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Reference Values for Serum Lipid Profiles in Iranian Adults: Tehran Lipid and Glucose Study

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Abstract

Background: Lipid abnormalities are major risk factors for cardiovascular diseases. In addition to age and sex, other variables can affect serum lipid levels, warranting the determination of population-specific reference values. This study aimed to determine age- and sex-specific reference values for serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) in healthy Tehranian adults.

Methods: TC, TG, and HDL-C were measured using the enzymatic colorimetric method and the Friedewald equation (LDL-C = TC – HDL-C – TG/5) was used to calculate LDL-C concentrations in individuals with TG <400 mg/dL. After applying the exclusion criteria, 1147 participants (548 men and 599 women) aged ≥20 years were included. For determining reference values, the International Federation of Clinical Chemistry guidelines (non-parametric method) and the robust method were used for sample sizes ≥120 and <120, respectively.

Results: Reference values for serum TC, LDL-C, HDL-C, and TG were 121.0–261.0, 54.1–175.2, 30.9–71.9, and 46.9–301.2 mg/dL in men and 117.8–235.9, 49.9–160.9, 36.0–83.9, and 38.1–184.2 mg/dL in women, respectively. All parameters except HDL-C were higher in men than women and showed an increasing trend with age.

Conclusion: Reference values for serum TC, LDL-C, HDL-C, and TG in healthy Tehranian adults were determined, and these values could provide the basis for better decision making in both prevention and clinical settings.

Keywords: Cholesterol, Coronary heart disease, Lipid, Reference values, Triglycerides

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Introduction

Lipid abnormalities, major risk factors for cardiovascular diseases (CVD) are the leading cause of mortality worldwide.[1-3] Circulating levels of total cholesterol (TC),[4] low-density lipoprotein-cholesterol (LDL-C),[4-6] high-density lipoprotein-cholesterol (HDL-C),[4,7] and triglycerides (TG)[5,8] are known to be associated with CVD. Reference values are the most widely used medical decision-making tools[9,10] that help clinicians in interpreting laboratory results and therefore play a vital role in clinical practice.[11,12] In addition to age and sex, other factors including genetic background, ethnicity, dietary habits, life style, and environment could also affect serum lipid profiles.[13] Ranges of serum lipid profiles vary in different populations,[14] warranting the importance of determining population-specific reference values as proposed by the International Federation of Clinical Chemistry (IFCC).[15] To the best of our knowledge, there is one study focused on the reference values for serum lipid profile in Iranians[16]; the aim of this study was, therefore, to establish age and sex-specific reference values of TC, LDL-C, HDL-C, and TG in a healthy sample of Tehranian adults from a population-based study.

Materials and Methods

Participants

Data were obtained from participants of the Tehran Lipid and Glucose Study (TLGS), a prospective study, initiated in 1999 with the aim of determining the prevalence of non-communicable disease risk factors.[17] In this study, 10 821 participants from phase 5 of the TLGS (September 2011 to January 2015), aged ≥ 20 years, were included. Smokers, diabetics and hypertensive patients, those with body mass index ≥25 kg/m^2, estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2, cancer, CVD, thyroid dysfunction, significant weight loss during past 6 months, hospitalization during the past 3 months, and those with any other chronic disease were excluded from the study. Pregnant and lactating

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women, those who received medications including hormone therapy, hormone replacement therapy, diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers, beta-blockers, steroids, thyroid drugs, aspirin or other anticoagulants, and other cardiac drugs were also excluded. After excluding those who had missing data, 1185 participants (551 men, 634 women) remained for analysis. Since LDL-C concentrations were calculated using the Friedewald equation, four participants (1 woman and 3 men) with TG ≥400 mg/dL were also excluded. In addition, separate analyses were done for 34 menopausal women. Finally, 1,147 participants (548 men and 599 women) were included for determining reference values.

