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## 37 Influence of fieldwork conditions and sample quality on DBS values

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- ▶ Fieldwork conditions and sample quality during blood collection significantly influence DBS values
  - ▶ Small spot size is the main challenge; in particular, TC, CysC and HbA1c are sensitive to small spot size
  - ▶ In turn, tHb, TG and CRP are relatively robust regarding fieldwork conditions and sample quality
  - ▶ Conversion formulae between DBS and the medical gold standard can be significantly improved when they include fieldwork conditions and sample quality
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### 37.1 Introduction

The collection of dried blood spot (DBS) samples is an innovative method aimed at collecting objective health data. DBS samples are increasingly used in large-scale surveys. However, because the samples are collected during regular fieldwork, they are exposed to varying field conditions that might influence the obtained sample quality.

In SHARE Wave 6, we collected DBS samples from approximately 27,000 respondents during face-to-face interviews at respondents' homes. A randomly drawn first batch of approximately 8,000 samples was analysed for up to seven biomarkers. They include high density lipoprotein (HDL), total haemoglobin (tHb), glycated haemoglobin (HbA1c), total cholesterol (TC), triglycerides (TG), C-reactive protein (CRP) and Cystatin C (CysC). The number and types of analysed markers for each single DBS sample depend on the amount of available blood material. We call the obtained analysis results 'raw' DBS values and obviously do not know the corresponding 'gold standard' values taken from venous blood.

This chapter explores the systematic associations of raw DBS values with a set of fieldwork conditions and quality measures. Our main result is that some of these associations are statistically significant and substantially large. Moreover, these associations cannot be measured in isolation because they interact with each other, such as short drying time and the lack of humidity protection. Therefore, understanding DBS results requires understanding the fieldwork process.

## 37.2 Fieldwork conditions and sample quality

Five dimensions of fieldwork conditions and sample quality have been measured or estimated during or after the fieldwork. To break down the complexity of the possible influences, they are coded as dummy variables.

**Outside temperature:** High temperatures could affect the integrity of the biomarker molecules that might, as a consequence, deteriorate more rapidly. The deterioration products might not be detected by the laboratory assay, leading to a spuriously decreased blood level result.

Interviewers were asked to estimate the actual outside temperature during the interview. A temperature variable for 'high outside temperature' was created that took the value of 1 if the estimated outside temperature reached 30°C or hotter and 0 otherwise (as long as any estimation was given).

**Spot size:** The diameter of a regular dried blood spot is approximately 1 cm. Sometimes, smaller spots occur for a variety of reasons, which might lead to a deviant distribution of the marker molecules inside the analysed material, resulting in spuriously increased analysis values.

The size of the analysed blood spot is estimated on the basis of the following information. For HbA1c, we know whether it has been analysed from an otherwise unsuitable spot (which is usually 'unsuitable' because of its small size) or from a regular spot that, in general, should be larger. For TC, TG, CRP and CysC, we know whether from the entire sample only this set of markers (the so-called 'B-markers') has been analysed (indicating that only one punch was possible at all and, thus, the used spot must be very small) or whether further markers have been analysed (and the used spot was probably larger). This information was used to create a binary variable for 'small spot size' with a value of 1 for small spots and 0 for larger ones. No spot size information is available to date for tHb and HDL ('A-markers' other than HbA1c).

**Drying time:** The main threat of insufficient drying time is that the blood on the filter paper might still be wet during shipment. Humidity can lead to the deterioration of biomarker molecules.

At the end of the computer-assisted interview, the interviewer was asked via a programmed item to prepare the DBS sample for shipment. The time from the end of the DBSS specific module to the end of the interview was calculated using key-stroke analysis, and the resulting time was used as estimated drying time (in minutes). This estimate was approximate because the interviewers might have ignored the request and might have packed the sample at an earlier or later point in time, leading to shorter or longer 'true' drying times. The dummy variable for 'short drying time' is 1 if the estimated drying time is shorter than 6 minutes. This value is very conservative; as far as we know, optimal drying times would be much longer.

**Shipment time:** Biomarker molecules might continue to deteriorate during shipment unless they are frozen (at delivery). Short shipment times, hence, are preferable.

