Atherosclerosis 410 (2025) 120484

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Sex differences in cholesterol levels among prepubertal children

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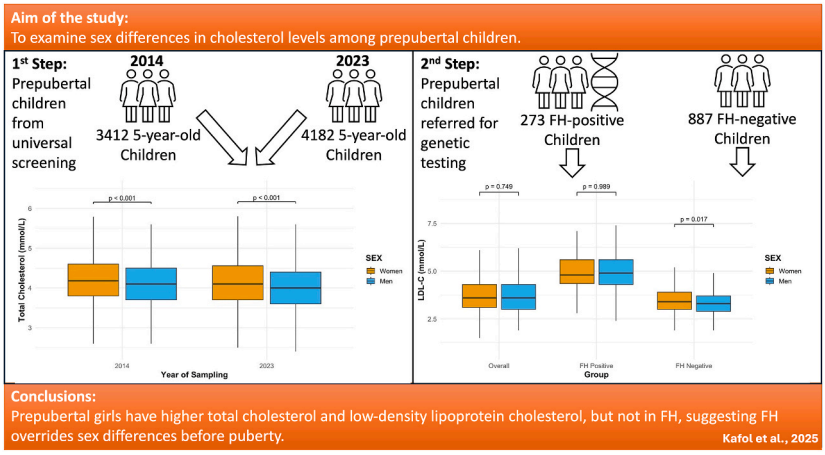
HIGHLIGHTS

- Prepubertal girls have higher total cholesterol than prepubertal boys.
- No significant sex differences in lipids among FH-positive prepubertal children.
- Genetic FH burden may override physiological sex differences before puberty.
- Results support universal cholesterol screening in prepubertal children.
- Sex-specific lipid references may aid risk assessment in prepubertal children.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Sex differences
Prepubertal children
Total cholesterol
Low-density lipoprotein cholesterol

ABSTRACT

Background and aims: Sex differences in cholesterol levels are well documented in adults and adolescents, but limited data exist for prepubertal children. This study aimed to evaluate innate sex differences in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels among prepubertal children, both in the general population and among those with familial hypercholesterolemia (FH).

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https://doi.org/10.1016/j.atherosclerosis.2025.120484
Received 11 May 2025; Received in revised form 17 July 2025; Accepted 7 August 2025
Available online 18 August 2025

Familial hypercholesterolemia
Universal screening

Methods: This cross-sectional study used data from Slovenia's Universal FH Screening Program. Two population-based random samples of children undergoing routine cholesterol screening at age 5 years were included from 2014 (N = 3412) and 2023 (N = 4182). In addition, a referred cohort from the Slovenian Hypercholesterolemia Registry (n = 1160, aged <10 years) who underwent genetic testing was analyzed.
Results: In both the 2014 and 2023 cohorts, girls had significantly higher TC levels than boys (median difference: 0.10–0.11 mmol/L; $p < 0.05$). Among FH-negative children in the Registry, girls had on average 0.14 mmol/L higher TC and 0.13 mmol/L higher LDL-C than boys (both $p < 0.05$). No sex differences were observed in FH-positive children ($p = 0.83$ for TC; $p = 0.82$ for LDL-C). In the overall Registry cohort, after adjusting for FH status, girls had 0.11 mmol/L higher TC and 0.10 mmol/L higher LDL-C (both $p < 0.05$).
Conclusion: Prepubertal girls have modestly higher TC and LDL-C than boys, a difference not observed in prepubertal FH-positive children, suggesting that the presence of a pathogenic FH variant may override innate physiological differences in lipid metabolism. These findings support universal early cholesterol screening and suggest that sex-specific reference values may improve early cardiovascular risk assessment in prepubertal FH-negative children.

1. Introduction

Sex plays a pivotal role in the development of atherosclerotic cardiovascular disease, yet its influence has long been underexplored, with most clinical studies focusing predominantly on men [1]. Robust clinical evidence highlights that female hormones confer significant cardiovascular protection, contributing to a lower disease risk in pre-menopausal women [2,3]. However, following menopause, the incidence of myocardial infarction in women not only increases but ultimately exceeds that observed in men, underscoring a dynamic, hormone-driven shift in cardiovascular risk over the lifespan [4]. Moreover, sex-based differences in lipid profiles—shaped by life events like menstruation, pregnancy, and menopause—affect atherosclerosis; for example, while prepubertal girls tend to have higher low-density lipoprotein cholesterol (LDL-C) than boys, women in early adulthood generally exhibit lower LDL-C and higher high-density lipoprotein cholesterol (HDL-C) levels, a balance that worsens after menopause [5].

Although previous reports have characterized cholesterol levels in prepubertal children, the extent of inherent sex-related differences in lipid profiles before puberty remains incompletely understood [6–9]. This study aims to establish robust baseline cholesterol levels in Slovenian 5-year-olds—prior to the hormonal influences of puberty—and to identify innate sex differences in this population. In addition, we assess sex differences in lipid profiles among prepubertal children genetically tested for familial hypercholesterolemia (FH). By comparing sex differences in a general prepubertal cohort, as well as in FH-positive and FH-negative subpopulations, our investigation seeks to determine whether early-life lipid levels exhibit consistent sex-specific patterns, thereby shedding light on the developmental origins of cardiovascular risk.

