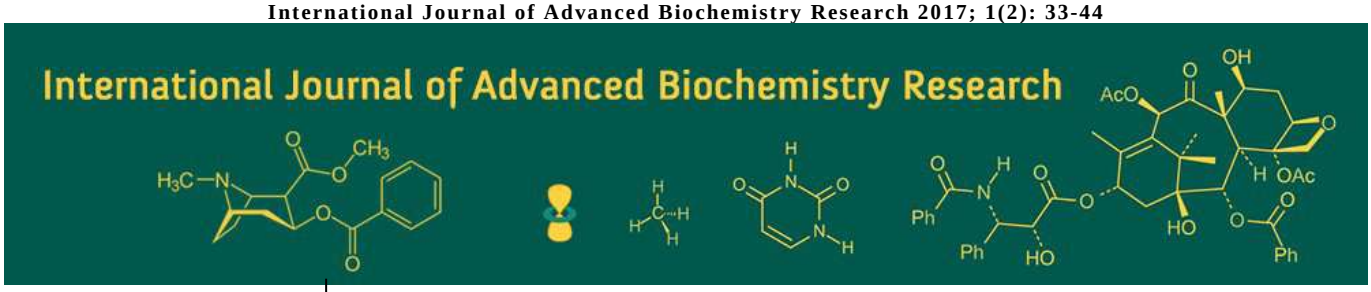

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Reference intervals for fasting lipid profiles for the adult and geriatric population of Taita-taveta county, Kenya

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Abstract
Fasting lipid profiles are affected by many factors including age, sex, geographical location, dietary habits (foods taken), life-style (sedentary or active, smoker or non-smoker, alcoholic or non-alcoholic), health status, environment, comprehensiveness of the selection criteria of the referent individual, socio-economic status of the referent individual, racial differences, genetics, and the method and reagents used to estimate the lipid profile analyte of interest. The aim of the study is therefore to establish age and sex specific fasting lipid profile reference interval limits for adults and geriatrics of Taita-Taveta County, Kenya and compare them with those previously reported in medical literature. This was a prospective cross-sectional study design involving 272 randomly sampled healthy adults and geriatrics consisting of 123 males and 148 females. The study followed CLSI EP28 A3c guidelines. Fasting blood samples were collected from 272 adults and geriatrics referent individuals from Taita-Taveta County, Kenya and analyzed using a Clinical Chemistry Auto Analyzer (Integra 400) for total cholesterol (T-Chol), higher density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), non-high density lipoprotein cholesterol (non-HDL-Chol), triacylglycerols (TG), total cholesterol (T-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio, triacylglycerols (TG): high density lipoprotein cholesterol (HDL-Chol) ratio, and low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio. Non-parametric analytical tools were used to establish the 2.5 percentile and 97.5 percentile reference interval based on gender and age. The established reference intervals for fasting lipid profiles were gender dependent for high density lipoprotein cholesterol, total cholesterol: high density lipoprotein cholesterol ratio and triacylglycerol (TG): high density lipoprotein cholesterol (HDL-Chol) ratio, gender independent for total cholesterol, low density lipoprotein cholesterol (LDH-Chol), triacylglycerols (TG), non-high density lipoprotein cholesterol (non-HDL-Chol), and low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio and were different from those reported in medical literature. In conclusion, the established age and gender specific reference intervals for fasting lipid profiles for adults and geriatrics of Taita-Taveta County, Kenya should be adopted for improved diagnostic interpretation and decision making by clinicians.

Keywords: Reference intervals, fasted lipid profile, adults, geriatrics, Taita-Taveta, Kenya

Introduction
Chronic Kidney Disease (CKD) is a progressive reduction in renal function [1]. It is a condition where the kidneys lose their normal function, especially excretory and regulatory functions which can be due to infections, autoimmune diseases, diabetes, hypertension, cancer and toxic chemicals [2]. CKD is heading towards becoming a major health problem [3] and is rapidly assuming epidemic proportions globally [4]. India has highest number of diabetics in the world having a prevalence of 3.8% in rural and 11.8% in urban adults [3]. It is associated with adverse outcomes in all stages of CKD [5]. It has been estimated that approximately 25-40% of diabetic and hypertensive patients usually develop CKD (Nephropathy) [3]. Studies conducted on renal patients revealed that up to 90% were found to have oral symptoms of uremia like ammonia like taste and smell, stomatitis, gingivitis, decreased salivary flow, xerostomia and parotitis [2]. The objectives of early diagnosis is identification of asymptomatic disease at that time when intervention has a reasonable potential of a positive impact on outcome [3].

Elevated fasting lipid profiles including total cholesterol (T-Chol), non-high density lipoprotein cholesterol (non-HDL-Chol), low density lipoprotein cholesterol (LDL-Chol), and triacylglycerol (TG), and low levels of high density lipoprotein cholesterol (HDL-Chol) are associated with the risk of cardiovascular diseases including heart attack, stroke and atherosclerosis (Slhessarenko *et al*., 2015) [13]. High density lipoprotein cholesterol (HDL-Chol; good cholesterol) controls the level of low density lipoprotein cholesterol (LDL-Chol; bad cholesterol) by transporting fat molecules out of the arterial walls, and decreasing macrophage accumulation resulting in preventing or regressing atherosclerosis. Cardiovascular diseases are the major cause of death in many countries, including Kenya (Slhessarenko *et al*., 2015) [13]. Estimation of the normal status or otherwise of a medical report of lipid profile analytes for individual patients is based on comparing the analytes value with the reference interval value. A reference interval is defined as a value of a parameter or analyte obtained by observation or measurement from a referent individual selected on the basis of fulfilling a specific inclusion criteria. Reference intervals are estimated using a minimum sample size of 120 referent individuals and spans 95% of the sample referent population. Where stratification is required on the basis of sex and age, the minimum sample size for each age and sex group is 120 referent individuals (CLSI {EP28 3c guideline}, 2010) [5]. Reference intervals for fasting lipid profiles are used by clinicians to assess the risk of cardiovascular diseases in patients using relative ratios such as total cholesterol: high density lipoprotein cholesterol, low density lipoprotein cholesterol: high density lipoprotein cholesterol, triacylglycerols: high density lipoprotein cholesterol of different fractions of plasma lipids to diagnose cardiovascular diseases, assess the stage of cardiovascular disease, assess toxicity to xenobiotics, and to monitor response to therapy used in managing cardiovascular diseases patients (Achila *et al*., 2017) [1].

Reference intervals of fasting lipid profiles for adults and geriatrics from different parts of the world are affected by factors such as age, sex, geographical location, dietary habits (foods taken), life-style (sedentary or active, smoker or non-smoker, alcoholic or non-alcoholic), health status, environment, climate, comprehensiveness of the inclusion criteria used in recruiting the referent individual, socio-economic status of the referent individual, ethnicity, genetics, and the method and reagents used to estimate the lipid profile analyte of interest of the referent individual (Slhessarenko *et al*., 2015) [13]. For this reason, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) requires that each clinical laboratory develops its own local reference intervals for fasting lipid profiles analytes for adults and geriatrics using its own local population for accurate early disease detection, monitoring response to therapy, and predicting clinical outcomes. Despite the fact that there cannot be universal reference interval limits for biological parameters, most African countries, including Kenya, use fasting lipid profile reference intervals for adults and geriatrics developed using the Caucasian/Western population (Europeans and North Americans) whose diets, lifestyle and genetics differ from that of Africans reported in medical literature (medical books, refereed research articles in medical journals, and diagnostic test manufacturer's insert reagent kits) (Liu *et al*., 2019) [11] which is inappropriate. For the Kenyan population including that of the Taita-Taveta County, there are no reported fasting lipid profile reference intervals for adults and geriatrics. There is therefore a dare need to develop fasting lipid profile reference interval for the adults and geriatrics of Taita-Taveta County, Kenya. The aim of this study therefore was to establish age and sex specific 95% reference interval limits for fasting lipid profiles for adults and geriatrics of Taita-Taveta County, Kenya and compare them with those previously reported in medical literature.