Anthropometric, Clinical, and Laboratory Assessments

Details for data collection in the TLGS have previously been published.[18] Height and weight were measured according to standard protocols and body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Waist circumference (WC) was measured at the level of the umbilicus and hip circumference at the widest point over light clothing. Systolic and diastolic blood pressures were measured twice on the right arm after a 15 minute rest. Measurements were performed in a sitting position, using a standardized mercury sphygmomanometer, and the mean of the two measurements was reported as the participant's blood pressure.

Blood samples, obtained between 7:00 and 9:00 AM after 12–14 hour overnight fasting using anticoagulant-free tubes, were centrifuged at 3000 rpm for 10 minutes, 30–45 minutes after collection. All blood analyses were done at the TLGS research laboratory on the day of collection. For oral glucose tolerance tests, blood samples were taken 2 hrs after oral administration of a solution containing 75 g anhydrous glucose. TC, TG, and HDL-C were measured using the enzymatic colorimetric method. For the TC assay, cholesteryl ester hydrolase was used to convert cholesteryl ester to cholesterol, which was then oxidized by cholesterol oxidase to cholesterol-4-en-3-one and H_2O_2. For the TG assay, TG was broken down to glycerol and free fatty acids using lipoprotein lipase and glycerol was then phosphorylated to glycerol phosphate by glycerokinase; glycerol phosphate was converted to dihydroxyacetone phosphate and H_2O_2 by glycerol phosphate oxidase.

Before measuring HDL-C, apolipoprotein B containing lipoproteins were precipitated with phosphotungstic acid and magnesium ions. The colorimetric indicator in all the aforementioned analyses is quinoneimine, which is prepared from 4-aminoantipyrine and phenol by H_2O_2 and is measured at 546 nm. The Friedewald equation (LDL-C = TC – HDL-C – TG/5) was used to calculate LDL-C concentrations in samples with TG <400 mg/dL.[19] Serum glucose was measured using the enzymatic colorimetric method; glucose was oxidized to glucuronic acid and H_2O_2 by glucose oxidase, the colorimetric indicator being quinoneimine. Serum creatinine was measured using the photometric Jaffe method in which creatinine reacts with picrate in an alkaline medium to yield an orange–red color, read at 505 nm.

Analyses were performed using commercial kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra Pro M auto-analyzer (Vital Scientific, Spankeren, the Netherlands). The quality of assays was monitored using assayed serum controls at two different concentrations. Intra- and inter-assay coefficients of variation (CV) were both 3.0% for TC, 2.3% for TG, 4.6% for HDL-C, and 2.1% for creatinine.

Determining Outliers

After defining the data set, individuals with unknown medical problems or "outliers" may still be included and lead to widening of reference values. Before estimating the reference values, outliers were removed from the data set using the Dixon outlier range statistic as recommended by the Clinical and Laboratory Standards Institute (CLSI).[9] In the Dixon test, D is defined as the absolute difference between the most extreme value and the next most extreme value, and R is the range of the values; if the D/R ratio exceeds 1/3, the extreme value is considered an outlier and must be deleted.[20]

Determining Reference Values

For determining reference values, the CLSI/IFCC guidelines (the non-parametric method) were used for sample sizes ≥120 and the robust method was used for sample sizes <120.[21] The retrospective (posteriori) selection of individuals from a population-based study was used as it is considered ideal for the study of exclusion and partitioning criteria according to IFCC.[22] For the IFCC non-parametric method, values were sorted in ascending order and rank numbers were assigned to values. Rank numbers of the 0.025 and 0.975 fractals were computed as $0.025 \times (N + 1)$ and $0.975 \times (N + 1)$, respectively and considered as reference intervals. To compute the 90% confidence intervals for reference values, we used rank numbers presented by IFCC according to sample size.[23] For determining reference values by the robust method we used bootstrap principle which consists of repeated random resampling of original data with replacement[24]; the software was generously provided by Professor Paul S. Horn (University of Cincinnati).

