Stability and reliability of plasma level of lipid biomarkers and their correlation with dietary fat intake

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Abstract. The reliability and stability of plasma lipid biomarkers and their association with dietary fat intake were evaluated among 48 subjects who were randomly chosen from the participants of a validation study of the population-based cohort, the Shanghai Men’s Health Study (SMHS). Four spot blood samples, one taken each season, were measured for total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol levels. The reliability and stability of these measurements were assessed by intraclass correlation coefficients (ICC) and by the correlations between a randomly chosen measurement with the mean of measurements across seasons using a bootstrap approach. The median levels for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were 177.5, 164.5, 41.0, and 102.5 (mg/dl), respectively. The ICCs of the biomarkers ranged from 0.58 (LDL-cholesterol) to 0.83 (HDL-cholesterol). The correlation between randomly chosen spot measurements and the mean measurement were 0.91, 0.86, 0.93, and 0.83 for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, respectively. The correlations of lipid biomarkers with dietary fat intake and other lifestyle factors were comparable to other previous reports. In conclusion, this study suggests that measurements of lipid biomarkers from a single spot blood sample are a good representation of the average blood levels of these biomarkers in the study population and could be a useful tool for epidemiological studies.

1. Introduction

Morbidity and mortality from cardiovascular disease (CVD), type 2 diabetes mellitus, and hypertension, important components of the metabolic syndrome, are expected to dramatically increase during the next 20 years in China and other developing countries [1]. CVD accounted for nearly 40% of all deaths in 1994 in China [2,3], and the prevalence of hypertension increased from 11.3% in 1991 to 27.2% in 2000 [4,5].

There is now convincing evidence that elevated blood levels of total cholesterol and low-density lipoprotein (LDL) cholesterol are associated with increased CVD risk, whereas elevated high-density lipoprotein (HDL) cholesterol concentrations appear to provide protection against premature CVD [6–8]. Although lipid biomarkers are often of primary interest in epidemiological studies as they relate to many chronic diseases such as metabolic syndrome and cancer, for economic and logistical reasons it is difficult for such studies to collect either fasting blood samples or multiple blood samples. The degree to which a single sample reflects long-term biomarker levels for an individual depends on the within-person variability of the biomarker over time [9]. The larger the variation, the larger the degree...
of measurement error associated with a single measurement and, as a consequence, observed associations (e.g., relative risk) could be attenuated. Therefore, knowledge of how well a single biomarker measurement reflects longer term levels is important for conducting and interpreting epidemiological studies investigating the associations between those biomarkers and the incidence/prevalence of chronic diseases [10].

We evaluated the reliability and stability of lipid biomarkers and the utility of a single spot blood sample for measuring lipid levels for future large epidemiological studies in a subset of men who participated in the dietary validation of the Shanghai Men’s Health Study (SMHS).

2. Methods

2.1. Subjects

The SMHS is a population-based prospective cohort study with a primary aim to investigate the effect of dietary, occupational, and lifestyle factors and genetic susceptibility on the risk of cancers and other major chronic diseases. Recruitment for the SMHS started in April 2002 and was completed in June 2006. A total of 61,582 men aged 40 to 74 years were recruited into the study, with a participation rate of 74.1%. The SMHS food frequency questionnaire (FFQ) was evaluated in a validation study that included 196 men who were randomly selected from SMHS participants between April 2003 and May 2004 [11]. The validation study included the administration of two FFQs one year apart, 12 monthly 24-hour dietary recalls, and the collection of a blood and urine sample in each quarter of the one-year study period. The overall response rate for the validation study was 69.3%. For the current study, 48 men were randomly selected from SMHS participants between April 2003 and May 2004 [11]. The validation study included the administration of two FFQs one year apart, 12 monthly 24-hour dietary recalls, and the collection of a blood and urine sample in each quarter of the one-year study period. The overall response rate for the validation study was 69.3%. For the current study, 48 men were randomly selected from validation study participants who had provided four blood samples throughout the year. Participants of the validation study and parent study were comparable in age, body mass index, waist-to-hip ratio, education, individual income, alcohol consumption, cigarette smoking rates, and total energy intake (data not shown). The study was approved by the Institutional Review Boards of all participating institutions, and all participants provided written informed consent.