Materials and Methods

Study Area

This study was conducted in four subcounties (Mwatate, Taveta, Wundanyi, and Voi) of Taita-Taveta County, Kenya between May, 2015 and December, 2017.

Study Population

The study population involved a total of 271 referent individuals who had fasted for between eight and twelve hours of age 18-93 years. They comprised 123 males and 148 females recruited from the four subcounties of Taita-Taveta County, Kenya who fulfilled the inclusion criteria. To participate in this study, the referent individual required to be above eighteen years, fulfilled the inclusion criteria, and consented to participate by signing the consent forms.

Study Design

This was a cross-section study design involving 271 randomly recruited male and female adults and geriatrics of Taita-Taveta County, Kenya.

Ethical Considerations

This study protocol was approved by Kenyatta University Ethical Review Committee (KU-ERC) Ref Number I84/31987/15/, National Commission for Science, Technology and Innovation (NACOSTI) Ref number 16/22096/14531, and Taita-Taveta county Medical director.

Recruitment of the Referent Individuals

The 271 referent subjects involved in this study were drawn from the four sub-counties of Taita Taveta, County, Kenya. The referent individuals were recruited by the researcher and his team mates by meeting the potential participants in the churches, chief barazas and door to door visits and explaining to them the purpose of the study and its importance to them. Those interested in participating in the study were then recruited and given instructions to fast after taking their super. The exclusion criteria used to select the referent individuals based on a self-reporting questionnaire and physical clinical observation by a physician included participants who consented but were not obese, pregnant, on strenuous exercise, suffering from diabetes mellitus, abnormal lipid profiles, and acanthosis nigerians, consumers of alcohol and medications that modify fasting lipid profiles including taking coffee. In addition, those with personal history of hypertension, thyroid hormone, renal or liver disease, acute myocardial infarction, angina, arrhythmia, and/or cerebrovascular disease were also excluded from participating in the study. Also excluded were potential participants of age below 18 years, and those positive for HIV/AIDS, syphilis, and hepatitis B and C.

Blood Sample Collection

After eight to twelve hours of fasting, five milliters syringe was used to draw five milliters of blood from the auxiliary

vein from each referent individual into plain vacutainer tubes labeled with the study number and the name of the referent individual. This blood sample was allowed to clot, and then placed into an ice box, and transported to the analysis laboratory where each sample was centrifuged at 3000g for five minutes. Serum was separated using a Pasteur pipette and then transferred into duplicate vials with bar codes for identification purposes and stored at -20°C until needed for analysis of fasting lipid profiles using Clinical Chemistry Autoanalyzer (Integra 400) machine within three months.

Laboratory Analysis

The fasting lipid profiles analyzed using the discrete Clinical Chemistry Autoanalyzer (Integra 400) machine included total cholesterol (T-Chol) using an enzymatic method involving cholesterol esterase, cholesterol oxidase and peroxidase, high density lipoprotein cholesterol (HDL-Chol) using an enzymatic method involving antihuman-β-lipoprotein, cholesterol esterase, cholesterol oxidase and peroxidase, low density lipoprotein cholesterol (LDL-Chol) using an enzymatic method involving cholesterol esterase, cholesterol oxidase, sodium azide, and peroxidase, and triacylglycerols (TG) using an enzymatic method involving lipase, glycerol kinase, glycerol-3-phosphate oxidase and peroxidase. Non-high density lipoprotein cholesterol was obtained by subtracting high density lipoprotein cholesterol (HDL-Chol) value from total cholesterol (T-Chol) value for each referent individual. The ratio of total cholesterol to high density lipoprotein cholesterol, low density lipoprotein cholesterol to high density lipoprotein cholesterol, and triacylglycerols to high density lipoprotein cholesterol were also calculated for each referent individual.

Data Management and Statistical Analysis

Laboratory obtained values for all the measured and calculated fasting lipid profile parameters were recorded into the laboratory notebook, entered into the Excel spreadsheet, checked for errors and cleaned. This clean data was exported into SPSS software and tested for normality using mean, standard error of mean, median, mode, variance, standard deviation, Skewness, standard error of skewness (SES), Kurtosis, standard error of kurtosis (SEK), range, minimum and maximum, and 2.5 and 97.5 percentiles. Outliers were removed using Box plots using the formula Q_3+1.5 (IQR) for the upper limit and Q_1-1.5 (IQR) for the lower limit, where IQR = Q_3-Q_1. IQR is the interquartile range. Q_1, and Q_3 are the interquartile 1 and interquartile 3, respectively. Q_2 is the median or interquartile 2 (Bauman, 2014) [4]. Since Clinical Laboratory Standards Institute (CLSI, 2010) [5] recommends that reference intervals be expressed as median and 95% range (2.5 and 97.5 percentiles), the difference between male and female values for each parameter for the referents were compared using Mann-Whitney U test. Further, the difference between the two age categories for this data for each gender was also compared using Mann-Whitney U test. A $\rho < 0.05$ was considered significant.

Results

Results of the Quality Control lipid profile material

The results of the daily analysis of the quality control material presented in Table i indicates that the study quality control results were similar to the assigned quality control values. This implies that the analytical process was operating optimally and therefore the results generated using this analytical process were accurate and reliable.

Table 1: Results for quality control material for lipid profiles

Analyte (unit)	Assigned QC report			Study QC report		
	Upper limit	Target	Lower limit	Mean	SD	%CV
T-CHOL (mmol/L)	6.99	6.61	6.63	6.59	0.13	1.97
HDL-CHOL (mmol/L)	2.01	1.61	1.43	1.62	0.03	2.85
LDL-CHOL (mmol/L)	2.6	2.3	2.1	2.2	0.05	2.27
TG (mmol/L)	2.18	2.10	1.95	2.10	0.03	1.43

Results of the normality statistics for fasting lipid profiles for adults and geriatrics of Taita-Taveta County, Kenya

The results of the normality statistics for fasting lipid profiles for adults and geriatrics of Taita-Taveta County, Kenya are presented in Table 2. Results indicate that the data for total cholesterol (T-Chol) and low density lipoprotein cholesterol are normally distributed for the whole sample, combined males and females, separate males and females for the two age categories (18-55 years and 56-95 years) and whole sample (18-95 years) based on the value for skewness [+1 through zero to -1] and kurtosis [+2 through zero to -2], in addition to the values for mean, median and mode. For high density lipoprotein cholesterol (HDL-Chol), the data for males in age category 18-55 years (skewness & kurtosis), males in age category 56-95 years (kurtosis), and males in whole sample (18-95 years) (kurtosis) are not normally distributed based on the value of mean, median, mode, skewness, and kurtosis. For non-high density lipoprotein cholesterol (non HDL-Chol), the data for males in age category 18-55 years (kurtosis), is not normally distributed while that for age category 56-95 years and whole sample (18-95 years) are normally distributed based on the value of mean, median, mode, skewness and kurtosis. For triacylglycerols (TG), the data for combined males and females, and separate males in age category 18-55 years are not normally distributed (skewness), that for combined males and females, and separate males and females in age category 56-95 years and whole sample (18-95 years) are not normally distributed (mean, median, mode, skewness and kurtosis). The data for total cholesterol: high density lipoprotein cholesterol ratio, triacylglycerols: high density lipoprotein cholesterol ratio, and low density lipoprotein cholesterol: high density lipoprotein cholesterol ratio in age category 18-55 years is not normally distributed (mean, median, mode, skewness and kurtosis), combined males and females and separate males for total cholesterol: high density lipoprotein cholesterol ratio and low density lipoprotein cholesterol: high density lipoprotein cholesterol ratio (mean, median, mode, skewness and kurtosis), and combined males and females and separate male and females in age category 56-95 years, and whole sample (18-95 years) are not normally distributed (mean, median, mode, skewness and kurtosis) based on the value of mean, median, mode, skewness and kurtosis (Table ii).