Definitions of Variables

In accordance with the definition of the American Diabetes Association, subjects were considered to have diabetes if they have fasting glucose ≥ 126 mg/dL or 2-hour glucose ≥200 mg/dL or taking anti-diabetic medications.[25] Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or pharmacological treatment for hypertension.[26] CVD was defined as history of coronary heart disease (CHD) and/or stroke. CHD was defined as symptomatic ischemic heart disease, including

myocardial infarction, acute coronary syndrome, myocardial ischemia demonstrated by noninvasive testing, and history of coronary artery procedures.[6] Thresholds for defining lipid disorders were those recommended by the ATPIII guidelines.[6] Smokers were defined as those smoking ≥1 cigarette per day or using a waterpipe.

Statistical Analysis

IBM SPSS software (SPSS Inc., Chicago, IL, version 20) was used for statistical analyses. Baseline variables including age, BMI, WC, systolic blood pressure, diastolic blood pressure, fasting serum glucose and serum creatinine were reported as mean and standard deviation. To compare baseline variables between men and women, the independent sample *t* test was used. The independent sample *t*-test was also used for comparing serum lipid profiles between men and women at different age groups. Linear regression analysis was run to determine trend of TC, LDL-C, HDL-C and TG changes with age in both sexes; median age in each quartile was used as the independent variable. *P* values < 0.05 were considered statistically significant.

Results

Table 1 shows characteristics of healthy participants used for determining reference values; men were older than women and had significantly higher values of BMI, WC, systolic and diastolic blood pressures, fasting serum glucose, and serum creatinine.

Table 1. Characteristics of Healthy Study Participants Used for Determining Reference Values

	Men (n = 548)	Women (n = 599)	*P* Value[b]
Age, year [a]	40.2 ± 16.2	30. ± 7.2	<0.001
Body mass index, kg/m²	22.6 ± 1.9	22.2 ± 2.0	<0.001
Waist circumference, cm	84.7 ± 6.5	76.6 ± 6.4	<0.001
Systolic blood pressure, mm Hg	110 ± 11	100 ± 10	<0.001
Diastolic blood pressure, mm Hg	73.7 ± 7.0	67.6 ± 7.8	<0.001
Fasting serum glucose, mg/dL	92.4 ± 7.6	88.8 ± 6.5	<0.001
Creatinine, mg/dL	1.18 ± 0.14	0.96 ± 0.09	<0.001

[a] Data are shown as mean ± SD; [b] by independent *t* test.

Sex- and age-specific reference values of TC, LDL-C, HDL-C and TG have been presented in Tables 2–5 respectively. Reference values for serum TC were 121.0–261.0 mg/dL in men and 117.8–235.9 mg/dL in women. Among men, the highest upper limit was registered in those aged ≥50 years; the corresponding value in non-menopausal women was documented between 40-50 years. The upper reference value was higher for menopausal-women compared with non-menopausal women (Table 2).

Reference values for serum LDL-C concentrations were 54.1–175.2 mg/dL in men and 49.9–160.9 mg/dL in women, and both upper and lower limits showed a tendency to increase in both sexes; the highest upper limits were registered for ≥50 and 40–50 year old men and women respectively. Menopausal women had the highest upper limits among both sexes and all age groups (Table 3).

Overall 95% reference values for serum HDL-C concentrations were 30.9–71.9 mg/dL in men and 36.0–83.9 mg/dL in women. In non-menopausal women, reference values for serum HDL-C were similar at all ages; a similar pattern was also observed for lower limits in men. Lower limits for HDL-C were higher in women compared to men, although it was nearly the same for men aged ≥ 50 and menopausal women (Table 4).

Reference values for serum TG concentrations were 46.9–301.2 mg/dL and 38.1–184.2 mg/dL in men and women respectively. In both sexes, the lowest and highest upper limits were registered for the 20–30 and 40–50 year age groups, respectively; the upper reference value was higher for menopausal women compared with their non-menopausal counterparts (Table 5).

