Relationship between serum cholesterol and indices of erythrocytes and platelets in the US population[S]

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Abstract **Whereas dyslipidemia has been associated with leukocytosis, the relationship between serum cholesterol and other hematopoietic lineages is poorly defined. Erythrocytes and platelets, anucleate cells relegated to nonspecific diffusional exchange of cholesterol with serum, have been proposed to have a distinct relationship to cholesterol from leukocytes. We examined the relationship between serum cholesterol and circulating erythrocyte/platelet indices in 4,469 adult participants of the National Health and Nutrition Examination Survey (NHANES) 2005–2006. In linear regression analyses, serum non-high density lipoprotein-cholesterol (non-HDL-C) was positively associated with mean erythrocyte number, hematocrit, hemoglobin concentration, platelet count, and platelet crit independently of age, gender, race/ethnicity, smoking, body mass index, serum folate, and C-reactive protein. The magnitude of the relationship was most marked for platelets, with lowest versus highest non-HDL-C quartile subjects having geometric mean platelet counts of 258,000/μl versus 281,000/μl, respectively (adjusted model, $P < 0.001$ for trend). These associations persisted in a sensitivity analysis excluding several conditions that affect erythrocyte/platelet and/or serum cholesterol levels, and were also noted in an independent analysis of 5,318 participants from NHANES 2007–2008. As non-HDL-C, erythrocytes, and platelets all impact cardiovascular disease risk, there is a need for advancing understanding of the underlying interactions that govern levels of these three blood components.**—Fessler, M. B., K. Rose, Y. Zhang, R. Jaramillo, and D. C. Zeldin. **Relationship between serum cholesterol and indices of erythrocytes and platelets in the US population.** ***J. Lipid Res.*** **2013.** 54: **3177–3188.**

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Several cross-sectional analyses have indicated that metabolic syndrome and dyslipidemia associate with leukocytosis in humans (1–3), however the relationship between serum cholesterol and other hematopoietic lineages is poorly defined. Erythrocytes and platelets are linked in their life cycle, deriving from a common progenitor in the bone marrow and ultimately undergoing clearance by the reticuloendothelial system (4–6). Unlike macrophages, they have minimal capacity for storage of cholesteryl ester and, as anucleate cells, lack the means for cholesterol synthesis (7, 8). As erythrocytes have no intracellular membranes and undergo nonspecific diffusional exchange of cholesterol with their milieu (7, 8), the cholesterol content of the erythrocyte plasma membrane is particularly susceptible to serum cholesterol (7, 9). Platelets undergo similar diffusional exchange of cholesterol with plasma (7). Given this, erythrocytes and platelets have been proposed to have a relationship to extracellular (serum) cholesterol distinct from that of leukocytes (7).

Reports using animal models have identified erythrocyte and platelet abnormalities associated with dyslipidemia (4, 6, 10–15). Loading of the plasma membrane with cholesterol, such as by elevated non-high density lipoprotein-cholesterol (non-HDL-C) in the setting of a high-fat diet, promotes erythrocyte hemolysis (10, 12) and reduces platelet survival (14). Elevated high density lipoprotein-cholesterol (HDL-C), such as seen in scavenger receptor class B type I (SR-BI)-deficient mice, has also been linked to impaired lifespan and number of erythrocytes and platelets, as well as to macrocytosis of both cell types (4, 6, 13). In vitro studies indicate that erythrocyte membrane fluidity is reduced and stability increased in parallel with exogenously induced increases in membrane cholesterol/phospholipid ratio (16), but that membrane stability may be maximal within a critical

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Abbreviations: BMI, body mass index; CRP, C-reactive protein; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; MCV, mean corpuscular volume; MPV, mean platelet volume; NHANES, National Health and Nutrition Examination Survey; non-HDL-C, non-high density lipoprotein-cholesterol; SR-BI, scavenger receptor class B type I; TC, total cholesterol.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of 24 tables.

window of cholesterol content, above which it, along with erythrocyte and platelet lifespan, is compromised (4, 6, 17).

In humans, multiple small clinical case series have identified reduced serum cholesterol as a common finding in a variety of hemolytic anemias, and it has been proposed that this may occur through cholesterol consumption by avid erythropoiesis (11). Reports such as these suggest that, in addition to serum cholesterol impacting the population kinetics of erythrocytes and platelets, erythrocytes may reciprocally impact serum cholesterol levels. In support of this postulate, in vitro studies indicate that human erythrocytes act as a reservoir of cholesterol for serum lipoproteins, presumably because of their high nonspecific cholesterol loading capacity (18, 19).

There have been very few studies of the relationship between serum cholesterol and indices of either erythrocytes or platelets in large human populations. While a few studies have shown positive correlations between serum cholesterol and either hematocrit or hemoglobin (20, 21), others have found no such relationship (22). Findings for serum cholesterol and platelets have similarly been disparate (23, 24). Given this, the nature of the relationships in humans between serum cholesterol and both erythrocytes and platelets remain undefined outside of disease extremes, as does the broader relevance of these relationships to public health. Given that hypercholesterolemia, erythrocytosis, and thrombocytosis, as well as the membrane cholesterol content of both erythrocytes and platelets are all risk factors for cardiovascular disease (25–28); there is a need for advancing our understanding of the underlying relationships between serum cholesterol and erythrocyte and platelet lineages in humans.