The shipment time is the time between the date of the interview (which equals the date of the blood collection) and the date of the DBS sample arriving at the biobank. A variable for 'long shipment time' takes the value of 1 for a DBS sample arriving at the biobank more than six days after the blood collection and the value of 0 for shorter durations.

**Humidity protection:** As previously described, humidity might lead to biomarker molecule deterioration. Therefore, all DBS samples should be protected from ambient humidity during shipment.

Interviewers were trained on how to protect the DBS samples against humidity during shipment. Namely, each sample should be put into a polyethylene (PE) bag together with a desiccant. The PE bag had to be closed tightly (via a ziplock system). These humidity protection measures have been evaluated for each sample at its arrival at the biobank (bag available and closed; desiccant available inside the bag). A variable for 'insufficient humidity protection' takes the value of 0 only if all described measures were taken, and 1 otherwise.

We also hypothesize that these fieldwork conditions interact, that is, the presence of one condition affects the strength of another condition:

- *Drying time X humidity protection:* Insufficient humidity protection might have a stronger impact on an incompletely dried sample.
- *Humidity protection X shipment time:* Biomarker molecules might deteriorate faster at high humidity levels. Hence, longer shipment times have a stronger impact on badly protected samples.
- *Outside temperature X shipment time:* Biomarker molecules might deteriorate faster at a high temperature. Hence, longer shipment times have a stronger impact when the outside temperature is high.
- *Outside temperature X drying time:* Samples might dry faster when the outside temperature is high. Hence, shorter drying times might have a weaker impact in this case.

## 37.3 Respondent characteristics

Analysis results from DBS will depend on respondents' health and other characteristics. For example, respondents with a high body mass index are more likely to have high levels of HbA1c. To control for this variation and to better isolate the effects of field conditions and sample quality, all associations will

be conditioned on age (standardized), body mass index score (BMI score; standardized), sex, smoking (yes or no), activity level (low, middle, high), education level (low, middle, high; according to ISCED 1997) and a set of self-reported conditions or diseases possibly related to the biomarkers: heart attack, high blood pressure, high cholesterol level, stroke, diabetes mellitus, rheumatoid arthritis, osteoarthritis and kidney disease.

## 37.4 Interviewer effects

Some field conditions and sample quality measures, such as drying time and humidity protection status, probably depend on the reliability and the care of our interviewers. Other conditions, such as shipment time and some biomarker levels themselves, are possibly clustered in regions. To account for unobserved heterogeneity at the interviewer level, we include interviewer fixed effects in all associations. Because respondents and interviewers are clustered on a regional basis, this also accounts for regional effects. As an example, regarding high density lipoprotein (HDL), the intraclass correlation for clustering in interviewers is only 0.14, but some coefficients and significance levels vary when calculating the same models with or without interviewer fixed effects.

