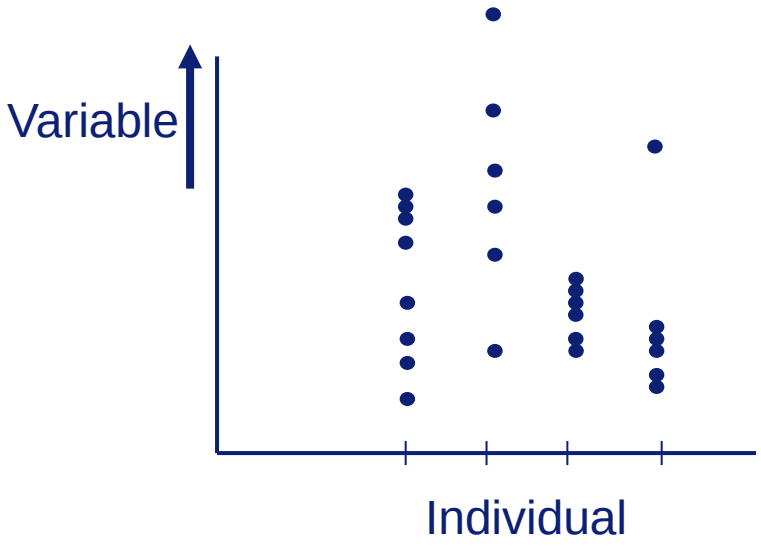
Biological variation of inflammatory and hemostatic markers

Longitudinal study in healthy subjects

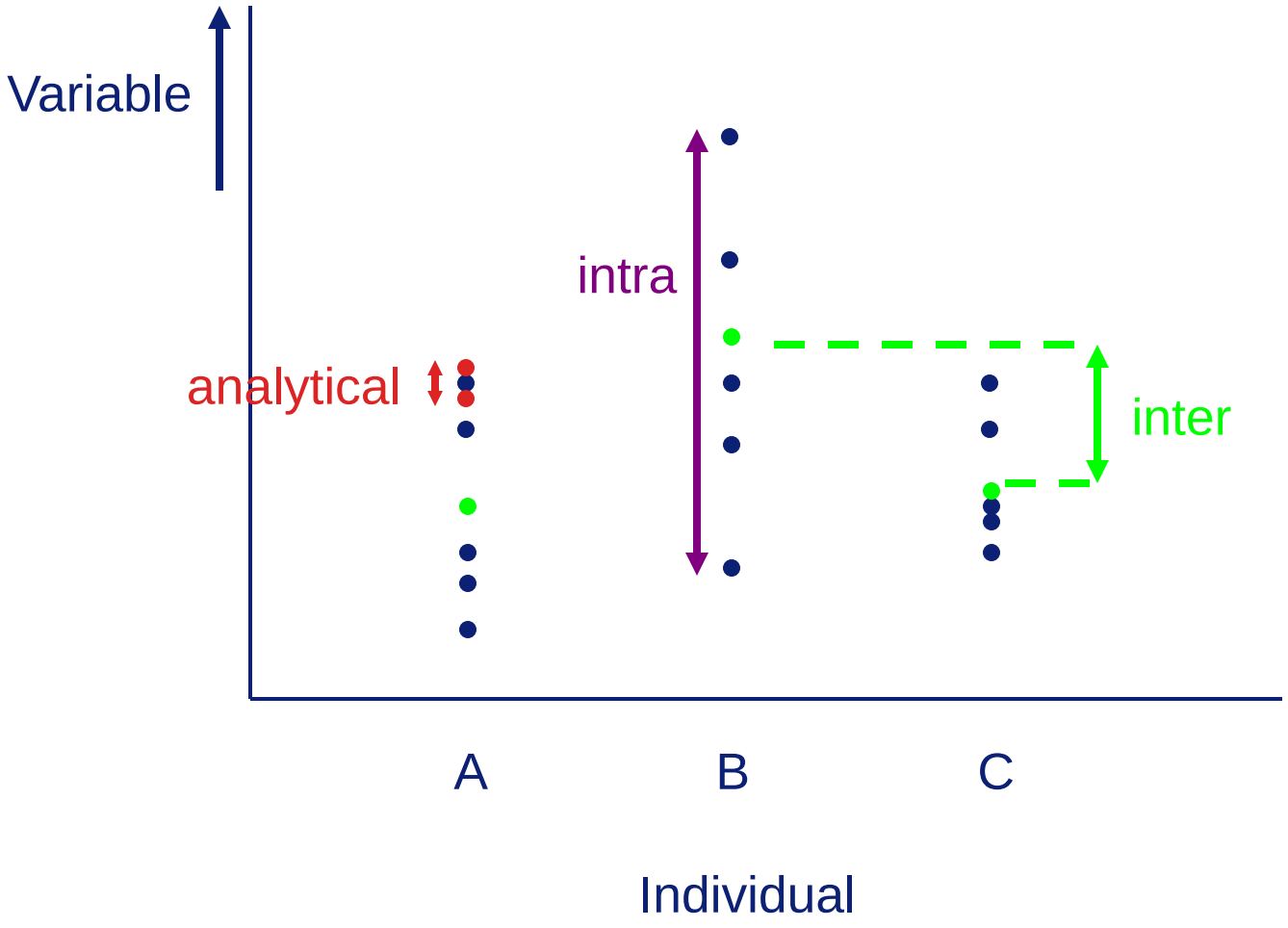
November 7, 2008
ECAT Participant meeting

Introduction

Biological variables can vary within individuals over time



Sources of variation



Inflammatory and thrombophilia markers

- Often only 1 blood sample is taken in studies – is this representative of the habitual level?
- Analytical quality specifications
  - Discriminative in a diagnostic setting, but also in cohort-studies?
- Is there a need to take seasonal variation into account?
  - Cardiovascular mortality varies over seasons

Research questions

1. Is there a need for multiple measurements of these variables over time?
2. What is the maximal recommended analytical variation of the corresponding assays?
3. Are the studied variables characterized by seasonal variation?
4. What is the effect of air pollution on the studied variables?