Table 2: Results of the normality statistics for fasting lipid profiles for adult and geriatric population of Taita-Taveta County, Kenya

	Normality statistics for fasting lipid profiles									
18-55 years	Sex	N	T-CHOL	HDL	LDL	Non-HDL	TG	T-CHOL:HDL	TG:HDL	LDL:HDL
Mean	M&F	138	5.14±1.35	1.20±0.48	3.19±1.27	3.94±1.31	1.78±1.00	4.50±1.84	1.79±1.40	2.77±1.47
	M	57	4.91±1.21	1.07±0.32	3.09±1.17	3.82±1.17	1.81±1.01	4.56±1.68	1.84±1.22	2.81±1.45
	F	81	5.31±1.43	1.28±0.55	3.26±1.33	4.02±1.41	1.75±1.01	4.46±1.96	1.75±1.52	2.74±1.50
Variance	M&F	138	1.833	0.232	1.599	1.719	1.008	3.391	1.963	2.165
	M	57	1.474	0.102	1.367	1.362	1.016	2.822	1.492	2.087
	F	81	2.041	0.306	1.769	1.974	1.013	3.826	2.313	2.244
SD	M&F	138	1.354	0.481	1.265	1.311	1.004	1.841	1.401	1.471
	M	57	1.214	0.320	1.169	1.167	1.008	1.680	1.222	1.445
	F	81	1.429	0.553	1.330	1.405	1.007	1.956	1.521	1.498
SEM	M&F	138	0.155	0.041	0.108	0.112	0.085	0.157	0.119	0.125
	M	57	0.161	0.042	0.155	0.155	0.133	0.223	0.162	0.191
	F	81	0.159	0.061	0.148	0.156	0.112	0.217	0.169	0.166
Median	M&F	138	5 (3-8.53)	1 (0-2)	3 (1-6)	4 (2-7.53)	2 (1-4)	4 (2-9.05)	1 (0-6)	3 (1-6.53)
	M	57	5 (2-8.10)	1 (0.45-2)	3 (0.45-6)	4 (1.45-7.10)	1 (1-4)	4 (1.90-10.55)	1 (1-6)	3 (0.45-8.10)
	F	81	5 (3-8.95)	1 (0-2)	3 (1-6)	4 (2-7.95)	2 (0.05-4.95)	4 (2-8)	1 (0-6.95)	3 (1-6)
Mode	M&F	138	5	1	3	4	1	4	1	2
	M	57	5	1	3	4	1	4	1	3
	F	81	6	1	3	4	1	4	1	2
Skewness	M&F	138	0.413	0.888	0.407	0.641	1.169	1.890	2.240	1.707
	M	57	0.297	1.574	0.033	0.634	1.055	1.576	1.835	1.713
	F	81	0.384	0.477	0.553	0.593	1.272	2.055	2.398	1.738
SES	M&F	138	0.206	0.206	0.206	0.206	0.206	0.206	0.206	0.206
	M	57	0.316	0.316	0.316	0.316	0.316	0.316	0.316	0.316
	F	81	0.267	0.267	0.267	0.267	0.267	0.267	0.267	0.267
Kurtosis	M&F	138	0.470	1.353	0.253	0.773	0.946	7.890	6.487	5.624
	M	57	1.819	6.457	0.538	2.302	-0.022	4.432	3.317	5.850
	F	81	-0.111	0.266	0.018	0.163	1.767	9.375	7.349	5.896
SEK	M&F	138	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.410
	M	57	0.623	0.623	0.623	0.623	0.623	0.623	0.623	0.623
	F	81	0.529	0.529	0.529	0.529	0.529	0.529	0.529	0.529
Range	M&F	138	7	3	7	7	5	14	9	10
	M	57	7	2	6	7	3	10	5	9
	F	81	6	3	6	6	5	14	9	9
Minimum	M&F	138	2	0	0	1	0	1	0	0
	M	57	2	0	0	1	1	1	1	0
	F	81	3	0	1	2	0	1	0	1
Maximum	M&F	138	9	3	7	8	5	15	9	10
	M	57	9	2	6	8	4	11	6	9
	F	81	9	3	7	8	5	15	9	10
Percentiles										
2.5	M&F	138	3	0	1	2	1	2	0	1
	M	57	2	0.45	0.45	1.45	1	1.9	1	0.45
	F	81	3	0	1	2	0.05	2	0	1
97.5	M&F	138	8.53	2	6	7.53	4	9.05	6	6.53
	M	57	8.1	2	6	7.1	4	10.55	6	8.1
	F	81	8.95	2	6	7.95	4.95	8	6.95	6
56-95 years	Sex	N	T-CHOL	HDL	LDL	Non-HDL	TG	T-CHOL:HDL	TG:HDL	LDL:HDL
Mean	M&F	133	4.89±1.31	1.20±0.52	2.89±1.119	3.59±1.32	1.79±1.02	4.41±2.19	1.81±1.71	2.74±1.64
	M	66	4.79±1.28	1.09±0.42	2.89±1.08	3.61±1.35	1.86±1.19	4.88±2.52	2.09±2.01	2.97±1.93
	F	67	4.99±1.33	1.31±0.58	2.88±1.16	3.57±1.29	1.72±0.83	3.96±1.72	1.54±1.71	2.51±1.26
Variance	M&F	133	1.707	0.269	1.252	1.729	1.046	4.805	2.911	2.680
	M	66	1.647	0.176	1.173	1.812	1.412	6.324	4.022	3.722
	F	67	1.773	0.340	1.349	1.673	0.691	2.953	1.707	1.587
SD	M&F	133	1.306	0.519	1.119	1.315	1.023	2.192	1.706	1.637
	M	66	1.283	0.420	1.083	1.346	1.188	2.515	2.006	1.929
	F	67	1.331	0.583	1.162	1.294	0.831	1.718	1.300	1.260
SEM	M&F	133	0.113	0.045	0.097	0.114	0.089	0.190	0.148	0.142
	M	66	0.158	0.052	0.133	0.166	0.146	0.310	0.247	0.237
	F	67	0.163	0.071	0.142	0.158	0.102	0.210	0.160	0.154
Median	M&F	133	5 (2-7)	1 (0-2)	3 (1-5)	4 (1-6)	2 (1-5)	4 (2-9.3)	1 (0-7)	3 (1-5.65)
	M	66	5 (2-8)	1 (0-2)	3 (1-6)	4 (1-6.33)	2 (0.68-6)	5 (2-16)	1 (0-9.3)	3 (0.68-10.63)
	F	67	5 (2-7)	1 (0-3)	3 (1-5)	4 (1-6)	2 (1-4.3)	4 (0.7-7.9)	1 (0-5.6)	2 (0.7-5.3)
Mode	M&F	133	5	1	3	4	1	4	1	3
	M	66	5	1	3	4	1	5	1	3
	F	67	5	1	3	4	1	4	1	2

