METHODOLOGY

Regression dilution methods for meta-analysis: assessing long-term variability in plasma fibrinogen among 27,247 adults in 15 prospective studies

The Fibrinogen Studies Collaboration*

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Background Within-person variability in measured values of a risk factor can bias its association with disease. The extent of this regression dilution bias for plasma fibrinogen was investigated using repeat measurement data collected at varying time intervals on 27,247 adults in 15 prospective studies.

Methods Regression dilution ratios (RDRs) were estimated from a linear regression of repeat measurements on baseline values in each study and for each time interval, and pooled allowing for within- and between-study heterogeneity. RDRs were estimated both without and with adjustment for confounders, and factors were investigated that might influence the RDRs.

Results The unadjusted overall RDR was 0.51 (95% CI: 0.47, 0.55), which decreased to 0.46 (95% CI: 0.42, 0.49) after adjustment for age, sex and measured values of other established vascular risk factors. The RDR did not vary materially by assay method, age, sex or smoking status, but decreased at higher levels of baseline fibrinogen.

Conclusion It is appropriate to use an RDR of 0.5 to correct approximately for regression dilution bias in plasma fibrinogen values; however, this correction factor may produce somewhat conservative hazard ratios in adjusted analyses, at higher fibrinogen concentrations and in follow-up beyond a decade. More generally, the methods described in this report have widespread applicability to quantifying regression dilution bias in repeatability data from multiple prospective studies.

Keywords Regression dilution bias, correction methods, measurement error, long-term variability, plasma fibrinogen, meta-analysis

Introduction

Many epidemiological studies aim to estimate the association between potential risk factors and the likelihood of disease. Because risk factors are usually measured with error and subject to fluctuations within individuals, analyses that use only a single measurement of the risk factor may produce biased estimates of any etiological association between average (or ‘usual’) risk factor levels and disease.1 The extent of such bias increases with the extent of (i) measurement error, (ii) short-term biological variability (including both transient fluctuations and any diurnal or seasonal variation) and (iii) longer-term within-person fluctuations (which may occur for several reasons, including physical activity, diet, treatment, disease, or age). These tend to cause associations with baseline measurements of a risk factor to underestimate the true strength of any etiological association with long-term average levels of the risk factor (i.e. ‘attenuation’ or ‘regression dilution bias’),2,3 whereas for multiple risk factors the biases may be in either direction.4 Ideally, the use of multiple measurements and optimized measurement techniques would avoid bias, but in practice some bias usually remains. It is, therefore, important to collect data on the variability in

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measured values of risk factors in order to help quantify it and correct for it.

The Fibrinogen Studies Collaboration (FSC)\(^5\) is a meta-analysis of individual data on 154 211 adults from 31 prospective studies with information on plasma fibrinogen and major disease outcomes.\(^5\) The FSC has previously reported moderately strong associations between plasma fibrinogen values and the risks of major vascular and non-vascular chronic disease outcomes, although the causal relevance of these associations remains uncertain.\(^7\) Variability in plasma fibrinogen measurements within individuals over time may be caused by several factors, notably measurement error in assay methods, transient fluctuations due to acute-phase reactions, chronic disease, ageing, changes in smoking or other habits, or medication use.\(^8\) As part of the FSC data collection, information was provided on repeat measurements of plasma fibrinogen (ie, values recorded after the initial ‘baseline examination’) from 27 247 participants in 15 of the prospective studies (Table 1). Study populations have been previously described.\(^5\) In this paper, these data are analysed to identify the determinants of fibrinogen repeatability and, more generally, to develop new methods to refine the assessment of regression dilution bias.