Study design

- 40 healthy subjects living or working in Rotterdam region
- 15 blood samples were taken during 1-year from each participant (between January 2005 and December 2006)

- A total of 520 samples collected on 197 different days

Standardization

- Pre-analytical
  - Blood collection while sitting and resting
  - Right antecubital vein
  - Participants were allowed to have a light breakfast
  - Medical questionnaire (smoking, medication, common influenza, etc.)
- Analytical
  - From each participant all samples were assayed in 1 run
- Circadian variation
  - Samples were collected between 9 – 11 AM

Inflammatory and thrombophilia markers

Inflammatory markers

- Fibrinogen
- CRP

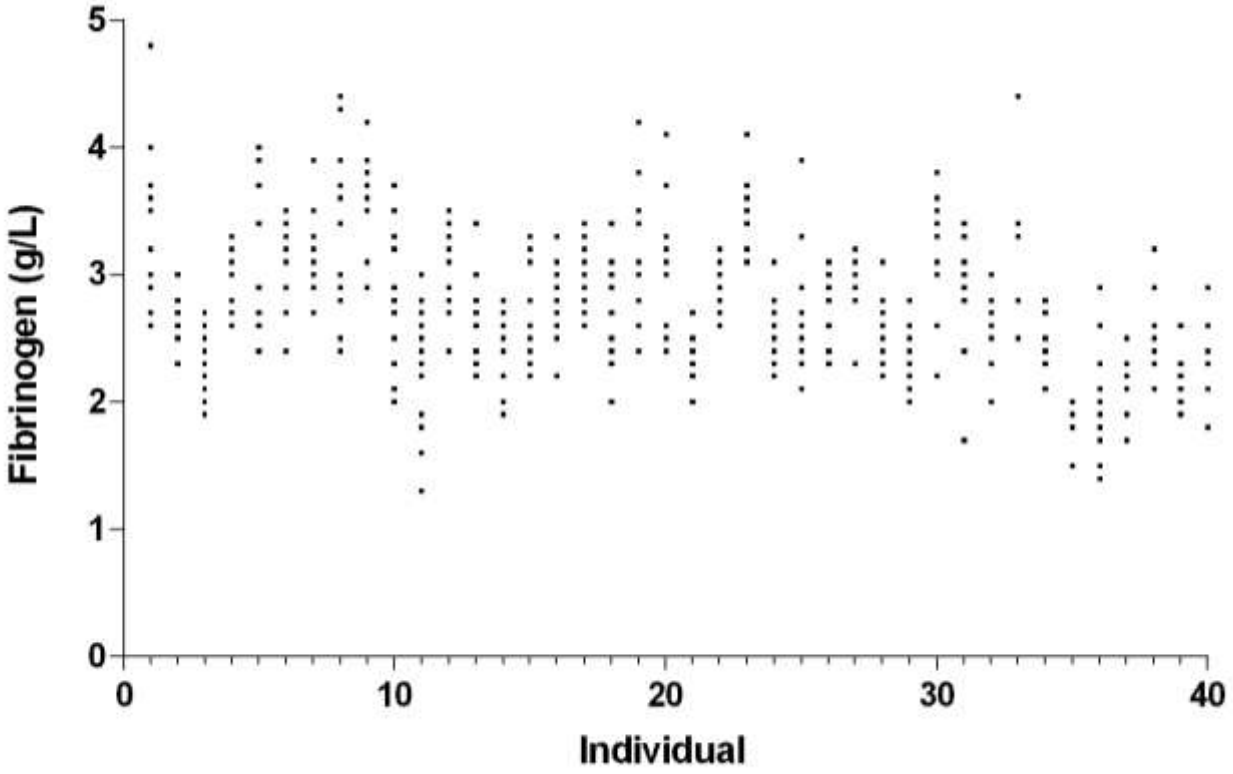
Thrombophilia markers

- Prothrombin time
- Thrombin generation
- Antithrombin
- Protein C
- Fibrinogen

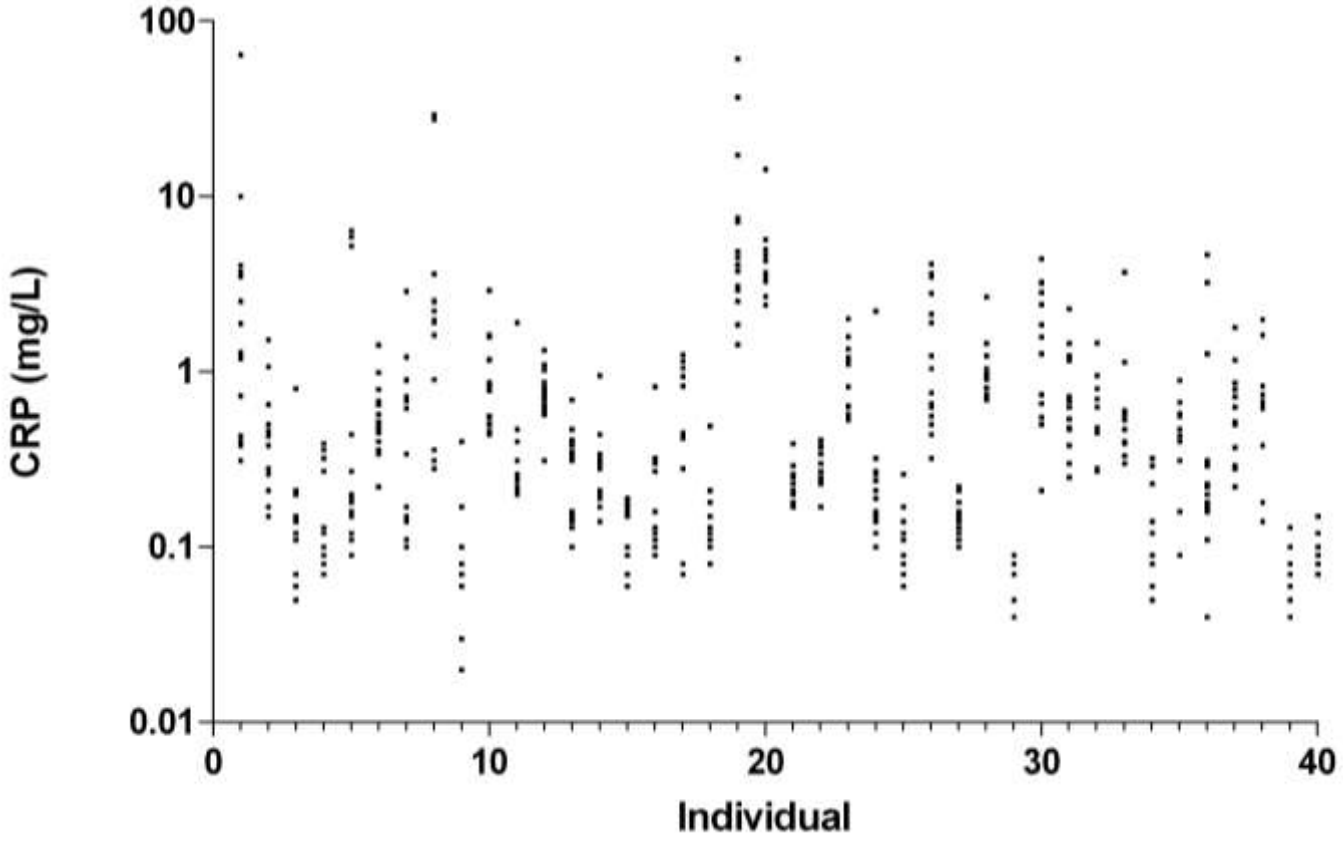
Characteristics of the study population

Variable	Study population (n=40)
Age (years)	41 ± 15
Females	26 (65%)
BMI (kg/m^2)	22.6 ± 2.0
Smokers	7 (18%)
Oral contraceptives	9 (23%)

