Title

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Authors

Sawant, Sonia
Tucker, Bradley
Senanayake, Praween
et al.

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Research paper

The association between lipid levels and leukocyte count: A cross-sectional and longitudinal analysis of three large cohorts

Sonia Sawant [a], Bradley Tucker [b,c], Praween Senanayake [c], David D. Waters [d], Sanjay Patel [a,b], Kerry-Anne Rye [c], Kwok Leung Ong [c,1], Blake J. Cochran [c,*,1]

[a] *Royal Prince Alfred Hospital, Sydney, Australia*
[b] *Heart Research Institute, Sydney, Australia*
[c] *School of Medical Sciences, UNSW Sydney, Sydney, Australia*
[d] *Division of Cardiology, San Francisco General Hospital and the University of California at San Francisco, San Francisco, CA, United States of America*

ABSTRACT

Background: Relationships between dyslipidaemia and leukocyte counts have been investigated in several studies, demonstrating limited evidence of associations in humans. As such, studying a diverse range of cohorts will ensure evidence is robust. This study focused on investigating cross-sectional and longitudinal relationships in three large-scale cohorts.
Methods: The cross-sectional analysis included a total of 27,566 participants with valid data on lipid measures and leukocyte counts from three study cohorts: National Health and Nutrition Survey (NHANES), Korean National Health and Nutrition Survey (KNHANES) and Treating to New Targets (TNT) trial. The longitudinal analysis included 9323 participants with valid data on lipid measures and leukocyte counts at baseline and one year with statin treatment. Associations between lipid levels and leukocyte counts were analysed by multivariable linear regression and adjusted for basic demographic and cardiovascular risk factors.
Results: Cross-sectional data from NHANES demonstrated the association of lower high-density lipoprotein (HDL) cholesterol and higher triglycerides with higher leukocyte count (0.9% lower and 0.3% higher count per 10 mg/dL increase in HDL cholesterol and triglycerides respectively, both $p < 0.001$). Similar trends were found in TNT trial (both $p < 0.001$), but not in KNHANES. In the TNT trial, 10 mg/dL increase in triglycerides over one year was also associated with a $0.09 \times 10^3/\mu L$ increase in leukocyte count over the same period.
Conclusions: The findings of this study are consistent with those of previous human studies, supporting weak yet noteworthy associations between dyslipidaemia and leukocytosis.

1. Introduction

Inflammation and more specifically leukocytes are essential in the development of atherosclerosis [1]. The pathogenesis of atherosclerotic disease is the product of immune-mediated lipid deposition within the intima of large- and medium-sized vessels. As such, defining the relationship between circulating leukocyte counts and serum lipid levels would provide further insight into the development of this complex disease.

Several animal studies have supported an association of dyslipidaemia with leukocytosis. In rabbits a positive relationship between leukocytosis and serum cholesterol has been identified [2], while

hyperlipemic diet has been proven to drive monocytopoiesis in swine [3]. Furthermore, deletion of the apolipoprotein E gene in mice is associated with monocytosis [4,5] and neutrophilia [6]. This is supported by Murphy et al. [7] who illustrated that in apoE$^{-/-}$ mice, reduced apolipoprotein-mediated cholesterol efflux leads to proliferation and expansion of hematopoietic stem and multipotential progenitor cells (HSPC), providing a mechanistic link between hypercholesterolemia and leukocytosis. Conversely, inhibition of HSPC proliferation has been described with elevated high-density lipoprotein (HDL) cholesterol [8].

Despite abundance of preclinical data, there is limited evidence that confirms such a robust relationship in humans. Moreover, there is only

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* Corresponding author at: School of Medical Sciences, UNSW Sydney, NSW 2052, Australia.
E-mail address: b.cochran@unsw.edu.au (B.J. Cochran).
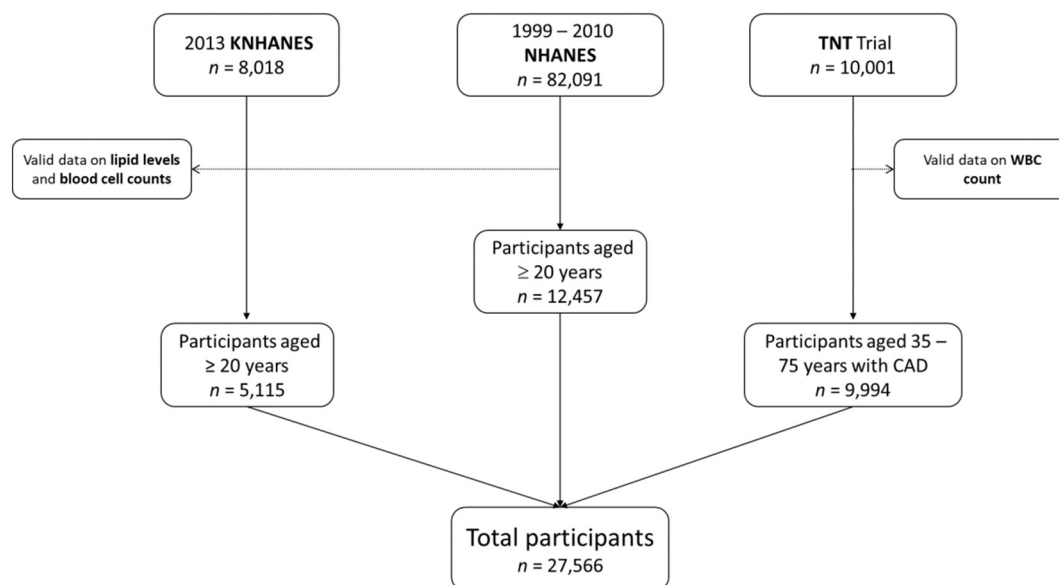[1] These authors contributed equally and share senior authorship.