A comparison of serum lipid profiles between men and women at different age groups is shown in Figure 1; in both sexes, serum TC concentrations increased significantly ($P < 0.001$) with age. Men in the age groups of 20–29 (*P*

Table 2. Reference Intervals for Serum Total Cholesterol Concentration (mg/dL) According to Age and Sex[a]

	n	95% Reference Intervals		Median	IQR	Min	Max
		Lower (90% CI)	Upper (90% CI)				
Men							
20–30	188	109.8 (83.1–119.9)	254.8 (249.0–278.8)	163.2	143.1–179.8	71.2	278.8
30–40	115	123.0 (119.1–126.8)	250.2 (243.2–259.1)	175.6	143.1–163.2	187.9	276.9
40–50	97	131.1 (121.8–138.8)	247.9 (239.0–259.9)	189.1	170.2–208.8	10.8	274.2
≥50	146	134.2 (97.8–141.1)	269.1 (257.2–281.9)	194.9	167.0–220.0	97.8	281.9
All	546[b]	121.0 (109.8–124.1)	261.0 (216.2–247.9)	177.9	155.8–204.1	71.2	317.1
Women							
20–30	320	107.9 (102.1–119.1)	233.9 (222.0–251.0)	157.0	1400–177.9	83.1	259.1
30–40	209	126.1 (107.1–131.1)	233.2 (222.0–262.7)	169.0	153.1–189.1	104.0	263.0
40–50	63	141.9 (126.8–148.1)	254.1 (242.8–266.0)	187.9	172.8–215.0	143.1	271.0
Menopause	34	138.0 (129.2–146-9)	271.1 (256.0–276.1)	199.2	169.0–223.1	139.9	278.0
All[c]	597[b]	117.8 (107.1–123.0)	235.9 (228.9–247.1)	165.1	165.1–148.1	83.1	271.2

[a]Clinical and Laboratory Standards Institute (CLSI)/International Federation of Clinical Chemistry (IFCC) criteria (nonparametric method) and robust methods were used for sample sizes ≥120 and ≤120 respectively.
[b]Outliers were excluded in each age group separately, therefore the total number is not equal to the sum of the number of participants in each group.
[c]Not including menopausal women.

Table 3. Reference Intervals for Serum LDL-C Concentration (mg/dL) According to Age and Sex[a]

	n	95% Reference intervals		Median	IQR	Min	Max
		Lower (90% CI)	Upper (90% CI)				
Men							
20–30	187	49.1 (37.1–53.0)	167.8 (152.0–199.2)	95.9	76.2–113.0	37.1	199.9
30–40	115	59.2 (54.9–63.0)	165.9 (158.9–172.8)	107.1	85.8–129.2	54.1	187.9
40–50	97	56.1 (48.0–64.2)	172.8 (165.1–170.9)	114.8	95.1–136.1	39.1	193.0
≥50	145	59.2 (44.9–73.1)	187.2 (172.8–199.2)	117.2	95.9–143.8	44.9	199.2
All	544[b]	54.1 (46.0–56.8)	175.2 (172.1–187.9)	107.1	85.1–129.9	37.1	20.1
Women							
20–30	320	44.9 (42.2–51.8)	153.1 (134.2–167.0)	85.8	71.9–104.0	20.9	187.2
30–40	209	54.1 (44.9–59.9)	158.9 (146.2–172.1)	95.1	82.0–114.1	27.8	172.1
40–50	63	71.9 (68.1–75.0)	172.1 (163.2–179.8)	109.8	95.9–136.1	73.1	170.9
Menopause	34	56.8 (47.6–66.9)	194.1 (182.1–206.1)	124.1	95.1–148.9	59.9	184.1
All[c]	599[b]	49.9 (44.9–53.0)	160.9 (148.9–167.0)	92.0	78.1–109.1	20.9	187.2

Abbreviations: LDL-C, Low-density lipoprotein–cholesterol; IQR, Inter Quartile Range; n, number; Min, Minimum; Max, Maximum.
[a]Clinical and Laboratory Standards Institute (CLSI)/International Federation of Clinical Chemistry (IFCC) criteria (nonparametric method) and robust methods were used for sample sizes ≥120 and ≤120 respectively.
[b]Outliers were excluded in each age group separately, therefore the total number is not equal to the sum of the number of participants in each group.
[c]Not including menopausal women.