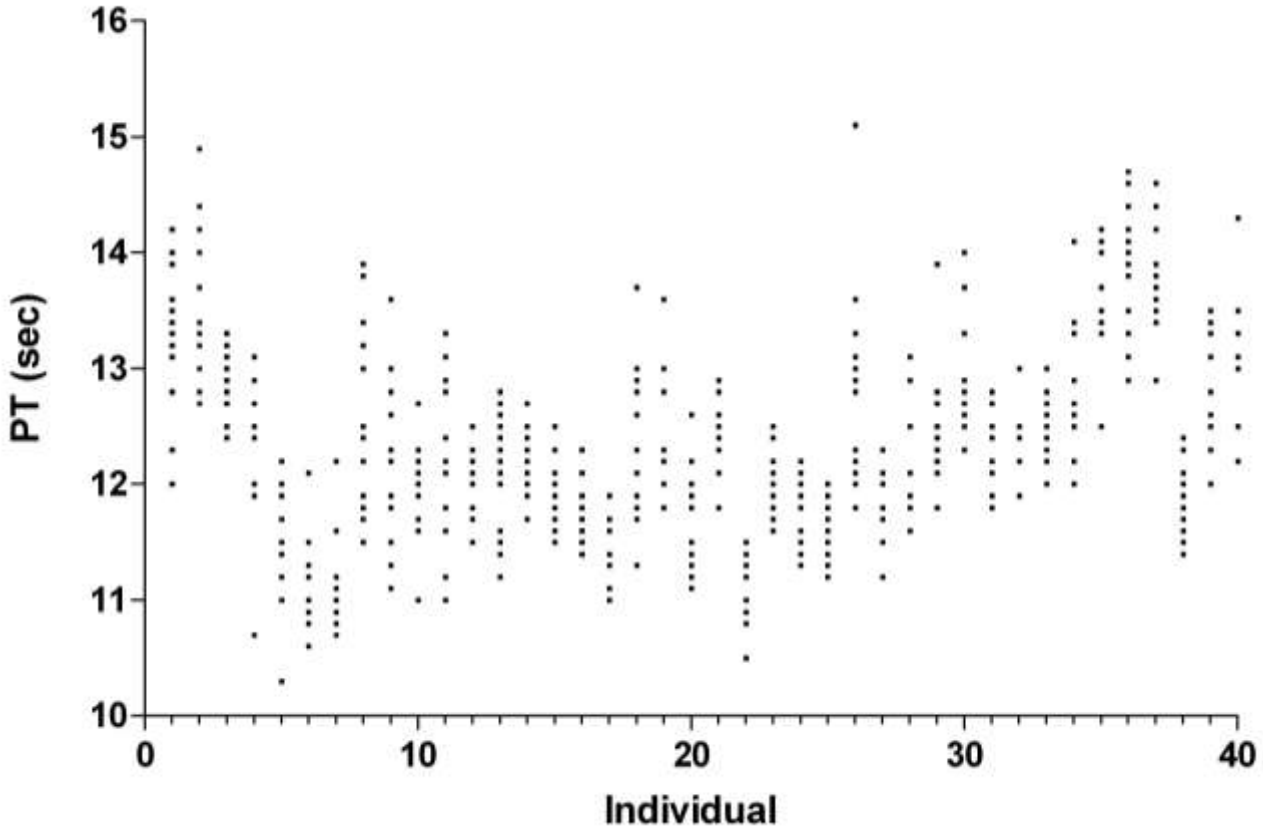
Biological variation of fibrinogen



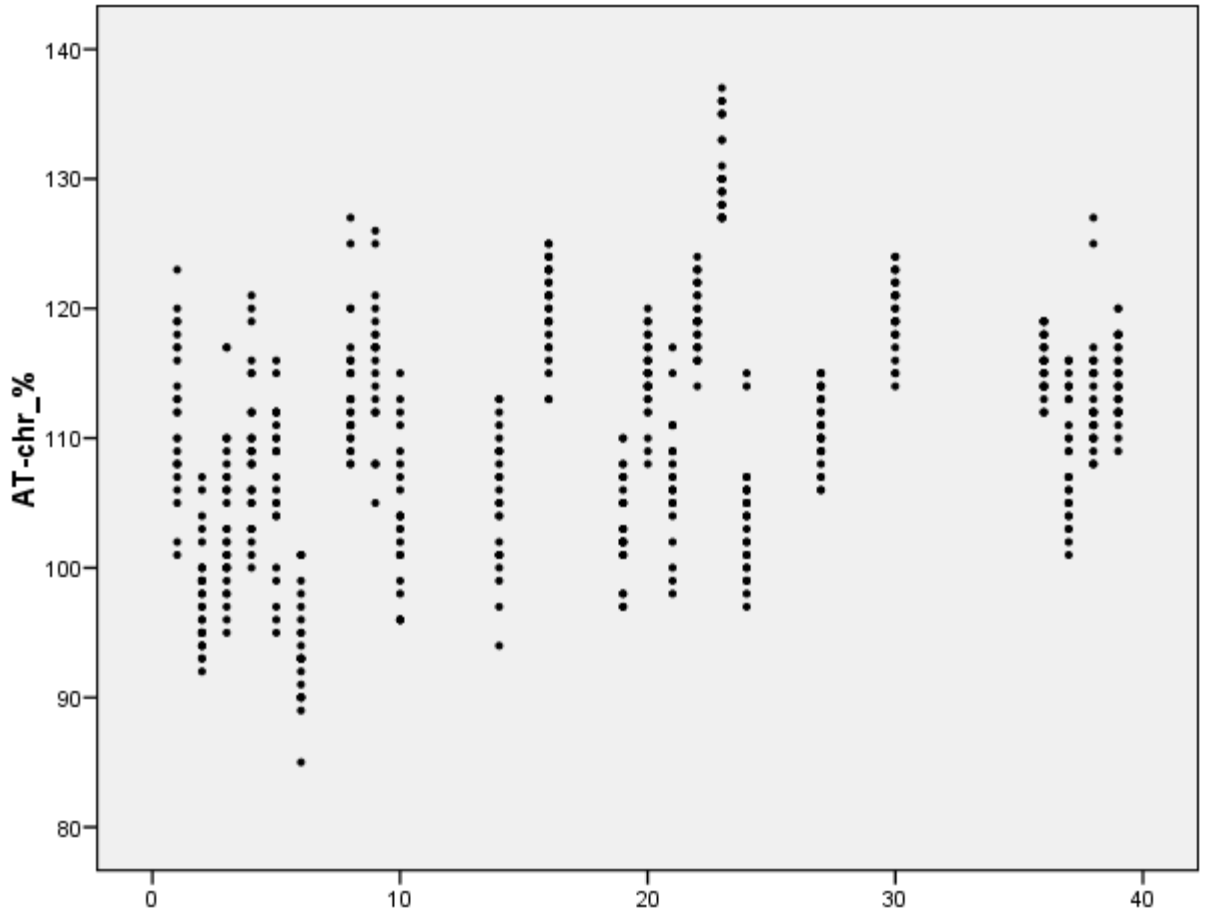
