

## Article

# Development and Validation of Multiple Equations for Low-Density Lipoprotein and Apolipoprotein B in Korean Patients Visiting Local Clinics and Hospitals

Rihwa Choi <sup>1,2</sup> , Sang Gon Lee <sup>1,\*</sup>  and Eun Hee Lee <sup>3,\*</sup>

<sup>1</sup> Department of Laboratory Medicine, Green Cross Laboratories, Yongin 16924, Republic of Korea; pirate0720@naver.com

<sup>2</sup> Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Republic of Korea

<sup>3</sup> Green Cross Laboratories, Yongin 16924, Republic of Korea

\* Correspondence: sglee@gclabs.co.kr (S.G.L.); ehlee@gclabs.co.kr (E.H.L.); Tel.: +82-31-260-9209 (S.G.L.); +82-31-260-9201 (E.H.L.)

**Abstract:** We investigated the utilization of apolipoprotein B (ApoB), an independent risk factor for cardiovascular disease, and developed and validated a translational equation for calculating low-density lipoprotein cholesterol (LDL-C) in the Korean population visiting local clinics and hospitals. Among a total of 469,520 data sets of the lipid profile panel (total cholesterol, triglycerides, and high-density lipoprotein cholesterol), 142,932 lipid test sets with data on LDL-C and/or ApoB were used for statistical analysis. Using linear regression analysis, we created ApoB percentile value-derived LDL-C equations in a creating set and validated them with previously reported equations (a total of 11 equations) in comparison to directly measured LDL-C using two independent validating sets. Among all lipid test sets, the simultaneously measured ApoB test only accounted for 2.0%, indicating its underutilization in Korea. The ApoB-derived equations, which were derived in this study and previous studies, showed an overall agreement of  $\geq 94.3\%$  for NCEP ATP III criteria. However, the accuracy of the equations varied among data sets of populations. Future studies are needed to validate translational equations for ApoB and LDL-C in different populations to clarify the clinical implications of these equations.

**Keywords:** apolipoprotein B; ApoB; dyslipidemia; utilization; translation; equation; Korea



**Citation:** Choi, R.; Lee, S.G.; Lee, E.H. Development and Validation of Multiple Equations for Low-Density Lipoprotein and Apolipoprotein B in Korean Patients Visiting Local Clinics and Hospitals. *Nutrients* **2023**, *15*, 2786. <https://doi.org/10.3390/nu15122786>

Academic Editor: Maret G. Traber

Received: 28 April 2023

Revised: 14 June 2023

Accepted: 16 June 2023

Published: 17 June 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Cholesterol levels are a leading modifiable risk factor and the target of treatment for atherosclerotic cardiovascular diseases [1,2]. Historically, total cholesterol (TC), triglycerides (TG), LDL cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) have been evaluated through lipid profile tests for the diagnosis and management of dyslipidemia and atherosclerotic cardiovascular diseases [3]. Apolipoprotein B (ApoB) is a key structural protein component of all major atherogenic lipoproteins such as chylomicron and its remnants, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and lipoprotein (a); ApoB-containing lipoproteins retained in the arterial wall may provoke atherosclerotic cardiovascular diseases [4,5]. In the last decades, ApoB has been highlighted as a more accurate marker of cardiovascular risk than LDL-C and non-high-density lipoprotein cholesterol [4,6]. Recent European and Canadian guidelines have stated the superiority of ApoB over LDL-C [4]. Multi-ethnic studies of atherosclerosis confirmed ApoB as an independent risk factor for atherosclerosis [7]. While ApoB has been extensively studied in Europeans, its importance has been relatively less emphasized in Korea, and Korean guidelines for dyslipidemia suggest ApoB evaluation in patients with diabetes and familial hypercholesterolemia [8]. According to recent guidelines from the European

Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS), as well as the American College of Cardiology (ACC)/American Heart Association (AHA) guidelines and the Canadian Cardiovascular Society (CCS) guidelines, it is recommended to include ApoB measurements alongside the traditional lipid panel (TC, TG, HDL-C, and LDL-C) to define risk estimation and establish therapeutic targets for treatment [4,9–11]. However, the current Korean clinical guidelines primarily focus on traditional lipid tests, such as TC, TG, HDL-C, and LDL-C, and provide established cutoffs for risk estimation and therapeutic targets [8]. Although ApoB is mentioned in the guidelines, there is a lack of detailed, specific recommendations or guidelines regarding its interpretation [8].

The available data suggest that there are differences in the lipid profiles of Asians compared to Western populations, which can be attributed to a combination of genetic, environmental, and lifestyle factors [8–13]. For example, multi-ethnic studies have reported that Asians exhibit distinct dyslipidemia subtypes compared to non-Hispanic Whites, and even among different ethnicities within the Asian population, variations in lipid patterns exist [8–13]. It has been observed that Asians have a higher prevalence of elevated TG levels compared to non-Hispanic Whites, while Japanese men show a higher prevalence of low HDL-C levels compared to non-Hispanic White men [13]. However, no significant differences in low HDL-C were found between Korean men and non-Hispanic Whites [14]. Additionally, the study showed no significant differences in high LDL-C levels between Korean men and non-Hispanic Whites [13]. Therefore, conducting clinical studies to investigate lipid profiles in diverse ethnicities across various geographical regions is essential for effective dyslipidemia management on a global scale [9–13].

In addition, LDL-C is the primary target of lipid-lowering treatment in current clinical practice guidelines [4,14,15]. Because clinicians are more familiar with LDL-C concentrations and their cutoffs for treatment, in order to increase the use of ApoB, a translational tool to convert data to a familiar format report would be useful [14]. Cole et al. recently provided a simple equation to report ApoB levels measured in mass concentration units of mg/dL as well as in transformed LDL-C equivalent units (ApoB LDL-CEq) [14]. Cole et al. calculated ApoB LDL-CEq alongside the Friedewald (LDL-C\_Friedewald) [16], Sampson (LDL-C\_Sampson/NIH) [17], and Martin/Hopkins (LDL-C\_Martin/Hopkins) equations [18]. However, these equations have not been validated in a large Korean population in multiple studies. Previous studies conducted in Korean populations have reported varying levels of agreement between equations for calculating LDL-C derived from different ethnic populations using various analytical methods and directly measured LDL-C [19].

Therefore, in this study, we aimed to investigate the utilization of the ApoB test in local clinics and hospitals in Korea and to develop and validate new translational equations for ApoB LDL-CEq in a large Korean population. In addition, the results of the proposed equations were compared with those of LDL-C\_Friedewald, LDL-C\_Sampson/NIH, LDL-C\_Martin/Hopkins, LDL-C\_Choi, and three equations by Cole et al. [14], as well as with the directly measured LDL-C (LDL-C\_Direct).

## 2. Materials and Methods

### 2.1. Subjects

We retrospectively obtained anonymized clinical laboratory results for lipid profiles from the laboratory information system of Green Cross Laboratories between 1 January 2021 and 31 December 2021 for a population of Korean adults (age > 20 years) who visited local clinics and hospitals and underwent serum TG, TC, HDL-C, LDL-C, and ApoB testing. Results with TG greater than 1000 mg/dL (11 mmol/L) were excluded to minimize confounding factors, as extremely high TG levels can interfere with accurate measurements of other lipid parameters and may indicate specific pathological states affecting lipid parameters and patterns [14,20]. To ensure calculation reliability and minimize errors, results with HDL-C levels higher than TC were excluded. This exclusion was based on the understanding that a normal lipid profile typically exhibits TC calculated as the sum of HDL-C and non-HDL-C components [14–19]. Because the aim of this study was to

compare calculated LDL-C in comparison with LDL-C\_Direct, test results with only TG, TC, or HDL-C without LDL-C\_Direct or ApoB measurements were excluded.

