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# Abnormal blood rheology and chronic low grade inflammation: Possible risk factors for accelerated atherosclerosis and coronary artery disease in Lewis negative subjects

Tamas Alexy [a, b, *], Eszter Pais [a], Rosalinda B. Wenby [a], Wendy J. Mack [a], Howard N. Hodis [a], Naoko Kono [a], Jun Wang [a], Oguz K. Baskurt [c], Timothy C. Fisher [a], Herbert J. Meiselman [a]

[a] University of Southern California, Keck School of Medicine, Los Angeles, CA 90033, USA
[b] Emory University, School of Medicine, Atlanta, GA 30322, USA
[c] Koc University, School of Medicine, Istanbul, Turkey

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ABSTRACT

*Objective:* To test the hypothesis that abnormal hemorheology and chronic low-grade inflammation are more prevalent in Lewis negative individuals, possibly contributing to premature atherosclerosis.
*Methods and results:* We enrolled 223 healthy subjects (154 females, mean age: 64yrs). Conventional risk factors, markers of inflammation and hemorheological profiles were measured; Lewis blood group was determined by serology. Conventional risk factors (age, gender, BMI, blood pressure, lipid profile, smoking habit) did not differ among Lewis phenotypes. However, markers of inflammation (WBC, hs-CRP, ESR) were significantly elevated and rheological parameters (RBC aggregation, plasma viscosity) were abnormal in Lewis negative subjects, especially when compared to the Le(a−b+) group.
*Conclusions:* With a prevalence of 33% in select populations, our data support the hypothesis that Le(a−b−) represents a pro-inflammatory phenotype that may contribute to the elevated cardiovascular risk in this group.



## 1. Introduction

The synthesis of Lewis A ($Le^a$) and Lewis B ($Le^b$) antigens is determined by the activity of multiple fucosyltransferase enzymes (FUT) encoded on chromosome 19 (19p13.3) [1]. Soluble $Le^a$ and $Le^b$ are released by exocrine epithelial cells into body fluids and adsorb passively onto the red blood cell (RBC) membrane [2] thereby enabling the use of immunoassays to determine an individual's Lewis phenotype: negative Le(a−b−); A + Le(a+b−); B + Le(a−b+) or A + B + Le(a+b+). Lewis negative individuals lack alpha (1–3/1–4) fucosyltransferase activity due to inactivating point mutations affecting the FUT3 locus [3].

Epidemiologic studies found the Lewis negative phenotype to be independently associated with a two-fold higher prevalence of coronary artery disease and a four-fold higher risk for fatal coronary events [4,5]. While a few subsequent studies failed to prove such association [6], several others re-confirmed Le(a−b−) as an independent risk factor for atherosclerosis [7,8]. The underlying mechanisms responsible for the increased cardiovascular risk have not been determined.

Abnormal hemorheological parameters lead to impaired hemodynamic profiles both in the macro- and microcirculation [9]. As a consequence, an extended area of the vascular endothelium is subjected to low-oscillatory shear stress inducing arterial wall remodeling, endothelial dysfunction [10] and adhesion molecule overexpression [11], ultimately promoting inflammation and atherosclerotic plaque formation [12]. The association between Lewis phenotypes, chronic low-grade inflammation and disturbed blood rheology has not been explored. The present study evaluated the hypothesis that the accelerated atherosclerosis in Lewis negative individuals is associated with a genetically determined pro-inflammatory state that exists on a permissive background of abnormal hemorheology.

* Corresponding author. Emory University, School of Medicine, 101 Woodruff Circle, WMB 308, Atlanta, GA 30322, USA.
*E-mail address:* talexy@emory.edu (T. Alexy).