Table 4. Reference Intervals for Serum HDL-C Concentration (mg/dL) According to Age and Sex[a]

	n	95% Reference Intervals		Median	IQR	Min	Max
		Lower (90% CI)	Upper (90% CI)				
Men							
20–30	190	30.9 (30.2–32.9)	75.4 (65.4–85.5)	47.6	42.54–54.52	24.75	86.2
30–40	115	30.9 (30.2–32.1)	67.3 (64.6–70.4)	45.6	38.67–38.67	29.78	76.2
40–50	97	27.1 (25.9–29.0)	64.6 (61.5–67.3)	43.7	35.58–35.58	23.98	71.5
≥50	146	32.1 (30.9–35.2)	73.5 (70.4–77.3)	48.7	39.83–48.72	30.55	74.2
All	548[b]	30.9 (30.2–30.9)	71.9 (69.2–75.4)	46.8	29.78–53.36	23.98	86.2
Women							
20–30	320	36.0 (32.9–37.9)	83.1 (78.5–88.2)	55. 7	47.6–64.6	27.8	92.0
30–40	211	36.0 (32.9–37.9)	83.1 (77.3–97.1)	53.4	46.8–61.5	29.8	97.1
40–50	63	36.0 (32.9–37.9)	82.4 (76.2–87.4)	53.4	47.6–63.4	29.8	92.0
Menopause	34	32.9 (30.9–36.0)	84.3 (78.5–91.3)	50.7	43.7–61.5	25.9	92.0
All[c]	599[b]	36.0 (35.2–37.9)	83.9 (80.4–87.4)	54.5	47.6–63.4	27.8	97.1

Abbreviations: HDL-C, High-density lipoprotein-cholesterol; IQR, Inter Quartile Range; n, number; Min, Minimum; Max, Maximum.
[a]Clinical and Laboratory Standards Institute (CLSI)/International Federation of Clinical Chemistry (IFCC) criteria (nonparametric method) and robust methods were used for sample sizes ≥120 and ≤120 respectively.
[b]Outliers were excluded in each age group separately, therefore the total number is not equal to the sum of the number of participants in each group.
[c]Not including menopausal women.

= 0.086) and 30-39 (P = 0.012) years had higher serum TC concentrations than women, values however were comparable between men and women, aged 40–49 years. Menopausal women had slightly higher values of serum TC than men ≥ 50 years, although the difference was not statistically significant. A similar pattern was observed for LDL-C concentrations. No trend was observed for changes in serum HDL-C concentrations with age in both sexes. Compared to men, women had significantly higher HDL-C concentrations in all age groups (P < 0.001 for age <50 and P = 0.020 for age ≥ 50 years). In both sexes, serum TG concentrations significantly (P < 0.001) increased with age; in addition, serum TG concentrations were significantly (P < 0.001) higher in men than women in all age groups except for those ≥ 50 years; however no such difference was observed between menopausal women and men > 50 years.

Prevalence of TC concentrations above the optimum level (≥ 200 mg/dL) was 28.4% and 14.0% in men and women, respectively. For 2.2% of women and 7.3% of men, TC concentrations were at high risk levels (≥240 mg/dL); in both sexes, the percentage of TC concentrations ≥ 200 mg/dL increased with age. Prevalence of TG levels ≥ 300 mg/dL was 26.8% in men and 6.8% in women. The prevalence of LDL-C concentrations ≥ 130 mg/dL) was 24.7% and 9.1% in men and women, respectively. For both sexes, the prevalence of high risk categories of LDL-C levels increased with age with a sharp increase in menopausal women compared to other age groups. Among men, 21.7% and among women, 6.2% were in the low HDL-C category (< 39.8 mg/dL); and 13.0% of men versus 34.5% of women had high HDL-C levels (≥ 60 mg/dL).