Skewness	M&F	133	-0.223	0.896	0.193	-0.150	1.728	2.289	2.658	2.862
	M	66	0.096	0.606	0.441	0.060	1.690	2.584	2.644	3.722
	F	67	-0.528	0.778	0.000	-0.391	1.395	0.736	1.723	0.358
SES	M&F	133	0.210	0.210	0.210	0.210	0.210	0.210	0.210	0.210
	M	66	0.295	0.295	0.295	0.295	0.295	0.295	0.295	0.295
	F	67	0.293	0.293	0.293	0.293	0.293	0.293	0.293	0.293
Kurtosis	M&F	133	-0.129	1.616	-0.207	-0.462	4.039	10.340	10.637	17.116
	M	66	0.191	2.507	0.716	-0.269	3.359	10.153	9.358	16.850
	F	67	-0.141	0.710	-0.859	-0.660	2.759	1.373	4.040	-0.409
SEK	M&F	133	0.417	0.417	0.417	0.417	0.417	0.417	0.417	0.417
	M	66	0.582	0.582	0.582	0.582	0.582	0.582	0.582	0.582
	F	67	0.578	0.578	0.578	0.578	0.578	0.578	0.578	0.578
Range	M&F	133	6	3	5	6	6	16	12	14
	M	66	6	2	5	6	6	14	12	14
	F	67	5	3	4	5	4	10	7	6
Minimum	M&F	133	2	0	1	1	0	0	0	0
	M	66	2	0	1	1	0	2	0	0
	F	67	2	0	1	1	1	0	0	0
Maximum	M&F	133	8	3	6	7	6	16	12	14
	M	66	8	2	6	7	6	16	12	14
	F	67	7	3	5	6	5	10	7	6
Percentiles										
2.5	M&F	133	2	0	1	1	1	2	0	1
	M	66	2	0	1	1	0.68	2	0	0.68
	F	67	2	0	1	1	1	0.7	0	0.7
97.5	M&F	133	7	2	5	6	5	9.3	7	5.65
	M	66	8	2	6	6.33	6	16	9.3	10.63
	F	67	7	3	5	6	4.3	7.9	5.6	5.3
18-95 years	Sex	N	T-CHOL	HDL	LDL	Non-HDL	TG	T-CHOL:HDL	TG:HDL	LDL:HDL
Mean	M&F	271	5.02±1.34	1.20±0.50	3.04±1.20	3.77±1.32	1.78±1.01	4.46±2.02	1.80±1.56	2.75±1.55
	M	123	4.85±1.25	1.08±0.38	2.98±1.12	3.71±1.27	1.84±1.10	4.73±2.17	1.98±1.69	2.89±1.72
	F	148	5.16±1.39	1.30±0.58	3.09±1.27	3.82±1.37	1.74±0.93	4.23±1.86	1.66±1.43	2.64±1.40
Variance	M&F	271	1.781	0.249	1.446	1.749	1.023	4.071	2.419	2.409
	M	123	1.558	0.141	1.262	1.602	1.219	4.690	2.844	2.948
	F	148	1.933	0.319	1.604	1.878	0.862	3.471	2.037	1.948
SD	M&F	271	1.335	0.499	1.203	1.323	1.011	2.018	1.555	1.552
	M	123	1.248	0.375	1,123	1.266	1.104	2.166	1.686	1.717
	F	148	1.390	0.565	1.267	1.370	0.928	1.863	1.427	1.396
SEM	M&F	271	0.081	0.030	0.073	0.080	0.061	0.123	0.094	0.094
	M	123	0.133	0.034	0.101	0.114	0.100	0.195	0.152	0.155
	F	148	0.114	0.046	0.104	0.113	0.076	0.153	0.117	0.115
Median	M&F	271	5 (2-8)	1 (0-2)	3 (1-6)	4 (1-6)	2 (1-4.2)	4 (2-8.4)	1 (0-6.2)	3 (1-6)
	M	123	5 (2-7.9)	1 (0-2)	3 (1-6)	4 (1-6)	2 (1-4.9)	4 (2-10.9)	1 (0-6.9)	3 (1-8.8)
	F	148	5 (2-8)	1 (0-2.28)	3 (1-6)	4 (1-6.28)	2 (1-4.28)	4 (1.73-8)	1 (0-6.28)	2 (1-6)
Mode	M&F	271	5	1	3	4	1	4	1	3
	M	123	5	1	3	4	1	4	1	3
	F	148	5	1	3	4	1	4	1	2
Skewness	M&F	271	0.126	0.891	0.359	0.242	1.443	2.136	2.531	2.539
	M	123	0.169	0.905	0.244	0.225	1.480	2.538	2.750	2.952
	F	148	0.044	0.622	0.403	0.238	1.326	1.585	2.179	1.316
SES	M&F	271	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.148
	M	123	0.218	0.218	0.218	0.218	0.218	0.218	0.218	0.218
	F	148	0.199	0.199	0.199	0.199	0.199	0.199	0.199	0.199
Kurtosis	M&F	271	0.297	1.480	0.181	0.342	2.463	9.599	9.596	12.349
	M	123	0.792	3.626	0.495	0.630	2.391	10.973	11.052	15.513
	F	148	0.064	0.459	-0.044	0.174	2.141	6.911	6.468	4.365
SEK	M&F	271	0.295	0.295	0.295	0.295	0.295	0.295	0.295	0.295
	M	123	0.433	0.433	0.433	0.433	0.433	0.433	0.433	0.433
	F	148	0.396	0.396	0.396	0.396	0.396	0.396	0.396	0.396
Range	M&F	271	7	3	7	7	6	16	12	14
	M	123	7	2	6	7	6	15	12	14
	F	148	7	3	6	7	5	15	9	10
Minimum	M&F	271	2	0	0	1	0	0	0	0
	M	123	2	0	0	1	0	1	0	0
	F	148	2	0	1	1	0	0	0	0
Maximum	M&F	271	9	3	7	8	6	16	12	14
	M	123	9	2	6	8	6	16	12	14
	F	148	9	3	7	8	5	15	9	10

Percentiles										
2.5	M&F	271	2	0	1	1	1	2	0	1
	M	123	2	0	1	1	1	2	0	1
	F	148	2	0	1	1	1	1.73	0	1
97.5	M&F	271	8	2	6	6	4.2	8.4	6.2	6
	M	123	7.9	2	6	6	4.9	10.9	6.9	8.8
	F	148	8	2.28	6	6.28	4.28	8	6.28	6

Reference interval limits for fasting lipid profile for adults and geriatrics of Taita Taveta County, Kenya

The established median reference interval for low density lipoprotein cholesterol (LDL-Chol), non-high density lipoprotein cholesterol (non-HDL-Chol), triacylglycerols (TG), and the ratio of low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) for male adult and geriatric population of Taita-Taveta County, Kenya were statistically similar to those of the female population of similar age range ($\rho > 0.05$). Therefore a combined reference interval of these parameters for this population was established. The established median reference interval limits for adults and geriatrics of Taita-Taveta County, Kenya for low density lipoprotein cholesterol (LDL-Chol) is 3 (1-6) mmol/L, non-high density lipoprotein cholesterol (non-HDL-Chol) is 4 (1-6) mmol/L, triacylglycerols (TG) is 2 (1-4.2) mmol/L, and low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio is 3 (1-6).

However, the established median reference interval for the adult and geriatric male population of Taita-Taveta County, Kenya for total cholesterol (T-Chol), high density lipoprotein cholesterol (HDL-Chol), total cholesterol (T-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio, and triacylglycerol (TG): high density lipoprotein cholesterol (HDL-Chol) ratio were statistically significantly different from that of the female population of similar age range ($\rho < 0.05$). The established median reference interval limits for the adult and geriatric population of Taita-Taveta County, Kenya for total cholesterol (T-Chol) for males (5 (2-7.9) mmol/L) with mean rank of 125.57 are significantly lower than those for females (5 (2-8) mmol/L) with mean rank of 144.67 ($U = 7819$, $z = -2.061$, $\rho = 0.039$, $r = 0.1252$), high density lipoprotein cholesterol (HDL-Chol) for males (1 (0-2) mmol/L) with mean rank of 121.81 are significantly lower than those for females (1 (0-2.28) mmol/L) with mean rank of 147.79 ($U = 7356.5$, $z = -3.579$, $\rho = 0.000$, $r = 0.2174$), total cholesterol (T-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio for males (4 (2-10.9)) with mean rank of 146.76 are significantly higher than those for females (4 (1.73-8)) with mean rank of 127.06 ($U = 7778.5$, $z = -2.099$, $\rho = 0.036$, $r = 0.1275$), and triacylglycerols (TG): high density lipoprotein cholesterol (HDL-Chol) ratio for males (1 (0-6.9)) with mean rank of 145.96 are significantly higher than those for females (1 (0-6.28)) with mean rank of 127.72 ($U = 7876.5$, $z = -2.065$, $\rho = 0.039$, $r = 0.1254$) (Table iii).