## 2.2. Analytical Methods

Serum HDL-C, TG, TC, and LDL-C were measured using well-established automated Roche Cobas 8000 c702 analyzers (Roche, Mannheim, Germany) [14,19]. Serum ApoB was measured using an automated immunoturbidimetric assay using a Tina-quant Apolipoprotein B ver.2 reagent kit (Roche) on c702 analyzers (Roche). The analytical measurement range of the serum ApoB assay was 20.0–400.0 mg/dL. The calibrator of this assay was traceable to the IFCC reference material SP3-07 [21,22]. The manufacturer's reference interval for ApoB in men ranged from 66.0 to 144.0 mg/dL and in women from 60.0 to 141.0 mg/dL. The accuracy of lipid measurements was assured through participation in accuracy-based external quality assurance programs by the Centers for Disease Control, USA; College of American Pathologists; and Korean External Quality Assessment Scheme. These quality assurance programs provide proficiency test materials with target values assigned by reference measurement methods. Participation in these quality assurance programs promotes standardization, validates testing methods, assesses proficiency, identifies errors and biases, and drives continuous improvement [23,24].

## 2.3. Definitions

In order to develop a translational equation for LDL-C based on ApoB in this study group (ApoB LDL-CEq\_Choi), data sets from specimens with TC, TG, HDL, and ApoB data without LDL-C\_Direct results were used (Creation Set). Linear regression analysis between calculated LDL using each equation (LDL-C\_Sampson/NIH, LDL-C\_Friedewald, LDL-C\_Martin/Hopkins, and LDL-C\_Choi) and ApoB percentile was performed [14].

Considering that the ApoB test has not been widely used in Korean patients, two independent data sets were used for validating calculated LDL-C; the first was for calculated LDL-C using equations derived from TC, TG, and HDL (LDL-C\_Sampson/NIH, LDL-C\_Friedewald, LDL-C\_Martin/Hopkins, and LDL-C\_Choi), and the second was for calculated LDL-C using all equations including the newly developed ApoB percentile-derived equation in this study population using the Creation Set (ApoB LDL-CEq\_Sampson/NIH, ApoB LDL-CEq\_Friedewald, ApoB LDL-CEq\_Martin/Hopkins, and ApoB LDL-CEq\_Choi) and equations previously reported by Cole et al. (Apo B LDL-CEq\_Cole\_Sampson/NIH, Apo B LDL-CEq\_Cole\_Friedewald, and Apo B LDL-CEq\_Cole\_Martin/Hopkins) [14].

In order to validate the calculated LDL-C in comparison with LDL-C\_Direct for the first analysis, test results with TC, TG, HDL, and LDL-C\_Direct without ApoB were defined as 'Validation Set 1'. To validate the ApoB-derived equations, test results with TC, TG, HDL, LDL-C\_Direct, and ApoB levels were defined as 'Validation Set 2'.

Criteria for the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) for LDL-C were used to investigate the agreement of CVD risk categorization by equation as optimal LDL-C < 100 mg/dL, above optimal 100–129 mg/dL, borderline high 130–159 mg/dL, high 160–189 mg/dL, and very high  $\geq$  190 mg/dL [3].

## 2.4. Statistical Analysis

Non-parametric analysis was used when the data did not show a normal distribution (age and lipid results). Chi-square tests were used to compare to categorical variables (sex and NCEP ATP III criteria). Linear regression analysis was used to create equations to obtain LDL-C with ApoB results [14]. Calculated LDL-C using each equation was compared quantitatively with LDL-C\_Direct using Bland–Altman plot analysis. The results were compared qualitatively based on agreement of NCEP ATP III categorization for LDL-C [19]. Systemic differences between calculated LDL-C and LDL-C\_Direct levels were calculated as 'calculated LDL-C minus LDL-C\_Direct'. The percentage of systemic difference (%difference) was calculated as 'calculated LDL-C and LDL-C\_Direct/LDL-C\_Direct  $\times$  100'. Absolute percentage error was calculated as  $100 \times \text{absolute value} [(y - \text{ref})/\text{ref}]$ , where 'y'

is the observation and 'ref' is the reference value (LDL-C\_Direct). Statistical analysis was executed using MedCalc Statistical Software Version 20.110 (MedCalc Software bv, Ostend, Belgium; <https://www.medcalc.org>; accessed on 29 March 2023). *p* values were considered significant at the 0.05 level.