2. Materials and methods

This study was approved by the National Medical Ethics Committee of Slovenia (No. 25/10/09; 22/01/2017; 0120-14/2017-2; 0120-14/2017-5; 0120-100/2019/5; 07120-1/2024/161) and conducted in accordance with the Declaration of Helsinki, following EQUATOR reporting guidelines. Informed consent for genetic analysis, was obtained from parents or legal guardians. An overview of the study design and main findings is presented in Fig. 1.

2.1. Slovenian universal familial hypercholesterolemia program

The data was obtained from the first and second phase of Slovenia's Universal FH screening program, which comprises three steps: 1) Measuring total cholesterol (TC) in all children attending the mandatory

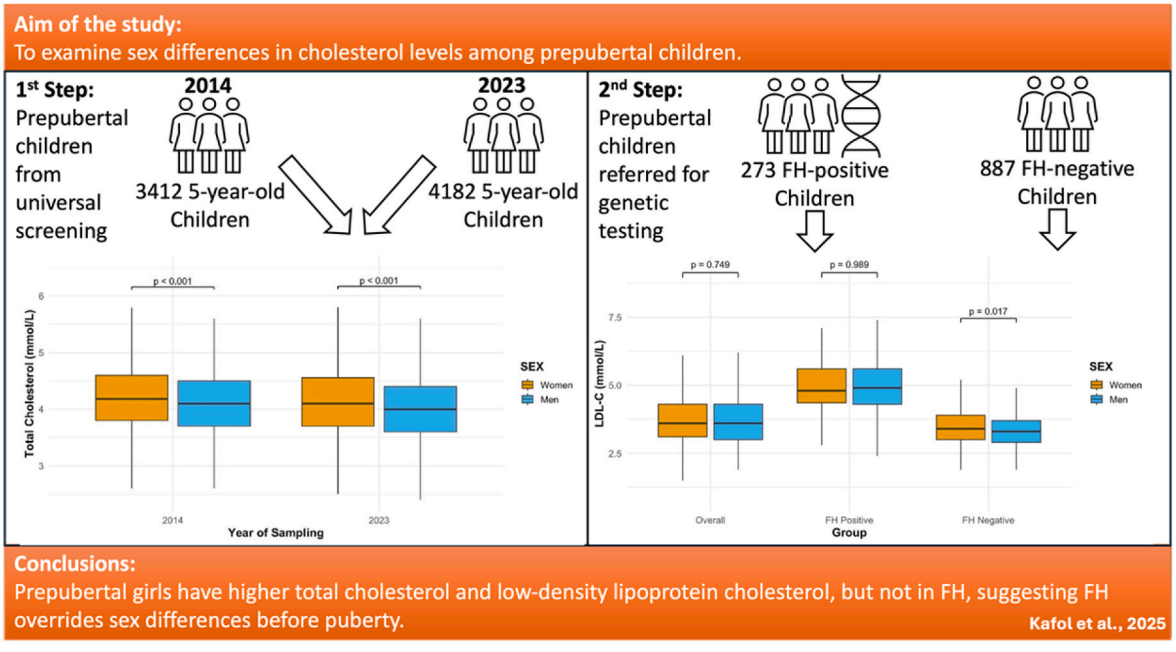

Fig. 1. Graphical summary of the study design and main findings.

preschool examination at primary care pediatricians. 2) Referring all eligible children—those with TC ≥ 6.0 mmol/L (≥232.0 mg/dL), or ≥5.0 mmol/L (≥193.4 mg/dL) combined with a family history of early cardiovascular disease—to the University Children's Hospital at the University Medical Center (UCH UMC) Ljubljana, which serves as the national reference center for FH diagnostics. 3) Performing cascade testing for parents and siblings if FH is confirmed [10,11].

2.2. Study population and sample selection

For our first analysis, we examined two random samples of children from 2014 and 2023. These data were collected during the initial step of Slovenia's universal FH screening program and included children from various regions across the country. The samples were obtained from multiple primary care centers, ensuring the cohort is geographically diverse and representative of the national pediatric population. The dataset included age, sex, body height (BH), body weight (BW), body mass index (BMI), and total TC levels. In the 2014 sample, 3782 children were initially included. Of these, 3638 had available TC measurements, and after excluding children with missing age data, 3412 remained for analysis [12]. According to the Statistical Office of the Republic of Slovenia, 21,856 children were born in 2009 [13]. Thus, our 2014 cohort represented 16.6 % of the entire birth cohort. In the 2023 sample, 4762 children were initially included. After excluding 4 with unclear values, 9 with missing height and weight data, and 576 with missing TC measurements, 4182 children remained for analysis. A total of 20,241 children were born in 2017, meaning that the 2023 sample accounted for 20.6 % of the birth cohort [13]. Taken together, these two samples comprise 7820 children, covering 18.5 % of the relevant birth cohorts (7820 out of 42,097 children born in 2009 and 2017).

For our second analysis, children from the Hypercholesterolemia registry were examined. These data were obtained from individuals who entered the referral and genetic testing phase (step two of screening). Initially, 1575 children and adolescents (<18 years) were included in the registry. After excluding those without LDL-C measurements (N = 7), those lacking height and weight data (N = 48), participants with variants of uncertain significance (VUS) (N = 101), homozygotes for FH (N = 4), and cascade cases (N = 88), the sample was reduced to 1333 subjects. We excluded cascade cases to focus on children identified through universal screening and direct referral, thereby minimizing family-based selection bias and ensuring a more population-representative assessment of early lipid profiles. We also verified that none of the included children were receiving lipid-lowering therapy at the time of their initial visit. For the final analysis, we further restricted the sample to include only children under 10 years of age at the time of their first visit. This cutoff was chosen because it approximates the average onset of puberty in girls—who tend to mature earlier than boys—thereby ensuring that all included subjects were prepubertal and the cohort was more homogeneous [14]. This final step resulted in a sample of 1160 children for analysis.