Discussion

This study presents age and sex-specific reference values for TC, LDL-C, HDL-C, and TG in a sample of healthy Tehranian adults selected from a population-based study.

Table 5. Reference Intervals for Serum Triglycerides Concentration (mg/dL) According to Age and Sex[a]

	n	95% Reference Intervals		Median	IQR	Min	Max
		Lower (90% CI)	Upper (90% CI)				
Men							
20–30	188	44.3 (31.9–46.1)	229.4 (190.4–257.8)	87.7	69.1–118.7	20.4	335.7
30–40	112	48.7 (54.9–0.6)	264.0 (240.0–287.0)	105.4	85.9–148.8	38.1	248.9
40–50	97	55.8 (51.4–60.2)	314.4 (287.0–340.1)	137.3	90.4–180.7	56.7	357.8
≥ 50	144	48.7 (44.3–54.0)	272.8 (253.3–281.7)	117.8	78.8–153.2	44.3	281.7
All	548[b]	46.9 (44.3–48.7)	301.2 (270.2–336.6)	123.1	86.8–171.7	20.4	398.6
Women							
20–30	320	38.1 (36.3–39.9)	171.0 (163.0–186.9)	70.9	55.8–94.8	35.4	224.1
30–40	211	37.2 (33.7–44.3)	194.9 (168.3–258.6)	77.1	61.1–108.1	33.7	258.6
40–50	62	53.1 (48.7–54.9)	227.6 (193.1–264.0)	91.2	76.2–124. 9	53.1	295.8
Menopause	34	47.8 (43.4–54.9)	230.3 (204.6–254.2)	105.4	84.1–156. 8	54.9	217.9
All[c]	597[b]	38.1 (37.2–39.9)	184.2 (168.3–224.1)	76.2	60.2–101.9	33.7	295.8

Abbreviations: IQR, inter quartile range; n, number; Min, Minimum; Max, Maximum.
[a] Clinical and Laboratory Standards Institute (CLSI)/International Federation of Clinical Chemistry (IFCC) criteria (nonparametric method) and robust methods were used for sample sizes ≥120 and ≤120 respectively.
[b] Outliers were excluded in each age group separately, therefore the total number is not equal to the sum of the number of participants in each group.
[c] Not including menopausal women.

Reference values for serum lipid profiles in the current study and values reported in some other countries are summarized in Table 6; in these studies, reference values are presented as the 2.5 and 97.5 percentiles, 5th and 95th percentiles, and mean ± 2SD. Results of a study conducted in Ahvaz city (in southern Iran) showed that "Lore" (a known ethnicity of Iran) participants had higher serum TC levels compared to Arab and Persian groups.[16] As seen in Table 6, large differences are observed between lipid reference values in different populations. According to this table, it seems that upper limits of reference values for TC, TG, and LDL-C in our population are lower than most western countries and higher than most Asian countries. Possible explanations for this dissimilarity include differences in ethnicity[27,32] dietary habits,[32,35] genetic background,[5,32] environmental factors,[27,32] and life styles.[31,32] Regarding LDL-C, the

Table 6. Reference Values for Serum Lipid Concentrations (mg/dL) in the Current Study and Some Other Countries