Table 3: Reference interval limits for fasting lipid profile for adult and geriatric population of Taita-Taveta County, Kenya

Analyte (unit)	Sex	N	Median	Percentile		Reference Interval	IV	Difference between M&F	
				2.5th	97.5th			z-value	Sig
T-Chol (mmol/L)	M&F	271	5.02±1.34					-2.061	ρ = 0.039
			5	2	8	2-8	6		
	F	148	5.16±1.39						
			5	2	8	2-8*	6		
	M	123	4.85±1.25						
			5	2	7.9	2-7.9	5.9		
HDL-Chol (mmol/L)	M&F	271	1.20±0.50					-3.579	ρ = 0.000
			1	0	2	0-2	2		
	F	148	1.30±0.58						
			1	0	2.28	0-2.28*	2.28		
	M	123	1.08±0.38						
			1	0	2	0-2	2		
LDL-Chol (mmol/L)	M&F	271	3.04±1.20					-0.492	ρ = 0.623
			3	1	6	1-6	5		
	F	148	3.09±1.27						
			3	1	6	1-6	5		
	M	123	2.98±1.12						
			3	1	6	1-6	5		
TG (mmol/L)	M&F	271	1.78±1.01					-0.671	ρ = 0.502
			2	1	4.2	1-4.2	3.2		
	F	148	1.74±0.93						
			2	1	4.28	1-4.28	3.28		
	M	123	1.84±1.10						
			2	1	4.9	1-4.9	3.9		
Non HDL-Chol (mmol/L)	M&F	271	3.77±1.32					-0.277	ρ = 0.782
			4	1	6	1-6	5		
	F	148	3.82±1.37						
			4	1	6.28	1-6.28	5.28		
	M	123	3.71±1.27						
			4	1	6	1-6	5		

Total Chol: HDL-Chol	M&F	271	4.46±2.02					-2.099	ρ = 0.036
			4	2	8.4	2-8.4	6.4		
	F	148	4.23±1.86						
			4	1.73	8	1.73-8*	6.27		
	M	123	4.73±2.17						
			4	2	10.9	2-10.9	8.9		
TG:HDL-Chol	M&F	271	1.80±1.56					-2.065	ρ = 0.039
			1	0	6.2	0-6.2	6.2		
	F	148	1.66±1.43						
			1	0	6.28	0-6.28*	6.28		
	M	123	1.98±1.69						
			1	0	6.9	0-6.9	6.9		
LDL-Chol: HDL-Chol	M&F	271	2.75±1.55					-1.298	ρ = 0.194
			3	1	6	1-6	5		
	F	148	2.64±1.40						
			2	1	6	1-6	5		
	M	123	2.89±1.72						
			3	1	8.8	1-8.8	7.8		

Results are expressed as median and range for the number of referent participants in the column labeled N. Statistical comparisons of the median values between male and female referent participants were carried out using Mann-Whitney U test. Differences were considered statistically significant at $\rho < 0.05$

Effect of age and gender on the reference intervals for fasting lipid profiles for adults and geriatrics of Taita Taveta County, Kenya

The effect of age and gender on the reference intervals limits for fasting lipid profiles for the adult and geriatric population of Taita Taveta County, Kenya was investigated by categorizing the results into two age groups: (a) age group 1 (18-55 years) and (b) age group 2 (56-95 years). The statistical difference between the reference intervals for fasting lipid profiles for adult and geriatric males and females were estimated using Mann-Whitney U test and ρ-values less than 0.05 was considered statistically significant. The Median and 95% range for males and females for the two age groups are presented in Table iv. Results indicate that all the measured and calculated fasting lipid profile analytes including total cholesterol (Chol), high density lipoprotein cholesterol (HDL-Chol), low density lipoprotein cholesterol (LDH-Chol), non-high density lipoprotein cholesterol (non-HDL-Chol), and the ratios of total cholesterol (Chol): high density lipoprotein cholesterol (HDL-Chol) ratio, low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio, and triacylglycerol (TG): high density lipoprotein cholesterol (HDL-Chol) ratio were statistically unaffected by advancement in age ($\rho > 0.05$; Table iv).

However, in the age category 18-55 years, males (1 (0.45-2 mmol/L)) with mean rank of 61.39 had a statistically significantly lower levels of high density lipoprotein cholesterol (HDL-Chol) compared to females (1 (0-2) mmol/L) with mean rank of 75.21 (U = 1846, z = -2.666, ρ = 0.008, r = 0.2269). Further, in age category 56-95 years, males (1 (0-2) mmol/L) with mean rank of 60.75 had a statistically significantly lower levels of high density lipoprotein cholesterol than females (1 (0-3) mmol/L) with mean rank of 73.16 (U = 1798.5, z = -2.416, ρ = 0.016, r = 0.2095), and males (5 (2-16)) with mean rank of 75.10 had a statistically significantly higher levels of total cholesterol (T-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio than females (4 (0.7-7.9)) with mean rank of 59.02 (U = 1676.5, z = -2.445, ρ = 0.014, r = 0.2120).

Table 4: Effect of age and gender on the reference intervals of the fasting lipid profile for adults and geriatrics of Taita Taveta County, Kenya

Analyte (unit)	Median reference intervals for fasting lipid profiles						
	Sex	**N**	**18-55 years**	**N**	**56-95 years**	**N**	**18-95 years**
T-Chol (mmol/L)	M&F	138	5.14±1.35	133	4.89±1.31	271	5.02±1.34
			5 (3-8.53)		5 (2-7)		5 (2-8)
	M	57	4.91±1.21	66	4.79±1.28	123	4.85±1.25
			5 (2-8.1)		5 (2-8)		5 (2-7.9)*
	F	81	5.31±1.43	67	4.99±1.33	148	5.16±1.39
			5 (3-8.95)		5 (2-7)		5 (2-8)
HDL-Chol (mmol/L)	M&F	138	1.20±0.48	133	1.20±0.52	271	1.22±0.46
			1 (0-2)		1 (0-2)		1 (0-2)
	M	57	1.07±0.32	66	1.09±0.42	123	1.08±0.38
			1 (0.45-2)*		1 (0-2)*		1 (0-2)*
	F	81	1.28±0.55	67	1.31±0.58	148	1.30±0.57
			1 (0-2)		1 (0-3)		1 (0-2.28)
LDL-Chol (mmol/L)	M&F	138	3.19±1.27	133	2.89±1.12	271	3.04±1.19
			3 (1-6)		3 (1-5)		3 (1-6)
	M	57	3.09±1.17	66	2.86±1.08	123	2.98±1.12
			3 (0.46-6)		3 (1-6)		3 (1-6)
	F	81	3.26±1.33	67	2.88±1.16	148	3.09±1.27
			3 (1-6)		3 (1-5)		3 (1-6)
TG	M&F	138	1.78±1.00	133	1.79±1.02	271	1.79±1.00