Fig. 1. Participant flow in the three datasets. Three data registries were used in this study. In the NHANES (1999–2010) and KNHANES (2013) a total of 82,091 and 8018 participants were included, respectively. After exclusion of participants with missing data on lipid levels and blood cell counts 12,457 participants aged ≥20 years were included in the NHANES final analysis; 5115 participants aged >20 years were included in the KNHANES final analysis. In the TNT trial, a total of 10,001 participants aged 35–75 with known coronary artery disease (CAD) were recruited. Of these, 9994 participants had valid data on leukocyte count and were included in the final analysis.

one study, to our knowledge, that has investigated the longitudinal relationship of leukocyte counts with lipid levels [9]. A recent study conducted on healthy participants of the Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated that increased total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol were associated with lower total leukocyte, monocyte and neutrophil counts [10]. However, there was an inverse relationship between HDL cholesterol and total leukocyte count, in agreement with animal studies. Additionally, higher triglyceride levels were associated with elevated total leukocyte and lymphocyte counts. Consistent with these findings, other studies have identified a positive correlation between triglyceride levels and total leukocyte, lymphocyte, neutrophil, monocyte and basophil counts [11–13], and a negative correlation between HDL cholesterol and total leukocyte as well as monocyte counts [11,14]. In contrast to the described inverse correlation of LDL cholesterol with total leukocyte, monocyte and neutrophil counts, LDL cholesterol may positively correlate with lymphocyte counts [15,16].

The results of studies in humans demonstrate a modest yet conflicting relationship when compared to animal studies, highlighting potential differences in biological processes between mice and humans that are not currently understood. Due to the central role of lipids and leukocytes in the development of atherosclerotic disease, it is important to delineate the relationship between these factors to better understand the basic pathobiology of atherosclerosis. By utilising a combination of two large-scale, population-based studies, and one clinical trial study, we sought to examine the relationship of serum lipid levels with total and differential leukocyte counts in humans.

2. Methods

This study assessed the relationship of lipid levels with leukocyte counts in three study cohorts (Fig. 1): National Health and Nutrition Survey (NHANES), Korean National Health and Nutrition Survey (KNHANES) and Treating to New Targets (TNT) trial.

2.1. NHANES

2.1.1. Study population

The NHANES is a continuous cross-sectional survey of the health and nutritional status of United States residents, citizens and non-industrialised individuals [17]. The NHANES collects data on approximately 5000 individuals each year using a complex sampling procedure, as previously described [18]. In 1999–2010, 82,091 participants were surveyed in NHANES, and 17,736 of them underwent both interview and examination, with fasting samples for lipids and lipids and leukocyte count measurements. Of these, 12,457 participants were included in the adult cohort of this analysis which comprised of those who were aged ≥20 years and had valid data on TC, LDL cholesterol, triglycerides, HDL cholesterol, differential leukocyte counts, red blood cell (RBC) count and platelet count.

2.1.2. Lipids and leukocyte count measurements

Fasting blood samples were obtained from participants to measure lipid levels. TC, HDL cholesterol and triglycerides were measured enzymatically (Hitachi 704 Analyzer, Roche Diagnostics). LDL cholesterol was calculated using the Friedewald formula [19] in participants with triglycerides <400 mg/dL, and otherwise measured directly if triglycerides were > 400 mg/dL. The complete blood count was performed using a Beckman Coulter MAXM (Beckman Coulter) as per the manufactures guidelines [18].

2.2. KNHANES

2.2.1. Study population

KNHANES is an annual, cross-sectional survey composed of health interview, health examination and nutrition surveys [20]. Participants included in this study were non-institutionalised Korean citizens selected using a stratified, multistage, cluster sampling study design with proportional allocation based on geographical area, sex and age group using data from the National Census Registry [20]. Data were extracted from the sixth KNHANES conducted in 2013, which included a total of 8018 participants. Of these, 5115 participants aged ≥20 years with valid data on TC, HDL cholesterol, LDL cholesterol and

triglycerides, total leukocyte count, RBC count and platelet count were included in the analysis.

2.2.2. Lipids and leukocyte count measurements

Fasting blood samples were obtained to measure TC, HDL cholesterol and triglycerides enzymatically using commercially available kits (Hitachi Automatic Analyzer 7600, Hitachi). LDL cholesterol was calculated using the Friedewald formula [19] for participants with triglycerides <400 mg/dL or measured directly in those with triglycerides >400 mg/dL. Leukocyte counts were quantified using an automated blood cell counter (ADVIA 120, Bayer).

2.3. TNT study

2.3.1. Study population

The design and results of the TNT study have been previously reported [21]. Briefly, the TNT cohort consisted of participants recruited from 14 countries, from 1998 to 1999. Men and women aged 35–75 years with stable coronary heart disease were recruited to the study, with exclusion criteria described in detail previously [21]. 10,001

Table 1
Baseline characteristics of NHANES study participants.