Biological variation of CRP



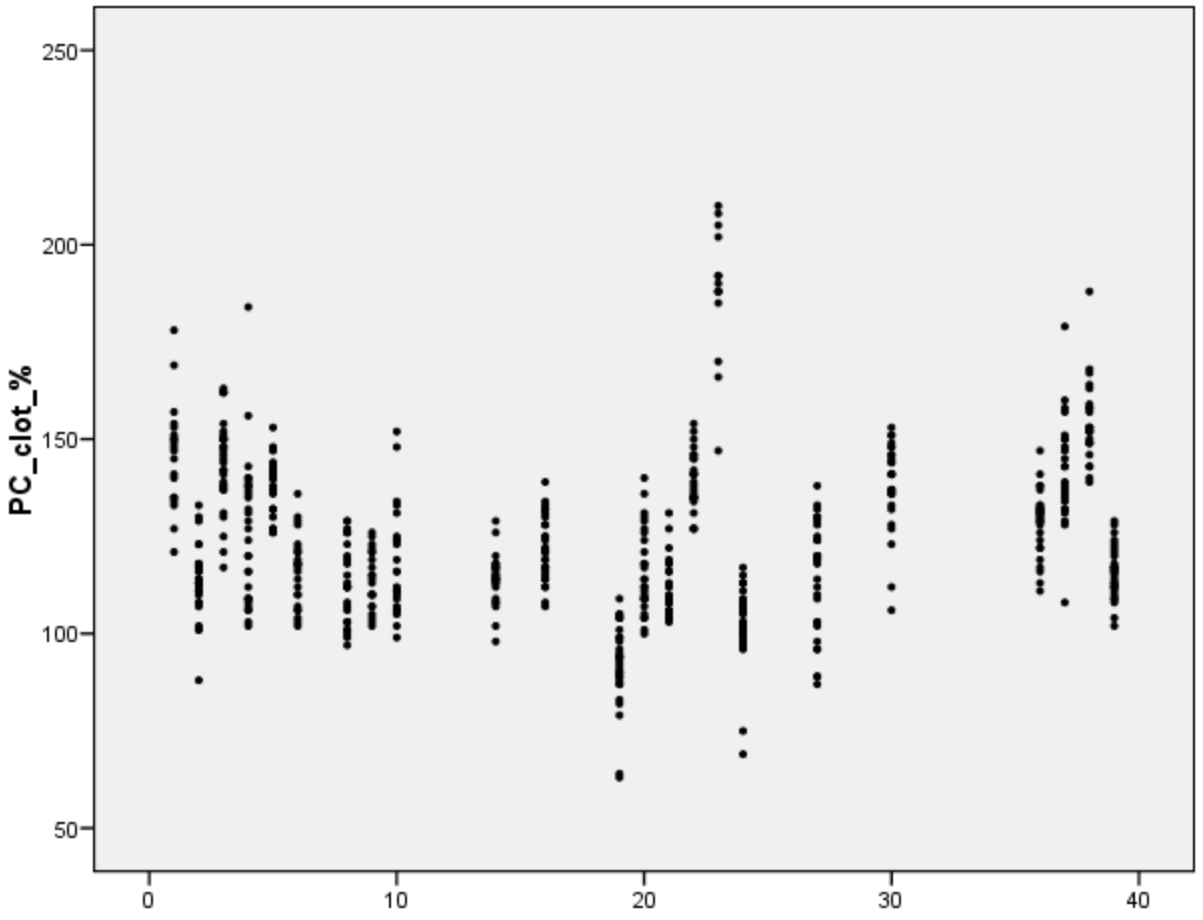
Biological variation of prothrombin time