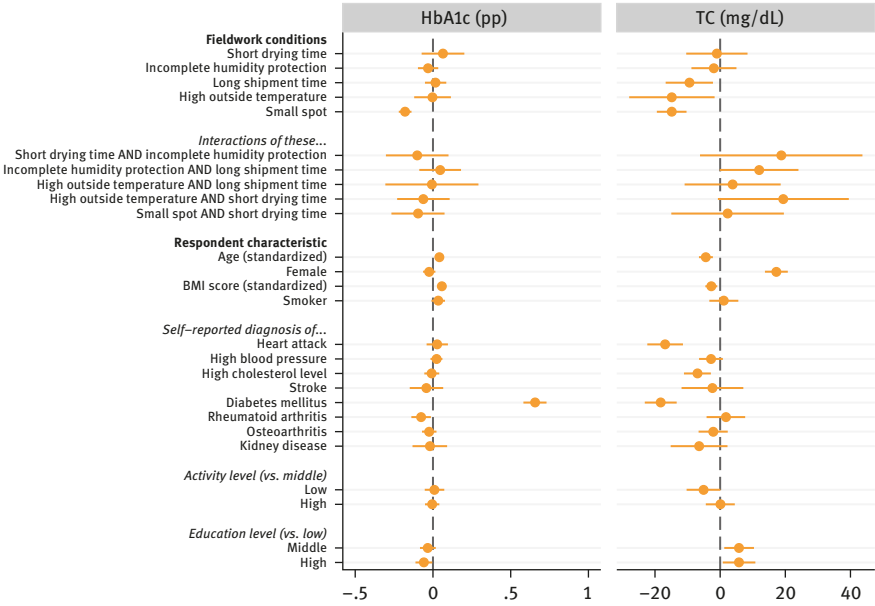
## 37.5 Results

We express our results for the seven SHARE biomarkers as coefficient plots. A dot at the left of the dashed line means a lower than expected DBS raw value; a dot to the right means the opposite. Whiskers represent 95 per cent confidence intervals. The biomarkers are described in groups of markers with similar patterns regarding their associations with field conditions and/or their interactions.

Figure 37.1 shows that HbA1c and TC are sensitive to some field conditions, but no significant associations exist with their interactions – at least not at the 5 per cent level. HbA1c is sensitive to small blood spot sizes; TC is sensitive to long shipment times, high outside temperatures or small spot size.

Regarding respondent characteristics, the HbA1c level is high in diabetics, as was to be expected. Furthermore, this level increases with age and BMI, is lower with rheumatoid arthritis and slightly lower in people with a high education level.

The TC level is associated with age and sex, as expected. A lower TC level is observed with higher BMI (driven by lower HDL levels). TC is also lower in

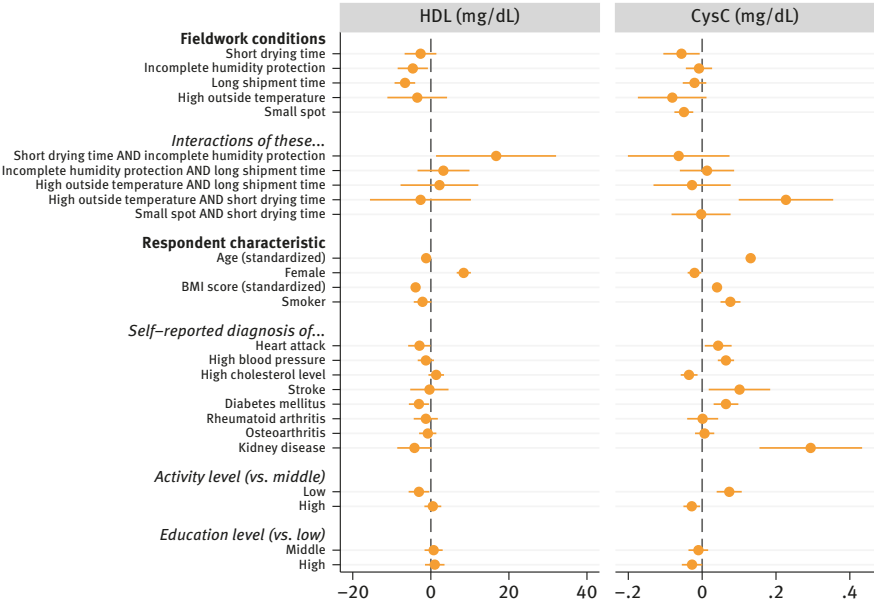


**Figure 37.1:** Assay results associated with field conditions.

**Note:** Mean (and median) levels: HbA1c 5.6 (5.5) %, TC 374 (370) mg/dL.

people who reported the diagnosis of a heart attack, diabetes mellitus or high cholesterol. Higher levels would have been expected in these patients. After the diagnosis, these people might be more likely to actively lower their cholesterol levels. Higher TC levels are observed in people with a middle and high level of education.

As illustrated in Figure 37.2, the analysis results of HDL and CysC not only show associations with single field conditions (incomplete humidity protection and long shipment times for HDL, short drying times and small spot sizes for CysC) – these biomarkers are also sensitive to interactions of these conditions: a short drying time, for example, seems to not be a problem for HDL as long as the samples are well protected against humidity during shipment. However, if this protection is insufficient, a short drying time increases the raw DBS value. Similarly, the measured level of CysC is lower after short drying times and in small blood spots. High outside temperatures do not show a significant effect as long as the used blood spot is large. However, for small spots, high outside temperatures have a significant effect on CysC (in the opposite direction as a small spot at lower temperatures). Note that no information is available on the size of the blood spots used for HDL analysis.