Various methods have been used in the literature to estimate the effect of regression dilution bias and to correct the disease association estimated from a single measurement of the risk factor.\(^1\)\(^,\)\(^9\) The present report adapts the approach of regression dilution ratios (RDRs), which is appealing due to its simplicity and its familiarity in the epidemiological literature.\(^2\)\(^,\)\(^10\)\(^–\)\(^13\) Investigation of the repeatability data in the FSC underscores the need to extend current methodology in the estimation of RDRs, including that used in the previous FSC analysis,\(^7\) in several ways. First, it might be expected that RDRs decrease with increasing time interval, reflecting increasing within-person variability. This issue has been considered previously,\(^1\)\(^,\)\(^14\) by estimating the RDR for each time interval and investigating whether these are constant or changing over time. Second, in such combined datasets, RDR estimates must be pooled across studies using some form of meta-analysis, allowing both for the precision of each RDR estimate and for heterogeneity between studies. Third, choice of an appropriate RDR may depend on the confounders used in a risk model,\(^1\)\(^5\) implying the need to investigate the importance of adjusting for covariates in the calculation of the RDR for fibrinogen. Fourth, it may be important to assess whether there are other factors that affect the variability in plasma fibrinogen, such as assay method, age, sex, and the level of fibrinogen itself.

### Methods

#### Fibrinogen data

In some contributing studies, the repeat fibrinogen measurements occurred in a narrow time interval, but in others, repeat measurements spanned a long period (Figure 1). A total of 8667 participants provided more than one repeat, and in such studies we identify each measurement as belonging to repeat 1, repeat 2, etc. according to cut-off times selected by inspection of Figure 1. Each repeat is represented by the mean time since baseline. As in previous FSC reports,\(^7\) baseline fibrinogen levels above 5.62 g/L (the highest 1% of values) are excluded due to the potential

### Table 1 Prospective studies of plasma fibrinogen and cardiovascular disease in general populations: characteristics of studies and individuals with repeat measurements

<table>
<thead>
<tr>
<th>Study</th>
<th>Assay method at baseline</th>
<th>No. of individuals with baseline plasma fibrinogen</th>
<th>Male %</th>
<th>Median baseline age years (IQR)</th>
<th>Mean baseline fibrinogen g/L (SD)</th>
<th>Number of repeats</th>
<th>No. of individuals with 2 or more repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC</td>
<td>Clotting time</td>
<td>14436</td>
<td></td>
<td>53 (49, 59)</td>
<td>3.00 (0.61)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bruneck</td>
<td>Clotting time</td>
<td>846</td>
<td></td>
<td>56 (48, 66)</td>
<td>2.59 (0.56)</td>
<td>2</td>
<td>650</td>
</tr>
<tr>
<td>Caerphilly</td>
<td>Clotting time</td>
<td>1696</td>
<td></td>
<td>52 (48, 55)</td>
<td>3.64 (0.72)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CHS original</td>
<td>Clotting time</td>
<td>3961</td>
<td></td>
<td>71 (68, 75)</td>
<td>3.14 (0.59)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Edinburgh Artery</td>
<td>Non-clot</td>
<td>1322</td>
<td></td>
<td>63 (59, 68)</td>
<td>2.61 (0.65)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>FINRISK</td>
<td>Non-clot</td>
<td>2026</td>
<td></td>
<td>53 (48, 59)</td>
<td>3.42 (0.70)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Goetheborg 1913</td>
<td>Clot weight</td>
<td>606</td>
<td></td>
<td>53 (53, 54)</td>
<td>3.22 (0.65)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Kuopio IHD Risk Factor Study</td>
<td>Clotting time</td>
<td>1927</td>
<td></td>
<td>48 (42, 54)</td>
<td>2.96 (0.54)</td>
<td>2</td>
<td>665</td>
</tr>
<tr>
<td>Northwick Park Heart 1</td>
<td>Clot weight</td>
<td>2367</td>
<td></td>
<td>41 (54)</td>
<td>2.92 (0.61)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Northwick Park Heart 2</td>
<td>Clotting time</td>
<td>2906</td>
<td></td>
<td>56 (53, 59)</td>
<td>2.75 (0.53)</td>
<td>5</td>
<td>2632</td>
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<td>PROCAM</td>
<td>Clotting time</td>
<td>9749</td>
<td></td>
<td>50 (40, 51)</td>
<td>2.61 (0.57)</td>
<td>13</td>
<td>537</td>
</tr>
<tr>
<td>Speedwell</td>
<td>Non-clot</td>
<td>2034</td>
<td></td>
<td>50 (50, 58)</td>
<td>3.51 (0.72)</td>
<td>3</td>
<td>1726</td>
</tr>
<tr>
<td>Strong Heart</td>
<td>Clotting time</td>
<td>4020</td>
<td></td>
<td>54 (49, 61)</td>
<td>2.98 (0.73)</td>
<td>2</td>
<td>2457</td>
</tr>
<tr>
<td>Thrombosis Prevention</td>
<td>Clotting time</td>
<td>22 638</td>
<td></td>
<td>50 (50, 62)</td>
<td>3.24 (0.58)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Whitehall II</td>
<td>Clotting time</td>
<td>7881</td>
<td></td>
<td>47 (53)</td>
<td>2.76 (0.58)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>78 415</td>
<td></td>
<td>54 (49, 60)</td>
<td>3.02 (0.95)</td>
<td>8667</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Clotting time = claus method, non-clot = turbidometry/nephelometry, Clot weight = Blomback method.