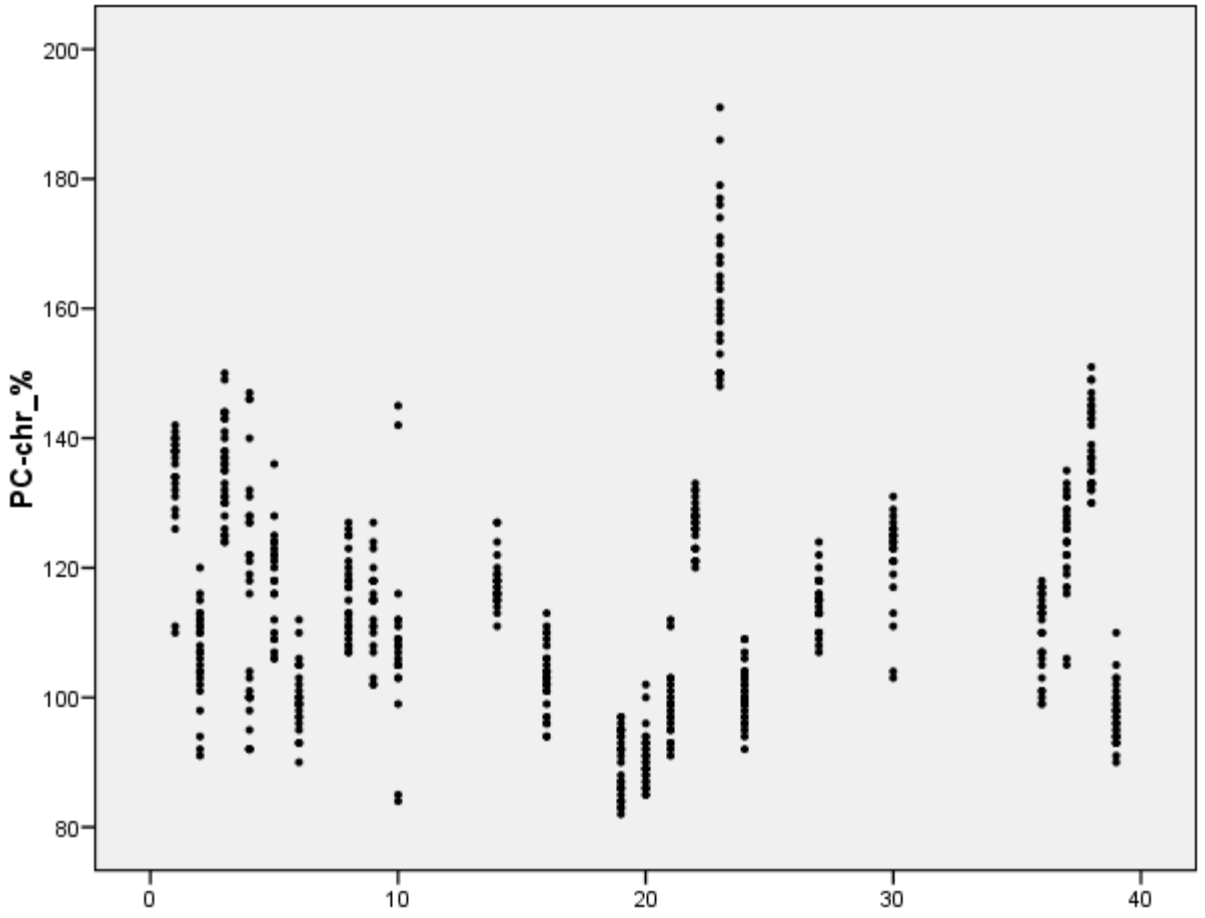
Biological variation of antithrombin



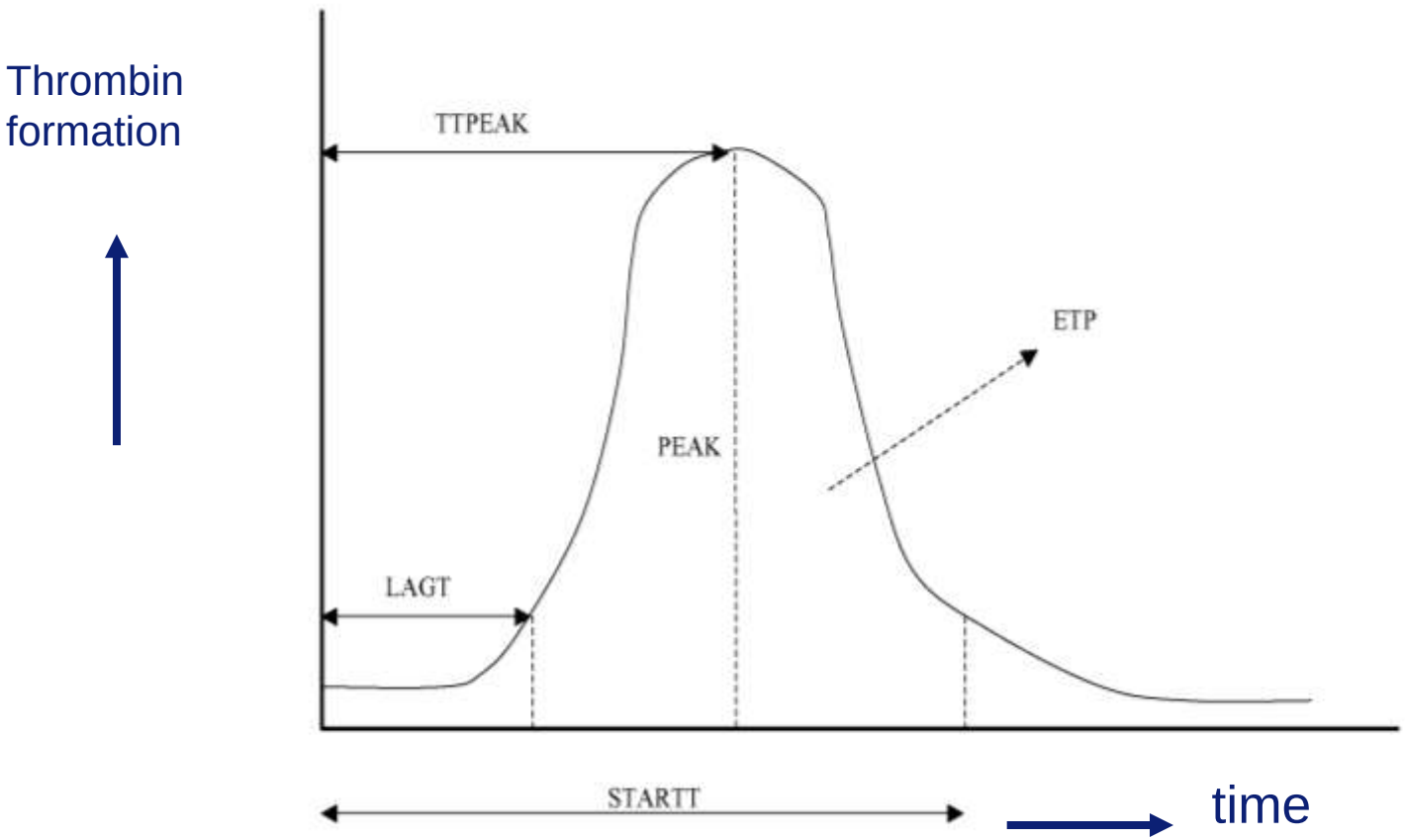
Biological variation of Protein C clotting