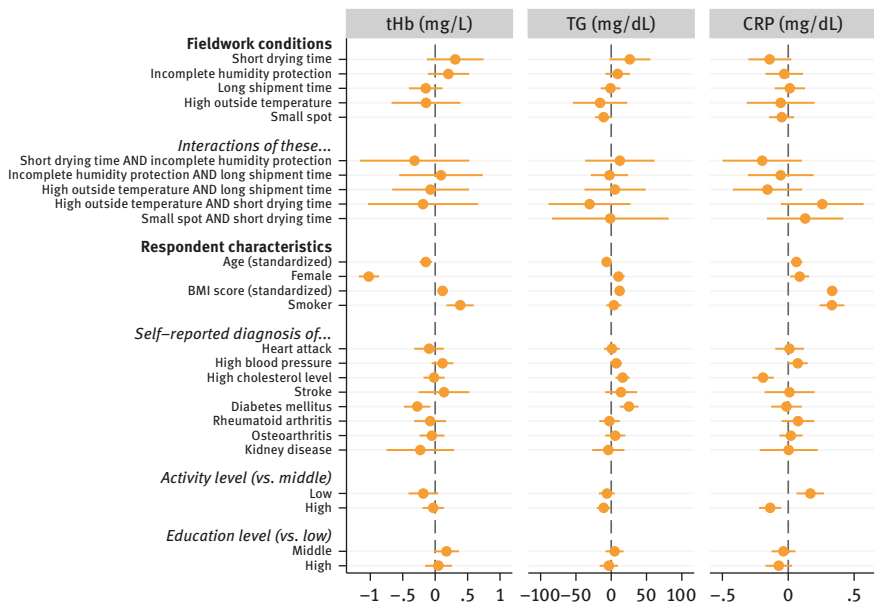
**Figure 37.2:** Assay results associated with interactions of field conditions.  
**Note:** Mean (and median) levels: HDL 107 (105) mg/dL, CysC 1.17 (1.11) mg/L.

Regarding respondent characteristics, the plots show expected associations: high BMI scores, diabetes and low activity levels are accompanied by lower HDL levels. Females generally show higher HDL levels.

The CysC level increases with age, BMI and less physical activity. CysC is lower in women and higher in smokers, and is negatively associated with the reported diagnosis of high cholesterol and positively with the reported diagnosis of a heart attack, high blood pressure, stroke or diabetes mellitus. Not surprisingly, CysC is also highly associated with reports of kidney disease.

Some markers do not show any significant sensitivity to fieldwork conditions, namely, tHb, TG and CRP (see Figure 37.3). Note that information on spot size is not available for tHb.

Women show remarkably lower tHb levels than men. Higher levels are associated with higher BMI scores and smoking. The TG level is associated with age, sex, BMI score and a reported diagnosis of high cholesterol or diabetes mellitus. TG is lowered in highly active people. CRP increases with age and BMI, and is higher in women and smokers. CRP decreases with a higher activity level and is lower with a reported diagnosis of high cholesterol.



**Figure 37.3:** Assay results showing no significant interactions with field conditions.  
**Note:** Mean (and median) levels: tHb 14.0 (13.9) mg/L, TG 263 (234) mg/dL, CRP 1.38 (1.0) mg/L.

## 37.6 Conclusions

Fieldwork conditions during blood collection and sample quality measures influence the raw DBS value obtained through laboratory analysis. Many of the effects are statistically significant, matter in terms of substance and interact with each other. Some biomarkers analysed for SHARE show sensitivity to a variety of fieldwork conditions, HDL and CysC additionally to interactions of these. Small spot size is the main challenge; TC, CysC and HbA1c are sensitive to small spots. Short drying times seem to affect CysC and HDL values, the latter only with high outside temperatures. Long shipment times are problematic for HDL and TC. High outside temperatures might affect the DBS values of TC and CysC, the latter only after a very short drying time. Some markers seem relatively robust regarding fieldwork conditions and sample quality, namely, tHb, TG and CP. Overall, the results presented in this chapter show that conversion formulae can be significantly improved when they include fieldwork conditions and sample quality.