Country	Year	n	TC	LDL-C	HDL-C	TG	R
Men							
Japan[a]	1991	677	136.1–232.0	NR	27.07–77.73	40.7–148.8	33
Canada[a]	1992	8348	118.7–282.7	54.14–194.90	34.42–69.22	91.2–394.1	34
USA†	1993	4572	143.8–290.8	80.05–208.04	29.00–68.06	54.9–311.8	5
Finland[a]	1994	292	163.6–326.0	103.63–254.45	30.94–74.25	46.0–248.9	35, 36
South India†	2009	1161	121.0–235.1	61.1–257.2	27.8–59.9	57.6–262.2	32
Assamen*	2009	840	96.7–234.0	41.0–172.8	24.0–73. 1	40.7–225.0	29
Ahvaz (Iran)[a]	2013	617	92.0–262.2	19.72–77.3	8.5–24.8	17.7–237.4	16
North India*	2013	865	123.7–235.1	63.0–157.8	32.1–58.0	47.8–205.5	27
Burkina Faso*	2015	139	113.3–224.7	45.6–154.3	25.1–68.4	NR	30
Netherlands[a]	2017	54065	174.0–223.9	47.2–67.7	19.0–25.1	71.7–144.4	46
Current study (Tehran)		548	121.0–261.0	54.1–175.2	30.9–71.9	46.9–301.2	
Women							
Japan‡	1991	467	135.0–230.9	NR	36.0–82.0	31.0–134.6	33
Canada‡	1992	8571	114.1–282.7	48.0–190.3	28.2–80.8	31.9–276.4	34
USA†	1993	4114	143.1–305.1	80.8–220.0	32.9–79.7	48.7–263.1	5
Finland‡	1994	299	158.9–332.2	97.1–253.3	36.7–85.1	40.7–202.0	35, 36
South India†	2009	762	119.1–235.1	56.8–157.0	32.1–67.0	54.0–217.9	32
Assamen*	2009	645	92.8–263.0	39.8–172.8	22.8–73.1	39.9–256.0	29
Ahvaz (Iran) ‡	2013	601	101.3–249.4	17.4–75.8	9.8–30.6	6.2–211.7	16
North India*	2013	662	125.7–-232.8	64.2–155.8	32.9–65.0	47.8–203.7	27
Burkina Faso*	2015	140	113.3–226.6	44.9–161.7	30.6–65.0	NR	30
Netherlands‡	2017	79475	166.3–199.9	42.5–62.3	21.7–31.7	57.6–105.4	46
Current study (Tehran)		594	117.8–235.9	49.9–160.9	36.0–83.9	38.1–184.2	

* 2.5-97.5 percentile; † 5-95 percentile; ‡ mean ± 2 SD.
Abbreviations: NR: Not reported; LDL-C, Low-density lipoprotein-cholesterol; HDL-C, High-density lipoprotein-cholesterol; TC, Total cholesterol; TG, Triglycerides; R, References.

measurement method (direct measurement vs. calculation by the Friedewald formula) could also affect results. It has been reported that the results of Friedewald's formula and the direct measurement of LDL-C are not identical, which could affect patient classification.[37]

Despite the stringent exclusion criteria used in our study to select the reference sample, the upper limits of TC (261.0 mg/dL in men and 235.9 mg/dL in women), LDL-C (175.2 mg/dL in men and 160.9 mg/dL in women) and TG (301.2 mg/dL in men and 184.2 mg/dL in women) were higher; the lower limits of HDL-C (30.9 mg/dL in men and 36.0 mg/dL in women) were lower than cut-off points recommended by NCEP-ATPIII guidelines for incident CHD. These findings are similar to those reported previously.[32,35] Cut-off points may be lower than the upper limit of reference intervals and should not be confused with the upper reference limits.[38,39] Reference values are obtained in a healthy population and have high specificity for health; however clinical decision limits or cut-off points consider both sensitivity and specificity of a disease and define thresholds for a specific disease associated with higher risk of the adverse clinical outcomes.[40] According to the ATPIII, 7.3% of men and 2.2% of women in our selected "healthy" sample were categorized as having high risk for their TC concentrations.

A comparison of serum lipid profiles between men and women at different age groups is shown in Figure 1. In both sexes, serum TC, TG and LDL-C concentrations significantly increased with age while no trend was observed for HDL-C. In addition, in all age groups, serum HDL-C and TG concentrations were, respectively, higher and lower in women than in men. Regarding changes in lipid profiles with age and sex, our results are consistent with those reported from Canada,[34] Finland,[35] and China,[31] which also reported higher TC and LDL-C concentrations in men, compared to women ≤50 years old and slightly higher values in menopausal women, possibly due to decreasing estrogen activity. These studies report an increasing trend in TC and LDL-C concentrations with age in both sexes, a finding again similar to our results.