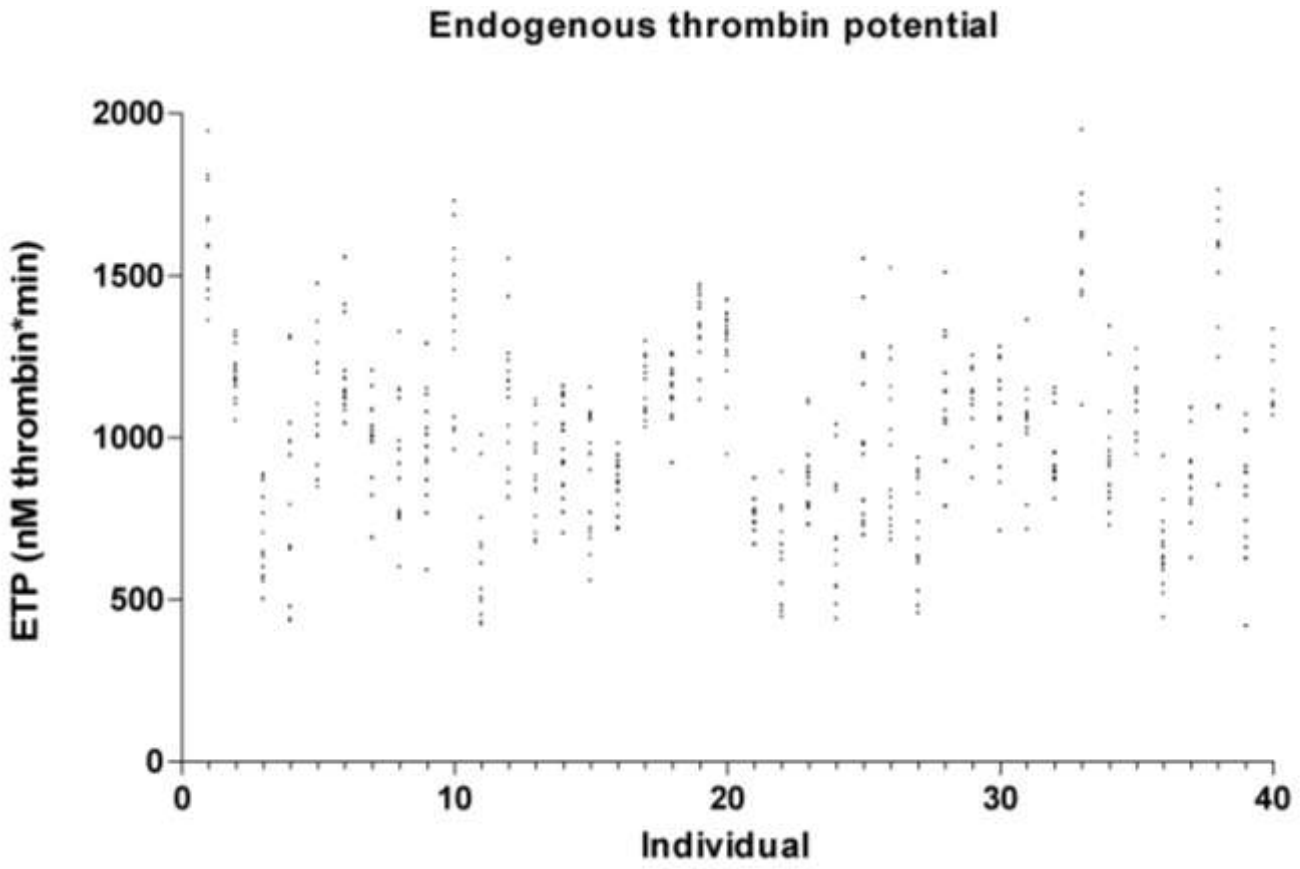
Biological variation of Protein C chromogenic



Thrombin generation



Biological variation of thrombin generation



Components of variation

$$y(ij) = \mu + \alpha(i) + \varepsilon(ij)$$

$$SD^2_{total} = SD^2_{between} + SD^2_{within} + SD^2_{analytical}$$

How to express variation:

- Variance SD^2
- SD $\sqrt{(SD^2)}$
- CV (SD / mean) * 100%

Components of variation

Variable	Mean	Coefficient of Variation			
		Total	Between-subject	Within-subject	Analytical
Fibrinogen (g/L)	2.8	20%	15%	13%	
CRP (mg/L)	0.37	132%	108%	78%	
Thrombin generation (ETP (nM*min))	1012	29%	24%	17%	
Prothrombin time (sec)	12.3	7.0%	5.7%	3.9%	
Antithrombin (%PP)	110.7	9.0%	7.9%	3.9%	2.1%
Protein C clotting (%PP)	124.6	18.4%	15.5%	8.8%	4.5%
Protein C chromogenic (%PP)	114.9	18.1%	16.1%	6.6%	4.7%

Subgroup analyses and adjustments

- The results for the between- and within-subject variation only slightly changed when:
  - Outliers were not included
  - The analyses were performed for nonsmokers only
  - in men and women separately
  - after excluding periods of reported disease (common flu)
  - or for nonusers of contraceptives

- Adjustments for age and BMI did not affect significantly the within-subject (biological) variation.