2.2. Estimation of dietary intake and other selected lifestyle factors

Baseline information including age, physical activity, occupation history, disease history, and dietary habits was collected by an in-person interview. The consumption of each food item or food group was measured with the validated FFQ based on frequency (daily, weekly, monthly, yearly, or never) and amount consumed in liang (one liang = 50 g) per unit of time. FFQ items used for estimation of fat intake included fat from animal foods (red meat, poultry, fish, eggs, and milk) and fat from plant foods (vegetables, soy, fruits, and other plant sources). Fatty acids were divided into saturated, monounsaturated, and polyunsaturated fatty acids. Consumption of α-linolenic acid (18:3), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6) were summed to derive crude omega-3 fatty acid intake, and consumption of linoleic acid (18:2), γ-linolenic acid (20:3), and arachidonic acid (20:4) were summed to derive crude omega-6 fatty acid intake.

Using a standard protocol, trained interviewers measured weight, circumferences of the waist and hips, sitting and standing height, and blood pressure for all participants. The anthropometric measurements were taken twice, and a third measurement was administered if the difference between the first two exceeded the tolerance limits (1 cm for height and 1 kg for weight). We calculated body mass index (BMI) as weight in kilograms divided by the square of height in meters.

2.3. Analysis of serum lipids

Blood samples were collected using EDTA-containing BD Vacutainer® tubes from study participants at the time of their in-person interview without a specific requirement that participants fast beforehand. Blood samples were immediately placed in a cooler with blue ice and processed within 6 hours. Plasma samples were stored at ~70°C. Among the 48 participants of this reliability study, 30 men provided four non-fasting blood samples; 18 men provided two to three fasting blood samples; and one subject provided four fasting blood samples. Fasting status was defined as an interval between the last meal and blood draw of 8 hours or longer. The levels of selected lipid biomarkers were measured by the Vanderbilt Lipid Laboratory, which is standardized by the Centers for Disease Control and Prevention for lipid analysis, using the ACE® Clinical Chemistry System (Alfa Wassermann,
Inc. West Caldwell, NJ). Levels of triglycerides, total cholesterol, and HDL-cholesterol were assayed using the ACE® Triglyceride Reagent (#SA1023), ACE® Cholesterol Reagent (#SA1010), and ACE® HDL-C Reagent (#SA1038) (Alfa Wassermann, Inc, West Caldwell, NJ), respectively, following the manufacturer’s protocols. LDL-cholesterol levels were calculated with the Friedewald equation [12] \([\text{LDL}-\text{cholesterol}] = [\text{Total cholesterol}] – [\text{HDL}-\text{cholesterol}] – ([\text{triglycerides}] / 5)\). In subjects with triglyceride levels above 400 mg/dL, LDL-cholesterol levels were measured directly using ACE® LDL-C Reagent (#SA1040). Coefficient variations (CVs) for intra-batch variation were 2.36, 1.24, 1.31, and 3.22 for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, respectively. The CVs for inter-batch variation were 3.36, 2.84, 2.13, and 4.65 for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, respectively. All four samples for each study participant were measured in the same analytic batch to avoid the influence of batch-to-batch variation.

2.4. Statistics

Demographic characteristics and selected lifestyle factors of participants in the validation study were compared with the parent cohort using the Z-test (data not shown). The Student’s t-test was employed to compare lipid biomarker levels between fasting and non-fasting blood, and the ANOVA test was used to compare levels across four seasons. To evaluate the stability and usefulness of a single spot blood measurement as an objective biomarker, we evaluated the Spearman correlation between randomly chosen spot blood measurements (4*48 = 192 measurements) and the average of the four measurements provided by each subject. The correlation estimates and their 95% confidence intervals, (95% CI) were derived using the bootstrap method with 2,000 repeats in each case. Finally, intraclass correlation coefficients (ICC) were estimated to evaluate the seasonal variability of blood lipid biomarkers using the following one-way random effect model:

\[
\text{ICC} (\rho) = \frac{\sigma_w^2}{(\sigma_b^2 + \sigma_w^2)}
\]

\[
= \frac{\text{between group variance}}{\text{between group variance + within group variance}}
\]