Characteristics	n	Estimate
Age, years	12,457	46.3 (0.3)
Women/girls, %	12,457	52.3 (0.4)
Race/ethnicity, %	12,457	
Non-Hispanic White		70.9 (1.3)
Non-Hispanic Black		10.9 (0.7)
Mexican American		7.8 (0.6)
Others		10.4 (0.8)
Education, %[a]	12,436	
<High school		19.3 (0.6)
High school graduate		25.0 (0.6)
>High school		55.7 (0.9)
Ratio of family income to poverty, %	12,355	
≤1.30		18.6 (0.7)
1.31–3.50		40.7 (0.8)
>3.50		40.7 (1.0)
BMI, kg/m^2	12,242	28.3 (0.1)
Smoking, %[a]	12,443	
Non-smoker		51.8 (0.8)
Former smoker		25.5 (0.7)
Current smoker		22.7 (0.6)
Alcohol drinker, %[a]	11,707	74.2 (0.9)
Engage in moderate/vigorous exercise, %	12,452	59.9 (0.9)
Systolic blood pressure, mm Hg	12,009	120.9 (0.2)
Hypertension, %[a]	12,140	31.4 (0.7)
Anti-hypertensive medication, %[a]	12,455	23.4 (0.6)
Cholesterol-lowering medication, %[a]	12,457	12.8 (0.5)
Diabetes, %	12,455	9.3 (0.3)
History of CVD, %[a]	12,385	8.2 (0.4)
CRP, mg/dL	12,454	1.9 (1.8, 1.9)
Lipid profile, mg/dL		
Total cholesterol	12,457	197.2 (0.5)
HDL cholesterol	12,457	53.6 (0.2)
LDL cholesterol	12,457	118.1 (0.5)
Triglycerides	12,457	127.4 (0.9)
Blood cell counts		
Total leukocyte count, ×10^3/μL	12,457	6.515 (6.457, 6.574)
Basophils, ×10^3/μL	12,457	1.033 (1.031, 1.034)
Eosinophils, ×10^3/μL	12,457	1.194 (1.192, 1.197)
Lymphocytes, ×10^3/μL	12,457	1.874 (1.859, 1.889)
Monocytes, ×10^3/μL	12,457	1.525 (1.520, 1.531)
Neutrophils, ×10^3/μL	12,457	3.747 (3.700, 3.794)
Red blood cells, ×10^6/μL	12,457	4.74 (0.01)
Platelets, ×10^3/μL	12,457	262.1 (0.9)

Data are expressed as mean (SE) or % (SE). For variables with skewed distribution, data are expressed as geometric mean (95% CI).
Abbreviations: BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; RBC, red blood cells.
[a] Data not available in adolescent cohort.

patients with an LDL cholesterol level off-therapy of 130–250 mg/dL (3.4–6.5 mmol/L), decreasing to <130 mg/dL (<3.4 mmol/L) after an 8-week run-in period on atorvastatin 10 mg/day, were randomised to treatment with either atorvastatin 10 or 80 mg/day. Of the 10,001 participants included in the TNT trial 9994 participants with valid data on baseline leukocyte count were included in the final analysis of the present study.

2.3.2. Lipids and leukocyte cell count measurements

TC, HDL cholesterol and triglycerides were measured on fasting blood samples at baseline and at 12 months by standard methodologies, as previously described [22]. LDL cholesterol was calculated using the Friedewald formula [19] when triglyceride levels were < 174 mg/dL (<4.5 mmol/L), above this LDL cholesterol was measured directly by ultracentrifugation, as previously described [22]. Leukocyte counts were quantified using a Beckman Coulter GEN S (Beckman Coulter) automated blood cell counter.

2.4. Covariates of interest

Common definitions of covariates among the three cohorts included those of smoking status and body mass index (BMI). Smoking status was categorised as 'non', 'former' or 'current'. Weight and height measured during clinical examination were used to calculate BMI. In the NHANES and KNHANES, diabetes was defined as a fasting glucose >125 mg/dL, measured using an automated analyzer and enzymatic assay (Hexokinase-mediated reaction Roche/Hitachi Modular P Chemistry Analyzer and Pureauto S GLU: Daiichi), or use of hypoglycaemic medication.

In the NHANES, data on demographics, smoking status, race/ethnicity, physical activity and medications was obtained from self-reported questionnaires. Ratio of family income to poverty was calculated by dividing family income by the poverty measure from Department of Health and Human Services' poverty guidelines [23]. History of cardiovascular disease (CVD) was defined as self-reported congestive heart failure, coronary artery disease, angina, myocardial infarction or stroke. Of three consecutive blood pressure readings, the average of the final two was used as the reported value. C-reactive protein (CRP) levels were analysed by latex-enhanced nephelometry with high sensitivity using a Dade Behring Nephelometer II Analyzer System (Dade Behring Diagnostics, Somerville, New Jersey).

With respect to the KNHANES, information on demographics, smoking status, alcohol consumption, education, medications and history of CVD was obtained using health interviews and clinical examinations. Regular alcohol consumption was defined as more than one glass of alcohol per month. Education was categorised as '<high school diploma', 'high school diploma' and '≥college'. HbA1c was measured using high performance liquid chromatography by Tosoh G8 (Tosoh) using reagent of HLC-723G8 HbA1c (Tosoh).

In the TNT trial, participants provided written informed consent, sociodemographic information was obtained, vital signs measured, concomitant medications documented and a complete medical history recorded [21]. At the follow up point of 12 months, vital signs, clinical end points, adverse events and concurrent medication use were again documented.