\(^b\)Values weighted by study size.
Regression dilution ratios

The RDR is a measure of within-person variability, that is, the extent to which an individual’s fibrinogen measurements vary around a long-term average fibrinogen level. We assume that knowledge of the long-term average fibrinogen level would completely capture the risk of disease associated with fibrinogen; this assumption permits nondifferential measurement error, so that additional information about observed fibrinogen measurements would tell us nothing more about the risk of disease. The RDR is defined as the regression coefficient of the true underlying fibrinogen level on the observed fibrinogen level. In the absence of within-person variability, these levels are equal and so the RDR is 1. Values closer to zero imply greater levels of within-person variability. A correction for regression dilution bias involves dividing the estimated disease association (e.g. the log hazard ratio and its 95% confidence limits) by the RDR.

Regression dilution correction based on RDRs is exactly correct when the regression model for disease risk is a linear regression or a Poisson regression; however, in general, it is an approximate method. The regression model for disease risk typically includes potential confounders as well as the risk factor of interest. In this case, correction by an RDR remains valid, provided that an adjusted RDR is used. This is defined as the regression coefficient of true underlying fibrinogen levels on measured fibrinogen levels adjusted for potential confounders. For simplicity, we will here assume that all confounders have fixed values, but, of course, factors such as cholesterol, blood pressure, and smoking are also measured with error. As data on repeat measurements on most confounders are available in the FSC, this information will enable extension to a multivariate approach.

Estimating regression dilution ratios

If the true underlying fibrinogen levels were known for a subsample, then the RDR could be directly estimated by regressing the true underlying fibrinogen levels on observed fibrinogen levels in this subsample. However, computing the RDR in practice usually requires one or more repeat fibrinogen measurements to be made on a subsample or on the whole sample. In this case, the RDR can be estimated by regressing a repeat fibrinogen measurement on the first. We call this the Rosner regression model. This is valid provided that the error in the second measurement is uncorrelated with error in the first measurement.

Other methods to calculate RDRs from repeat measurements have been described. If the repeats have the same error variance then the RDR may be estimated as the correlation between the first and repeat measurement or as the intra-class correlation. In MacMahon’s method, individuals are grouped according to their first fibrinogen measurement (usually into fifths), and the mean fibrinogen is calculated for each group at each repeat. The MacMahon’s RDR is the ratio of the range of means at the repeat to the range of means at the first.

Figure 1 Levels and timings of baseline and repeat fibrinogen measurements in the 15 studies. Each point represents the observed fibrinogen measurements per person-visit.
measurement. We use the Rosner method in this paper because it: (i) avoids the assumption of equal error variances, (ii) is more efficient than MacMahon’s method, and (iii) can be extended to multivariate settings.