(mmol/L)			2 (1-4)		2 (1-5)		2 (1-4.2)
	M	57	1.81±1.01	66	1.86±1.19	123	1.84±1.10
			1 (1-4)		2 (0.68-6)		2 (1-4.9)*
	F	81	1.75±1.01	67	1.72±0.83	148	1.74±0.93
			2 (0.05-4.95)		2 (1-4.3)		2 (1-4.28)
Non HDL-Chol (mmol/L)	M&F	138	3.94±1.31	133	3.59±1.32	271	3.77±1.30
			4 (2-7.53)		4 (1-6)		4 (1-6)
	M	57	3.82±1.17	66	3.61±1.35	123	3.71±1.27
			4 (1.45-7.1)		4 (1-6.33)		4 (1-6)
	F	81	4.02±1.41	67	3.57±1.29	148	3.82±1.37
			4 (2-7.95)		4 (1-6)		4 (1-6.28)
T-Chol: HDL-Chol	M&F	138	4.50±1.84	133	4.41±2.19	271	4.48±2.00
			5 (2-9.05)		4 (2-9.30)		4 (2-8.4)
	M	57	4.56±1.68	66	4.88±2.52	123	4.73±2.17
			4 (1.9-10.55)		5 (2-16)*		5 (2-10.9)*
	F	81	4.46±1.96	67	3.96±1.72	148	4.23±1.86
			4 (2-8)		4 (0.7-7.9)		4 (1.73-8)
TG:HDL-Chol	M&F	138	1.79±1.40	133	1.81±1.71	271	1.80±1.56
			1 (0-6)		1 (0-7)		1 (0-6.2)
	M	57	1.84±1.22	66	2.09±2.01	123	1.98±1.69
			1 (1-6)		1 (0-9.3)		1 (0-6.9)*
	F	81	1.75±1.52	67	1.54±1.30	148	1.66±1.43
			1 (0-6.95)		1 (0-5.6)		1 (0-6.28)
LDL-Chol: HDL-Chol	M&F	138	2.77±1.47	133	2.74±1.64	271	2.75±1.55
			3 (1-6.53)		3 (1-5.65)		3 (1-6)
	M	57	2.81±1.45	66	2.97±1.93	123	2.89±1.72
			3 (0.45-8.1)		3 (0.68-10.63)		3 (1-8.8)
	F	81	2.74±1.50	67	2.51±1.26	148	2.64±1.40
			3 (1-6)		2 (0.7-5.3)		2 (1-6)

Results are expressed as Mean ± standard deviation (SD), and Median and range of the number of subjects indicated in the column labeled N. *$\rho < 0.05$ when male median reference interval limits are significantly different when compared to the female median reference interval limits within each age category (down the columns) by Mann-Whitney U test, a$\rho < 0.0167$ when median reference interval limits in age category 18-55 years are significantly different when compared to the reference intervals in age category 56-95 year (along the rows) by Mann-Whitney U test for each gender.

Comparison of the developed reference interval limits for fasting lipid profile for adults and geriatrics of Taita-Taveta County, Kenya with those reported in medical literature

A comparison of the developed reference intervals for fasting lipid profile for adults and geriatrics of Taita-Taveta County, Kenya with those reported in medical literature are presented in Table v. For this study, the lower limit of the reference interval for fasting total cholesterol (T-Chol) levels is similar to that of the Haryana population but the upper limit is greater than that of the Haryana population. However, the reference intervals for fasting total cholesterol were similar for males and females of both populations. The lower limit for the reference interval of fasting total cholesterol for Punjab population is higher than that generated in this study for the Taita-Taveta county population while the upper limit are lower than that generated in this study. Further, the reference interval for fasting total cholesterol for the Punjab population differ by sex while those generated in this study are similar. The lower reference interval limit for fasting total cholesterol for the Assamese population of India were similar to those generated for the Taita-Taveta County population while the upper reference interval limit is lower than that generated in this study. However, the Assamese population reference interval for fasting total cholesterol differs by sex. For the Western Maharashtra population of India, the lower and upper reference interval limit for fasting total cholesterol are lower than those developed for the Taita-Taveta County population of this study. Further, the Western Maharashtra population had different reference interval for fasting total cholesterol for males and females. For the Venezuelan Maracaibo population, the lower reference interval for fasting total cholesterol is higher than that established for Taita-Taveta County population in this study, while the upper limit for the same population is lower than that of the Taita-Taveta County population. However, the Venezuelan Maracaibo population has different reference intervals for fasting total cholesterol for males and females (Table v).

For the Punjab, Western Maharashtra, Assamese, and Taita-Taveta populations, all had different reference interval limits for fasting lipid profiles for males and females. For the Punjab population, the lower reference interval limit for fasting high density lipoprotein cholesterol (HDL-Chol) is higher than that established for the Taita-Taveta County population, while the upper reference interval limit is lower. For the Western Maharashtra and Assamese populations, the lower reference interval limit for fasting high density lipoprotein cholesterol are higher than those established for the Taita-Taveta County population, while the upper reference interval limits were lower. For the Haryana, and Venezuelan Maracaibo populations, the combined (male and female) lower reference interval limits for fasting high density lipoprotein cholesterol are higher than the established (separate for males and females) for the Taita-Taveta County population, while the upper reference interval limit is lower (Table v).

Punjab, Western Maharashtra, and Haryana population reported combined reference intervals for TG for adult and geriatric males and females just like that established for the adult and geriatric male and female population of Taita-Taveta County, Kenya. However, the lower combined

established reference interval limits for triacylglycerol for adult and geriatric male and female population of Taita-Taveta County, Kenya is lower than that reported for Punjab population, and the upper reference interval limit is higher; the lower combined established reference interval limits for triacylglycerol for adult and geriatric male and female population of Taita-Taveta County, Kenya is higher than that reported for Western Maharashtra population, and upper reference interval limit is lower; the lower combined established reference intervals for TG for adult and geriatric male and female population of Taita-Taveta County, Kenya is similar to that reported for the Haryana population, and the upper reference interval limit is lower. Further, the lower combined established reference interval limits for triacylglycerol for adult and geriatric male and female population of Taita-Taveta County, Kenya is higher than the reported separate (male and female) reference interval limit for Venezuelan Maracaibo, and Assamese population, and the upper separate reference intervals for the two populations are lower. In addition, the established lower separate (male and female) reference interval limit for total cholesterol: high density lipoprotein cholesterol ratio for the adult and geriatric population of Taita-Taveta County, Kenya is lower than that reported for the combined reference interval for the Punjab population, and the upper separate reference interval limit is lower (Table v).

Table 5: Comparison of the developed reference intervals for fasting lipid profile for adult and geriatric population of Taita-Taveta County, Kenya with those reported in literature

Analyte	Sex	This study RI	Durgawale *et al*., 2009. [7]	Das and Saikia, 2009 [6]	Kaur *et al*., 2012 [8]	Bermudez *et al*., 2012	Agrawal *et al*., 2014 [2]
T-Chol (mmol/L)	M&F	2-8				2.98-6.99	2.21-5.49
	F	2-8*	0.48-3.95	2.40-6.80	4.70-4.84	3.14-7.02	
	M	2-7.9	0.00-5.54	2.51-6.05	4.57-4.73	2.79-6.99	
HDL-Chol (mmol/L)	M&F	0-2				0.78-1.98	0.52-1.63
	F	0-2.28*	0.65-1.74	0.62-1.89	1.15-1.18	0.79-1.96*	
	M	0-2	0.58-1.63	0.59-1.53	1.10-1.13	0.78-1.98	
LDL-Chol (mmol/L)	M&F	1-6			2.9-3.0	1.39-4.80	1.30-3.81
	F	1-6					
	M	1-6					
TG (mmol/L)	M&F	1-4.2	0.31-1.69		1.36-1.41	0.35-2.25	0.69-1.76
	F	1-4.28		0.46-2.54		0.35-1.93*	
	M	1-4.9		0.45-2.89		0.37-2.35	
Non HDL-Chol (mmol/L)	M&F	1-6					
	F	1-6.28					
	M	1-6					
T-Chol: HDL-Chol	M&F	2-8.4			4.15-4.26		
	F	1.73-8*					
	M	2-10.9					
TG:HDL-Chol	M&F	0-6.2					
	F	0-6.28*					
	M	0-6.9					
LDL-Chol: HDL-Chol	M&F	1-6			2.58-2.69		
	F	1-6					
	M	1-8.8					

Punjab population by Kaur *et al*. (2012) [8], Western Maharashtra population by Durgawale *et al*. (2009) [7], Haryana population by Agrawal *et al*. (2014) [2], Venezuelan Maracaibo Population by Bermudez *et al*. (2012), Assamese Population by Das and Saikia (2009) [6].