2.5. Statistical analysis

To provide accurate variance estimates, data analysis for the NHANES and KNHANES utilised sample weights that accounted for the unequal probabilities of sampling selection, oversampling of some participants with certain characteristics, and nonresponse. Data from these studies were presented as mean or percent and standard error. Variables with skewed distribution are expressed as geometric mean (95% confidence interval). Data from the TNT trial are expressed as mean (standard deviation) or frequency (percentage) where appropriate. All data analyses were performed using SPSS 25 (IBM, Armonk, NY, USA) or STATA
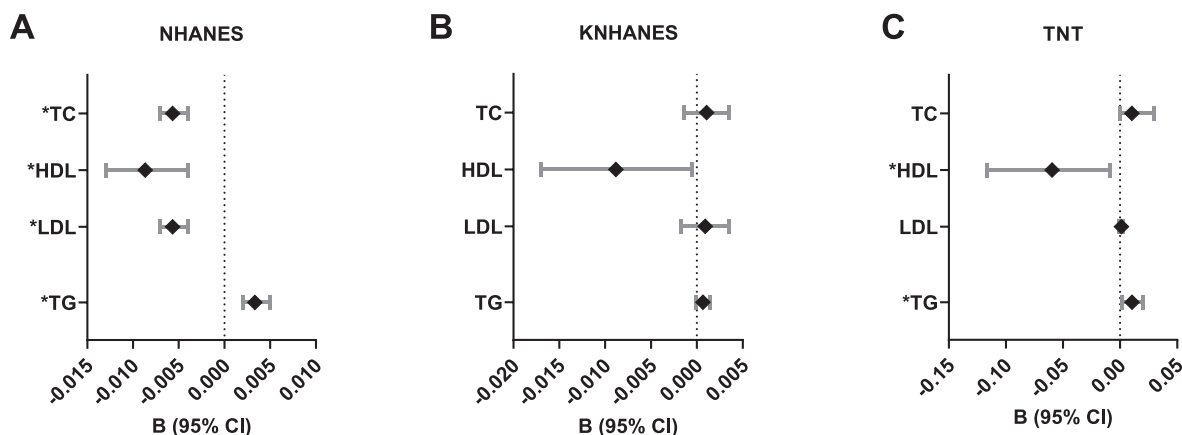
Fig. 2. Association of lipid levels with total leukocyte count in the NHANES, KNHANES and TNT trial. For (A) NHANES and (B) KNHANES, regression coefficient B (95% CI) is expressed as ln-transformed cell counts ($\times 10^3$/μL) per 10 mg/dL increase in lipid measures. In the (C) TNT study, a pilot analysis using diagnostic plot of residues does not suggest any improvement in model fitting after taking ln-transformation of leukocyte count. Therefore, the regression coefficient B (95% CI) is expressed as leukocyte count ($\times 10^3$/μL) per 10 mg/dL increase in lipid measures. Data from all three registries were adjusted for age, gender, BMI, smoking status, diabetes, LDL cholesterol (except for analysis of total cholesterol and LDL cholesterol), HDL cholesterol (except for analysis of HDL cholesterol) and triglycerides (except for analysis of triglycerides). Data in the NHANES and KNHANES were further adjusted for education, alcohol consumption, systolic blood pressure, use of anti-hypertensive medication, use of lipid-lowering medication, history of CVD, RBC count and platelet count. Beyond this the NHANES was further adjusted for race/ethnicity, ratio of family income to poverty, physical activity and ln-transformed CRP, and the KNHANES was further adjusted for HbA1c. Data in the TNT study were further adjusted for race/ethnicity, hypertension and blood urea nitrogen.

Abbreviations: B, regression coefficient; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

*Remained significant after adjusting for multiple comparisons using a false discovery rate of <0.05.

14 (StataCorp).

Multivariable linear regression analysis was performed with each lipid parameter (TC, HDL cholesterol, LDL cholesterol and triglycerides) as independent predictor variables and total or differential (neutrophil, monocyte, lymphocyte, basophil and eosinophil) leukocyte counts as the dependent variables. For the NHANES and KNHANES the regression coefficient was estimated as the ln-transformed leukocyte counts ($\times 10$ [3]/μL) per 10 mg/dL increase in lipid level. The regression coefficient was converted to a percentage change using the following formula: (EXP (B)-1) $\times$ 100; where EXP is the exponent and B is the regression coefficient. For the TNT trial, the regression coefficient was estimated as the leukocyte count ($\times 10$ [3]/μL) per 1 mg/dL increase in lipid level as a pilot analysis using diagnostic plot of residues did not suggest any improvement in model fitting after taking ln-transformation of leukocyte count. Follow-up data in the TNT trial facilitated longitudinal analysis, whereby the absolute change in leukocyte count and lipid levels were included in the final regression model as the dependent and independent variables respectively.

Across all three studies data were adjusted for basic demographics and cardiovascular risk factors. More specifically, data from all three datasets were adjusted for age, sex, BMI, smoking status, diabetes, LDL cholesterol (except for analysis of TC and LDL cholesterol), HDL cholesterol (except for analysis of HDL cholesterol) and triglycerides (except for analysis of triglycerides). Data in the NHANES and KNHANES were further adjusted for education, alcohol consumption, systolic blood pressure, use of anti-hypertensive medication, use of lipid-lowering medication, history of CVD, RBC count and platelet count. Beyond this the NHANES was further adjusted for race/ethnicity, ratio of family income to poverty, physical activity and ln-transformed CRP, and the KNHANES for HbA1c. Data in the TNT trial were further adjusted for race/ethnicity, hypertension and blood urea nitrogen. In the longitudinal analysis of the TNT trial, data were further adjusted for baseline leukocyte count and treatment allocation. Multiple testing correction was performed using false discovery rate with the study-wide false discovery rate at 0.05. A two-tailed $p < 0.05$ was considered statistically significant.