2.3. Screening protocol and genetic evaluation

In the first screening step, children undergo a clinical examination by a pediatrician during which anthropometric measurements are recorded. Fasting venous blood samples are then collected and analyzed for TC at accredited laboratories using standardized protocols. In the first step of universal screening (2014 and 2023 cohorts), only total cholesterol was measured to ensure simplicity, lower costs, and maximize participation. Detailed lipid profiles, including HDL-C, triglycerides, and LDL-C, were obtained only in children referred for further evaluation as part of the hypercholesterolemia registry. In the second screening step, children are evaluated by a lipid specialist at the UCH UMC Ljubljana, where additional fasting venous blood samples are obtained. LDL-C is calculated using the Friedewald formula when triglyceride levels are below 4 mmol/L, or measured directly when they exceed this threshold. TC, HDL-C, directly measured LDL-C, and triglycerides are analyzed using the Abbott Alinity C analyzer (Abbott Laboratories, USA). Lipoprotein(a) (Lp [a]) was determined by immunonephelometry using the Siemens Atellica Neph 630 (Siemens Healthineers, Ireland). Additionally, children referred to the second step undergo genetic analysis at the same center. Patients are classified as FH-positive if a pathogenic or likely pathogenic variant is detected in one of the three primary FH-associated genes (*LDLR, APOB, or PCSK9*), with genetic variants interpreted according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) [15,16]. The genetic methods used have been described in detail in our previous publications, and the overall structure of the screening program, including diagnostic and referral criteria, is further elaborated in our recent manuscript [10,17].

2.4. Statistical methods

Data were collected using Excel 365 (Microsoft Corporation, USA) and analyzed in R version 4.4.1 (R Foundation for Statistical Computing, Austria). Subjects with missing values were excluded from the analysis, while outlier data points were retained. BMI Z-scores were computed using the British 1990 reference data, employing the LMS Growth add-in for Excel (available at http://www.healthforallchildren.com/shop-base/shop/software/lmsgrowth/) [18,19]. Normality was assessed using the Shapiro-Wilk test, and due to the non-normal distribution of variables, data are presented as medians with interquartile ranges (Q1–Q3). The Wilcoxon test was used for two-group comparisons. A univariate linear regression model was used to assess the relationship between sex and TC. Additionally, Spearman correlation was applied to evaluate the monotonic association between sex and TC. Interaction effects between sex and other covariates were further tested within multivariate regression models to explore whether the association between sex and TC varied across levels of these factors. All tests were two-tailed, with $p < 0.05$ considered statistically significant, and the Benjamini-Hochberg correction was applied to control the false discovery rate.

3. Results

3.1. Baseline characteristics and sex differences in the 2014 and 2023 universal screening cohort

In 2014, the sample included 1638 females (48.0 %) with a median child age of 5.1 years (5.0–5.2), and in 2023, 1962 females (46.9 %) with a median age of 5.7 years (5.1–6.0) were observed. In both samples, no significant age differences were noted between sexes ($p = 0.277$ in 2014; $p = 0.758$ in 2023).

Baseline anthropometric measures also slightly differed by sex in both cohorts. In 2014, females had lower BW (19.2 kg [17.6–21.2]) and BH (112.5 cm [109.5–116.0]) compared to males (BW: 19.6 kg [18.0–21.5]; BH: 113.0 cm [110.0–116.6]; both $p < 0.001$), while BMI was slightly lower in females (15.2 kg/m^2 [14.3–16.3] vs. 15.4 kg/m^2 [14.6–16.3]; $p = 0.005$). The 2023 sample revealed similar trends with females showing lower BW (20.3 kg [18.4–22.6]) and BH (115.5 cm [111.5–119.4]) than males (BW: 20.9 kg [19.0–23.0]; BH: 116.5 cm [112.6–120.5]; both $p < 0.001$) and nearly identical BMI values (15.3 kg/m^2 [14.4–16.3] in females vs. 15.3 kg/m^2 [14.5–16.3] in males; $p = 0.050$).

Median TC levels were consistently higher in females (see Fig. 2A). In 2014, females had a median TC of 4.2 mmol/L (3.8–4.6) versus 4.1 mmol/L (3.7–4.5) in males ($p < 0.001$), and in 2023, the corresponding values were 4.1 mmol/L (3.7–4.6) for females and 4.0 mmol/L (3.6–4.4) for males ($p < 0.001$).