Stratified Rosner regression model

We use the Rosner regression model to obtain an estimated RDR for each repeat measurement in each study. We explore these study- and time-specific RDRs using meta-analysis techniques to obtain a single overall RDR if appropriate. Let the kth repeat fibrinogen measurement for the jth individual in the ith study be \( Y_{ijk} \) (i = 1, . . . , n; j = 1, . . . , ni; k = 0, . . . , mi) where s is the number of studies, ni is the number of individuals in the ith study, and mi is the number of repeat measurements in the ith study. \( Y_{ijk} \) represents the baseline fibrinogen value for the jth individual in study i. The stratified Rosner regression model is

\[
Y_{ijk} = a_{ik} + b_{ik} Y_{ij0} + e_{ik}
\]  

for each study i and each repeat k > 0, where \( e_{ik} \sim N(0, \sigma_e^2) \) and \( b_{ik} \) is the study- and time-specific RDR. To obtain a single overall RDR we use the model:

\[
b_{ik} = b + v_{ik} + u_i
\]

where b is the overall RDR and \( v_{ik} \sim N(0, \Sigma_v) \) and \( u_i \sim N(0, \Sigma_u) \) allow for both within- and between-study heterogeneity, respectively. Within-study heterogeneity is measured by \( \Sigma_v: \Sigma_v = 0 \) corresponding to the true RDRs at different repeats in the same study being equal while \( \Sigma_v > 0 \) means that true RDRs at different repeats in the same study differ more than would be expected by chance. Between-study heterogeneity is measured by \( \Sigma_u: \Sigma_u = 0 \) corresponding to true RDRs in different studies being equal while \( \Sigma_u > 0 \) means that true RDRs in different studies differ more than would be expected by chance. We report the standard deviation of the true RDRs using the total heterogeneity, \( \sqrt{(\Sigma_v + \Sigma_u)} \), and the within-study correlation between true RDRs at different repeats, \( \Sigma_v/(\Sigma_v + \Sigma_u) \).

Fitting the model

It is convenient to fit this model in two stages. First, the model defined by Equation 1 is fitted separately for each i and k using standard regression software, giving estimates \( b_{ik} \) of the \( b_{ik} \) along with their standard errors \( s_{ik} \), so that we can write

\[
b_{ik} = b_{ik} + e_{ik}
\]

where \( e_{ik} \sim N(0, \sigma_e^2) \). Secondly, the three-level random effects model defined by Equations 2 and 3 are fitted; for this stage we use multilevel modelling software. The errors \( e_{ik} \) are typically correlated across repeats k for the same study i, because the same individuals are used to estimate different \( b_{ik} \). Our estimation procedure above ignores this correlation. Any correlation between the \( e_{ik} \) is likely to be taken up by the study random effect \( u_i \), with the result that the between-study heterogeneity term \( \Sigma_u \) is biased upwards and the within-study heterogeneity term \( \Sigma_v \) is biased downwards. The total heterogeneity may be little affected. For comparison, we fit the model in a one-stage estimation approach defined by Equations 1 and 2 directly, using multilevel modelling software. Inserting a random effect \( w_{ij} \) in Equation 1 correlates different observations on the same individual.

Adjusting RDRs for confounders in the risk model

Potential confounders can be added to Equation 1. For example, if the risk of coronary heart disease associated with fibrinogen is adjusted for baseline smoking, then a smoking-adjusted RDR should be used to correct it. Adjusted RDRs estimated from Equation 1 are combined in the same way as unadjusted RDRs.

Time trends in RDRs

It is possible that earlier fibrinogen measurements are more associated with the baseline measurement than are measurements taken many years later. We estimate the time trend in the RDRs across repeats by including a time effect in Equation 2:

\[
b_{ik} = b + d_{i1} \text{Assay}_{i1} + d_{i2} \text{Assay}_{i2} + v_{ik} + u_i
\]

where \( f_{ik} \) represents the mean time at which the kth repeat was made in the ith study. The model defined by Equations 2a and 3 is fitted using multilevel modelling software. For comparison, the one-stage estimation approach is similarly adapted to include a time trend in the model defined by Equation 1. This approach can use the actual times of measurements instead of assuming a common measurement time for all observations at the same repeat in the same study.