3. Results

3.1. NHANES

In the NHANES 70.9% of participants were non-Hispanic white, the mean age was 46.3 years, 52.3% were female and the mean BMI was 28.3 kg/m^2. 51.8% were non-smokers while 74.2% were alcohol drinkers and 9.3% had diabetes (Table 1).

Each 10 mg/dL increment in total, HDL and LDL cholesterol was associated with a 0.6% (95% CI -0.7%, −0.4%; $p < 0.001$), 0.9% (95% CI -1.3%, −0.4%; $p < 0.001$) and 0.6% (95% CI -0.7%, −0.4%; $p < 0.001$) lower total leukocyte count, respectively (Fig. 2A; Supplementary Table 1). Conversely, each 10 mg/dL increment in triglycerides was associated with a 0.3% (95% CI 0.2%, 0.5%; $p < 0.001$) higher total leukocyte count.

To further delineate this relationship, the association of lipid levels with differential leukocyte counts was explored (Fig. 3; Supplementary Table 2). After adjusting for all covariates, each 10 mg/dL increment in TC, LDL cholesterol and triglycerides was associated with a 0.4% (95% CI [0.3%, 0.6%], [0.3%, 0.6%], [0.3%, 0.5%] respectively; $p < 0.001$) higher lymphocyte count, a 0.1% (CI 95% [−0.2%, 0.0%], [−0.2%, 0.0%], [−0.1%, 0.0%]; $p = 0.002$, $p = 0.003$, $p < 0.001$) lower monocyte count and 0.1% (CI 95% [−0.2%, 0.0%], [−0.2%, 0.0%], [−0.2%, −0.1%]; $p = 0.027$, $p = 0.026$, $p < 0.001$) lower neutrophil count. Further, each 10 mg/dL increment in HDL cholesterol was associated with a 0.2% (CI 95% -0.4%, 0.0%; $p = 0.017$) lower eosinophil count. None of the lipid parameters in this study were associated with basophil count.

In a separate analysis, exclusion of 1788 participants taking any lipid-lowering medication showed similar results. In addition, the association of triglycerides with eosinophil count (B = -0.001; CI 95% -0.001, 0.000; $p = 0.029$) and the association of HDL cholesterol with lymphocyte count (B = -0.005; CI 95% -0.010, 0.000; $p = 0.032$) became significant (Supplementary Table 3).

3.2. KNHANES

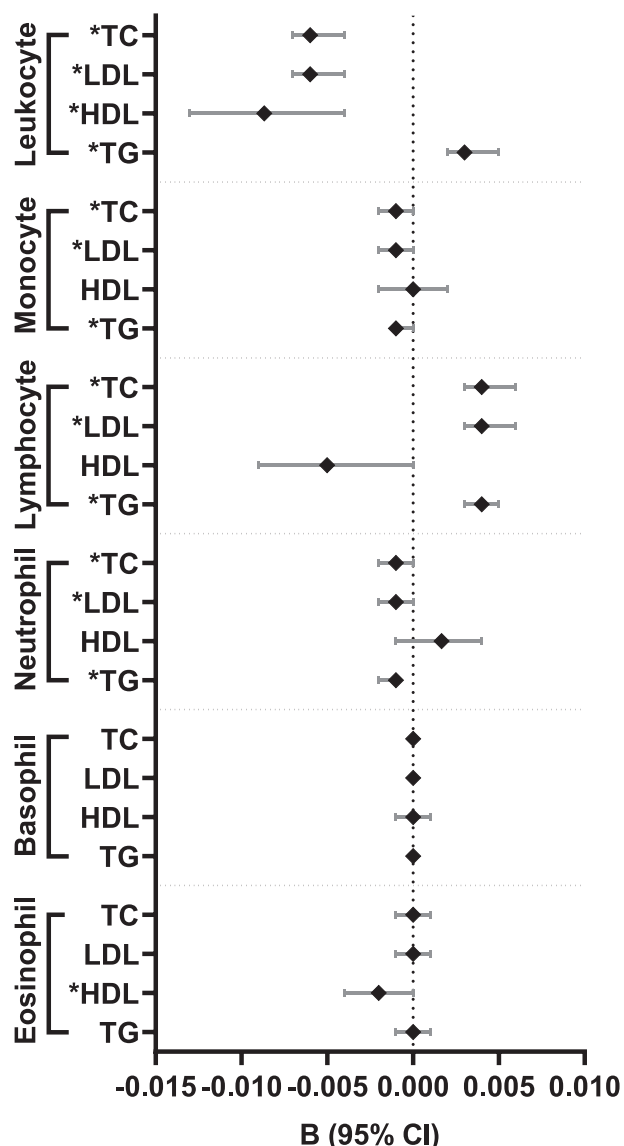In the KNHANES, the mean age of study participants was 45.5 years,

Fig. 3. Association of lipid levels with differential cell counts in the NHANES study. The regression coefficient B (95% CI) are expressed as ln-transformed cell counts ($\times 10^3$/μL) per 10 mg/dL increase in lipid measures. Data were adjusted for ln-transformed total leukocyte count (except for analysis of total leukocyte count), age, sex, race/ethnicity, education, ratio of family income to poverty, BMI, smoking, alcohol consumption, exercise, systolic blood pressure, use of hypertensive medication, use of cholesterol lowering medication, diabetes, history of CVD, ln-transformed CRP, LDL cholesterol (except for analysis of total and LDL cholesterol), HDL cholesterol (except for analysis of HDL cholesterol), triglycerides (except for analysis of triglycerides), RBC count and platelet count.