The National Health and Nutrition Examination Survey (NHANES) is a biennial, cross-sectional population-based survey of the US population that includes measurements of erythrocyte and platelet indices, and serum cholesterol. We hypothesized that, in humans, as observed in rodent models, HDL-C would have an inverse association with abundance indices of erythrocytes (erythrocyte number, hematocrit, hemoglobin concentration) and platelets (platelet crit, platelet count). Given that non-HDL-C may possibly promote both production and destruction of both cell types, we had no clear a priori hypothesis regarding the relationship between non-HDL-C and erythrocyte/platelet indices.

METHODS

Study population

Data were obtained from the NHANES 2005–2006 and NHANES 2007–2008, which used a complex multistage design to assess the health and nutritional status of the civilian noninstitutionalized US population. NHANES uses a randomization scheme to select US counties and, within them, households for survey each year, and thus by design minimizes the likelihood of resampling individuals across 2 year survey installments. To ensure adequate sample sizes of certain subgroups of the population, NHANES oversampled persons of low income, elderly subjects (≥60 years), African Americans, and Mexican Americans, among others. All study participants who completed the household interview were also invited to participate in the Health Examination Component that was conducted in the mobile examination center. Detailed description of the survey design and implementation may be found online. NHANES 2005–2006 was treated as the primary study population for our analyses, and NHANES 2007–2008 as a replication study population. All participants aged ≥20 years who visited the NHANES mobile examination center, and for which data were available for total cholesterol (TC), HDL-C, erythrocyte count, hemoglobin concentration, hematocrit, and platelet count were included in our analyses.

Serum cholesterol and blood cell measurements

Serum TC and HDL-C were measured using a Roche Hitachi 717 or 912 (NHANES 2005–2006) or a Roche Modular P chemistry analyzer (NHANES 2007–2008). For TC, coupled enzymatic reactions were used involving cholesteryl ester hydrolase, cholesterol oxidase, and peroxidase, followed by phenazone absorbance detection. HDL-C measurement was by the Roche/Boehringer-Mannheim Diagnostics direct HDL method. For blood cell analysis, a Beckman Coulter MAXM (NHANES 2005–2006) or Beckman Coulter HMX (NHANES 2007–2008) was used. Erythrocyte count (RBCC) was measured directly. Hemoglobin concentration was determined by absorbance found through photocurrent transmittance. Mean corpuscular volume (MCV) was derived from the erythrocyte histogram, and used in NHANES to compute hematocrit as: RBCC × MCV/10. Platelet count and mean platelet volume (MPV) were both derived from the platelet histogram. Platelet crit (%) was calculated as follows: (platelet count × MPV)/10,000.

Covariates and other laboratory measurements

Covariates were obtained from a questionnaire (age, race/ethnicity, gender, smoking), lab analyses [serum C-reactive protein (CRP), erythrocyte and serum folate], and physical examination (height, weight). CRP was measured by latex-enhanced nephelometry. Serum and erythrocyte folate were measured by radioassay (2005–2006) or microbiologic assay of *Lactobacillus rhamnosus* by turbidometry at 590 nm (2007–2008). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Glycohemoglobin (hemoglobin A1C) was measured on either a Tosoh A1c 2.2 Plus or Tosoh G7 automated HPLC system (commenced in 2007) glycohemoglobin analyzer.

Statistical analysis

To account for the complex sampling design used in NHANES and to assure unbiased variance estimates, all analyses were conducted using SAS Survey statistical software (Version 9.3, SAS, Cary, NC). Descriptive statistics were generated [means or percentages and associated standard errors (SEs)]. All blood parameters were assessed for normality. Hemoglobin and platelets were not normally distributed; thus, geometric means are presented. Linear regression analyses were run, assessing the association of the blood parameters with quartiles of HDL-C and non-HDL-C; least squares means of the blood parameters, by quartiles of cholesterol, and associated 95% confidence intervals were generated from the regression coefficients and variance estimates. Cholesterol quartiles derived from NHANES 2005–2006 were used to analyze both surveys: *1*) HDL-C [low (≤41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), very high(>62.93 mg/dl)]; and *2*) non-HDL-C [low (≤114.42 mg/dl), medium (>114.42–140.1 mg/dl), high

(>140.1–168.28 mg/dl), very high (>168.28 mg/dl)]. Cholesterol quartiles for NHANES 2007–2008 were similar, as follows: *1*) HDL-C [low (≤40.04 mg/dl), medium (>40.04–49.14 mg/dl), high (>49.14–60.61 mg/dl), very high (>60.61 mg/dl)]; and *2*) non-HDL-C [low (≤115.06 mg/dl), medium (>115.06–140.67 mg/dl), high (>140.67–170.08 mg/dl), very high (>170.08 mg/dl)]. Five sets of models were run: *1*) unadjusted; *2*) adjusted for age, race/ethnicity, gender, smoking, and BMI; *3*) adjusted for age, race/ethnicity, gender, smoking, BMI, and fasting time; *4*) adjusted for age, race/ethnicity, gender, smoking, BMI, and CRP; and *5*) adjusted for age, race/ethnicity, gender, smoking, BMI, CRP, and fasting time. Because adjustment for fasting time and CRP did not affect the observed associations, only the crude models (Model 1) and the models adjusting for age, race/ethnicity, gender, smoking, and BMI (Model 2) are presented in the results section. A test for trend was used to statistically evaluate variations in the blood parameters across quartiles of cholesterol. A *P* value of ≤0.001 was set as a cutoff for statistical significance. This value was chosen given the large NHANES sample sizes and to account for the multiple blood parameters examined.