Univariate linear regression estimated that males had lower TC by 0.090 mmol/L in 2014 and 0.127 mmol/L in 2023 (both $p < 0.001$), and Spearman's correlation indicated weak yet significant negative correlations ($\rho = -0.070$ and -0.084, respectively; both $p < 0.001$). Multivariate linear regression further identified sex, age, BH, and BW as

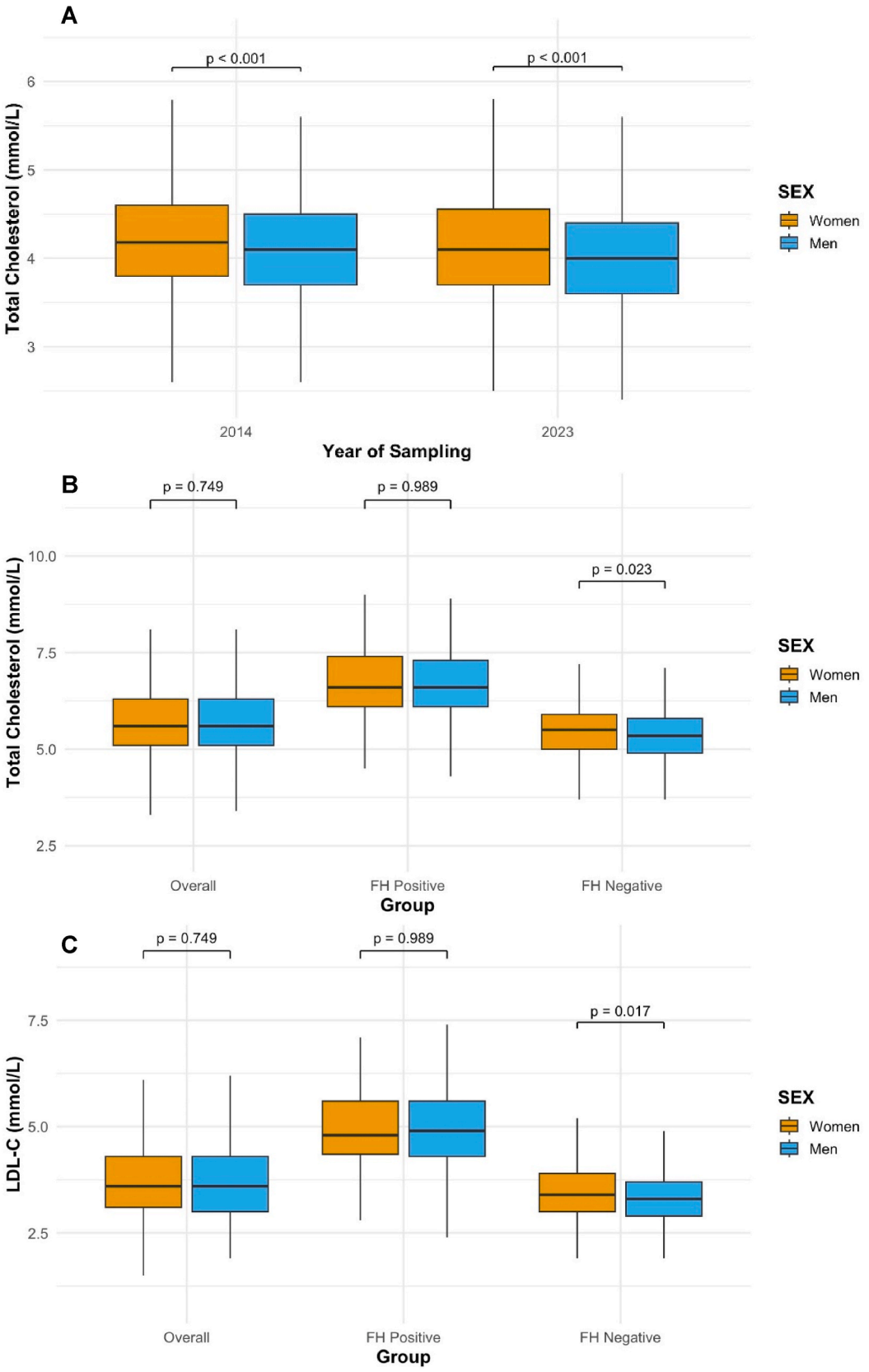

Fig. 2. (A) Boxplots of total cholesterol (TC) by sex for two random samples of children from the years 2014 and 2023. (B) Boxplots of total cholesterol by sex for individuals in the Hypercholesterolemia registry, categorized into three groups: overall, Familial Hypercholesterolemia (FH)-positive, and FH-negative. (C) Boxplots of low-density lipoprotein cholesterol (LDL-C) by sex for the Hypercholesterolemia registry, similarly divided into three groups. p-values are adjusted using the Benjamini–Hochberg method.

significant predictors of TC in both cohorts (see Fig. 3). In 2014, the coefficients were: sex (β = −0.080), age (β = 0.183), BH (β = −0.014), and BW (β = 0.017); in 2023, they were: sex (β = −0.119), age (β = −0.078), BH (β = −0.011), and BW (β = 0.008) (all $p < 0.001$, except BW in 2023 at $p = 0.028$). Nonetheless, these models accounted for only a modest fraction of the variation in TC (adjusted $R^2 = 0.012$ in 2014 and 0.022 in 2023). Notably, tests for interactions between sex and the other covariates (age, body height, and body weight) were non-significant in both samples, suggesting that the association between sex and TC does not vary across levels of these factors.

3.2. Baseline characteristics and sex differences in the Slovenian Hypercholesterolemia Registry

Table 1 presents the median (Q1–Q3) values for age, anthropometric measures, and lipid parameters by sex for the overall cohort, as well as for the FH-positive and FH-negative subgroups. Although the descriptive differences between females and males are modest in the overall and FH-positive groups, the FH-negative subgroup shows statistically significant sex differences in TC and LDL-C (Fig. 2B and C). In contrast, no significant sex differences were observed for other lipid parameters, including HDL-C, Lp(a), and triglycerides.