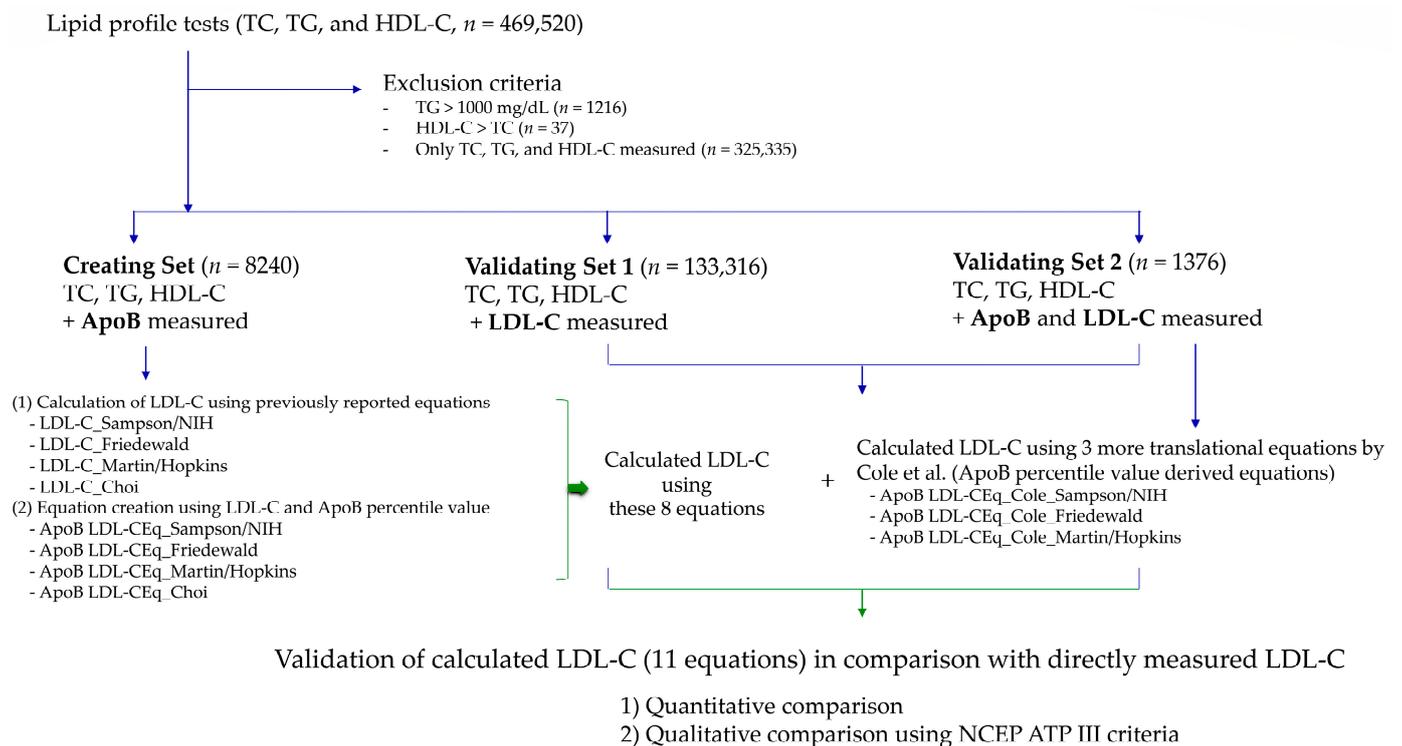
2.5. Ethical Approval

This study was conducted according to the guidelines outlined in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Institutional Review Board (IRB) of Green Cross Laboratories (GCL-2023-1010-01, 17 February 2023). A waiver of informed consent was approved by the IRB as the study was retrospective and involved no risk to subjects.

3. Results

3.1. Characteristics of Study Subjects and Lipid Results

Between 1 January 2021 and 31 December 2021, 469,520 Korean adults (213,637 men and 255,883 women) with a mean age of 55.6 years (SD 14.95) were tested for a lipid profile panel (TC, TG, and HDL-C). After applying exclusion criteria, 142,932 lipid test sets were used for statistical analysis; 8240 test sets for the Creation Set; 133,316 test sets for Validation Set 1; and 1376 test sets for Validation Set 2. The study scheme and baseline characteristics of each group of patients are summarized in Figure 1 and Table 1. The three datasets showed significant differences in age, sex distribution, and cholesterol levels except for LDL-C.



**Figure 1.** Study scheme. The development and validation of apolipoprotein B derived equations for calculating LDL-C were performed in comparison with previously reported equations by Cole et al. [14]. Abbreviations: ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

**Table 1.** Baseline characteristics.

| Characteristics          | Total (n = 142,932)    | Creating Set (n = 8240)                 | Validating Set 1st (n = 133,316) | Validating Set 2nd (n = 1376) | p-Value |
|--------------------------|------------------------|---|----------------------------------|-------------------------------|---------|
| Analytes                 | Sum of all data sets   | TC, TG, HDL-C, ApoB                     | TC, TG, HDL-C, LDL-C             | TC, TG, HDL-C, ApoB, LDL-C    |         |
| Age, years (median, IQR) | 54.6 (44.2 to 64.1)    | 59.3 (48.4 to 67.2)                     | 54.3 (44.0 to 63.9)              | 54.5 (42.8 to 64.5)           | <0.0001 |
| Sex (n, %)               |                        |   |                                  |                               | <0.0001 |
| Male                     | 68,240 (47.7%)         | 3869 (47.0%)                            | 63,801 (47.9%)                   | 570 (41.4%)                   |         |
| Female                   | 74,692 (52.3)          | 4371 (53.0%)                            | 69,515 (52.1%)                   | 806 (58.6%)                   |         |
|                          |                        | Lipid test results, mg/dL (median, IQR) |                                  |                               |         |
| TC                       | 185.0 (157.0 to 215.0) | 175.0 (150.0 to 205.0)                  | 186.0 (157.0 to 215.0)           | 187.0 (157.0 to 221.5)        | <0.0001 |
| TG                       | 120.0 (84.0 to 178.0)  | 123.0 (87.0 to 182.0)                   | 120.0 (84.0 to 177.0)            | 114.0 (77.5 to 174.0)         | <0.0001 |
| HDL-C                    | 53.0 (44.0 to 63.0)    | 53.0 (44.0 to 63.0)                     | 53.0 (44.0 to 63.0)              | 56.0 (44.6 to 67.0)           | <0.0001 |
| Non-HDL-C                | 129.0 (102.0 to 160.0) | 119.0 (95.0 to 150.0)                   | 129.0 (102.0 to 160.0)           | 127.0 (98.0 to 164.0)         | <0.0001 |
| LDL-C <sup>1</sup>       | 109 (84.0 to 136.0)    | -                                       | 109.0 (84.0 to 136.0)            | 108.0 (81.0 to 142.0)         | NS      |
| ApoB <sup>1</sup>        | 89.6 (73.5 to 110.6)   | 89.1 (73.5 to 109.6)                    | -                                | 92.2 (74.1 to 118.8)          | <0.0001 |

<sup>1</sup> The total number of LDL-C measurements was 134,692 and the total number of ApoB measurements was 9616. Abbreviations: ApoB, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Non-HDL-C, non-HDL cholesterol calculated from TC minus HDL-C; NS, not significant; TC, total cholesterol; TG, triglycerides.

3.2. Equations for apoB LDL-CEq

Equations for ApoB LDL-CEq using linear regression analysis between the ApoB percentile of calculated LDL-C and the previously reported calculated LDL-C are summarized in Table 2. For regression analysis between the ApoB percentile and LDL-C level, 8240 patients with measurements of TC, TG, HDL-C, and ApoB without LDL-C\_Direct were selected and categorized as the Creation Set.

**Table 2.** Equations for calculated LDL-C.

| Abbreviation of Equation         | Equation Driven           | Equation (y) = LDL-C  |
|----------------------------------|---------------------------|---|
| LDL-C_Sampson/NIH                | By Sampson et al. [17]    | $y = (TC/0.948) - (HDL-C/0.971) - [(TG/8.56) + \{(TG \times Non-HDL-C)/2140\} - (TG)^2/16100] - 9.44$ |
| LDL-C_Friedewald                 | By Friedewald et al. [16] | $y = TC - HDL-C - (TG/5)$   |
| LDL-C_Martin/Hopkins             | By Martin et al. [18]     | $y = TC - HDL-C - (TG/\text{different adjustable factors})$   |
| LDL-C_Choi                       | By Choi et al. [19]       | $y = TC - 0.87 \times HDL-C - 0.13 \times TG$   |
| ApoB LDL-CEq_Sampson/NIH         | This study                | $y = 1.352 \times (ApoB) - 27.163$  |
| ApoB LDL-CEq_Friedewald          | This study                | $y = 1.382 \times (ApoB) - 33.756$  |
| ApoB LDL-CEq_Martin/Hopkins      | This study                | $y = 1.327 \times (ApoB) - 23.669$  |
| ApoB LDL-CEq_Choi                | This study                | $y = 1.393 \times (ApoB) - 17.113$  |
| ApoB LDL-CEq_Cole_Sampson/NIH    | By Cole et al. [14]       | $y = 1.38 \times (ApoB) - 29$   |
| ApoB LDL-CEq_Cole_Friedewald     | By Cole et al. [14]       | $y = 1.385 \times (ApoB) - 32.2$  |
| ApoB LDL-CEq_Cole_Martin/Hopkins | By Cole et al. [14]       | $y = 1.348 \times (ApoB) - 26.4$  |