RESULTS

The characteristics of the NHANES 2005–2006 and 2007–2008 study populations are shown in **Table 1**. The NHANES 2005–2006 study population was approximately equally divided between genders, with a mean ± SE age of 46.8 ± 0.7 years, and was predominantly (72.4%) non-Hispanic White, with the remainder represented by non-Hispanic Black, Mexican American, and Other categories. A little under half of the subjects had fasted (i.e., ≥9 h) at the time of laboratory analysis, and 13.3 ± 0.8% reported using a statin drug within the past 30 days. TC and HDL-C were measured in both fasting and nonfasting NHANES

TABLE 1. Characteristics of the NHANES 2005–2006 and 2007–2008 study populations, aged ≥20 years

	NHANES 2005–2006			NHANES 2007–2008		
	N[c]	Mean (%)	SE	N[c]	Mean (%)	SE
Total	4,469	100.0	—	5,318	100.0	—
Gender						
Male	2,148	48.1	0.6	2,608	48.2	0.6
Female	2,321	51.9	0.6	2,710	51.8	0.6
Race/ethnicity						
White non-Hispanic	2,254	72.4	2.7	2,537	70.0	3.6
Black non-Hispanic	996	11.1	1.9	1,018	10.3	1.8
Mexican American	910	8.0	1.0	941	8.5	1.5
Other[a]	309	8.5	1.1	822	11.2	1.8
Mean age (years)	4,469	46.8	0.7	5,318	47.0	0.4
BMI (kg/m^2)						
Underweight (<18.5)	142	2.9	0.3	155	2.6	0.3
Healthy (≥18.5 to 25)	1,261	30.7	1.3	1,432	30.0	0.9
Overweight (≥25 to 30)	1,507	32.3	0.8	1,826	34.3	0.8
Obese (≥30)	1,559	34.0	1.4	1,905	33.0	1.1
Education attainment[b]						
Less than 9th grade	523	6.4	0.7	681	7.0	0.7
9th–11th grade	648	11.6	1.3	876	12.9	1.4
High school graduate or GED	1,052	24.9	1.0	1,317	25.6	1.3
Some college	1,248	30.9	1.1	1,299	27.9	1.0
College graduate and above	879	26.3	2.1	1,034	26.6	2.1
Mean CRP (mg/l)	4,468	4.3	0.2	5,315	4.0	0.1
Smoking status						
Never	2,348	51.1	1.3	2,788	52.9	1.7
Past	1,135	24.9	1.0	1,344	24.6	0.7
Current	983	24.0	1.2	1,180	22.5	1.3
Fasting (≥9 h)	2,065	46.2	0.7	2,475	46.1	1.2
HDL-C (mg/dl)	4,469	54.6	0.3	5,318	52.0	0.5
Non-HDL-C (mg/dl)	4,469	144.4	0.9	5,318	145.3	0.6
Hematocrit (%)	4,469	42.7	0.2	5,318	41.6	0.2
Hemoglobin (g/dl)[d]	4,469	14.4	0.1	5,318	14.3	0.1
Erythrocyte count (million/ul)	4,469	4.7	0.02	5,318	4.7	0.02
Erythrocyte MCV (fL)	4,469	90.2	0.2	5,318	88.6	0.3
Platelets (1,000 cells/ul)[d]	4,469	272.1	2.0	5,318	258.2	1.3
MPV (fL)	4,469	8.1	0.03	5,318	7.7	0.05
Platelet crit (%)	4,469	0.225	0.002	5,318	0.204	0.002
Statin use, past 30 days	645	13.3	0.8	972	15.1	0.6
Hemoglobin A1C ≥6.5%[e]	385	6.2	0.5	611	7.8	0.7

[a]Includes Hispanics other than Mexican Americans, other race/ethnic groups, and persons reporting a race/ethnicity in more than one category.

[b]Based on education of the referent household member.

[c]The sum of the Ns for levels of individual characteristics may be slightly lower than the total N due to a small percentage of missing values.

[d]Geometric mean.

[e]Hemoglobin A1C ≥6.5% was proposed as diagnostic of diabetes by (58). There were changes in the equipment used to measure hemoglobin A1C from NHANES 2005–2006 to NHANES 2007–2008 (see Methods).

participants, whereas low density lipoprotein-cholesterol (LDL-C) was only measured in subjects who had been instructed to fast. Non-HDL-C (i.e., TC minus HDL-C), a composite measure of atherogenic LDL-C and very low density lipoprotein-cholesterol (VLDL-C), has comparable or better predictive value than LDL-C for cardiovascular disease (29, 30), and both fasting and nonfasting non-HDL-C are predictive of cardiovascular disease (31). Thus, all primary analyses were based upon non-HDL-C (derived as TC minus HDL-C) and HDL-C measured in a combined fasting and nonfasting study population, as previously reported (32). The mean ± SE serum non-HDL-C in the 2005–2006 study population was 144.4 ± 0.9 mg/dl, and the mean serum HDL-C was 54.6 ± 0.3 mg/dl. Mean ± SE values for hematocrit, hemoglobin, platelet count, and platelet crit were 42.7 ± 0.2%, 14.4 ± 0.1 g/dl, 272.1 ± 2.0 × 10^3/μl, and 0.225 ± 0.002%, respectively. For the 2007–2008 study population, the mean ± SE serum non-HDL-C was 145.3 ± 0.6 mg/dl, and the mean serum HDL-C was 52.0 ± 0.5 mg/dl. Mean ± SE values for hematocrit, hemoglobin, platelet count, and platelet crit were 41.6 ± 0.2%, 14.3 ± 0.1 g/dl, 258.2 ± 1.3 × 10^3/μl, and 0.204 ± 0.002%, respectively.