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## 2. Methods

A blinded cross-sectional study was designed to evaluate our hypothesis. Blood samples were collected from 318 randomly selected males and postmenopausal females participating in one of two randomized controlled trials at the University of Southern California (USC): 1) B-Vitamin Atherosclerosis Intervention Trial (BVAIT; n = 145) [13] and 2) Women's Isoflavone Soy Health trial (WISH; n = 173) [14]. For both trials, exclusion criteria included known atherosclerotic vascular disease, diabetes mellitus, untreated hypertension, chronic kidney disease, untreated hypo- or hyperthyroidism and five or more alcoholic beverages per day. For the current study, additional exclusion criteria included acute illness within four weeks and transfusion of blood products within 90 days of sampling. All participants provided written informed consent and the Institutional Review Board of USC approved all protocols.

After collecting demographic information, risk factor profile and hemorheological data from 100 individuals blinded to their Lewis phenotype, the study protocol was expanded to include the following surrogate inflammatory markers: white blood cell count (WBC), high-sensitivity C reactive protein (hs-CRP), plasma fibrinogen concentration and insulin level. In addition, plasma homocysteine was determined in all BVAIT subjects. An additional 123 subjects were enrolled in this second study phase. Given the low prevalence of Lewis negatives in the general population, a third phase of sampling was performed aiming to enrich for Le(a−b−) subjects. All 95 trial participants in this third phase were tested for their Lewis status but only blood from the five Le(a−b−) subjects was utilized. Individuals who typed Le(a−b+) (n = 80) or Le(a+b−) (n = 10) were not added to the analytic dataset.

BVAIT and WISH participants were recruited at the 30-month and pre-randomization visits, respectively. Samples for hs-CRP, fibrinogen and insulin measurements were stored at −80 °C for a maximum of three months and were assayed in batches. All other tests were completed within 6 h of collection. Complete blood counts were determined using an automated hematology analyzer (Micros, Horiba-ABX, Irvine, CA); microhematocrit (Hct) for each suspension was also confirmed using a tabletop centrifuge. A Coulter Plasma Viscometer II (Coulter Electronics, Luton, UK) was utilized to measure plasma viscosity (PV) at 25 °C. RBC aggregation and aggregability (i.e., the intrinsic tendency of RBC to aggregate in a standard 70 kDa dextran medium) were determined with a Myrenne MA-2 aggregometer (Myrenne GmBH, Roetgen, Germany) according to the current guidelines for hemorheological laboratory techniques [15]. Erythrocyte sedimentation rate (ESR) was measured in a subset of subjects at native and at 40% Hct using standard Westergren tubes. Plasma fibrinogen was determined by the STart benchtop hemostasis system (Diagnostica Stago, Parsippany, NJ) for 79 individuals. hs-CRP (Zymutest, Hyphen BioMed, Neuville-sur-Oise, France) and insulin levels (Linco Research, St. Charles, MO) were measured by ELISA in 120 and 97 samples, respectively. Homocysteine was determined by reverse phase high performance liquid chromatography in 87 BVAIT samples. RBC Lewis phenotype was determined by two independent investigators by serology according to standard laboratory procedures [16]. Monoclonal anti-Le$^a$ and anti-Le$^b$ antibodies were purchased from Ortho Clinical Diagnostics, Rochester, NY.

Demographic and laboratory characteristics were compared between Lewis phenotype groups using analysis of variance for continuous, and chi-square tests for categorical measures. The associations between hemorheological parameters and Lewis phenotype were evaluated with adjustment for age, gender, race/ethnicity and smoking habits using analysis of covariance (ANCOVA). These comparisons of mean differences of hemorheological measures among Lewis phenotype groups were also adjusted for a 3-level trial group variable that included WISH (all participants were sampled at pre-randomization), B-vitamin-treated BVAIT, and placebo-treated BVAIT. Variables not normally distributed were log transformed for ANCOVA; results are presented as mean ± SEM by Lewis phenotype.

## 3. Results

318 individuals were Lewis phenotyped; 49 (15.4%) were Lewis negative, 68 (21.4%) were Le(a+b−) and 201 (63.2%) tested Le(a−b+). Consistent with literature [17], Lewis negative phenotype was most prevalent among African Americans (25.0%) followed by Hispanics (19.0%), Caucasians (13.9%) and Asians (13.9%). The hemorheological profile was evaluated for 223 subjects with the following Lewis phenotype distribution: 19.7% Le(a−b−); 26.0% Le(a+b−); 54.3% Le(a−b+).