Predictors of variability

Some factors may increase or decrease the variability in fibrinogen concentrations and thus affect the RDR. How this possibility is explored depends on whether the factor is defined at study level (such as assay method in the FSC) or at individual level (such as smoking habits). For example, assay method can be entered in Equation 2:

\[
b_{ik} = b + d_{i1} \text{Assay}_{i1} + d_{i2} \text{Assay}_{i2} + v_{ik} + u_i
\]

where \( \text{Assay}_{i1} \) and \( \text{Assay}_{i2} \) are dummy variables for the assay type in study i (the third assay type being the reference category—see Table 1). The model, defined by Equations 2b and 3, is again fitted using multilevel modelling software and significance assessed using likelihood ratio tests. Potential predictors of variability at the individual level, such as smoking habits, cannot be assessed using the estimated \( b_{ik} \). Instead, an interaction term between the predictor such as smoking and baseline fibrinogen is added to Equation 1:

\[
Y_{ijk} = a_{ik} + b_{ik} Y_{ij0} + c_{ik} \text{Smoking}_i + d_{ik} Y_{ij0} \text{Smoking}_i + e_{ik}
\]

The significance of the interaction term is assessed by fitting the model defined by Equations 2 and 3 with \( b_{ik} \) replaced by \( d_{ik} \). Within-person variability of many biochemical factors increases as the true level of the risk factor increases. If this were the case for fibrinogen, the RDR would decrease as the baseline fibrinogen level increases. To explore this possibility, we replace smoking with baseline fibrinogen in Equation 1a to give a quadratic term.

Results

Repeat measurements

A total of 27 247 out of 78 415 individuals (35%) in 15 studies had one or more repeat measurements (Table 1). The
participants with repeat measurements were not formally random samples from each cohort, although in general they were selected with the intention of being fairly representative (Supplementary table 1). Baseline characteristics are summarized in Table 1. Individuals in the 15 studies, which had repeat fibrinogen measurements, were generally older and more likely to be male than individuals in the studies without repeat fibrinogen measures (results available on request). Individuals with repeat measurements generally had lower baseline fibrinogen measures, were younger, and somewhat more likely to be women and non-smokers than individuals in the same studies without repeats (results available on request). A total of 45,806 repeat measurements were available (Figure 1, Supplementary table 2) derived from 36 different repeat examinations in the 15 studies. Repeat plasma fibrinogen measures generally had higher variances than baseline measures (Supplementary table 2).

**Study- and time-specific RDRs**

The estimated 36 study- and time-specific RDRs ranged from 0.20 to 0.68 (Figure 2). An overall RDR, combined across all studies and time intervals, was 0.51 (95% CI: 0.47, 0.55). The total heterogeneity between RDRs had a standard deviation of 0.091 ($P < 0.001$) with estimated within-study correlation of 0.25. The one-stage estimation approach gave similar results. When all heterogeneity was ignored the overall unadjusted RDR was 0.50 (95% CI: 0.49, 0.51). Table 2 shows that the overall RDR decreased modestly to 0.46 (95% CI: 0.42, 0.49) with increasing adjustment for several possible confounders.

**Time trends in RDRs**

The overall RDRs decreased by about 0.05 (95% CI: 0.02, 0.09; $P = 0.01$) per 5 years between repeat measures. From the fitted values, the mean overall RDR was 0.57 at 1 year, declining to 0.48 at 10 years and 0.37 at 20 years. After adjusting for the trend, the standard deviation of the total heterogeneity between RDRs was little changed at 0.089, but the within-study correlation increased to 0.49. A similar time trend was estimated from the one-stage approach, which used actual measurement times. The RDRs estimated after 5 years of follow-up were dominated by the PROCAM study, which had lower estimated RDRs than most other studies, and by the Göteborg study, which had a relatively high estimated RDR (Figure 2). Excluding the PROCAM study, the decline over time in the overall RDR shallowed to 0.02 per 5 years (95% CI: −0.01, 0.06); excluding the Göteborg study, the decline over time in the overall RDR steepened to 0.07 per 5 years (95% CI: 0.03, 0.12). The overall RDR obtained from combining across all studies and repeats made within the first 10 years was 0.52 (95% CI: 0.48, 0.55); there was no evidence of a decrease in the RDR over this time period ($P = 0.25$).