To evaluate the influence of the observed measurement error in plasma lipid biomarkers on relative risk estimates for future etiological studies, we estimated the relative risk that would be observed given true relative risks of 1.5, 2.0, and 2.5 by multiplying the natural logarithm of the specified true relative risks with the ICC and exponentiated the result [14]. The correlation of lipid biomarkers with dietary fat intake and other lifestyle factors were evaluated using Spearman correlation coefficients adjusting for the time interval between the last meal and blood collection, medicine use, number of cigarettes smoked within the last 24 hours, and total energy intake. All statistical analyses were carried out using SAS, version 9.1 (SAS Institute, Inc, Cary, NC).

3. Results

The levels of lipid-related biomarkers from nonfasting blood samples were similar to those from fasting samples with the exception of triglyceride levels (Table 1). The median levels were 177.5, 164.5, 41.0, and 102.5 (mg/dl) for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, respectively, and levels did not appear to vary greatly by season (0.10 \(\leq p \leq 0.95\)). The correlations between the average intake of these biomarkers across four seasons and during a single season based on bootstrap analysis were also high, ranging from 0.83 (LCL-cholesterol) to 0.93 (HDL-cholesterol) (Table 2).

The ICCs of the measurements across four seasons for total cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were 0.75, 0.65, 0.83, and 0.58, respectively (Table 3). Given the observed ICC and assuming that the true relative risks of the disease-lipid association were 1.5, 2.0, 2.5, the observed relative risk would be attenuated from 68.0% to 93.3% (Table 3).

Total cholesterol levels were positively correlated with age (\(r = 0.30\)). Triglyceride levels were associated with BMI (\(r = 0.56\)) and systolic blood pressure (\(r = 0.33\)). On the other hand, HDL-cholesterol levels were inversely associated with BMI (\(r = -0.43\)). No obesity-related markers were associated with LDL-cholesterol levels (Table 4).

The patterns of correlation between fat and blood lipid biomarkers differed by fat sources. Total cholesterol was positively associated with fat from red meat (\(r = 0.15\)) and poultry (\(r = 0.18\)), but inversely associated with fat from fish (\(r = -0.20\)) and soy (\(r = -0.27\)). Triglycerides were related to fat from eggs (\(r = -0.18\)), milk (\(r = 0.19\)), and other plant sources (\(r = -0.21\)). High HDL-cholesterol levels were associated with high fat intake from fish (\(r = 0.17\)) and eggs (\(r = 0.16\)), but was inversely associated with vegetable oil intake (\(r = -0.18\)). LDL-cholesterol levels were positively associated with poultry fat (\(r = 0.24\)),...
but inversely associated with fat from fish (r = -0.23) and soy (r = -0.28). Although total polyunsaturated fatty acids were not associated with lipid biomarkers, omega-3 fatty acid was inversely related to total cholesterol (r = -0.25) and LDL-cholesterol (r = -0.26). Omega-6 fatty acid was positively associated with HDL-cholesterol (r = 0.18), but inversely associated with LDL-cholesterol (r = -0.16). Total cholesterol intake was positively correlated with HDL-cholesterol level (r = 0.17), total triglycerides (r = 0.37), and LDL-cholesterol (r = 0.72). Triglycerides were negatively correlated with HDL-cholesterol (r = -0.47) (Table 4).

4. Discussion

This study found no statistically significant differences in the blood levels of lipid biomarkers between fasting blood and non-fasting blood with the exception of triglycerides. In addition, a single spot blood lipid biomarker measurement correlated well with average levels and could be used to evaluate lipid status in large epidemiological studies.