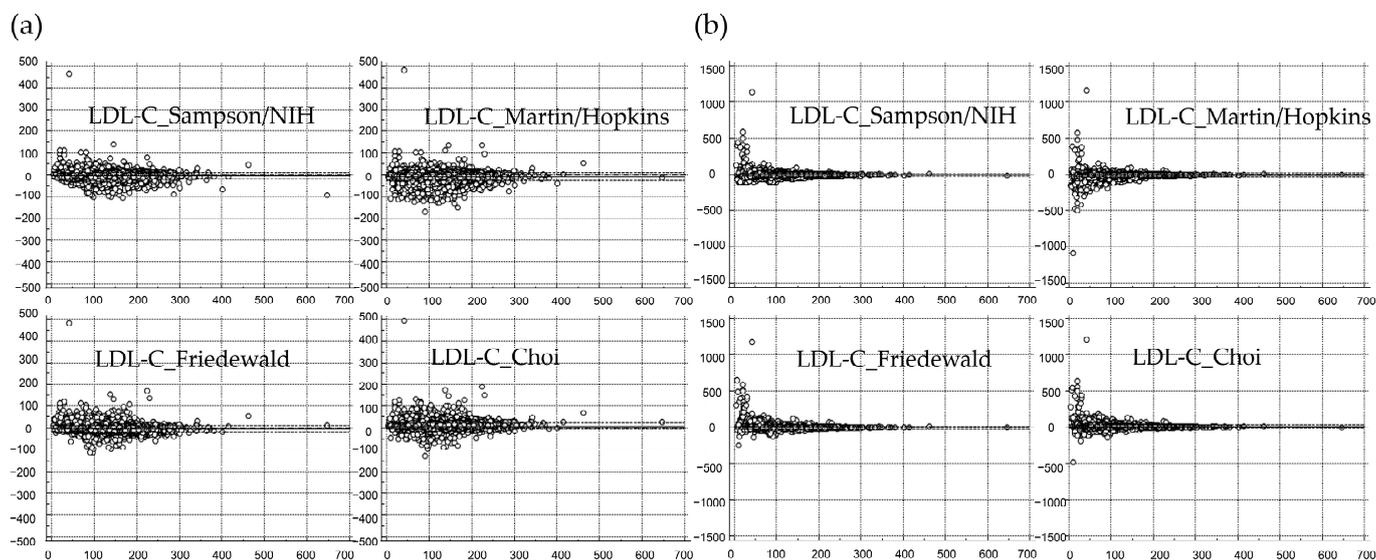
Unit for lipoprotein is mg/dL.

3.3. Validating Equations for Calculated LDL-C

Equations for calculating LDL-C using the non-ApoB-derived percentile (LDL-C\_Sampson/NIH, LDL-C\_Friedewald, LDL-C\_Martin/Hopkins, and LDL-C\_Choi) were obtained from each data set. A comparison between the calculated LDL-C and LDL-

C\_Direct was performed for Validation Sets 1 and 2 (LDL-C\_Direct was not available for the Creation Set).

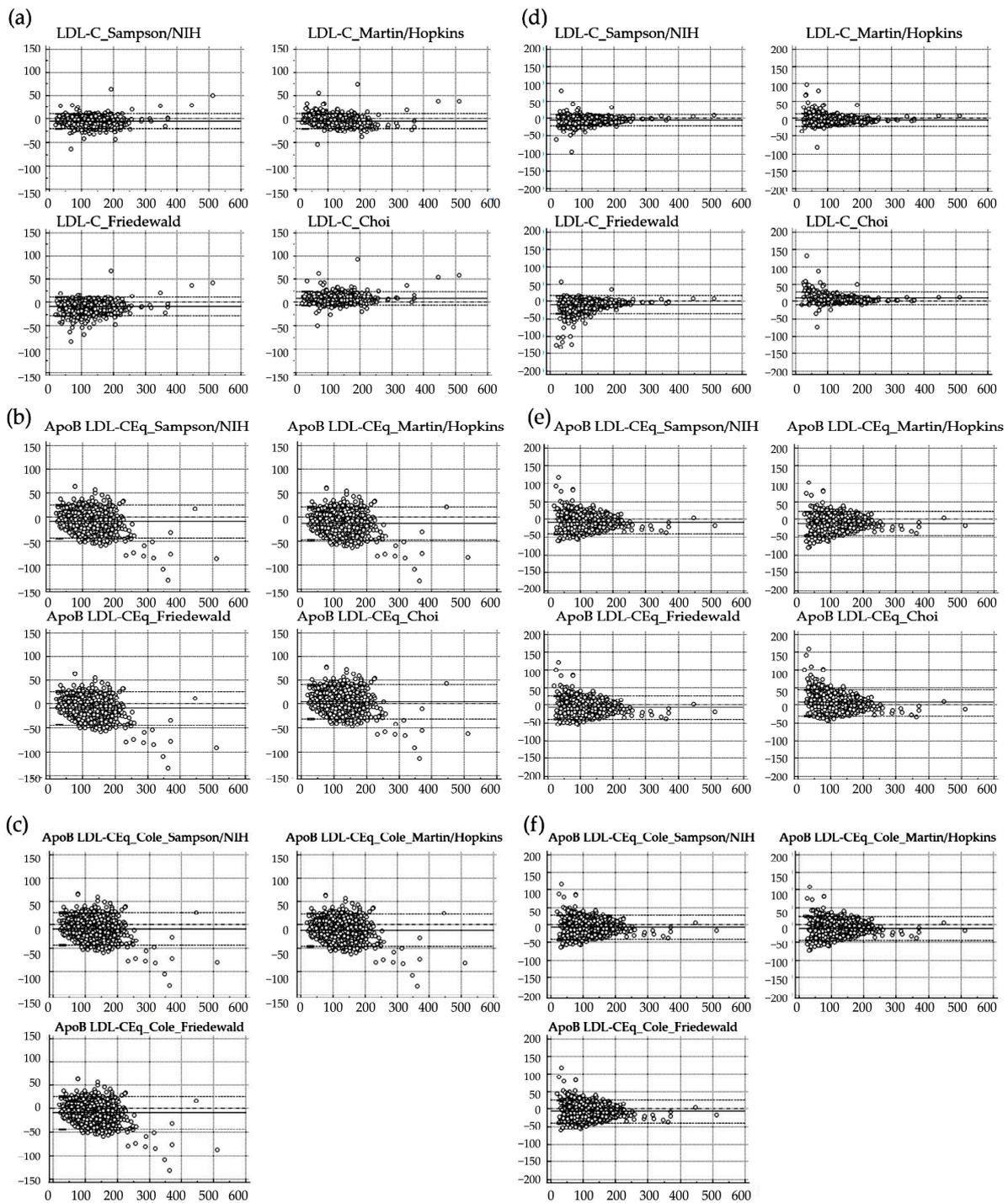
The systemic difference and %difference of the quantitative LDL-C level between LDL-C\_Direct and the calculated LDL-C using each equation in Validation Set 1 are summarized using Bland–Altman plot analysis as in Figure 2.



**Figure 2.** Comparison of calculated LDL-C using previously reported non-ApoB percentile-derived equations in comparison with directly measured LDL-C (LDL-C\_Direct) in Validation Set 1 ( $n = 133,316$ ). (a) Difference between LDL-C\_Direct and calculated LDL-C. The Y-axis represents the difference between calculated LDL-C and directly measured LDL-C (mg/dL). (b) The %difference between LDL-C\_Direct and calculated LDL-C using previously reported equations. The Y-axis represents the %difference between calculated LDL-C using each equation and LDL-C\_Direct. The X-axis represents LDL-C\_Direct. The maximum scale for the Y-axis is consistent across all plots, set at  $\pm 500$  mg/dL for (a) and at  $\pm 1500\%$  for the percentage difference in (b). The horizontal line represents the line of equality (with a difference of 0), while the dashed horizontal line represents the 95% confidence interval for the limits of agreement.