**Predictors of variability**

There was no good evidence that the variability in fibrinogen levels was affected by the assay method used, sex, baseline smoking habit, baseline age (Table 3) or assay timing, study population, geographical location, body mass index, or levels of cholesterol and triglycerides (Supplementary table 3). There was, however, evidence that RDRs decreased at higher baseline...
Fibrinogenc (per 1 g/l increase)
Age (per 20 year
Sex Male 0.50 (0.47, 0.54) 0.50 b
Age, sex, smoking status, systolic blood
pressure, body mass index, and
total cholesterol
Age, sex, smoking status, systolic blood
pressure, body mass index, a alcohol
consumption, history of diabetes,
high-density lipoprotein, low-density
lipoprotein, triglycerides
a Excludes data from Go¨teborg 1913 study due to different assay methods
b Calculated among the smaller sample of 10 928 individuals with 15 538
repeats from 7 studies which collected complete information on age, sex,
smoking status, cholesterol (total, high density lipoprotein, and low density
lipoprotein), systolic blood pressure and body mass index, alcohol
consumption, history of diabetes, triglycerides (RDRs, unadjusted and
adjusted for fewer confounders, were similar to those shown for full data).

Discussion
The present report involves individual data on repeat fibrino-
gen measurement from over 27 000 participants in 15
prospective studies, providing the most comprehensive and
detailed assessment so far of the determinants of long-term
variability in plasma fibrinogen. The main finding is that there
is an overall regression dilution ratio of around 0.5 that is
robust to different model assumptions. This value is similar to
that derived by simpler methods for the main FSC analyses.7
Fibrinogen variability does not vary materially by the assay
method used, age, sex, or smoking status, although it increases
at higher baseline fibrinogen values. In the course of
conducting these analyses, existing methodology on the
assessment of variability in risk factors in longitudinal studies
has been extended, particularly for use in the setting of
individual participant meta-analyses.

Time trends in RDRs
The present data suggest a decline in RDRs for plasma
fibrinogen over time, from 0.57 at 1 year to 0.37 after
20 years. This suggestion should, however, be interpreted
cautiously because it is highly dependent on the results of the
PROCAM study. The apparent time trends are nearly abolished
when the PROCAM study is excluded from the analyses, and it
may be appropriate to do so given the comparatively high
variability also noted in this study for levels of total cholesterol
over prolonged periods,11,13 (analysis available on request).

Corrections for regression dilution bias require stronger
assumptions when RDRs vary substantially over time,16 as for
blood pressure where the RDR declines from two-third in the
first decade to one-third in the third decade.14 Given the
available data, however, it is not possible to assess reliably
fibrinogen variability beyond the first decade of follow-up.

Adjustment for covariates
The estimated RDRs for fibrinogen decreased modestly when
confounders were added into the regression dilution model
(Table 3). This is likely to reflect a general finding for
epidemiological risk markers, because any characteristic cross-
sectionally associated with a risk factor is also likely to be
 prospectively associated with future levels of that risk factor.
Using an unadjusted RDR to estimate the adjusted association
of underlying fibrinogen level with disease risk will underes-
timate the corrected association by up to ~10%. This finding
suggests that use of unadjusted RDRs for correcting relative risk
associations may generally be conservative.