The use of fasting lipoprotein analysis for the measurement of total cholesterol, triglyceride, HDL-cholesterol, and calculated LDL-cholesterol levels is recommended for clinical diagnosis and monitoring of CVD, diabetes, and hypertension. However, for use in large population-based studies, economic and logistical reasons make the collection of fasting blood samples difficult and impractical. In the present study, we found that the levels of lipid biomarkers obtained from non-fasting blood samples were similar to those obtained from fasting blood. Our finding is consistent with a previous report from a cross-sectional study that showed non-fasting HDL-cholesterol levels were similar to fasting HDL levels, and the agreement in classification of patients into desirable- and high-cholesterol groups using either fasting or non-fasting blood samples was high [15]. LDL-cholesterol and triglyceride levels are more prone to the influence of dietary cholesterol intake. We observed higher within-person variation (lower ICCs) for these two lipid biomarkers than for HDL-cholesterol when test samples were mixed with fasting and non-fasting samples. This suggests that a fasting blood sample may more desirable if LDL-cholesterol and triglyceride levels are to be used in epidemiological studies.

In the present study, total cholesterol level was positively correlated with age, and triglyceride levels increased with high BMI and systolic blood pressure.

Table 1

|                 | Fasting blood sample (n = 32) | Non-fasting sample (n = 160) | Wilcoxon rank sum test |
|-----------------|------------------------------|-----------------------------|-----------------------|
|                 | Mean ± SD | Median (Q1, Q3) | Mean ± SD | Median (Q1, Q3) | |
| Cholesterol (mg/dl) | 176.3 ± 22.4 | 175.0 (161.5, 185.5) | 180.3 ± 31.2 | 178.0 (162.5, 196.5) | 0.39 |
| Triglycerides (mg/dl) | 138.4 ± 71.1 | 113.5 (94.5, 177.0) | 218.5 ± 198.2 | 169.5 (120.0, 238.0) | 0.01 |
| HDL-cholesterol (mg/dl) | 44.5 ± 10.2 | 43.0 (36.5, 49.5) | 41.7 ± 9.5 | 40.0 (35.0, 47.0) | 0.15 |
| LDL-cholesterol (mg/dl) | 104.2 ± 24.2 | 105.5 (89.5, 116.0) | 99.8 ± 26.8 | 102.0 (82.5, 115.0) | 0.29 |

*Fasting* refers to blood samples collected 8 hours or more after the most recent meal.

Table 2

|                 | Median (mg/dl) | Correlation (95% CI) | r(95%CI)* |
|-----------------|----------------|---------------------|-----------|
|                 | Winter | Spring | Summer | Fall | Average | P**<sub>corr</sub> | Winter | Spring | Summer | Fall | |
| Cholesterol | 179.5 | 181.2 | 175.1 | 177.3 | 177.5 | 0.39 | 0.89 | 0.91 | 0.90 | 0.92 | 0.91 |
| Triglycerides | 137.5 | 165.0 | 176.5 | 164.5 | 155.5 | 0.95 | 0.91 | 0.86 | 0.82 | 0.84 | 0.86 |
| HDL-cholesterol | 42.0 | 41.0 | 38.5 | 42.5 | 41.0 | 0.34 | 0.94 | 0.96 | 0.93 | 0.95 | 0.93 |
| LDL-cholesterol | 106.0 | 101.0 | 96.0 | 89.0 | 102.5 | 0.10 | 0.80 | 0.82 | 0.83 | 0.85 | 0.83 |

*Spearman correlation coefficient between log transformed individual plasma lipid-related biomarkers and log transformed mean of plasma lipid-related biomarkers after adjustment for interval between last meal and blood collection, medicine use, number of cigarettes smoked within the previous 24 hours, and total energy intake.

**Correlations between a randomly chosen individual measurement and averaged measurements, estimated using the bootstrap method.