Abbreviations: B, regression coefficient; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

*Remained significant after adjusting for multiple comparisons using a false discovery rate of <0.05.

49.1% were female and the average BMI was 23.8 kg/m². 55.7% participants were non-smokers and 10.1% had diabetes (Table 2).

After adjusting for sociodemographic information and cardiovascular risk factors, TC, LDL cholesterol and triglycerides were not associated with total leukocyte count (Fig. 2B, Supplementary Table 1). In contrast, a 10 mg/dL increment in HDL cholesterol was associated with a 1% lower total leukocyte count (CI 95% -1.8%, −0.1%; $p = 0.027$) although this relationship was not significant after adjusting for multiple comparisons. In a separate analysis (Supplementary Table 4), exclusion of

Table 2
Baseline characteristics of KNAHNES study participants.

Characteristics	n	Estimate
Age (years)	5115	45.5 (0.4)
Females, %	5115	49.1 (0.6)
BMI, kg/m²	5108	23.9 (0.1)
Household income, %	5076	
Quartile 1	926	14.8 (1.0)
Quartile 2	1340	25.9 (1.1)
Quartile 3	1338	28.5 (1.0)
Quartile 4	1472	30.8 (1.5)
Education, %	4822	
<High school	1533	25.3 (1.1)
High school	1707	38.7 (1.1)
>High school	1582	36.0 (1.2)
Smoking, %	4808	
Non-smoker	2886	55.7 (0.8)
Former smoker	928	19.5 (0.6)
Current smoker	994	24.8 (0.9)
Regular drinker, %	4813	59.5 (0.9)
Hypertension, %	4871	25.6 (0.9)
Cholesterol-lowering medication, %	4827	6.0 (0.4)
HbA1c	5115	5.9 (0.02)
Diabetes, %	4854	10.1 (0.6)
Stroke, %	4827	1.8 (0.2)
Myocardial Infarction, %	4827	0.6 (0.1)
Lipid levels		
TC (mg/dL)	5115	187.7 (0.7)
HDL-C (mg/dL)	5115	50.8 (0.2)
LDL-C (mg/dL)	5115	112.0 (0.5)
TG (mg/dL)	5115	138.7 (2.0)
Blood cell counts		
RBC ($\times 10^6$/μL)	5115	4.63 (0.01)
Leukocyte count ($\times 10^3$/μL)	5115	5.920 (5.857, 5.983)
Platelets ($\times 10^3$/μL)	5115	253.3 (1.1)

Data are expressed as mean (SE) or % (SE). For variables with skewed distribution, data are expressed as geometric mean (95% CI).

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; RBC, red blood cells.

participants taking a lipid-lowering medication ($n = 372$) did not dramatically influence the aforementioned relationships.

3.3. TNT

The mean age of TNT participants was 61.0 years, with 94.1% white population, 19% females and an average BMI of 28.5 kg/m². 23.4% were non-smokers and 15% had diabetes (Table 3).

In the cross-sectional analysis at baseline, after adjustment for all covariates, total and LDL cholesterol were not associated with leukocyte count (Fig. 2C; Supplementary Table 1). In contrast, each 10 mg/dL increment in HDL cholesterol was associated with a decrease in leukocyte count of 0.09×10^3 cells/μL (CI 95% -0.117, −0.053; $p < 0.001$). Whereas each 10 mg/dL increment in triglycerides was associated with an increase in leukocyte count of 0.02×10^3 cells/μL (CI 95% 0.010, 0.020; $p < 0.001$).

Over the follow up period of 12 months, participants in the atorvastatin 10 mg cohort had an average increase in TC, LDL cholesterol and triglycerides of 2.9, 2.8 and 6.0 mg/dL respectively (all $P < 0.001$) (Table 3). HDL cholesterol decreased by 1.1 mg/dL on average ($P < 0.001$). In the atorvastatin 80 mg cohort, there was an average decrease in TC, HDL cholesterol, LDL cholesterol and triglycerides of 27.5, 1.4, 22.2 and 19.5 mg/dL respectively (all $P < 0.001$). Over the same period leukocyte count increased by 0.1×10^3 and 0.3×10^3 cells/μL in the atorvastatin 10 mg and 80 mg cohorts, respectively (both $P < 0.001$). In the longitudinal analysis (Fig. 4; Supplementary Table 5), increases in TC (0.002; CI 95% 0.001, 0.004; $p < 0.001$), HDL cholesterol (0.007; CI 95% 0.003, 0.011; $p = 0.002$) and triglycerides (0.001; CI 95% 0.000, 0.001; $p < 0.001$) over one year were associated with an increase in leukocyte count over the same period. A significant interaction with

Table 3
Characteristics of TNT trial participants at baseline and at one year.