In a gene-stratified analysis of FH-positive children, no significant sex differences were observed in LDL-C or TC levels among carriers of either *LDLR* (N = 189) or *APOB* (N = 84) variants. Median LDL-C concentrations were comparable between boys and girls within each gene group (e.g., *LDLR*: 5.2 [4.3–5.9] vs. 4.9 [4.4–5.7] mmol/L; *APOB*: 4.6 [4.3–5.0] vs. 4.7 [4.3–4.9] mmol/L; ($p = 0.720$ for both). No significant differences were found for age, anthropometric measures, HDL-C, triglycerides, or Lp(a). Notably, no *PCSK9* variants were identified in this cohort.

For LDL-C, univariate linear regression showed no significant sex differences in the overall cohort ($p = 0.649$) or in the FH-positive subgroup ($p = 0.992$). However, in the FH-negative subgroup, males had significantly lower LDL-C levels than females by 0.120 mmol/L ($p = 0.011$). In the case of TC, univariate tests also indicated no significant differences in the overall ($p = 0.950$) or FH-positive groups ($p = 0.985$), whereas in the FH-negative subgroup, females exhibited modestly higher TC levels than males by 0.131 mmol/L ($p = 0.011$).

Spearman's correlation analyses further supported these findings. In the overall cohort, LDL-C was not significantly correlated with sex ($p = 0.744$) and a similar pattern was observed for TC (0.711). Notably, within the FH-negative subgroup, correlations between lipid levels and sex were significant (LDL-C: $\rho = -0.090$, $p = 0.007$; TC: $\rho = -0.083$, $p = 0.013$), while in the FH-positive subgroup, no significant correlations with sex were observed (LDL-C: $p = 0.907$; TC: $p = 0.974$).

Multivariate regression models were constructed to adjust for age and BMI Z-score (Fig. 4). In the overall cohort the sex was insignificant predictor for both LDL-C ($p = 0.706$) and TC ($p = 0.987$). But when further adjusting for FH status, the model for LDL-C demonstrated that sex was an independent predictor, with males having on average 0.099 mmol/L lower LDL-C than females ($p = 0.038$). Similarly, after adjustment for FH status, the model for TC in the overall cohort revealed that males had significantly lower TC by 0.105 mmol/L compared with females ($p = 0.037$). Subgroup analyses indicated that, in the FH-negative subgroup, sex remained a significant predictor for both LDL-C (β = −0.134, $p = 0.004$) and TC (β = −0.141, $p = 0.006$), whereas in the FH-positive subgroup, the effect of sex on both LDL-C ($p = 0.815$) and TC ($p = 0.832$) was not significant. Notably, tests for interactions between sex and the other covariates (age, body height, and body weight) were non-significant in both samples, suggesting that the association between sex and TC remains consistent across different levels of these factors.

4. Discussion

In this study, we examined inherent sex differences in lipid profiles among prepubertal children with hypercholesterolemia, with a particular focus on how the presence of FH may influence these differences.

Our analyses, employing a range of statistical tests—including the Wilcoxon test, regression, and correlation techniques—clearly demonstrate that TC is significantly higher in girls than in boys ($p < 0.001$) in two independent samples of Slovenian 5-year-olds collected a decade

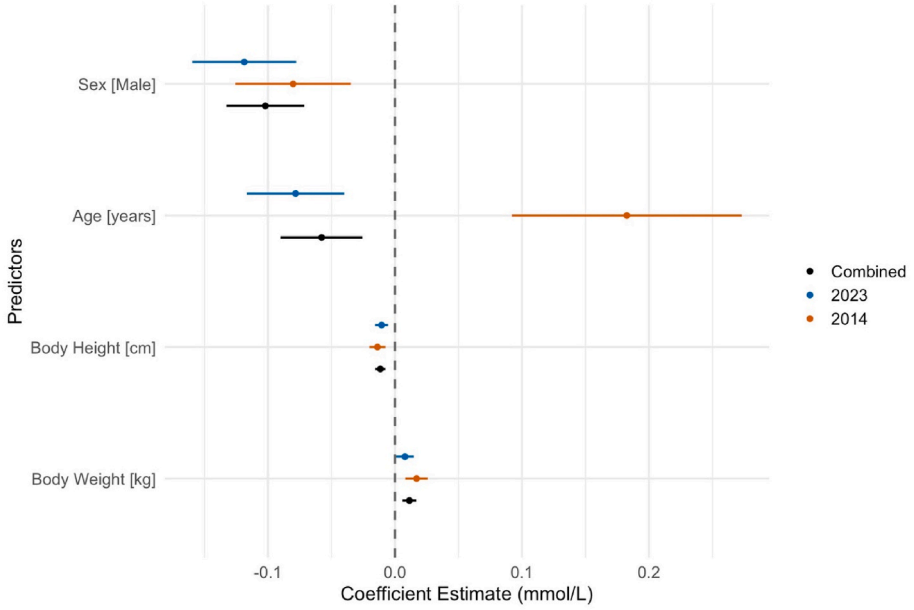

Fig. 3. Dot–whisker plot of regression coefficient estimates for total cholesterol (TC). The figure shows coefficient estimates (with 95 % confidence intervals) for key predictors of TC from three linear regression models: one for the 2014 sample, one for the 2023 sample, and one pooled model. The x-axis indicates the estimated change in TC (mmol/L) per unit increase in each predictor. The outlier in the age data from the 2014 cohort reflects a small number of children who were slightly older than the target age for screening due to delayed attendance at the mandatory preschool examination. These cases were retained to maximize cohort size and maintain representativeness. Notably, the median age was higher in the 2023 cohort, reflecting a general trend toward later screening and delayed school entry in recent years.