In Validation Set 1 ( $n = 133,316$ ), among four equations for the non-ApoB-derived value, LDL-C\_Choi showed a higher LDL-C level than LDL-C\_Direct with the largest mean difference (9.5 mg/dL, 95% CI 9.4 to 9.5) and mean %difference (9.7%, 95% CI 9.7 to 9.8). The LDL-C\_Martin/Hopkins equation showed the smallest difference ( $-3.7$  mg/dL, 95% CI  $-3.7$  to  $-3.6$ ) and %difference ( $-3.0\%$ , 95% CI  $-3.0$  to  $-2.9$ ) from LDL-C\_Direct, followed by LDL-C\_Sampson/NIH ( $-4.3$  mg/dL and  $-4.1\%$ ), LDL-C\_Friedewald ( $-8.0$  mg/dL and  $-8.0\%$ ), and LDL-C\_Choi. For the absolute percentage error, LDL-C\_Sampson/NIH showed the lowest (median 4.9%, 95% CI 4.8 to 4.9), followed by LDL-C\_Martin/Hopkins (5.0%), LDL-C\_Friedewald (6.5%), and LDL-C\_Choi (8.9%). The calculated LDL-C values using some of these equations showed negative values, resulting in significant differences and percentage differences (Figure 2).

The systemic difference and %difference of quantitative LDL-C level between LDL-C\_Direct and that of each equation in Validation Set 2 are summarized using Bland–Altman plot analysis as in Figure 3.



**Figure 3.** Comparison of calculated LDL-C with directly measured LDL-C (LDL-C\_Direct) in Validation Set 2 ( $n = 1376$ ). (a) Difference between LDL-C\_Direct and calculated LDL-C using previously reported non-ApoB-derived equations. (b) Difference between LDL-C\_Direct and calculated LDL-C using the proposed ApoB-derived equations. (c) Difference between LDL-C\_Direct and calculated LDL-C using ApoB-derived equations by Cole et al. (d–f) The %difference data for (a–c). The X-axis represents LDL-C\_Direct. The maximal value of the Y-axis is  $\pm 150$  mg/dL for the difference from (a–c) and  $\pm 200\%$  for the %difference from (d–f). The horizontal line represents the line of equality (with a difference of 0), while the dashed horizontal line represents the 95% confidence interval for the limits of agreement.

In Validation Set 2 ( $n = 1376$ ), 11 equations for the calculated LDL-C were compared with LDL-C\_Direct. Among these equations, the smallest systemic difference and %difference were observed for LDL-C\_Martin/Hopkins ( $-4.7$  mg/dL and  $3.9\%$ , respectively), followed by LDL-C\_Sampson/NIH ( $-5.0$  mg/dL and  $-4.9\%$ ). The LDL-C\_Choi equation showed an  $8.9$  mg/dL mean systemic difference and a  $9.3\%$  difference. The equation of ApoB LDL-CEq\_Choi showed a  $5.1$  mg/dL mean systemic difference and a  $7.0\%$  difference. The calculated LDL-C level was highest using LDL-C\_Choi and ApoB LDL-CEq\_Choi (positive mean systemic differences), while those using the other equations were lower than LDL-C\_Direct (negative mean systemic differences).

Overall, ApoB percentile-derived equations showed higher absolute percentage errors than non-ApoB-derived equations. The maximum systemic difference and %difference were observed for ApoB LDL-CEq\_Friedewald ( $-12.7$  mg/dL and  $-11.3\%$ , respectively), followed by ApoB LDL-CEq\_Cole\_Friedewald ( $-10.8$  mg/dL and  $-9.4\%$ ). For the absolute percentage error, the lowest median error was observed for LDL-C\_Martin/Hopkins ( $5.3\%$ ), followed by LDL-C\_Sampson/NIH ( $5.6\%$ ). The highest median error was observed for ApoB LDL-CEq\_Friedewald ( $14.1\%$ ) and then ApoB LDL-CEq\_Cole\_Friedewald ( $13.1\%$ ). Detailed results for the systemic difference, %difference, and absolute percentage error for a comparison of the calculated LDL-C and LDL-C\_Direct are summarized in Supplementary Tables S1 and S2.

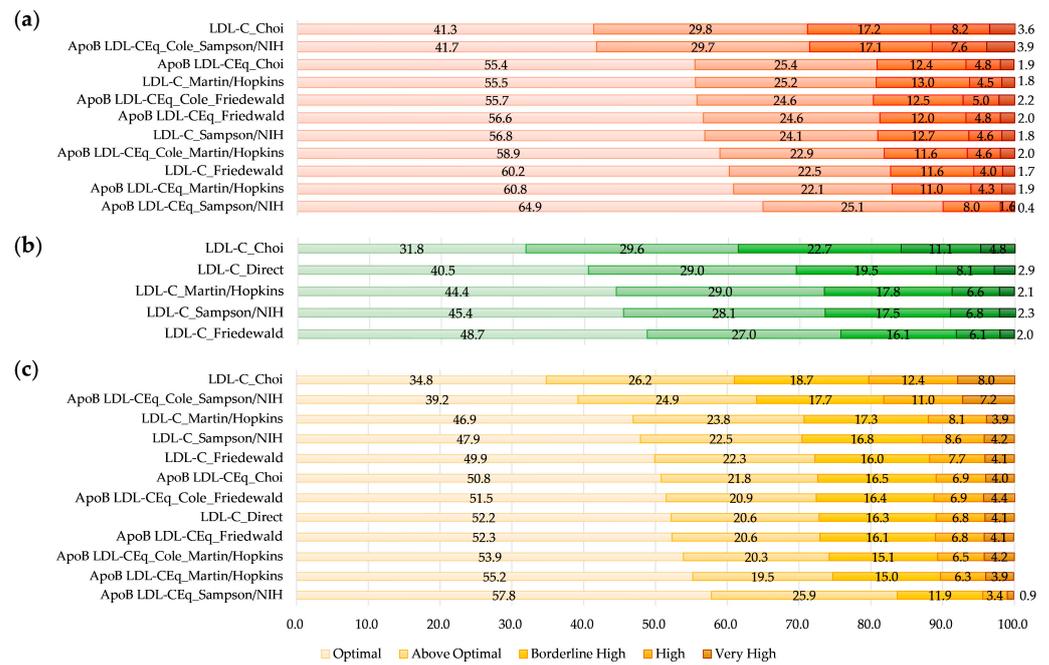
Qualitative results using NCEP ATP III criteria were also compared among equations for LDL-C as summarized in Figure 4. The proportion of patients with optimal LDL-C was different among sets. Among the equations, LDL-C\_Choi showed the smallest proportion of optimal LDL-C level for all data sets. Overall, the ApoB-derived equations had larger proportions of optimal LDL-C level than non-ApoB-derived equations except for the Friedewald equations for the Creation Set. Among all ApoB-derived equations, ApoB LDL-CEq\_Sampson/NIH had the largest proportion of optimal LDL-C, followed by ApoB LDL-CEq\_Martin/Hopkins. The proportion of patients with hyper-LDL-cholesterolemia based on NCEP ATP III criteria ( $\text{LDL-C} \geq 160$  mg/dL) was different among equations, ranging from  $2.0\%$  by LDL-CEq\_Sampson/NIH to  $11.8\%$  by LDL-C\_Choi in the Creation Set and from  $4.3\%$  by ApoB LDL-CEq\_Sampson/NIH to  $20.4\%$  by LDL-C\_Choi in Validation Set 2.