Characteristic	n	Total	n	10 mg	n	80 mg
Age, years	9994	61.0 (8.8)	5004	60.9 (8.9)	4990	61.2 (8.8)
Female, n (%)	9994	1902 (19.0)	5004	961 (19.2)	4990	941 (18.9)
Race/ethnicity, n (%)	9994		5004		4990	
White		9403 (94.1)		4709 (94.1)		4694 (94.1)
Black		289 (2.9)		154 (3.1)		135 (2.7)
Asian		102 (1.0)		54 (1.1)		48 (1.0)
Other		200 (2.0)		87 (1.7)		113 (2.3)
BMI, kg/m²	9960	28.5 (4.6)	4993	28.64, 4.66	4967	28.41, 4.46
Smoking status, n (%)	9994		5004		4990	
Non-smoker		2336 (23.4)		1167 (23.3)		1169 (23.4)
Former smoker		6318 (63.2)		3165 (63.2)		3153 (63.2)
Current smoker		1340 (13.4)		672 (13.4)		668 (13.4)
Diabetes, n (%)	9994	1500 (15.0)	5004	752 (15.0)	4990	748 (15.0)
Systolic blood pressure, mmHg	9990	130.7 (16.8)	5002	130.9 (16.8)	4988	130.5 (16.8)
Blood Urea Nitrogen, mg/dL	9994	16.9 (5.0)	5004	16.8 (4.9)	4990	16.9 (5.0)
Baseline values[a] Lipid levels (mg/dL)						
Total cholesterol	9987	174.7 (23.9)	5002	174.7 (24.0)	4985	174.8 (23.8)
HDL cholesterol	9987	47.3 (11.0)	5002	47.1 (10.8)	4985	47.5 (11.1)
LDL cholesterol	9986	97.5 (17.6)	5001	97.7 (17.6)	4985	97.3 (17.6)
Triglycerides	9987	150.6 (70.9)	5002	150.5 (71.7)	4985	150.6 (69.8)
Leukocyte count (×10³/μL)	9994	6.3 (1.6)	5004	6.3 (1.7)	4990	6.3 (1.6)
Follow up values Lipid levels (mg/dL)						
Total cholesterol	9441	162.3 (31.5)	4737	177.4 (27.2)	4704	147.0 (28.0)
HDL cholesterol	9440	46.1 (11.0)	4736	46.1 (10.8)	4704	46.1 (11.1)
LDL cholesterol	9431	87.7 (24.5)	4730	100.3 (20.8)	4701	75.0 (21.1)
Triglycerides	9441	143.3 (78.3)	4737	155.9 (83.3)	4704	130.5 (70.7)
Leukocyte count (×10³/μL)	9323	6.5 (1.8)	4685	6.4 (1.8)	4638	6.5 (1.8)

Data are expressed as mean (SD) or frequency (%) where appropriate.
Abbreviations: BMI, body mass index; n, valid number of participants.

[a] At baseline all patients had all been on atorvastatin 10 mg for 8 weeks.

treatment allocation was detected for the association of change in total, HDL, and LDL cholesterol with change in leukocyte count (all $p < 0.01$). Stratification by treatment allocation revealed that these relationships were significant only in the atorvastatin 10 mg cohort (all $p < 0.001$).

4. Discussion

Dyslipidaemia and inflammation are two of the hallmarks of CVD. By utilising data from three large cohorts, the present study analyses the relationship of lipid levels with leukocyte counts in a total of 27,566 individuals. Data of NHANES and TNT demonstrate an inverse
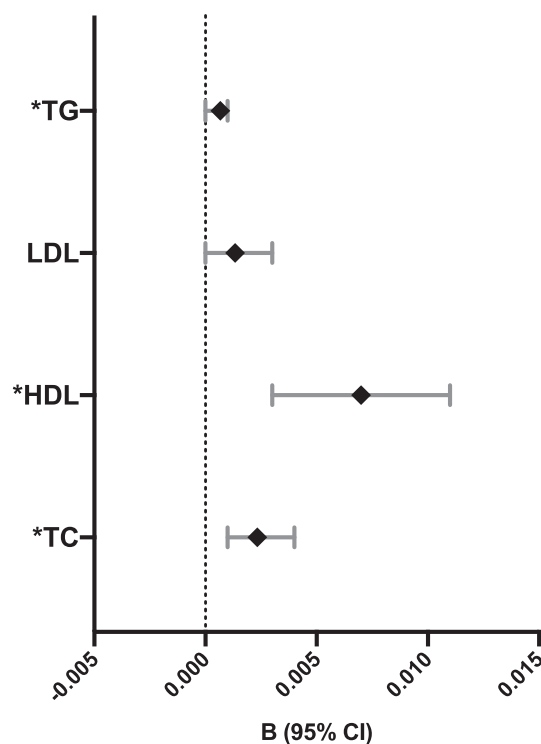

Fig. 4. Association of change in lipid levels with change in leukocyte count in TNT trial cohort over 12 months. This data reflects all participants ($n = 9318$), including those who took 10 mg atorvastatin ($n = 4683$) and 80 mg atorvastatin ($n = 4635$). The regression coefficient (B) and 95% confidence intervals (CI) were expressed as increase in cell counts ($\times 10^3/\mu L$) over one year per one mg/dL increase in lipid levels over the same period.

Data were adjusted for age, sex, Caucasian race, BMI, smoking status, hypertension, diabetes, LDL cholesterol (except for analysis of total cholesterol and LDL cholesterol), HDL cholesterol (except for analysis of HDL cholesterol), triglycerides (except for analysis of triglycerides), blood urea nitrogen, leukocyte count at baseline and treatment allocation.

association of HDL cholesterol with leukocyte count – a finding supported by previous cross-sectional studies [10,11,14]. Collectively, cross-sectional as well as longitudinal data of NHANES and TNT support a positive correlation between triglycerides and leukocyte count, in accordance with other cross-sectional studies in humans [10–13,15,16]. Due to heterogeneity among cohorts and differences in available variables, such as differential cell counts, a meta-analysis of cross-sectional results was not performed.