Table 2 presents mean hematocrit by quartiles of HDL-C and non-HDL-C. In the unadjusted model, mean hematocrit decreased as HDL-C increased. However, upon adjustment for age, race/ethnicity, gender, smoking status, and BMI, this inverse association did not persist. By contrast, mean hematocrit increased across ascending quartiles of non-HDL-C, and this association persisted after controlling for age, race/ethnicity, gender, smoking status, and BMI. Additional adjustment for fasting time, erythrocyte folate, and CRP did not appreciably impact results (data not shown). No relationship was found between ascending categories of non-HDL-C and transferrin saturation or serum folate (data not shown). As shown in Table 2, we repeated these analyses using data from NHANES 2007–2008, and while absolute mean values varied, the associations were replicated.

As shown in **Table 3**, patterns of association of cholesterol with mean hemoglobin concentration, an alternate clinically used metric of erythrocyte mass, were similar to those observed for hematocrit. In adjusted analyses, there was no significant association between HDL-C and hemoglobin, while there was a significant increase in hemoglobin across increasing quartiles of non-HDL-C. As for hematocrit, the associations of hemoglobin to HDL-C and non-HDL-C observed in NHANES 2005–2006 were replicated in NHANES 2007–2008.

Consistent with the findings for hematocrit and hemoglobin, a significant increase was also observed in unadjusted and adjusted erythrocyte number across ascending categories of non-HDL-C in both surveys (**Table 4**). By contrast, while the inverse relationships of HDL-C to hematocrit

TABLE 2. Mean hematocrit by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	Hematocrit Mean (%)	95% CI	Hematocrit Mean (%)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	44.37	43.98, 44.76	42.77	42.34, 43.21
Medium	43.00	42.45, 43.56	42.06	41.55, 42.58
High	42.15	41.64, 42.65	41.24	40.55, 41.93
Very high	41.39	40.82, 41.95	40.09	39.40, 40.78
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	42.85	42.45, 43.25	41.56	41.15, 41.97
Medium	42.57	41.98, 43.15	41.74	41.19, 42.30
High	42.83	42.27, 43.39	41.67	41.12, 42.22
Very high	42.54	42.08, 43.01	41.58	41.01, 42.16
Trend *P* value	0.212		0.962	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	41.82	41.27, 42.37	40.56	40.10, 41.01
Medium	42.32	41.74, 42.91	41.38	40.69, 42.07
High	42.87	42.39, 43.35	41.87	41.15, 42.59
Very high	43.66	43.23, 44.09	42.64	42.29, 42.99
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	42.12	41.57, 42.67	40.80	40.42, 41.18
Medium	42.42	41.90, 42.95	41.51	40.85, 42.18
High	42.88	42.40, 43.36	41.83	41.27, 42.39
Very high	43.32	42.82, 43.82	42.36	41.91, 42.81
Trend *P* value	<0.001		<0.001	

Cholesterol quartiles were determined from NHANES 2005–2006 and are as follows: HDL-C [low (≤41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and non-HDL-C [low (≤114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high (>168.28 mg/dl)]. CI, confidence interval.

[a] Unadjusted model.

[b] Adjusted for age, race/ethnicity, gender, smoking, and BMI.

TABLE 3. Geometric mean of hemoglobin by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	Hemoglobin Mean (g/dl)	95% CI	Hemoglobin Mean (g/dl)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	15.00	14.88, 15.11	14.78	14.57, 14.98
Medium	14.48	14.32, 14.65	14.43	14.21, 14.64
High	14.17	14.01, 14.32	14.11	13.85, 14.38
Very high	13.94	13.76, 14.11	13.70	13.44, 13.95
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	14.45	14.34, 14.56	14.31	14.12, 14.50
Medium	14.33	14.16, 14.51	14.31	14.09, 14.55
High	14.41	14.24, 14.58	14.27	14.06, 14.48
Very high	14.33	14.21, 14.45	14.24	14.03, 14.45
Trend *P* value	0.196		0.183	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	14.03	13.84, 14.22	13.87	13.68, 14.06
Medium	14.26	14.10, 14.42	14.16	13.89, 14.44
High	14.46	14.33, 14.59	14.36	14.07, 14.65
Very high	14.74	14.60, 14.88	14.72	14.55, 14.90
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	14.14	13.98, 14.30	13.96	13.81, 14.12
Medium	14.29	14.16, 14.43	14.21	13.95, 14.48
High	14.46	14.34, 14.58	14.34	14.12, 14.57
Very high	14.61	14.46, 14.77	14.61	14.43, 14.80
Trend *P* value	<0.001		<0.001	

Cholesterol quartiles were determined from NHANES 2005–2006 and are as follows: HDL-C [low (⩽41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and nonHDL-C [low (⩽114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high (>168.28 mg/dl)]. CI, confidence interval.

[a]Unadjusted model.

[b]Adjusted for age, race/ethnicity, gender, smoking, and BMI.

and hemoglobin did not persist after adjustment for covariates, an inverse relationship of HDL-C to erythrocyte number was observed in both unadjusted and adjusted analyses of NHANES 2005–2006. This relationship was attenuated after adjustment in NHANES 2007–2008, marginally missing the significance threshold.

Increasing MCV was observed across ascending quartiles of HDL-C in both adjusted and unadjusted models; this was observed in both NHANES surveys (**Table 5**). This relationship persisted after controlling for transferrin saturation and erythrocyte folate (data not shown). No relationship was observed between non-HDL-C and MCV.