Discussion

Results indicating statistically similar fasting reference intervals for total cholesterol (T-Chol), low density lipoprotein cholesterol (LDL-Chol), and non-high density lipoprotein cholesterol (non-HDL-Chol), triacylglycerols (TG), and low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio for adult and geriatric male and female population of Taita-Taveta County, Kenya implies that these parameters are sex independent. This observation is contrary to the well-known fact that females have a higher fat body content than males (Bermúdez *et al.*, 2012) [3]. The developed sex independent reference intervals for adults and geriatrics of Taita-Taveta County, Kenya is 2-8 mmol/L for total cholesterol, 1-6 mmol/L for low density lipoprotein cholesterol (LDL-Chol), 1-6 mmol/L for non-high density lipoprotein cholesterol (non-HDL-Chol), 1-4.2 mmol/L for triacylglycerols (TG), and 1-6 for low density lipoprotein cholesterol (LDL-Chol): high density lipoprotein cholesterol (HDL-Chol) ratio. This is in agreement with sex independent reference intervals for total cholesterol reported by Achila *et al*. (2017) [1] of 3-7.71 mmol/L, Agrawal *et al*. (2014) [2] of 2.21-5.49 mmol/L, and Bermudez *et al*. (2012) of 2.98-6.99 mmol/L for Asmara, Haryana, and Venezuelan Maracaibo population, respectively. These results, however, contrast the sex dependent reference intervals for total cholesterol reported by Durgawale *et al*. (2009) [7] of 0-5.54 mmol/L for males and 0.48-3.95 mmol/L for females, Das and Saika (2009) [6] of 2.51-6.05 mmol/L for males and 2.40-6.80 mmol/L for females, and Kaur *et al*. (2012) [8] of 4.57-4.73 mmol/L for males and 4.70-4.84 mmol/L for females for Western Maharashtra, Assamese, and Punjab populations, respectively.

The sex independent reference intervals for low density lipoprotein cholesterol (LDL-Chol) reported in this study agree with those previously reported by Kaur *et al*. (2012) [8] of 2.9-3.0 mmol/L, Bermudez *et al*. (2012) of 1.39-4.80 mmol/L, and Agrawal *et al*. (2014) [2] of 1.30-3.81 mmol/L for Punjab, Venezuelan Maracaibo and Haryana populations, respectively. Further, the sex independent

reference intervals for triacylglycerols reported in this study agree with those previously reported by Durgawale *et al*. (2009) [7] of 0.31-1.69 mmol/L, Kaur *et al* (2012) [8] of 1.36-1.41 mmol/L, and Agrawal *et al*. (2014) [2] of 0.69-1.76 mmol/L for Western Maharashtra, Punjab and Haryana populations, respectively. In contrast, these results differ from the sex dependent reference intervals for triacylglycerols reported by Das and Saika (2009) [6] of 0.45-2.89 mmol/L for males and 0.46-2.54 mmol/L for females, and Bermudez *et al*. (2012) of 0.37-2.35 mmol/L for males and 0.35-1.93 mmol/L for females with males having higher values than females for Assamese, and Venezuelan Maracaibo populations, respectively. The higher fasting lipid profiles and the associated ratios of the Taita-Taveta County, Kenya population reported in this study compared to those reported by Durgawale *et al*. (2009) [7], Das and Saika (2009) [6], Kaur *et al* (2012) [8], Bermudez *et al*. (2012) and Agrawal *et al*. (2014) [2] could be associated with the consumption of high carbohydrate and high fat diets including wheat products (sold at the markets), sweet potatoes, maize, sorghum and millet, and fish, broilers, eggs, red meat and milk from cattle, goats and sheep all of which are available in Taita-Taveta County, Kenya.

Results indicating statistically different reference intervals for high density lipoprotein cholesterol, and total cholesterol: high density lipoprotein cholesterol, and triacylglycerols: high density lipoprotein cholesterol ratios for adult and geriatric males and females of Taita-Taveta County, Kenya imply that theses parameters are sex dependent. The separate male and female reference intervals for high density lipoprotein cholesterol is 0-2 mmol/L for males and 0-2.28 mmol/L for females with males having a lower value than females, total cholesterol: high density lipoprotein cholesterol ratio is 2-10.9 for males, and 1.73-8 for females with males having a higher value than females, and triacylglycerols (TG): high density lipoprotein cholesterol (HDL-Chol) ratio is 0-6.90 for males and 0-6.28 for females with males having a higher value than females. The sex dependent reference interval limits for higher density lipoprotein cholesterol results agree with those reported by Durgawale *et al*. (2009) [7] of 0.58-1.63 mmol/L for males and 0.65-1.74 mmol/L for females, Das and Saikia (2009) [6] of 0.59-1.53 mmol/L for males and 0.62-1.89 mmol/L for females, and Kaur *et al*. (2012) [8] of 1.10-1.13 mmol/L for males and 1.15-1.18 mmol/L for females with males having lower values than females for Western Maharashtra, Assamese, and Punjab populations, respectively. In contrast, these results differ from the sex independent reference intervals for high density lipoprotein cholesterol reported by Bermudez *et al*. (2012) of 0.78-1.98 mmol/L, Agrawal *et al*. (2014) [2] of 0.52-1.63 mmol/L, and Achila *et al*. (2017) [1] of 0.81-2.07 mmol/L for Venezuelan Maracaibo, Haryana, and Asmara, Eritrea populations, respectively. The sex dependent reference interval for total cholesterol: higher density lipoprotein cholesterol ratio reported in this study contrasts the sex independent reference interval of this parameter reported by Kaur *et al*. (2012) [8] of 4.15-4.26 for Punjab population. The sex dependent reference interval limits for triacylglycerol (TG): high density lipoprotein cholesterol (HDL-Chol) ratio of 0-6.9 for male and 0-6.28 for female adults and geriatrics of Taita-Taveta County, Kenya has no previously developed and reported comparative data elsewhere in literature.

The developed higher fasting lipid profile reference intervals for the male and female adults and geriatrics population of Taita-Taveta County compared to the previously reported medical literature reference intervals developed from other referent populations from different parts of the world implies that this population is at a higher risk of developing coronary heart disease whose major risk factor is dyslipidemia (Bermúdez *et al*., 2012) [3]. These differences could be accounted for by differences in dietary habits (eating foods rich in saturated fatty acids and high carbohydrate levels), lifestyle characteristics such as sedentary lifestyles (lack of exercise), consumption of alcohol and smoking. In addition, socio-economic status, geographical location, inclusion criteria used to recruit the referents, ethnicity, race and genetics of the studied population could also be contributors. Populations eating foods rich in saturated fatty acids and high carbohydrate levels such as broilers, eggs, red meat and milk from cattle, goats and sheep, and high carbohydrate foods such as sweet potatoes, maize, sorghum, and millet which are all available and consumed in Taita-Taveta County, and having sedentary lifestyles especially people living in urban areas have higher levels of fasting lipid profiles (Lee and An., 2020; O'Neal *et al*., 2019, Turley *et al*., 1998) [10, 12, 14].