<table>
<thead>
<tr>
<th>Table 2 Overall regression dilution ratios for plasma fibrinogen adjusted progressively for possible confounding factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline characteristics</td>
</tr>
<tr>
<td>Assay methodc Non-clot</td>
</tr>
<tr>
<td>Clotting time</td>
</tr>
<tr>
<td>Clot weight</td>
</tr>
<tr>
<td>Sex Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Smoking status Non or former</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Age (per 20 year increase)</td>
</tr>
<tr>
<td>Lower third (25–50 years)</td>
</tr>
<tr>
<td>Middle third (51–59 years)</td>
</tr>
<tr>
<td>Upper third (60–89 years)</td>
</tr>
<tr>
<td>Fibrinogenc (per 1 g/l increase)</td>
</tr>
<tr>
<td>Lower third (~&lt;2.7 g/l)</td>
</tr>
<tr>
<td>Middle third (~2.7–3.25 g/l)</td>
</tr>
<tr>
<td>Upper third (~&gt;3.25 g/l)</td>
</tr>
</tbody>
</table>

a Excludes data from Göteborg 1913 study due to different assay methods used at baseline and follow-up.
b Comparison made between 20 240 participants with 14 848 repeats from 9 mixed gender studies.
c Tertiles formed on baseline fibrinogen levels.

fibrinogen levels (Table 3). Conversely, there was some limited
evidence that RDRs increased in individuals with a known
history of diabetes or with higher systolic blood pressure levels
(Supplementary table 3). These findings were little changed
when data were restricted to the first ten years of follow-up or
after adjustment for confounders. The RDR increased only by
0.01 over the inter-quartile range of systolic blood pressure levels,
but the decline in the RDR was more substantial among those in
the upper third of fibrinogen levels (Table 3). This non-linear
relationship between baseline fibrinogen and repeat measure-
ments was removed on log-transformation of all fibrinogen
measures. The overall RDR for log fibrinogen was 0.49 (95% CI:
0.45, 0.53), the standard deviation of the total heterogeneity
was 0.088 and the within-study correlation was 0.20.

<table>
<thead>
<tr>
<th>Table 3 Crude overall regression dilution ratios for fibrinogen according to potentially important baseline predictors of fibrinogen variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline characteristics</td>
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<tr>
<td>Assay methodc Non-clot</td>
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<tr>
<td>Sex Male</td>
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<td>Female</td>
</tr>
<tr>
<td>Smoking status Non or former</td>
</tr>
<tr>
<td>Current</td>
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<tr>
<td>Age (per 20 year increase)</td>
</tr>
<tr>
<td>Lower third (25–50 years)</td>
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<tr>
<td>Middle third (51–59 years)</td>
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<tr>
<td>Upper third (60–89 years)</td>
</tr>
<tr>
<td>Fibrinogenc (per 1 g/l increase)</td>
</tr>
<tr>
<td>Lower third (~&lt;2.7 g/l)</td>
</tr>
<tr>
<td>Middle third (~2.7–3.25 g/l)</td>
</tr>
<tr>
<td>Upper third (~&gt;3.25 g/l)</td>
</tr>
</tbody>
</table>
Predictors of variability

Fibrinogen variability increased at higher baseline fibrinogen values, maybe in part due to acute-phase reactions, suggesting that there is a non-linear relationship between repeats of fibrinogen levels (in which case, simple RDR corrections may not be optimal). RDR corrections in linear regression are valid without any assumption of linearity between repeat measures, but similar results hold only approximately for non-linear regression. As increasing fibrinogen levels are continuously associated with the risks of coronary (and other) outcomes and as the RDR for fibrinogen is lower at higher fibrinogen levels, use of an overall RDR may produce an underestimate of the true relative risk. The non-linear relationship between repeats of fibrinogen levels may also result in misleading conclusions about the shape of the underlying relationship between fibrinogen and disease risk. For example, if the true relationship is linear, then the observed relationship will appear weaker at higher risk factor levels. Regression calibration can allow for the non-linear relationship between repeats of fibrinogen measures. A suitable non-linear regression model would be selected and used to predict the true underlying fibrinogen level from the baseline fibrinogen level (and other predictors of variability and confounders, if in the disease model). A log transformation is also a useful tool for any variable whose variability increases with its level by removing the non-linear relationship, as in the present data. The RDR for log-transformed fibrinogen would only be appropriate for regression dilution correction if fibrinogen is also log transformed in the risk regression model.