An agreement of NCEP ATP III criteria for LDL-C between the calculated LDL-C and LDL-C\_Direct in the Validation Sets is summarized in Figure 5. In Validation Set 1, LDL-C\_Sampson/NIH and LDL-C\_Martin/Hopkins showed similar agreement with LDL-C\_Direct ( $83.8\%$  and  $83.4\%$ , respectively). In the same set, LDL-C\_Choi showed an overestimation of LDL-C in comparison with LDL-C\_Direct ( $24.2\%$ ).

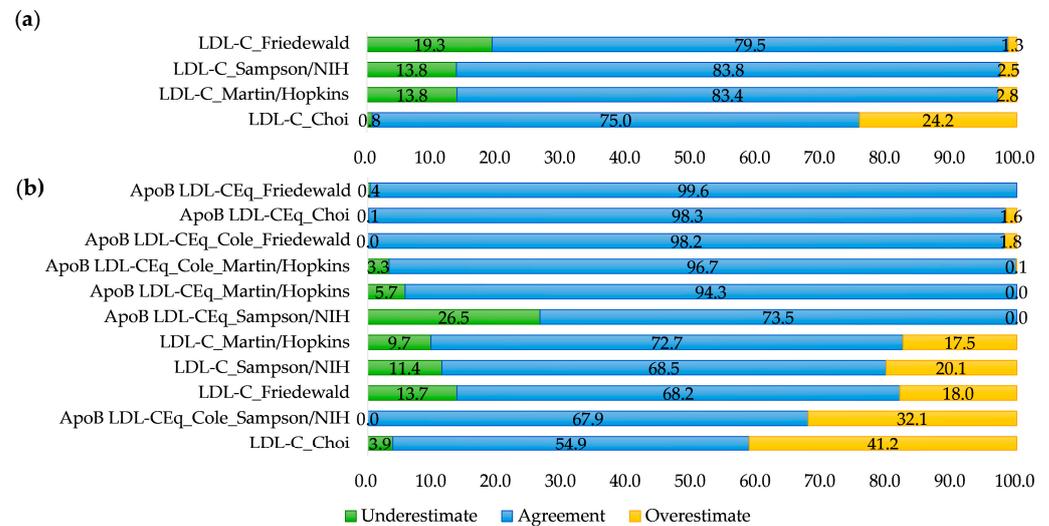
In Validation Set 2, ApoB LDL-CEq\_Friedewald showed the greatest agreement with LDL-C\_Direct ( $99.6\%$ ), followed by ApoB LDL-CEq\_Choi ( $98.3\%$ ). In Validation Set 2, ApoB LDL-CEq\_Sampson/NIH showed an underestimation of NCEP ATP III criteria in comparison with LDL-C\_Direct ( $26.5\%$ ). In Validating Set 2nd, ApoB-derived equations showed greater agreement for NCEP ATP III criteria with LDL-C\_Direct than did non-ApoB-derived equations, except for ApoB LDL-CEq\_Sampson/NIH (overestimation of  $32.1\%$ ). Non-ApoB-derived equations showed an overestimation of NCEP ATP III categorization compared to LDL-C\_Direct (range  $17.5\%$  to  $41.2\%$ ) in Validation Set 2.

### 3.4. ApoB Levels to Predict LDL-C

Table 3 summarizes the ranges of ApoB levels, based on each ApoB-derived equation, for predicting optimal LDL-C levels ( $<100$  mg/dL) and high LDL-C levels ( $\geq 160$  mg/dL), as well as the ranges of LDL-C levels for predicting ApoB levels at or above the upper limit of the reference range for men ( $>144$  mg/dL) and women ( $>141$  mg/dL). Overall, if LDL-C levels were  $<160$  mg/dL (the threshold for high LDL-C according to NCEP ATP III criteria), it appeared likely that ApoB levels would be  $<140$  mg/dL (close to the upper limit of the reference interval for women).



**Figure 4.** Proportion of data sets classified by NCEP ATP III Criteria for calculated LDL-C and directly measured LDL-C (%) in the Creation Set (a), Validation Set 1 (b), and Validation Set 2 (c). Darker colors indicate higher LDL-C levels.



**Figure 5.** Proportion of agreement of NCEP ATP III criteria categorization for calculated LDL-C in comparison with directly measured LDL-C (LDL-C\_Direct, %). (a) Validation Set 1 and (b) Validation Set 2. For creating sets, LDL-C\_Direct was not measured and agreement could not be evaluated.

**Table 3.** Apolipoprotein B levels to predict LDL-C.

| Abbreviation of Equation         | Equation Driven     | Equation (y) = LDL-C                      | ApoB Levels for LDL-C Target |     | LDL-C Levels for ApoB Level |                  |
|----------------------------------|---------------------|---|------------------------------|-----|-----------------------------|------------------|
|                                  |                     |   | 100                          | 160 | 141 <sup>1</sup>            | 144 <sup>2</sup> |
| ApoB LDL-CEq_Sampson/NIH         | This study          | $y = 1.352 \times (\text{ApoB}) - 27.163$ | 94                           | 138 | 163                         | 168              |
| ApoB LDL-CEq_Friedewald          | This study          | $y = 1.382 \times (\text{ApoB}) - 33.756$ | 97                           | 140 | 161                         | 165              |
| ApoB LDL-CEq_Martin/Hopkins      | This study          | $y = 1.327 \times (\text{ApoB}) - 23.669$ | 93                           | 138 | 163                         | 167              |
| ApoB LDL-CEq_Choi                | This study          | $y = 1.393 \times (\text{ApoB}) - 17.113$ | 84                           | 127 | 179                         | 183              |
| ApoB LDL-CEq_Cole_Sampson/NIH    | By Cole et al. [14] | $y = 1.38 \times (\text{ApoB}) - 29$      | 94                           | 137 | 166                         | 170              |
| ApoB LDL-CEq_Cole_Friedewald     | By Cole et al. [14] | $y = 1.385 \times (\text{ApoB}) - 32.2$   | 96                           | 139 | 163                         | 167              |
| ApoB LDL-CEq_Cole_Martin/Hopkins | By Cole et al. [14] | $y = 1.348 \times (\text{ApoB}) - 26.4$   | 94                           | 138 | 164                         | 168              |

<sup>1</sup> Upper limit of reference interval for women; <sup>2</sup> Upper limit of reference interval for men. Unit for lipoprotein is mg/dL.

#### 4. Discussion

In this study, we proposed translational equations for ApoB and LDL-C levels and validated them using independent validation sets in comparison with previously reported equations to assess the calculated LDL-C level in a large Korean population who visited local clinics and hospitals.