Table 1
Characteristics of subjects from hypercholesterolemia registry by sex and familial hypercholesterolemia (FH) status (N = 1160).

Parameter	Group	Female (N = 680)	Male (N = 480)	p-value
Age [years]	Overall	6.14 (5.67–6.82)	6.18 (5.65–7.02)	0.749
	FH Positive	5.99 (5.60–6.55)	5.86 (5.54–6.67)	0.739
	FH Negative	6.19 (5.70–6.94)	6.27 (5.74–7.11)	0.224
Body height [cm]	Overall	119 (114–125)	120 (115–126)	0.026
	FH Positive	119 (114–124)	119 (114–124)	0.989
	FH Negative	120 (115–125)	121 (116–128)	0.012
Body weight [kg]	Overall	22.2 (19.9–26.3)	23.1 (20.4–26.9)	0.014
	FH Positive	21.4 (19.7–26.2)	22.6 (20.0–25.1)	0.602
	FH Negative	22.4 (19.9–26.4)	23.3 (20.5–27.6)	0.012
BMI [kg/m^2]	Overall	15.6 (14.7–17.1)	15.9 (14.9–17.3)	0.022
	FH Positive	15.4 (14.7–17.0)	15.8 (15.1–17.2)	0.214
	FH Negative	15.7 (14.7–17.2)	15.9 (14.8–17.3)	0.044
BMI Z-Score	Overall	0.01 (−0.60–0.87)	0.21 (−0.55–1.11)	0.014
	FH Positive	−0.08 (−0.55–0.80)	0.17 (−0.44–1.11)	0.214
	FH Negative	0.03 (−0.64–0.90)	0.24 (−0.57–1.11)	0.029
Total Cholesterol [mmol/L]	Overall	5.6 (5.1–6.3)	5.6 (5.1–6.3)	0.749
	FH Positive	6.6 (6.1–7.4)	6.6 (6.1–7.3)	0.989
	FH Negative	5.5 (5.0–5.9)	5.4 (4.9–5.8)	0.023
LDL-C [mmol/L]	Overall	3.6 (3.1–4.3)	3.6 (3.0–4.3)	0.749
	FH Positive	4.8 (4.35–5.6)	4.9 (4.3–5.6)	0.989
	FH Negative	3.4 (3.0–3.9)	3.3 (2.9–3.7)	0.017
HDL-C [mmol/L][a]	Overall	1.5 (1.3–1.8)	1.5 (1.3–1.8)	0.252
	FH Positive	1.4 (1.2–1.5)	1.4 (1.2–1.6)	0.684
	FH Negative	1.6 (1.3–1.8)	1.5 (1.3–1.8)	0.634
Lp(a) [mg/dL][b]	Overall	13.5 (<9.9–44.3)	12.4 (<9.9–40.0)	0.348
	FH Positive	10.7 (<9.9–36.1)	10.9 (<9.9–31.6)	0.684
	FH Negative	14.7 (<9.9–45.4)	12.8 (<9.9–43.4)	0.634
Triglycerides [mmol/L][c]	Overall	0.9 (0.7–1.3)	0.8 (0.6–1.3)	0.252
	FH Positive	0.8 (0.7–1.1)	0.8 (0.6–1.3)	0.745
	FH Negative	0.9 (0.7–1.3)	0.9 (0.6–1.3)	0.622

Data are presented as median (first quartile–third quartile). Mann-Whitney Test was used for comparison of variables due to non-normal distribution. To control the false discovery rate, p-values were adjusted using the Benjamini-Hochberg method, with significance set at an adjusted $p < 0.05$.
Legend: BMI = Body mass index; LDL-C = Low-density lipoprotein cholesterol; HDL-C = High-density lipoprotein cholesterol; Lp(a) = Lipoprotein(a).
Footnotes: FH positive: N (females) = 139, N (males) = 134; FH negative: N (females) = 541, N (males) = 346; [a] Missing 3 values; [b] Missing 130 values; [c] Missing 4 values.

apart (n = 3412 in 2014 and n = 4182 in 2023). These differences persisted even after adjusting for age, height, and weight. Considering that each generation in Slovenia includes roughly 20,000 children [13], these robust sample sizes reinforce the reliability of our findings, suggesting that these sex-specific lipid profiles have early developmental origins.

While boys in the FH-negative and universal cohorts had slightly higher body weight, height, and BMI than girls, these differences were modest and not present in the FH-positive group. Importantly, our regression analyses—including sex and anthropometric measures—showed that sex remained a significant predictor of cholesterol levels in FH-negative children, independent of body size. Thus, the observed sex differences in cholesterol are not explained by differences in growth parameters, but reflect a true physiological distinction that is absent in FH-positive children.

Our results corroborate previous studies indicating that, after infancy, girls tend to have higher TC levels (around 0.1 mmol/L) compared to boys—a finding consistently observed in various cohorts [6–9]. However, our study offers a novel contribution by focusing on a large, randomly sampled group of prepubertal 5-year-olds, drawn from a universal FH screening program. This approach minimizes selection bias and the influence of pubertal hormonal changes, thereby providing a clearer picture of innate, sex-specific lipid profiles. This early establishment of sex-specific cholesterol levels aligns with previous reports, suggesting that the foundation for later cardiovascular risk differences is laid well before adolescence [5].