Serum cholesterol-platelet relationships were next examined. As for hematocrit, a significant increase in platelet crit was observed across ascending categories of non-HDL-C in unadjusted and adjusted models (**Table 6**). Similar to the findings for erythrocyte number, a significant increase in mean platelet count was also observed across ascending categories of non-HDL-C in unadjusted and adjusted models (**Table 7**). This relationship was seen in both NHANES surveys, and also persisted after adjustment for fasting time, CRP, and erythrocyte folate (data not shown). However, no consistent relationship was seen between either HDL-C or non-HDL-C and MPV (**Table 8**).

In order to evaluate the robustness of these associations, we performed a sensitivity analysis of the NHANES 2005–2006 study population in which we excluded subjects (N = 1,375) with one or more of the following conditions known to impact serum cholesterol levels and/or blood cell counts: *1*) history of liver disease; *2*) history of cancer; *3*) statin use within past 30 days; *4*) current pregnancy; and *5*) treatment for anemia within the past 3 months. Neither the magnitude nor the statistical significance of any of the relationships of HDL-C and non-HDL-C to hematocrit, hemoglobin, erythrocyte count, MCV, platelet crit, and platelet count was changed after these exclusions (supplementary Tables I–VI).

Analysis of the lipoprotein strata in both surveys revealed a significant decline in males across ascending HDL-C quartiles. Conversely, an increase in males across ascending non-HDL-C quartiles was observed in NHANES 2005–2006 (supplementary Table VII). Given this, in order to address possible persisting effects of gender upon our analysis, we also repeated the analyses within gender strata. The significant increases in hematocrit, hemoglobin, erythrocyte count, and platelet count seen in both surveys across increasing quartiles of non-HDL-C were also observed in gender-stratified analyses (i.e., within both males and females separately) (supplementary Tables VIII–XV). The significant increase in MCV across increasing quartiles of HDL-C was seen within both genders in NHANES 2005–2006 and within males in NHANES 2007–2008, but fell just short of significance ($P = 0.004$)

TABLE 4. Mean erythrocyte count by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	RBC Mean (million/ul)	95% CI	RBC Mean (million/ul)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	4.99	4.94, 5.03	4.87	4.84, 4.91
Medium	4.81	4.75, 4.87	4.77	4.72, 4.82
High	4.68	4.64, 4.73	4.64	4.58, 4.71
Very high	4.52	4.47, 4.57	4.49	4.42, 4.56
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	4.83	4.79, 4.87	4.74	4.71, 4.77
Medium	4.76	4.70, 4.82	4.73	4.68, 4.79
High	4.74	4.70, 4.79	4.69	4.64, 4.74
Very high	4.65	4.60, 4.69	4.66	4.59, 4.72
Trend *P* value	<0.001		0.002	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	4.64	4.60, 4.69	4.57	4.52, 4.62
Medium	4.69	4.63, 4.76	4.68	4.62, 4.75
High	4.78	4.73, 4.82	4.74	4.67, 4.82
Very high	4.85	4.81, 4.89	4.82	4.79, 4.85
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	4.67	4.62, 4.72	4.60	4.56, 4.64
Medium	4.70	4.64, 4.76	4.70	4.63, 4.76
High	4.78	4.74, 4.82	4.74	4.68, 4.79
Very high	4.81	4.77, 4.86	4.79	4.76, 4.83
Trend *P* value	<0.001		<0.001	

Cholesterol quartiles were determined from NHANES 2005-2006 and are as follows: HDL-C [low (⩽41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and nonHDL-C [low (⩽114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high (>168.28 mg/dl)]. CI, confidence interval.

[a]Unadjusted model.

[b]Adjusted for age, race/ethnicity, gender, smoking, and BMI.

within females in the adjusted model in NHANES 2007–2008 (supplementary Tables XVI–XVII).

Differences in the percentage of fasting subjects were also noted across HDL-C and non-HDL-C quartiles, although most differences fell short of statistical significance (supplementary Table XVIII). In order to more confidently exclude confounding by fasting, we evaluated the relationship of HDL-C and non-HDL-C to blood cell parameters among the subset of the study population that had fasted (i.e., ⩾9 h). As shown in supplementary Tables XIX–XXIV, we obtained very similar results to those obtained in the mixed fasting-nonfasting study population.

DISCUSSION

Examining US national data from NHANES 2005–2006, we report that serum non-HDL-C is positively related to abundance measures of both erythrocytes (erythrocyte number, hematocrit, hemoglobin concentration) and platelets (platelet crit, platelet count). This is independent of age, race/ethnicity, gender, smoking status, and BMI. Conversely, an inverse relationship was found between HDL-C and erythrocyte number. We also report that HDL-C is directly related to erythrocyte MCV, whereas no relationships were found between either HDL-C or non-HDL-C and MPV. Consistent findings were observed in an independent study population from NHANES 2007–2008.

Erythrocytes and platelets, anucleate blood cells with no significant capacity for cholesterol storage but with high capacity for diffusional exchange of cholesterol with plasma, have long been proposed to have a relationship to extracellular cholesterol that differs substantially from that of leukocytes (7). Coordinate abnormalities of erythrocytes and platelets have been observed in gene-targeted rodent models of dyslipidemia (4, 6, 13). Conversely, low serum cholesterol has been reported in several types of anemia and found to reverse upon treatment of anemia (11). However, the broader relevance of these relationships to human health has remained undefined.