In this study, the simultaneous measurement of LDL-C was performed in 28.7% of all tests, while ApoB testing was performed in only 2.0% of tests using the traditional lipid panel (TC, TG, and HDL-C). This demonstrates the underutilization of these tests in Korean patients visiting local clinics and hospitals. According to the Korean Guidelines for the Management of Dyslipidemia, ApoB measurements are recommended for diabetes patients [8]. According to the diabetes factsheet in Korea 2021, the age-standardized prevalence of hyper-LDL-cholesterolemia (LDL-C  $\geq$  160 mg/dL) in Korea is 76.5% in diabetes patients, and the prevalence of diabetes in 2021 was 16.7% among Korean adults aged 30 years or older. This indicates that 12.6% of clinical specimens could be calculated for ApoB [25]. According to the public database Healthcare Bigdata Hub by the Health Insurance Review & Assessment Service Korea (HIRA), 400,764 patients had apolipoprotein data but it did not differentiate between apolipoprotein A and ApoB [26] in 2021. This number was about 2.7% of all patients who underwent reimbursable TC tests, which were performed using an automated enzymatic assay in 2021 (14,990,233 patients, code D2611), confirming underutilization (2.0%). The use of the test could be improved with more clinical evidence to confirm its clinical implications in patient management in Korean populations and with appropriate educational programs targeted toward physicians [27].

In the present study, ApoB percentile-derived LDL-C equations showed comparable slope and intercept with those from equations provided by Cole et al. (Table 2) [14]. Overall, if ApoB levels are <140 mg/dL (close to the upper limit of the reference interval for women), LDL-C levels were predicted to be <160 mg/dL (the threshold for high LDL-C according to NCEP ATP III criteria). Clinicians can use this information to easily predict lipoprotein levels (Table 3). A translational tool for converting ApoB and LDL-C may promote the use of this test, as with the application of glycated hemoglobin and estimated average glucose level [14]. In the present study, ApoB percentile-derived equations in the Creation Set showed comparable results for qualitative NCEP ATP III LDL-C criteria to Validation Set 2. However, non-ApoB-derived equations in previously reported and well-validated equations including LDL-C\_Sampson/NIH, LDL-C\_Friedewald, and LDL-C\_Martin/Hopkins showed underestimated and overestimated LDL-C levels in comparison with LDL-C\_Direct in Validation Set 2. The characteristics of the populations were different among data sets except for LDL-C cholesterol levels. These results suggest that the performance of equations estimating LDL-C can be influenced by the characteristics of specific populations. In the same vein, the LDL-C\_Choi equation derived using data from the same laboratory in a different study period showed overestimated LDL-C levels

in comparison with LDL-C\_Direct in the present study [19]. The LDL-C\_Choi equation was not the best predictor for LDL-C in ApoB-measured groups in the same laboratory. These findings emphasize the importance of equation validation in different populations. Additional studies with detailed clinical information on lipid results and comorbidities affecting the accuracy of equations are needed.

The strength of this study was its large study population with lipid results from local clinics and hospitals. Considering that dyslipidemia is usually managed in such institutions, the study population of the present study included more real-world data than previous university-hospital-based studies [4,28]. In addition, the created and validated ApoB-derived LDL-C equation and previously reported equations provided robust results.

The limitations of this study include a lack of detailed clinical data about dyslipidemia, such as familial history, comorbidities, and lipid-lowering medications [4,19,29]. The physiological relevance of the prediction model is constrained by the limited availability of detailed clinical information, including data on cardiovascular disease endpoints such as heart attacks, emphasizing the necessity for further exploration in future research. The generalizability of this study may be limited to specific patients who visited local clinics and hospitals and had available LDL-C\_Direct and ApoB measurements. Future studies based on detailed clinical information regarding dyslipidemia are needed to clarify the clinical implications of various equations estimating LDL-C and its relationship with ApoB. The cost–benefit analysis of incorporating an ApoB test into a traditional lipid panel analysis was not within the scope of this study. However, considering that ApoB has been suggested as a risk enhancer and therapeutic target for treatment in international guidelines, future studies should investigate the cost–benefit of the ApoB test along with its clinical utility [2,4,10,12,30,31].

## 5. Conclusions

In conclusion, we investigated the utilization of the ApoB test in local clinics and hospitals in Korea and developed and validated new translational equations for ApoB LDL-CEq in a large Korean population along with other previously reported equations for calculated LDL-C in comparison with LDL-C\_Direct. The created ApoB-derived LDL-C equations (ApoB LDL-CEq) in this study showed comparable results with previously reported ApoB-derived equations as a translational tool. The results of this study will expand basic knowledge about equations for LDL-C and predictive changes in the prevalence of LDL-C according to the equation used. Considering that the accuracy of the equations varied by population set, future studies are needed to validate their accuracy and performance with detailed clinical information.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu15122786/s1>, Table S1: Comparison of calculated LDL-C Levels with directly measured LDL-C in the Validating Set 1 ( $n = 133,316$ ); Table S2: Comparison of calculated LDL-C levels with directly measured LDL-C in Validating Set 2nd ( $n = 1376$ ).

**Author Contributions:** Conceptualization, R.C.; methodology, R.C.; software, R.C.; validation, R.C. and S.G.L.; formal analysis, R.C.; investigation, R.C. and S.G.L.; resources, R.C., S.G.L. and E.H.L.; data curation, R.C.; writing—original draft preparation, R.C.; writing—review and editing, R.C., S.G.L. and E.H.L.; visualization, R.C.; supervision, S.G.L. and E.H.L.; project administration, R.C.; funding acquisition, S.G.L. and E.H.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Institutional Review Board of Green Cross Laboratories (GCL-2023-1010-01, 17 February 2023).

**Informed Consent Statement:** A waiver of informed consent was approved by the IRB as this study was retrospective and involved no more than minimal risk to subjects (GCL-2023-1010-01, 17 February 2023).