*Average of four seasonal samples.
while HDL-cholesterol was inversely associated with BMI similar to other previous reports [16]. The correlation between dietary fat intake and blood lipid biomarkers varied according to the fat source in this study. Blood lipids and lipoproteins have been shown to be associated with dietary fat intake. Several studies have indicated that low-fat and high-carbohydrate diets reduce both LDL-cholesterol and HDL-cholesterol [17–20], and may also raise triglycerides [17,19], an independent risk factor for the development of CHD [21]. In our study, levels of total and LDL-cholesterol were inversely correlated with intake of omega-3 and omega-6 fatty acids, although total saturated or unsaturated fatty acids were not associated with any lipid biomarker-

### Table 3

| Plasma lipid-related biomarker | ICC | Observed relative risk given specified true relative risk |
|-------------------------------|-----|--------------------------------------------------------|
| Cholesterol                   | 0.75| 1.4 (93.3) 1.7 (85.0) 2.0 (80.0) |
| Triglycerides                 | 0.65| 1.3 (86.7) 1.6 (80.0) 1.8 (72.0) |
| HDL-cholesterol               | 0.83| 1.4 (93.3) 1.8 (90.0) 2.1 (84.0) |
| LDL-cholesterol               | 0.58| 1.3 (86.7) 1.5 (75.0) 1.7 (68.0) |

*a* From log-transformed data, based on data from 48 men who participated in the SMHS dietary validation study. Levels of selected lipid-related biomarkers are represented as mg/dl of plasma.

*b* True relative risk.

*c* Observed relative risk.

*d* Percent of attribution.

### Table 4

| Fat from animal foods | Cholesterol (Bivariate analysis) | Triglycerides (Bivariate analysis) | HDL-cholesterol (Bivariate analysis) | LDL-cholesterol (Bivariate analysis) |
|-----------------------|---------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| Age                   | 0.30 (0.02, 0.54)               | 0.19 (−0.10, 0.45)               | 0.09 (−0.20, 0.36)                   | 0.17 (−0.12, 0.43)                   |
| Body mass index       | 0.24 (−0.05, 0.49)              | 0.56 (0.33, 0.73)                | −0.43 (−0.64, −0.17)                 | 0.01 (−0.27, 0.29)                   |
| Waist-to-hip ratio    | 0.02 (−0.37, 0.30)              | 0.22 (−0.07, 0.47)              | −0.26 (−0.51, 0.03)                   | −0.03 (−0.31, 0.26)                   |
| Systolic blood pressure | 0.23 (−0.06, 0.48)          | 0.33 (0.06, 0.56)               | −0.15 (−0.42, 0.14)                   | 0.05 (−0.24, 0.33)                   |
| Diastolic blood pressure | 0.11 (−0.18, 0.38)                | 0.28 (−0.01, 0.53)               | −0.23 (−0.48, 0.06)                   | −0.05 (−0.34, 0.23)                   |
| Physical activity     | 0.05 (−0.24, 0.33)              | −0.01 (−0.29, 0.27)             | −0.01 (−0.29, 0.27)                   | 0.04 (−0.25, 0.32)                   |
| Total energy intake   | −0.11 (−0.38, 0.18)             | −0.24 (−0.49, 0.05)             | 0.08 (−0.21, 0.36)                   | 0.10 (−0.90, 0.37)                   |
| Total Fat             | 0.02 (−0.27, 0.30)              | 0.07 (−0.22, 0.35)              | −0.12 (−0.39, 0.17)                   | 0.06 (−0.23, 0.34)                   |