In the Hypercholesterolemia registry, our analyses reveal that sex differences in lipid parameters are significant only among FH-negative children, with girls showing higher TC ($p = 0.023$) and LDL-C ($p = 0.017$) than boys—mirroring the trends observed in our random population samples. In contrast, among FH-positive subjects, TC and LDL-C levels were virtually identical between girls and boys ($p = 0.989$ for both), suggesting that the presence of a pathogenic variant is the dominant factor driving high cholesterol levels, thereby overshadowing any inherent sex differences. Consequently, when FH-positive and FH-negative individuals are combined, these sex differences become statistically non-significant ($p = 0.749$ for TC and LDL-C). Notably, however, when we adjusted for FH status in our multivariable regression models in overall cohort, sex emerged as an independent predictor of both LDL-C ($p = 0.038$) and TC ($p = 0.037$), reaffirming that, in the absence of genetic dyslipidemia, girls have higher cholesterol levels than boys. These findings, derived from multiple statistical approaches—including nonparametric tests, regression, and correlation analyses—underscore the necessity of stratifying by FH status to accurately discern the developmental origins of sex-specific lipid profiles.

Our findings differ from those of Holven et al. and Johansen et al. in that we observed no significant sex differences in TC and LDL-C among FH-positive subjects, whereas both of those studies reported that FH positive girls exhibited higher lipid levels than FH positive boys [20,21]. One explanation for this discrepancy may lie in the distinct study populations and designs. Our cohort, derived from a universal FH screening program, focused exclusively on prepubertal 5-year-olds and younger children—a group that is less affected by hormonal and environmental influences—thereby allowing us to better observe inherent sex differences and assess the impact of FH. In contrast, the above studies included a broader age range—including pubertal and post-pubertal subjects—when sex hormones and differences in body fat distribution begin to play a more prominent role in lipid metabolism. Notably, in the study by Holven et al., sex differences were insignificant among children younger than 5 years but became significant in those aged 5–9 years, highlighting that these differences may emerge with increasing age [21].

This supports our interpretation that the absence of sex differences among FH-positive children in our cohort likely reflects the overriding effect of the pathogenic FH variant in early childhood, which may mask subtle physiological differences otherwise observed in FH-negative peers. We hypothesize that with the onset of puberty, when hormonal influences begin to shape lipid metabolism more strongly, sex-related differences among FH-positive individuals would also emerge, consistent with the patterns described in broader and older FH cohorts [21,22]. Additionally, it is possible that our sample did not have sufficient statistical power to detect subtle sex differences in the FH-positive

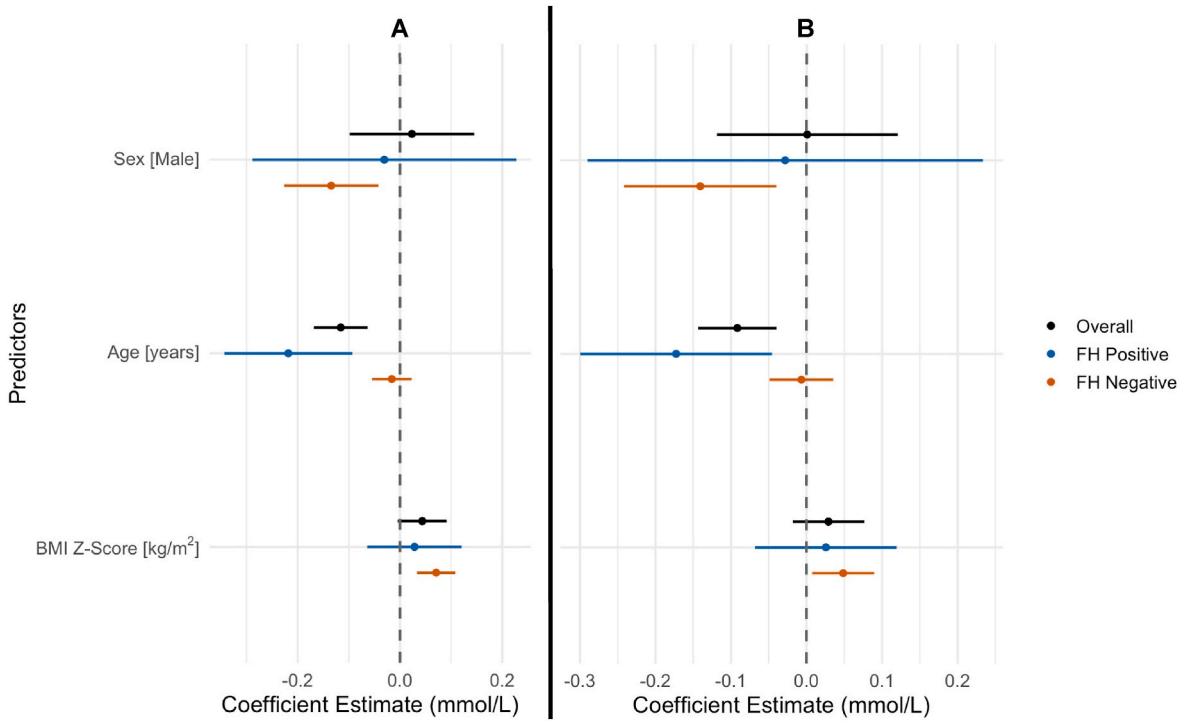

Fig. 4. Dot-whisker plot of regression coefficient estimates (with 95 % confidence intervals) for (A) low-density lipoprotein cholesterol (LDL-C) and (B) total cholesterol (TC) in a Hypercholesterolemia registry. The coefficients are derived from three separate linear regression models (overall, Familial Hypercholesterolemia (FH)-positive, and FH-negative). The x-axis indicates the estimated change in LDL-C or TC (mmol/L) per unit increase in each predictor.