Studies extending back over 30 years have elegantly shown that in vitro incubation of erythrocytes and platelets with cholesterol-enriched lipid dispersions or LDL leads to cholesterol incorporation into the cell membrane, and that cholesterol incorporation may regulate cell populations through impacting membrane stability (16, 33). Membrane fluidity is reduced and order increased in parallel with increases in membrane cholesterol/phospholipid ratio (16). This may explain clinical reports, generally consistent with the present one, that LDL-C, erythrocyte membrane stability, and hematocrit

TABLE 5. Erythrocyte MCV by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	Cell Volume Mean (fL)	95% CI	Cell Volume Mean (fL)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	89.14	88.72, 89.56	87.97	87.33, 88.61
Medium	89.55	88.96, 90.14	88.33	87.73, 88.93
High	90.15	89.61, 90.68	88.95	88.18, 89.71
Very high	91.74	91.27, 92.20	89.47	88.56, 90.39
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	88.96	88.50, 89.41	87.90	87.30, 88.50
Medium	89.58	89.03, 90.13	88.34	87.75, 88.93
High	90.41	89.88, 90.94	89.01	88.23, 89.78
Very high	91.69	91.29, 92.09	89.49	88.65, 90.34
Trend *P* value	<0.001		<0.001	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	90.32	89.60, 91.04	88.91	88.02, 89.81
Medium	90.32	89.72, 90.93	88.49	87.60, 89.37
High	89.91	89.41, 90.40	88.45	87.89, 89.02
Very high	90.19	89.66, 90.73	88.65	88.03, 89.28
Trend *P* value	0.526		0.376	
Adjusted[b]				
Low	90.36	89.75, 90.97	88.95	88.11, 89.79
Medium	90.37	89.85, 90.89	88.54	87.73, 89.36
High	89.92	89.33, 90.51	88.51	87.91, 89.11
Very high	90.17	89.62, 90.72	88.54	87.96, 89.11
Trend *P* value	0.360		0.101	

Cholesterol quartiles were determined from NHANES 2005-2006 and are as follows: HDL-C [low (≤41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and nonHDL-C [low (≤114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high (>168.28 mg/dl)]. CI, confidence interval.

[a] Unadjusted model.

[b] Adjusted for age, race/ethnicity, gender, smoking, and BMI.

are all positively correlated (34). It has also been proposed that the stability of erythrocytes may be maximal within an optimal range of membrane fluidity (and thus membrane cholesterol) (17). Thus, under cholesterol-loading conditions that exceed the critical range, erythrocyte membrane stability is impaired, likely accounting for the increased erythrocyte osmotic fragility and spur cell and hemolytic anemias seen in experimental animals fed a high-cholesterol diet (10, 12), and the erythrocyte membrane damage observed in hypercholesterolemic humans (9). Conversely, LDL-C reduction in hypercholesterolemic multiple sclerosis patients with statin therapy has been shown to increase erythrocyte stability (17).

As has been proposed for erythrocytes, it is possible that non-HDL-C may impact platelet number in part through effects on membrane stability. Interestingly, however, recent studies have suggested the potential for additional mechanisms. Thus, it has been reported that high LDL-C induces thrombocytosis in mice in part through delocalization of megakaryocytes in the bone marrow due to an altered gradient of stromal cell-derived factor-1 (35). It is also reported that cholesterol loading of megakaryocyte progenitors induces thrombocytosis through enhancing cell surface expression and activation of the thrombopoietin receptor, c-MPL (36).

Several case series have documented hypocholesterolemia as a common finding in a wide variety of anemias, including megaloblastic anemia, hereditary spherocytosis, sickle cell disease, aplastic anemia, glucose-6-phosphate deficiency, and anemia associated with liver disease (11). These studies suggest that, in addition to effects of serum cholesterol on erythrocyte populations, erythrocyte kinetics may reciprocally affect cholesterol status. Remarkably, following treatment of several disparate anemias, ranging from B12/folate repletion for megaloblastic anemia, to splenectomy for hereditary spherocytosis, to red blood cell transfusion for sickle cell disease or aplastic anemia, an increase in serum cholesterol has been noted that parallels the correction in hematocrit (11, 37). While this has led some investigators to hypothesize that serum cholesterol may be reduced during anemia by hemodilution, low serum cholesterol during hemolytic anemias has been attributed by others to consumption by avid erythropoiesis in the bone marrow (38, 39). Additional hypotheses for anemia-associated hypocholesterolemia have included reduced cholesterol biosynthesis by the liver and increased cholesterol clearance by the reticuloendothelial system. There have been no studies, to our knowledge, that have investigated serum cholesterol levels during primary platelet disorders.

While it is plausible that disease extremes such as hemolytic anemia may serve to reveal some of the mechanisms that govern blood cell-cholesterol relationships during health, it is likely that additional mechanisms may be at work in large human populations. For example, malnutrition

TABLE 6. Mean platelet crit by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	Platelet Crit Mean (%)	95% CI	Platelet Crit Mean (%)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	0.222	0.219, 0.225	0.204	0.198, 0.210
Medium	0.227	0.222, 0.232	0.203	0.199, 0.206
High	0.229	0.224, 0.233	0.206	0.202, 0.210
Very high	0.224	0.220, 0.228	0.204	0.200, 0.208
Trend *P* value	0.186		0.747	
Adjusted[b]				
Low	0.226	0.223, 0.229	0.205	0.200, 0.211
Medium	0.228	0.224, 0.233	0.203	0.200, 0.207
High	0.226	0.222, 0.230	0.205	0.200, 0.209
Very high	0.222	0.218, 0.226	0.201	0.197, 0.206
Trend *P* value	0.083		0.362	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	0.216	0.210, 0.222	0.195	0.191, 0.199
Medium	0.224	0.220, 0.227	0.203	0.199, 0.206
High	0.230	0.225, 0.235	0.208	0.203, 0.212
Very high	0.232	0.228, 0.236	0.210	0.206, 0.215
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	0.215	0.209, 0.221	0.194	0.190, 0.198
Medium	0.223	0.221, 0.226	0.202	0.199, 0.206
High	0.230	0.226, 0.235	0.207	0.203, 0.211
Very high	0.233	0.230, 0.236	0.210	0.207, 0.214
Trend *P* value	<0.001		<0.001	

Cholesterol quartiles were determined from NHANES 2005-2006 and are as follows: HDL-C [low (⩽41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and nonHDL-C [low (⩽114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high (>168.28 mg/dl)]. CI, confidence interval.