The statistically significantly higher total cholesterol: high density lipoprotein cholesterol, and triacylglycerol: high density lipoprotein cholesterol ratios in males compared to females in this study may imply that males of Taita-Taveta County, Kenya are at a greater risk of coronary artery disease than females. However, the risk of coronary heart disease and especially in males was not investigated in this Taita-Taveta population. These high values of the two ratios (T-CHOL: HDL-CHOL and TG: HDL-CHOL) may indicate that the males of Taita-Taveta County are consumers of high carbohydrate and high fat diets with minimal exercise (Agrawal *et al*., 2014) [2]. Most Taita-Taveta County males are businessmen. Such carbohydrate rich diets may include wheat products (sold in the markets), sweet potatoes, maize, sorghum, and millet while the high fat diets may include broilers, eggs, fish, red meat and milk (butter, ghee) from cattle, goats and sheep, all of which are available and consumed in Taita-Taveta County. Consumption of fish which is rich in omega-3-fatty acids leads to increased levels of high density lipoprotein cholesterol (HDL-Chol; good cholesterol) in the blood (Agrawal *et al*., 2014) [2]. At the family level (at home) males may also be consuming more broilers without removing the skin which has high fat content and eggs, and more red meat (animal fat) and milk (ghee, butter) from cattle, goats and sheep. The males could also be taking medications which modify the fasting lipid profile levels and their ratios, as well as being alcoholics and smokers despite the use of the self-reported questionnaire to eliminate such participants in the exclusion criteria (KPHC IV, 2019) [9]. This study did not investigate the foods consumed, the amounts, and their composition by Taita-Taveta population. High intake of high carbohydrate low fat diet leads to reduced levels of total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol (HDL) and increased levels of triacylglycerols (Lee and An., 2020; Turley *et al*., 1998) [10, 14]. Intake of low carbohydrate high fat diet leads to increased levels of total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol (O'Neal *et al*., 2019) [12].

Results of this study indicating statistically similar reference interval limits for total cholesterol, high density lipoprotein cholesterol, non-high density lipoprotein cholesterol, triacylglycerols and total cholesterol: high density lipoprotein cholesterol ratio for adult and geriatric population of Taita-Taveta County, Kenya with advancement of age implies that these parameters are not affected by age. These results contrast the well-known fact that body fat content increases with advancement of age because of reduced overall energy expenditure caused by reduced activity with age (Buffa *et al.* 2011). These observations are in agreement with those reported by Kaur *et al.* (2012) [8] for combined reference intervals for total cholesterol, high density lipoprotein cholesterol, triacylglycerols and total cholesterol: high density lipoprotein cholesterol ratio for adults and geriatrics of Punjab with advancement of age. However, Kaur *et al.* (2012) [8] also reported increasing reference interval limits for triacylglycerols (0.73-2.03 mmol/L at age 51-60 years and 0.93-2.08 mmol/L at age above 70 years for females) and decreasing reference interval limits for total cholesterol (3.17-6.46 mmol/L at age 51-60 years and 2.77-6.76 mmol/L at above 70 year in females) with advancement in age. Further, the reference interval limits for total cholesterol (3.17-6.46 mmol/L for females versus 2.78-6.63 mmol/L for males at age 51-60 years, 2.91-6.88 mmol/L for females versus 2.74-6.31 mmol/L for males at age 61-70 years, and 2.77-6.76 mmol/L for females versus 3.15-5.85 mmol/L for males at above 70 years), high density lipoprotein cholesterol (0.93-1.46 mmol/L for females versus 0.75-1.49 mmol/L for males at age 51-60 years, 0.84-1.56 mmol/L for females versus 0.67-1.49 mmol/L for males at age 61-70 years, and 0.72-1.49 mmol/L for females versus 0.82-1.34 mmol/L for males at above 70 years), low density lipoprotein cholesterol (1.20-4.79 mmol/L for females versus 1.22-4.47 mmol/L for males at age 51-60 years, 1.12-4.89 mmol/L for females versus 1.16-4.44 mmol/L for males at age 61-70 years, and 1.40-4.56 mmol/L for females versus 1.60-4.17 mmol/L for males at above 70 years), and triacylglycerols (0.73-2.03 mmol/L for females versus 0.57-2.17 mmol/L for males at age 51-60 years, 0.66-2.30 mmol/L for females versus 0.75-2.04 mmol/L for males at age 61-70 years, and 0.93-2.08 mmol/L for females versus 0.55-1.89 mmol/L for males at above 70 years) were high in females than males at the three age categories reported by Kaur *et al.* (2012) [8]. In addition, Kaur *et al.* (2012) [8] reported that the total cholesterol to high density lipoprotein cholesterol (2.43-5.57 for females versus 2.34-5.86 for males at age 51-60 years, 2.14-6.06 for females versus 2.14-6.46 for males at age 61-70 years, and 2.73-5.87 for females versus 2.63-5.77 for males at above 70 years), and low density lipoprotein cholesterol to high density lipoprotein cholesterol (0.93-4.07 for females versus 1.03-4.17 for males at age 51-60 years, 0.74-4.26 for females versus 0.84-4.36 for males at age 61-70 years, and 1.52-3.88 for females versus 1.33-4.07 for males at above 70 years) ratios were not affected by sex and advancement with age.

This study findings contrast those reported by Das and Saikia (2009) [6] who demonstrated increasing reference interval limits for total cholesterol (4.33 (2.69-6.0) mmol/L for males versus 4.84 (3.23-6.54) mmol/L for females at 51-60 years and 4.09 (2.51-5.97) mmol/L for males versus 5.12 (3.52-6.34) mmol/L for females at above 70 years) and high density lipoprotein cholesterol (0.98 (0.67-1.37) mmol/L for males versus 1.14 (0.73-1.61) mmol/L for females at 51-60 years and 1.06 (0.62-1.53) mmol/L for males versus 1.30 (0.73-1.89) mmol/L for females at above 70 years), and decreasing values for triacylglycerols (1.34 (0.49-2.55) mmol/L for males versus 1.28 (0.51-2.33) mmol/L for females at 51-60 years and 1.16 (0.59-1.99) mmol/L for males versus 1.11 (0.72-2.54) mmol/L for females at above 70 years) for adult and geriatric population for Indian Assamese with advancement in age.

A number of limitations were associated with this study. The first of these limitations is that the eating habits and the physical activity which can affect the fasting lipid profile levels of the Taita-Taveta County referent population used in this study were not investigated. The second limitation is that the inclusion criteria used was based on self-reporting questionnaire by the referent subjects who may not have given their true health status knowingly or unknowingly. This may have allowed participants who had diabetes mellitus, endocrine disorders, dyslipidemia, renal disease, hypertension, liver obstruction, cardiovascular disease, and those on medications which could influence the levels of fasting lipid profiles. It may also have allowed smokers, alcohol abusers, caffeine abusers, and pregnant women to participate in the study. However, participants with excessive body weight and those on strenuous exercise would have easily been identified and stopped from participating in the reference interval development study. In addition, because of differences in diet and geographic locations in the coastal counties, these reference intervals may not be adopted for all the coastal counties and the rest of Kenyan counties. Further, the sample size for the two age categories were lower than the recommended minimum sample size for reference interval development of 120 referent individuals by CLSI {EP28 3c guideline}, (2010) [5].

In conclusion, this study generated sex and age specific fasting lipid profile reference interval limits for adults and geriatrics of Taita-Taveta County, Kenya which was different from those previously reported in medical literature from other parts of the world. These developed fasting lipid profiles reference intervals for adult and geriatric population of Taita-Taveta County, Kenya can be adopted and used to make appropriate clinical decisions leading to improved diagnosis of cardiovascular diseases.

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