As shown in Table 1, the average age, BMI, fasting plasma glucose, lipid profile, systolic/diastolic blood pressure and the number of current/ex-smokers were equivalent among the three Lewis groups. The prevalence of the individual Lewis phenotypes did not differ among subjects enrolled from the WISH and BVAIT trials. Mean values for platelet count (data not shown) and hemoglobin/Hct were similar among Lewis phenotypes while WBC count was elevated in Lewis negatives ($p = 0.05$). ESR values were strikingly different between the groups both at native and at 40% Hct: ESR was in the normal range for all Le(a−b+) subjects while almost one-half of Le(a-b-) individuals had values exceeding the upper limit of the age-adjusted normal range (<30 mm/h; $p < 0.001$; Table 2). Results obtained by the Myrenne aggregometer were consistent with the ESR data. Mean group differences were attenuated when testing RBC aggregability but plasma viscosity was significantly elevated in Lewis negatives compared to the Lewis positive population ($p < 0.0001$). A trend for elevated fibrinogen and hs-CRP values was also noted such that Le(a−b−)>Le(a+b−)

**Table 1**
Demographic data and conventional risk factors for the study population.

| | Le(a−b−) n = 44 | Le(a+b−) n = 58 | Le(a−b+) n = 121 | p value* | p value** |
|---|---|---|---|---|---|
| Age (years) | 65.5 ± 1.4 | 63.9 ± 1.2 | 63.5 ± 0.9 | 0.50 | 0.24 |
| Females | 32 (73%) | 46 (79%) | 76 (63%) | 0.07 | 0.24 |
| BMI (kg/m$^2$) | 27.7 ± 0.8 | 26.1 ± 0.7 | 26.9 ± 0.5 | 0.28 | 0.43 |
| Fasting glucose (mg/dL) | 98.7 ± 1.7 | 95.3 ± 1.5 | 97.7 ± 1.0 | 0.25 | 0.62 |
| Total cholesterol (mg/dL) | 214.2 ± 5.4 | 223.7 ± 4.7 | 215.3 ± 3.2 | 0.28 | 0.86 |
| LDL cholesterol (mg/dL) | 132.3 ± 4.8 | 138.6 ± 4.2 | 132.3 ± 2.9 | 0.43 | 0.99 |
| HDL cholesterol (mg/dL) | 58.8 ± 2.5 | 63.6 ± 2.2 | 59.1 ± 1.5 | 0.19 | 0.93 |
| Triglycerides (mg/dL) | 115.2 ± 8.4 | 107.0 ± 7.2 | 119.3 ± 5.0 | 0.38 | 0.70 |
| Systolic BP (mmHg) | 125.8 ± 2.4 | 119.9 ± 2.1 | 124.5 ± 1.4 | 0.11 | 0.66 |
| Diastolic BP (mmHg) | 75.2 ± 1.3 | 76.1 ± 1.1 | 76.8 ± 0.8 | 0.57 | 0.31 |
| Smoking (ex and current) | 21 (48%) | 24 (41%) | 47 (39%) | 0.61 | 0.32 |
| Trial[a] | | | | | |
| BVAIT: B-vitamin treated | 15 (34%) | 11 (19%) | 37 (31%) | 0.15 | |
| BVAIT: placebo treated | 11 (25%) | 14 (24%) | 38 (31%) | | |
| WISH | 18 (41%) | 33 (57%) | 46 (38%) | | |

Values in tables are mean ± SEM or n (percent).
*p value for comparison of differences among Lewis phenotype groups ANOVA or chi-square test.
**p value for comparison of Lewis(a-b-) vs. Lewis(a−b+) group using ANOVA.
[a] Trial distribution by Lewis phenotype frequencies, n (%), chi-square p-value.