between Apo-B and LDL-C, it can be argued that the same percentiles may serve as appropriate epidemiological guidelines for Apo-B action limits (Table III). Until now, however, most published reports on Apo-A1 and Apo-B levels have provided only statistical reference ranges of the populations investigated.[10] In order for cut-off points to be used on a national basis, evaluation and specifications of the methods involved are essential. Various immunochemical methods which differ in principle and sensitivity have been used in these studies. At present, efforts are underway to develop reference material and standardisation programmes for apolipoproteins.[12] The two methods most commonly used by pathology laboratories in this country, radio-immunoassay (RIA) and immunonephelometric assay (INA), show a good correlation (under optimal conditions) over a broad range of Apo-A1 and Apo-B values with the specially prepared reference materials of the Standardisation Committee of the IUIS-CDC.[16] It is only to be expected that inter-laboratory methodological differences in routine laboratories may be larger than those reported,[16] since these assays are not usually as rigorously standardised as in a research setting. Despite these reservations, a recent authoritative comparison[17] has shown an excellent agreement between the Behring nephelometrically determined Apo-B and a highly standardised RIA Apo-B method ($r = 0{,}91$, standard error of the estimate 0,012 g/l, slope 1,03 and the intercept 3,3). However, using another commercial nephelometer and method, a bias of approximately −30% for Apo-B was demonstrated.

A comparison of TC, LDL-C and Apo-B of South Africans with that of a low-risk group, such as the Italians,[18] and a recently surveyed high-risk group, such as the British,[19] corroborates the suggestion[13] that white South Africans may have a particularly severe lipid problem (Table IV). The comparative increase in Apo-B over the same age interval for the Mediterranean population was approximately 0,2 g/l and for the South Africans 0,4 g/l. This difference in Apo-B concentration corresponds with the magnitude of change in the lipid concentrations. This unfavourable disposition towards lipid-related risk factors may, in addition, be aggravated by the smaller/denser LDL particles (lower LDL-C/Apo-B ratio) in South Africans, which remained remarkably constant throughout the different age categories. The South African LDL-C/Apo-B ratio was approximately 3,0 v. 3,5 for Italians.[18] However, these apparent differences in the LDL particle size should be further investigated before the impression that this may also be a population characteristic, predisposing to IHD, can be confirmed. The age-related deterioration of South African lipid risk factors is also borne out when the lipid (TC and LDL-C) levels are compared with UK communities known to have an unacceptably high IHD risk (Table IV). When the South Arican epidemiological limits for TC are used to designate the British and Italian populations to one of the three risk categories (Table V), it is evident that a smaller percentage of British and a very small percentage of Italians (< 1%) would fall into the high-risk category for South Africans (calculations by P.J.B.). This comparison further suggests that the action limits for South Africans previously determined[6] are not unrealistic and, indeed, attainable in that < 1% of an age-matched Italian population would fall into the high-risk category of South Africans (Table V). Drug treatment is usually reserved for non-responders in this category.

In their recent report on a prospective study in the UK, Pocock *et al.*[20] have once again shown that the increases in risk associated with elevated TC and depressed HDL-C levels seemed to operate independently of one another. The proposed[6] HDL-C action limit for South Africa is 1,0 mmol/l, below which risk increases. In this study the mean Apo-A1 concentration, which corresponds with a HDL-C value of 1,0 mmol/l for the population as a whole, is 1,31 g/l. In contrast with the good agreement between RIA and INA-determined Apo-B levels, there is a divergence between these two methods with respect to Apo-A1.[17]

In conclusion, a comparison of the present epidemiological survey with the CORIS study shows that the TC levels in the different age categories are very similar which implies: (*i*) that the epidemiological action limits based on the CORIS data are also applicable to other communities with a similar lifestyle in

South Africa; and (*ii*) that the TC levels in South Africa have probably not decreased despite various campaigns to increase public awareness about the risk associated with high cholesterol levels. Previous comparisons[6] with other high-risk populations, e.g. the MRFIT study,[3] showed that TC distributions in South Africans were upwardly displaced. A comparison of the refinement of lipid risk factors (LDL-C and Apo-B) with those obtained from UK and Italian populations confirms this upward displacement of lipid risk factors and emphasises the need for strategies to reduce the extent of this major risk factor in white, Asian and urban coloured South Africans. Since a large percentage of South Africans with a First-World lifestyle would be further investigated by means of LDL-C and even Apo-B determinations, we believe that epidemiological age-related cut-off points for these two parameters are prerequisites, since it has been adequately documented that statistically 'normal' values for TC, LDL-C and Apo-B are a contradiction in terms.

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