In agreement with our results, higher HDL-C values in women, with no significant trend among age groups, have been reported in studies conducted in Canada,[34] Finland,[35] China,[31] and India.[27,28,32] Higher HDL-C concentrations in women are attributed to the effects of sex hormones since estrogen increases and testosterone decreases serum HDL-C concentrations.[41,42] TG concentration trends show great diversity among different studies. South India[32] reported a pattern similar to ours. Canada and China[31,34] reported a decrease in TG concentration in older age groups, a finding similar to our results in men; however, they also reported slightly higher values in women compared to men in these age groups, a pattern not seen in our study.

Regarding the strengths of this study, we determined the reference values in healthy individuals selected from a population-based study, which may provide the best reference values for use in preventive medicine.[43] In addition, although health is a relative concept difficult to validate,[20] we applied restrict exclusion criteria. One limitation of this study is that we calculated LDL-C using the Friedewald formula; direct measurement of LDL-C is possible and is recommended by the NCEP Working Group on Lipoprotein Measurement;

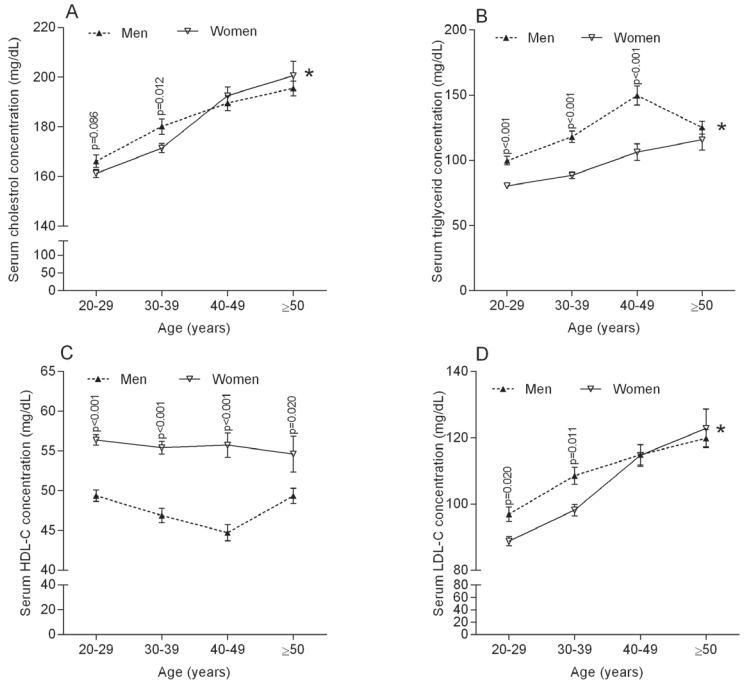

Figure 1. Comparison of Serum Lipid Profile Between Men and Women in Different Age Groups. LDL-C, Low-density lipoprotein-cholesterol; HDL-C, High-density lipoprotein-cholesterol. * *P* for trend < 0.001.

all available direct methods, however, have limitations for general use[44,45] and currently, Friedewald's formula is the most commonly used method for determining LDL-C concentrations in clinical laboratories.

In conclusion, this study reported reference values for lipid profile in healthy Tehranian adults; values which could definitely help decision-makers in both prevention and clinical settings.

Authors' Contribution

MR and AG designed the study; Data collection and data analysis were performed by MR, MG, AM and AG; MR and AG wrote the first draft of the paper; SJ and AG revised the paper and prepared the final draft. Final approval was given to the final manuscript by all authors.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

The proposal of the manuscript approved by the local ethics committee of Research Institute of Endocrine Sciences (RIES), Shahid Beheshti University of medical Sciences (Code: IR.SBMU.RIES.REC, 1394.129).

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