Individuals with a reported history of diabetes appear to have a somewhat decreased degree of fibrinogen variability, despite their generally higher fibrinogen levels. Further studies are required as this finding was not very convincing \( (P = 0.05) \) and observed in the context of multiple testing (Supplementary table 3). Time-dependent factors such as season may also affect levels of fibrinogen and, potentially, its variability (e.g. it is known, and can also be shown from the present data, that fibrinogen levels are higher in winter months than in summer months). Hence, the impact of seasonal trend was estimated from the data and subtracted from the repeat measurements, but this correction made little difference to the findings (results available upon request).

Using RDRs in meta-analyses

A common approach to meta-analysis of disease risk associations is to estimate a single RDR from one study and apply it to all studies. Such approaches are preferable to failure to attempt any correction for regression dilution bias, but may oversimplify if RDRs differ considerably between studies either because (i) within-person variability differs considerably or (ii) between-person variability differs considerably. Ideally, a reliable RDR would be available for each study, but when RDRs are available in only a subset of studies, as in the FSC, it is necessary to find ways to transfer the RDRs to the other studies. When considerable heterogeneity exists, an overall RDR may not be an appropriate summary, and careful account must be taken of any factors associated with the heterogeneity. If the heterogeneity can be explained by covariates (predictors of variability), then RDRs for studies without repeat measurements can be applied from studies with similar measurements. If the heterogeneity cannot be explained by covariates, then the overall RDR may be appropriate for the studies without repeats measurements as a main analysis. However, a sensitivity analysis should use other RDRs drawn to reflect the heterogeneity, such as Empirical Bayes estimates for studies with repeat measurements. Once RDRs have been identified for all studies, an overall estimate of the association of risk with true underlying fibrinogen levels can be obtained by first correcting the study-specific measures of association for regression dilution, and then using standard meta-analysis procedures on the corrected measures of association.

Limitations of RDR corrections

RDR methods of correction for measurement error make several critical assumptions. Firstly, they assume that confounders are perfectly measured. Otherwise, the exposure-disease association will be subject to residual confounding. An extension to the multivariate Rosner regression model allows correction for multiple error-prone risk factors. Extension of this approach to assess study heterogeneity and time trends is a topic for future research. Our initial results show that there are no dramatic changes in the hazard ratios for coronary heart disease with fibrinogen levels after making such multivariate corrections, the confounding effects being not particularly strong.

Secondly, RDR correction methods assume that disease risk depends on a single underlying long-term exposure. In a more realistic model with time-dependent true underlying exposure, RDR correction methods are valid if disease risk depends only on current true underlying exposure or if RDRs are constant over the life course, but otherwise they typically overcorrect. If, for example, the risk of disease depends on the temporal rate of change in the exposure, then an RDR correction would be invalid: life course methods would be more appropriate, although they have greater data requirements.

Thirdly, the observed exposure-disease association may reflect unmeasured confounding rather than a causal association. RDR corrections amplify such an association: this may correctly estimate the non-causal association between disease and true underlying exposure levels, but may not be a useful quantity. Strategies such as Mendelian randomization can reduce confounding due to unmeasured confounders.

Conclusions

A correction factor of 0.5 is approximately appropriate to adjust associations between fibrinogen levels and disease risk for within-person variability, at least within the first decade of follow-up, based on repeat fibrinogen measurements on 27 247 adults in 15 prospective studies. The present data demonstrate that adjusting for confounders reduces the RDR and highlight the usefulness of investigating possible predictors of variability, including an assessment of a non-linear relationship between repeats of the risk factor and exploration of heterogeneity over time and across studies.
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KEY MESSAGES

- We provide the most comprehensive and detailed assessment so far of the extent and determinants of long-term variability in plasma fibrinogen.
- Based on repeat measurement data from 27,247 adults in 15 prospective studies, the overall regression dilution ratio was 0.51 (95% CI 0.47–0.55).
- The long-term variability of fibrinogen did not vary materially with assay method used, age, sex, or smoking habit, but was greater at higher levels of baseline fibrinogen.
- The novel methods presented can be used to quantify regression dilution bias in repeatability data from multiple prospective studies.

References