Elevated triglyceride levels [24,25] and reduced HDL cholesterol levels [26,27] are known to be associated with an increased risk of CVD. Although dyslipidaemia is commonly known to be a combination of hypertriglyceridemia, elevated LDL cholesterol and low HDL cholesterol, these factors do not always occur simultaneously, as supported by NHANES and longitudinal TNT results. Mechanistic links between these lipid parameters and atherosclerosis remain poorly understood [28,29]. CANTOS showed that Canakinumab suppresses CRP and IL-6 levels in patients with elevated inflammatory markers and atherosclerotic disease, and potentially reduces risk of recurrent myocardial infarction, supporting a role for inflammation in the pathobiology of atherosclerosis [30,31]. Correspondingly, leukocytosis is known to be associated with incidence of coronary artery disease and CVD mortality [32,33], with strong evidence that leukocytes directly augment atherosclerosis and thrombosis [34]. Whilst the inflammatory action of LDL on leukocyte activity is well described, this study, together with our previous work [9,10] indicates that higher LDL levels are not correlated with increased leukocyte count, despite this phenomenon being well described in mice.

An association between metabolic syndrome and leukocytosis has been described [35] and therefore a possible explanation is that visceral adipose tissue produces pro-inflammatory cytokines which stimulate leukocyte production, supporting an indirect link between dyslipidaemia and leukocytosis as release of fatty acids stored in visceral adipose tissue results in concomitant hypertriglyceridaemia and low HDL cholesterol levels. A causal relationship is supported by animal studies whereby HDL cholesterol inhibits proliferation of HSPC [8]. Although this mechanistic link has not been identified in human studies, our cross-sectional data would be consistent with similar phenomena.

As CVD develops over decades, assessment of longitudinal relationships provides useful information regarding interaction of lipids and leukocytes over time [36]. Based on evidence to date, it is plausible that hypercholesterolaemia as well as hypertriglyceridemia directly and indirectly enhance leukocytosis, by stimulating proliferation of HSPC [7] and associated pro-inflammatory cytokine production by adipose tissue [35]. Positive temporal associations identified in TNT data support this. Notably however, elevated HDL cholesterol was found to be temporally associated with leukocytosis. This could reflect progression of atherosclerotic disease over 12 months despite improvement in HDL cholesterol while on lipid-lowering therapy throughout the trial.

Higher dose of statin appears to weaken positive temporal associations of TC, HDL cholesterol and LDL cholesterol with leukocyte count, as these are only significant in those taking only 10 mg atorvastatin following stratification by treatment. This suggests that aggressive management of hypercholesterolaemia may in fact lessen the extent to which proliferation and expansion of HSPC is augmented, even if dyslipidaemia remains. However, sensitivity analyses of cross-sectional NHANES data suggest that these relationships are independent of treatment with lipid-lowering medication. Furthermore, this study did not account for a history of familial and non-familial hypercholesterolaemia in participants. This would be a potential confounder, particularly if not managed by lipid-regulating medications. The absolute temporal increase in leukocyte count while on statin treatment is interesting yet challenging to interpret in terms of whether it reflects disease progression, as while leukocyte recruitment and adhesion to atherosclerotic lesions has been investigated in detail, information regarding leukocyte egress is lacking [37,38].

This study is unique in that it includes a cross-sectional and longitudinal analysis of data from a large, diverse population. Particular associations identified in NHANES were not supported by KNHANES and TNT data, perhaps due to smaller sample size and inconsistent study designs, with clinically differing cohorts. Moreover, we could not exclude the possibility of the ethnic difference in such association, since the KNHANES cohort was comprised of South Koreans (Asians), whereas NHANES was comprised of mainly non-Hispanic Whites, African Americans and Mexican Americans. Overall, our study supports meaningful cross-sectional and longitudinal relationships between lipid levels and leukocyte counts. In general, triglycerides and HDL cholesterol are robust predictors of leukocyte count across the three cohorts analysed in this study. In NHANES, data were adjusted for the well-known inflammatory marker, CRP. However, data on CRP was not available in the full cohort of KNHANES and TNT, which limits further study on the role of inflammation in the relationship of these lipid species with leukocyte. Although it is difficult to draw conclusions on causation based on this observational study, together with the evidence from previous studies this data suggests that triglycerides and HDL cholesterol may be directly involved in leukogenesis, although the precise mechanism of this relationship and direction of causation is currently ill-defined.

Overall, by utilising a large, comprehensive dataset this study highlights significant cross-sectional and longitudinal relationships of lipid and lipoprotein parameters with leukocyte counts. However, unlike in murine models the majority of these relationships are modest, with the most robust association being between triglycerides and differential leukocyte counts.

This study provides further evidence that major differences exist in the relationships between lipids and leukocyte numbers in mediating atherosclerosis in mice and humans. The impact of lipoproteins on the atherogenic nature of immune cells in humans is likely due to modification of cellular function, rather than alteration of leukogenesis and resulting changes in cell number.

CRediT authorship contribution statement

Sonia Sawant: Validation, Writing – original draft. **Bradley Tucker:** Formal analysis, Writing – original draft. **Praween Senanayake:** Formal analysis. **David D. Waters:** Validation, Resources. **Sanjay Patel:** Supervision, Resources. **Kerry-Anne Rye:** Supervision, Resources. **Kwok Leung Ong:** Validation, Supervision, Writing – reviewing & editing. **Blake J Cochran:** Conceptualisation, Supervision, Writing – reviewing & editing.

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Declaration of competing interest

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

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Appendix A. Supplementary data

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