**Fat from plant foods**

| Fat from plant foods | Cholesterol (Bivariate analysis) | Triglycerides (Bivariate analysis) | HDL-cholesterol (Bivariate analysis) | LDL-cholesterol (Bivariate analysis) |
|---------------------|---------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| Vegetables          | −0.04 (−0.32, 0.45)             | 0.05 (−0.24, 0.33)               | −0.18 (−0.44, 0.11)                   | 0.09 (−0.20, 0.36)                   |
| Soy                 | −0.27 (−0.51, 0.02)             | 0.12 (0.17, 0.39)                | −0.07 (−0.34, 0.22)                   | −0.28 (−0.53, 0.00)                   |
| Fruits              | −0.01 (−0.29, 0.27)             | −0.06 (−0.34, 0.23)              | −0.01 (−0.29, 0.27)                   | 0.01 (−0.27, 0.29)                   |
| Other               | −0.06 (−0.34, 0.23)             | −0.21 (−0.47, 0.08)              | 0.12 (0.17, 0.39)                     | 0.06 (−0.23, 0.34)                   |
| Saturated FA        | 0.14 (−0.15, 0.41)              | 0.02 (−0.27, 0.30)              | 0.01 (−0.27, 0.29)                   | 0.09 (−0.20, 0.37)                   |
| Monounsaturated FA  | 0.11 (−0.18, 0.38)              | −0.02 (−0.30, 0.27)             | 0.01 (−0.27, 0.29)                   | 0.11 (−0.18, 0.38)                   |
| Polyunsaturated FA  | −0.08 (−0.36, 0.21)             | −0.01 (−0.29, 0.27)             | 0.05 (−0.24, 0.33)                   | −0.06 (−0.34, 0.23)                   |
| ω−3 fatty acids     | −0.25 (−0.50, 0.04)             | 0.02 (−0.27, 0.30)              | 0.04 (−0.25, 0.32)                   | −0.26 (−0.51, 0.03)                   |
| ω−6 fatty acids     | −0.15 (−0.42, 0.14)             | −0.07 (−0.35, 0.22)             | 0.18 (−0.11, 0.44)                   | −0.16 (−0.42, 0.13)                   |
| Dietary cholesterol | 0.01 (−0.27, 0.29)              | −0.12 (−0.39, 0.17)             | 0.17 (−0.12, 0.43)                   | 0.01 (−0.27, 0.29)                   |

### Table 4

| Blood lipid biomarkers | Cholesterol (Bivariate analysis) | Triglycerides (Bivariate analysis) | HDL-cholesterol (Bivariate analysis) | LDL-cholesterol (Bivariate analysis) |
|-----------------------|---------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| Total cholesterol     | 1.00                            |                                  |                                     |                                     |
| Triglycerides         | 0.37 (0.10, 0.59)                |                                  |                                     |                                     |
| HDL-cholesterol       | 0.21 (−0.08, 0.47)               | −0.47 (−0.67, −0.21)             | 1.00                                 |                                     |
| LDL-cholesterol       | 0.72 (0.55–0.83)                 | −0.18 (−0.44, 0.11)             | 0.24 (−0.05, 0.49)                   | 1.00                                 |

*a* Spearman correlation coefficient after adjustment for interval between last meal and blood collection, medicine use, number of cigarettes smoked within the previous 24 hours, and total energy intake. Dietary information, blood pressure and anthropometrics were based on baseline survey data.

Bold indicates statistical significance (p < 0.05) for Spearman correlation coefficient analyses.
ers. The absolute concentration of LDL-cholesterol achieved in humans [22] or hamsters [23] has previously been shown to be positively associated with a diet predominated by saturated fatty acids but inversely associated with a diet predominated by unsaturated fatty acids. It should be noted that the number of subjects included in the current study is small, and estimates of the associations between dietary fatty acids and plasma lipid biomarker levels were not stable. Further studies on this topic are needed.

It is a common practice in epidemiological studies to classify individuals into different groups according to their exposure levels. If the within-person variability of biomarker levels is random, then the correlation of a single measurement with the average of multiple measurements in a population can be used to ‘correct’ the attenuation of relative risk estimates by using a single measurement [9]. In our study, all lipid-related biomarkers were shown to have reasonably high ICC values, suggesting small within-person variability and large between-person variability. An ICC of 0.65 or larger results in relatively modest decreases in the estimated relative risk, although the degree of attenuation that can be tolerated depends on the size of the study and the magnitude of the risk [14]. Correction for measurement error is particularly important in the case of exposure measurements, such as LDL-cholesterol (ICC = 0.58), where the level of error can result in appreciable attenuation of relative risk estimates.

In conclusion, this study suggests that levels of lipid biomarkers from a single spot blood sample are relatively stable in this cohort of Chinese men. Thus, a single spot blood sample can serve as a useful resource for future studies of associations between lipid levels and health.

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