subgroup, despite including nearly 300 FH-positive children (139 girls and 134 boys). Emerging evidence suggests that early-life epigenetic modifications, such as DNA methylation of estrogen receptor genes, can differ between sexes and influence lipid metabolism [23]. Furthermore, variations in sex chromosome composition, particularly the presence of two X chromosomes, have been associated with differences in lipid levels, independent of gonadal hormones [24]. These findings highlight the potential role of developmental and genetic factors in shaping sex-specific lipid profiles from an early age.

Interestingly, our observation that pathogenic FH variants may override natural sex differences in lipid levels is supported by recent findings in individuals with homozygous familial hypercholesterolemia (HoFH). In a large international HoFH registry, Mulder et al. reported no significant sex differences in untreated LDL-C levels between men and women with HoFH, despite a large sample size and global representation [25]. Together, these findings suggest that the presence of FH-related pathogenic variants—whether in heterozygous or homozygous form—can override physiological sex-based variation in lipid metabolism.

Although small, the observed sex differences in cholesterol levels may help refine early cardiovascular risk assessment. Clinicians should be aware that prepubertal girls typically have slightly higher TC and LDL-C than boys, which may inform interpretation of lipid results in this age group. Importantly, the lack of sex differences among FH-positive children supports uniform diagnostic thresholds regardless of sex. These findings reinforce the value of universal early screening and suggest that future guidelines might consider incorporating sex-specific reference values to improve risk stratification and guide preventive efforts.

While this study provides valuable insights, several limitations warrant mention. The study population was predominantly of Central European origin, which may limit the applicability of the findings to other ethnic groups. Additionally, the exact onset of puberty was not precisely determined in the Hypercholesterolemia registry, potentially affecting the classification of prepubertal status. Diet and lifestyle data were not recorded in the Slovenian FH registry. All children receive standardized counselling and written advice, but adherence is unknown. Consequently, between-cohort differences in dietary quality may have contributed to differences in LDL-C we observed compared with Norwegian FH children, who have documented healthier food choices and lower saturated-fat intake following long-term counselling [26,27]. Prospective collection of diet and physical-activity variables has been prioritized for the next registry update. The cross-sectional design restricts causal inferences, and the absence of data on diet, physical activity, and socioeconomic status—known determinants of lipid levels that may differ by sex—limits our ability to fully account for potential confounding. This is particularly relevant in the FH-negative subgroup, where observed sex differences in cholesterol may partly reflect unmeasured lifestyle or environmental influences. Furthermore, while our findings suggest that FH status attenuates sex differences in LDL-C, the precise biological mechanisms underlying this effect remain uncertain and warrant further investigation.

5. Conclusion

Our analysis of representative prepubertal cohorts confirms that girls have higher TC levels than boys, consistent with findings from other studies. Furthermore, when examining prepubertal children with FH, this inherent sex difference persists only in prepubertal FH-negative subjects, whereas prepubertal FH-positive children exhibit no significant sex-related differences in cholesterol levels. These results suggest that the presence of a pathogenic FH variant may override the innate sex differences in lipid metabolism. While the observed differences are small and unlikely to impact individual clinical decisions, they may hold relevance at the population level for optimizing screening strategies. These findings support the use of universal early cholesterol screening and suggest that sex-specific reference values could enhance risk assessment in prepubertal FH-negative children, potentially informing future preventive guidelines and research on individualized pediatric care.

Ethics considerations

The study was approved by the Slovenian National Medical Ethics Committee (No. 25/10/09; 22/01/2017; 0120-14/2017-2; 0120-14/2017-5; 0120-100/2019/5; 07120-1/2024/161). The study was conducted in accordance with the Declaration of Helsinki. Informed consent for genetic analysis and publication of anonymized data, was obtained from parents or legal guardians.

Originality of content

We confirm all information and materials in the manuscript are original.

Author contribution (CRediT):

Conceptualization: U.G., J.Ka.; Methodology: J.Ka., M.B., J.S., J.Ko.; Data Curation: M.B., B.C.K., M.M., K.S., M.K.; Formal Analysis: J.Ka., J. S., J.Ko.; Investigation: M.B., B.C.K., K.S., M.K., B.C.K., A.D.T.; Supervision: U.G., T.B., A.D.T.; Funding Acquisition: U.G., J.Ko., T.B.; Writing – Original Draft: J.Ka., U.G.; Writing – Review & Editing: All authors.

Financial support

This work was supported by the Slovenian Research and Innovation Agency (grants: P3-0343, J3-2536). The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We sincerely thank all the children and families who participated in this study. Additionally, we acknowledge the contributions of all clinicians, researchers, and collaborators who assisted in data collection, analysis, and manuscript preparation.

U.G. has had full access to all study data and takes responsibility for its integrity and accuracy. We acknowledge the use of artificial intelligence (AI)-based natural language processing tools for language editing and minor assistance with coding and data processing. These tools were used to improve clarity and readability and to facilitate certain computational tasks. No AI tool was involved in generating original scientific content, analysis, or interpretation.

Data availability

Data is available from the corresponding author upon reasonable request.

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