**Data Availability Statement:** The datasets generated and analyzed during the current study are available from the corresponding authors on reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Vaduganathan, M.; Mensah, G.A.; Turco, J.V.; Fuster, V.; Roth, G.A. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. *J. Am. Coll. Cardiol.* **2022**, *80*, 2361–2371. [CrossRef] [PubMed]
2. Behbodikhah, J.; Ahmed, S.; Elyasi, A.; Kasselmann, L.J.; De Leon, J.; Glass, A.D.; Reiss, A.B. Apolipoprotein B and Cardiovascular Disease: Biomarker and Potential Therapeutic Target. *Metabolites* **2021**, *11*, 690. [CrossRef] [PubMed]
3. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* **2002**, *106*, 3143–3421. [CrossRef]
4. Mach, F.; Baigent, C.; Catapano, A.L.; Koskinas, K.C.; Casula, M.; Badimon, L.; Chapman, M.J.; De Backer, G.G.; Delgado, V.; Ference, B.A.; et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Eur. Heart J.* **2020**, *41*, 111–188. [CrossRef]
5. Glavinovic, T.; Thanassoulis, G.; de Graaf, J.; Couture, P.; Hegele, R.A.; Sniderman, A.D. Physiological Bases for the Superiority of Apolipoprotein B Over Low-Density Lipoprotein Cholesterol and Non-High-Density Lipoprotein Cholesterol as a Marker of Cardiovascular Risk. *J. Am. Heart Assoc.* **2022**, *11*, e025858. [CrossRef]
6. Sniderman, A.D.; Navar, A.M.; Thanassoulis, G. Apolipoprotein B vs Low-Density Lipoprotein Cholesterol and Non-High-Density Lipoprotein Cholesterol as the Primary Measure of Apolipoprotein B Lipoprotein-Related Risk: The Debate Is Over. *JAMA Cardiol.* **2022**, *7*, 257–258. [CrossRef]
7. Cao, J.; Nomura, S.O.; Steffen, B.T.; Guan, W.; Remaley, A.T.; Karger, A.B.; Ouyang, P.; Michos, E.D.; Tsai, M.Y. Apolipoprotein B discordance with low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol in relation to coronary artery calcification in the Multi-Ethnic Study of Atherosclerosis (MESA). *J. Clin. Lipidol.* **2020**, *14*, 109–121. [CrossRef] [PubMed]
8. Committee of Clinical Practice Guideline of the Korean Society of Lipid and Atherosclerosis. Korean Guidelines for the Management of Dyslipidemia, the 5th ed. 2022. Available online: [https://www.lipid.or.kr/eng/file/guidelines\\_2022.pdf](https://www.lipid.or.kr/eng/file/guidelines_2022.pdf) (accessed on 17 April 2023).
9. Grundy, S.M.; Stone, N.J.; Bailey, A.L.; Beam, C.; Birtcher, K.K.; Blumenthal, R.S.; Braun, L.T.; de Ferranti, S.; Faiella-Tommasino, J.; Forman, D.E.; et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* **2019**, *139*, e1082–e1143. [PubMed]
10. Arnett, D.K.; Blumenthal, R.S.; Albert, M.A.; Buroker, A.B.; Goldberger, Z.D.; Hahn, E.J.; Himmelfarb, C.D.; Khera, A.; Lloyd-Jones, D.; McEvoy, J.W.; et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* **2019**, *140*, e596–e646. [CrossRef]
11. Pearson, G.J.; Thanassoulis, G.; Anderson, T.J.; Barry, A.R.; Couture, P.; Dayan, N.; Francis, G.A.; Genest, J.; Grégoire, J.; Grover, S.A.; et al. 2021 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in Adults. *Can. J. Cardiol.* **2021**, *37*, 1129–1150. [CrossRef]
12. Aygun, S.; Tokgozoglu, L. Comparison of Current International Guidelines for the Management of Dyslipidemia. *J. Clin. Med.* **2022**, *11*, 7249. [CrossRef] [PubMed]
13. Frank, A.T.; Zhao, B.; Jose, P.O.; Azar, K.M.; Fortmann, S.P.; Palaniappan, L.P. Racial/ethnic differences in dyslipidemia patterns. *Circulation* **2014**, *129*, 570–579. [CrossRef] [PubMed]
14. Cole, J.; Otvos, J.D.; Remaley, A.T. A Translational Tool to Facilitate Use of Apolipoprotein B for Clinical Decision-Making. *Clin. Chem.* **2023**, *69*, 41–47. [CrossRef]
15. Wilson, P.W.F.; Polonsky, T.S.; Miedema, M.D.; Khera, A.; Kosinski, A.S.; Kuvin, J.T. Systematic Review for the 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* **2019**, *139*, e1144–e1161. [CrossRef] [PubMed]
16. Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **1972**, *18*, 499–502. [CrossRef]
17. Sampson, M.; Ling, C.; Sun, Q.; Harb, R.; Ashmaig, M.; Warnick, R.; Sethi, A.; Fleming, J.K.; Otvos, J.D.; Meeusen, J.W.; et al. A New Equation for Calculation of Low-Density Lipoprotein Cholesterol in Patients with Normolipidemia and/or Hypertriglyceridemia. *JAMA Cardiol.* **2020**, *5*, 540–548. [CrossRef] [PubMed]
18. Martin, S.S.; Blaha, M.J.; Elshazly, M.B.; Toth, P.P.; Kwiterovich, P.O.; Blumenthal, R.S.; Jones, S.R. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA* **2013**, *310*, 2061–2068. [CrossRef]

19. Choi, R.; Park, M.J.; Oh, Y.; Kim, S.H.; Lee, S.G.; Lee, E.H. Validation of multiple equations for estimating low-density lipoprotein cholesterol levels in Korean adults. *Lipids Health Dis.* **2021**, *20*, 111. [CrossRef]
20. CLSI. Interference Testing in Clinical Chemistry. In *CLSI Guideline EP07*, 3rd ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.
21. Marcovina, S.M.; Albers, J.J.; Dati, F.; Ledue, T.B.; Ritchie, R.F. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. *Clin. Chem.* **1991**, *37*, 1676–1682. [CrossRef]
22. Albers, J.J.; Marcovina, S.M.; Kennedy, H. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B. II. Evaluation and selection of candidate reference materials. *Clin. Chem.* **1992**, *38*, 658–662. [CrossRef]
23. Centers for Disease Control and Prevention. Lipids Standardization Program. Available online: <https://www.cdc.gov/labstandards/csp/lsp.html> (accessed on 14 June 2023).
24. Kim, S.; Lee, K.; Park, H.-D.; Lee, Y.-W.; Chun, S.; Min, W.-K. Schemes and Performance Evaluation Criteria of Korean Association of External Quality Assessment (KEQAS) for Improving Laboratory Testing. *Ann. Lab. Med.* **2021**, *41*, 230–239. [CrossRef] [PubMed]
25. Bae, J.H.; Han, K.D.; Ko, S.H.; Yang, Y.S.; Choi, J.H.; Choi, K.M.; Kwon, H.S.; Won, K.C. Diabetes Fact Sheet in Korea 2021. *Diabetes Metab. J.* **2022**, *46*, 417–426. [CrossRef]
26. Health Insurance Review and Assessment Service, Korea. Statistics for Test Service. Healthcare Bigdata Hub. Available online: <http://opendata.hira.or.kr/op/opc/olapDiagBhvInfo.do> (accessed on 24 April 2023).
27. CLSI. Developing and Managing a Medical Laboratory (Test) Utilization Management Program. In *CLSI Report GP49*, 1st ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2017.
28. Visseren, F.L.J.; Mach, F.; Smulders, Y.M.; Carballo, D.; Koskinas, K.C.; Bäck, M.; Benetos, A.; Biffi, A.; Boavida, J.M.; Capodanno, D.; et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. *Eur. Heart J.* **2021**, *42*, 3227–3337. [CrossRef]
29. Lee, C.J.; Yoon, M.; Kang, H.J.; Kim, B.J.; Choi, S.H.; Jeong, I.K.; Lee, S.H. 2022 Consensus Statement on the Management of Familial Hypercholesterolemia in Korea. *J. Lipid Atheroscler.* **2022**, *11*, 213–228. [CrossRef] [PubMed]
30. Elshazly, M.B.; Quispe, R. The Lower the ApoB, the Better: Now, How Does ApoB Fit in the Upcoming Era of Targeted Therapeutics? *Circulation* **2022**, *146*, 673–675. [CrossRef] [PubMed]
31. Hagström, E.; Steg, P.G.; Szarek, M.; Bhatt, D.L.; Bittner, V.A.; Danchin, N.; Diaz, R.; Goodman, S.G.; Harrington, R.A.; Jukema, J.W.; et al. Apolipoprotein B, Residual Cardiovascular Risk after Acute Coronary Syndrome, and Effects of Alirocumab. *Circulation* **2022**, *146*, 657–672. [CrossRef] [PubMed]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.