[a]Unadjusted model.

[b]Adjusted for age, race/ethnicity, gender, smoking and BMI.

has the potential to reduce both serum cholesterol and circulating numbers of erythrocytes (e.g., through iron deficiency) and platelets (e.g., through folate or vitamin B12 deficiency) (40). Similarly, inflammation has complex effects, as it can be associated with anemia (41), altered serum cholesterol transport (42), and thrombocytosis (43). Arguing against an important role for nutritional factors in our findings, the relationships persisted after adjustment for red blood cell folate and BMI, and no relationship was detected between non-HDL-C and either transferrin saturation (a marker of iron status) or serum folate. In addition, our finding that the relationships persisted after adjustment for CRP argues against confounding by inflammation.

To date, there have been very few systematic evaluations of red cell abundance across varying levels of serum cholesterol in large human populations. In 1972, Böttiger and Carlson reported a positive correlation between serum cholesterol and hemoglobin in 2,458 nonanemic subjects (20); a similar correlation between serum cholesterol and hematocrit was noted by another group 20 years later (21). More recently, no relationship was found between serum cholesterol and erythrocyte number, hematocrit, hemoglobin concentration, or MCV (22). However, this study involved just 463 subjects, all of whom were elderly and from South Korea. Another small study documented increased erythrocyte membrane cholesterol in patients with primary hypercholesterolemia, but did not report on erythrocyte abundance indices (44). The few studies of platelet number during cholesterol disorders have yielded disparate findings. Pathansali, Smith, and Bath (24) reported no alteration in platelet count in eight patients with primary hypercholesterolemia. By contrast, in a Japanese study of 387 men and 550 women, platelet counts in women correlated negatively with HDL-C but were unrelated to non-HDL-C, whereas in men they were unrelated to HDL-C but were positively correlated to non-HDL-C (23). The latter result is consistent with our finding in NHANES. Last, of interest but of uncertain significance, thrombocytopenia has been described in Tangier disease, a condition of low HDL-C due to mutation of the ATP binding cassette transporter A1 (45).

Interestingly, we found that HDL-C was positively related to MCV, although apparently unrelated to MPV, suggesting important differences in the relationship of HDL to erythrocytes and platelets. HDL-C was also inversely related to erythrocyte count. These findings are somewhat reminiscent of the SR-BI-null mouse, which, along with marked increases in HDL-C, is reported to have macrocytic anemia (6, 13). In that setting, it is thought that increased erythrocyte membrane cholesterol deriving from HDL may both impair erythrocyte maturation (13) and promote hemolysis through effects on osmotic fragility

TABLE 7. Geometric mean platelet count by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	Platelet Mean (1,000 cells/ul)	95% CI	Platelet Mean (1,000 cells/ul)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	266.77	262.27, 271.35	254.34	249.15, 259.64
Medium	272.99	267.17, 278.94	258.86	255.19, 262.59
High	274.79	268.12, 281.63	260.47	255.32, 265.73
Very high	273.28	268.62, 278.02	259.96	255.30, 264.71
Trend *P* value	0.024		0.073	
Adjusted[b]				
Low	271.46	267.00, 276.00	256.08	251.13, 261.13
Medium	274.84	269.27, 280.53	259.89	256.56, 263.26
High	271.50	266.01, 277.10	258.73	252.44, 265.17
Very high	270.45	265.91, 275.08	257.08	251.18, 263.12
Trend *P* value	0.492		0.852	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	259.68	251.66, 267.96	244.00	239.61, 248.47
Medium	270.31	265.28, 275.43	256.39	251.18, 261.71
High	279.11	274.45, 283.84	263.99	259.95, 268.10
Very high	279.51	273.91, 285.23	268.28	264.20, 272.43
Trend *P* value	<0.001		<0.001	
Adjusted[b]				
Low	257.58	249.80, 265.59	242.74	238.06, 247.51
Medium	270.06	265.51, 274.69	256.27	250.50, 262.17
High	280.11	275.75, 284.54	263.91	260.87, 266.99
Very high	280.99	276.08, 285.99	268.62	265.17, 272.11
Trend *P* value	<0.001		<0.001	

Cholesterol quartiles were determined from NHANES 2005-2006 and are as follows: HDL-C [low (≤41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and nonHDL-C [low (≤114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high (>168.28 mg/dl)]. CI, confidence interval.

[a]Unadjusted model.

[b]Adjusted for age, race/ethnicity, gender, smoking, and BMI.

and deformability (6). The analogy to our study may be imperfect, however, given that SR-BI-null mice have abnormally large HDL particles as well as increased free cholesterol, and their elevated MCV may in part derive from reticulocytosis (6, 13).

Our study has limitations. Importantly, the cross-sectional design of the NHANES precludes inferences of causality between blood cell abundance and serum cholesterol. It is possible that serum cholesterol and erythrocyte/platelet levels, rather than causally impacting each other, may track together as biomarkers of a separate underlying condition. Our sensitivity analysis, nonetheless, indicates that the relationships do persist after several common conditions affecting blood cell levels and cholesterol are excluded. Although the ratio of free cholesterol:TC has been linked to platelet abnormalities in mouse and man (4, 46), we were unable to analyze free cholesterol as it was not measured in the NHANES. Also, the ABO blood group type, recently shown in a genome-wide association study to be associated with LDL-C (47), was not determined in NHANES. Two additional variables that we did not analyze, but that have important effects upon erythrocyte membrane stability, are albumin concentration (48) and glucose concentration (49). Strengths of our analysis include the large size of our study population and our replication of results in a separate cohort. In addition, we analyzed HDL-C and non-HDL-C, and not just TC (the sum of HDL-C and non-HDL-C), allowing us to identify distinct relationships for these different lipoprotein categories.

The effect size of the adjusted relationships of non-HDL-C to erythrocyte parameters is modest when considered in isolation. A somewhat more impressive relationship was found for platelets, where a nearly 10% difference in mean platelet count was observed between the lowest and highest non-HDL-C quartiles in the adjusted model. While these relationships are of uncertain clinical significance, erythrocytosis, thrombocytosis, and hypercholesterolemia are all risk factors for thrombosis (50, 51). If, as our data suggest, these variables track together in human subjects, it is possible that they may synergize in promoting cardiovascular disease. Indeed, emerging data suggest that erythrocytes, and erythrocyte membrane cholesterol, in particular, are independently associated with clinical instability in coronary artery disease patients (26, 52). Interestingly, red blood cell distribution width, a strong prognostic marker in cardiovascular disease, is positively associated with erythrocyte membrane cholesterol (53). Similarly, platelet cholesterol overload correlates with platelet activation and coronary artery disease (28).

Atorvastatin is reported to decrease erythrocyte membrane cholesterol in human subjects (54). The lack of a

TABLE 8. MPV by quartiles of serum cholesterol measures in adult participants in NHANES 2005–2008

	NHANES Survey 2005–2006 (N = 4,469)		NHANES Survey 2007–2008 (N = 5,318)	
	MPV Mean (fL)	95% CI	MPV Mean (fL)	95% CI
HDL-C (mg/dl)				
Unadjusted[a]				
Low	8.13	8.04, 8.22	7.83	7.71, 7.94
Medium	8.13	8.06, 8.19	7.69	7.59, 7.79
High	8.14	8.08, 8.21	7.74	7.62, 7.86
Very high	8.06	8.00, 8.12	7.68	7.58, 7.78
Trend *P* value	0.113		0.015	
Adjusted[b]				
Low	8.13	8.03, 8.23	7.83	7.72, 7.94
Medium	8.12	8.06, 8.19	7.69	7.59, 7.79
High	8.13	8.07, 8.20	7.74	7.61, 7.86
Very high	8.06	8.00, 8.12	7.68	7.57, 7.79
Trend *P* value	0.207		0.006	
Non-HDL-C (mg/dl)				
Unadjusted[a]				
Low	8.13	8.05, 8.22	7.76	7.67, 7.85
Medium	8.09	8.02, 8.16	7.75	7.63, 7.87
High	8.08	8.01, 8.16	7.72	7.61, 7.83
Very high	8.15	8.08, 8.22	7.71	7.60, 7.82
Trend *P* value	0.728		0.168	
Adjusted[b]				
Low	8.14	8.05, 8.23	7.76	7.66, 7.86
Medium	8.09	8.02, 8.16	7.76	7.63, 7.88
High	8.08	8.01, 8.15	7.72	7.61, 7.82
Very high	8.14	8.07, 8.21	7.71	7.61, 7.82
Trend *P* value	0.993		0.163	

Cholesterol quartiles were determined from NHANES 2005-2006 and are as follows: HDL-C [low (≤41.83 mg/dl), medium (>41.83–51.38 mg/dl), high (>51.38–62.93 mg/dl), and very high (>62.93 mg/dl)]; and nonHDL-C [low (≤114.42 mg/dl), medium (>114.42–140.1 mg/dl), high (>140.1–168.28 mg/dl), and very high [>168.28 mg/dl)]. CI, confidence interval.

[a]Unadjusted model.

[b]Adjusted for age, race/ethnicity, gender, smoking and BMI.

change in our results after excluding statin-treated subjects may suggest that pharmacologic reduction of cholesterol is associated with similar effects on blood cell indices as other environmental or genetic influences on cholesterol present in the general population. Nonetheless, whether interventions upon serum cholesterol such as statins impact population kinetics of erythrocytes and/or platelets is an important question that may require investigation using a prospective study design. Finally, our findings at least raise the possibility that non-HDL-C may act as a disease modifier of primary disorders of erythrocytes and platelets. For example, elevated non-HDL-C could conceivably attenuate anemia from iron deficiency and/or aggravate erythrocytosis in polycythemia vera. Conversely, our findings raise the possibility that circulating erythrocyte and/or platelet counts could modify the expression of primary hypercholesterolemia.

In closing, we report for the first time that non-HDL-C is directly related to abundance measures of circulating erythrocytes and platelets in the US population, whereas HDL-C is directly related to MCV. Given that elevated erythrocytes, elevated platelets, and hypercholesterolemia are all established risk factors for coronary disease and that hypercholesterolemia impairs erythrocyte deformability (55) and activates platelets (56, 57), our findings suggest an important need for characterizing possible mechanisms by which serum cholesterol and the population kinetics of erythrocytes and platelets may impact one another.

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