Contribution of biological to total variation after N repeated measurements

Variable	1	3
Fibrinogen	44%	21%
ln[CRP]	34%	15%
ETP	35%	15%
Prothrombin time	32%	13%
Antithrombin	18%	7%
Protein C clotting	23%	9%
Protein C chromogenic	13%	5%

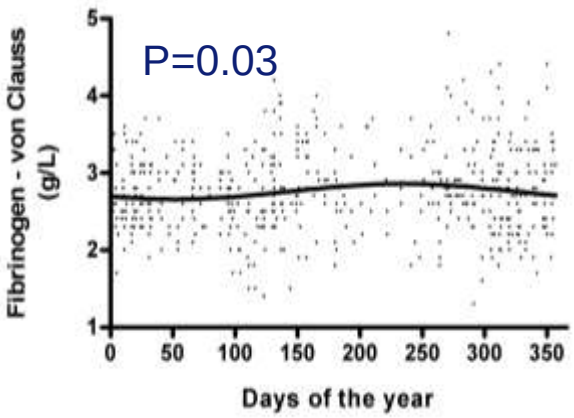
Seasonal variation

$$y = a + b*\sin(2\pi(t-1)/365) + c*\cos(2\pi(t-1)/365)$$

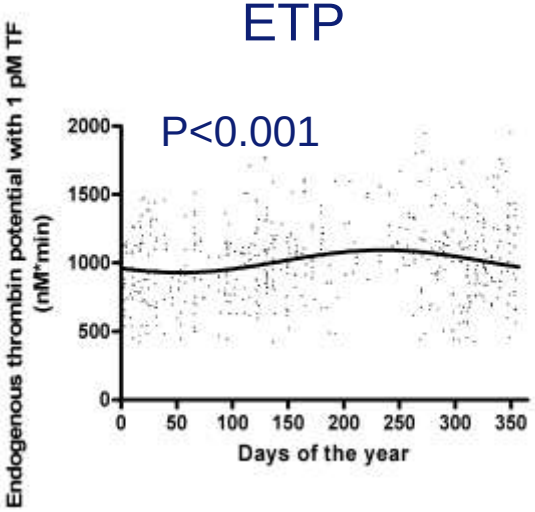
- *a*: annual mean
- *b* and *c*: components of seasonal variation
- *t*: day of the year

Seasonal variation

Fibrinogen



ETP



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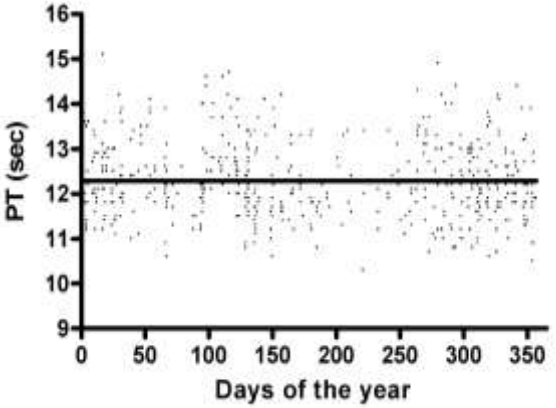
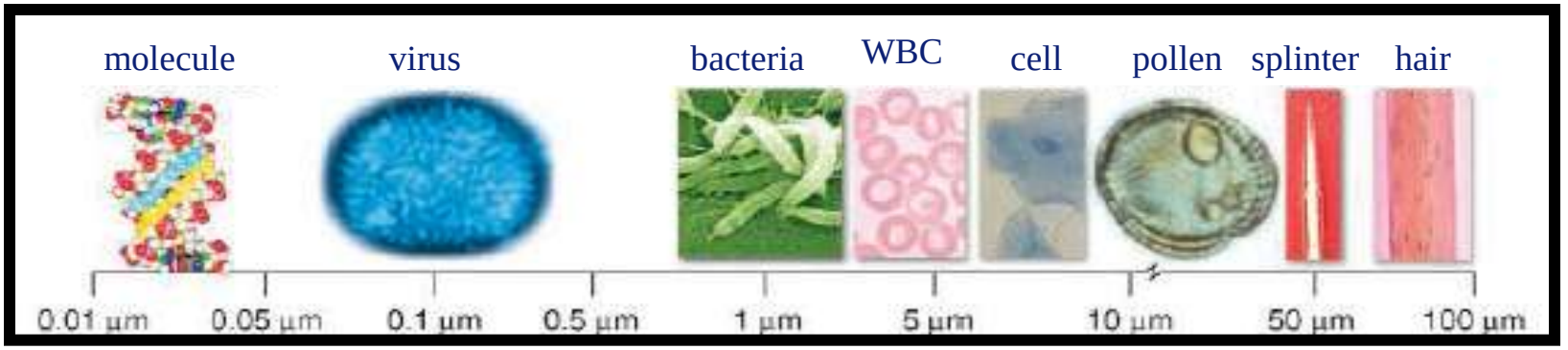
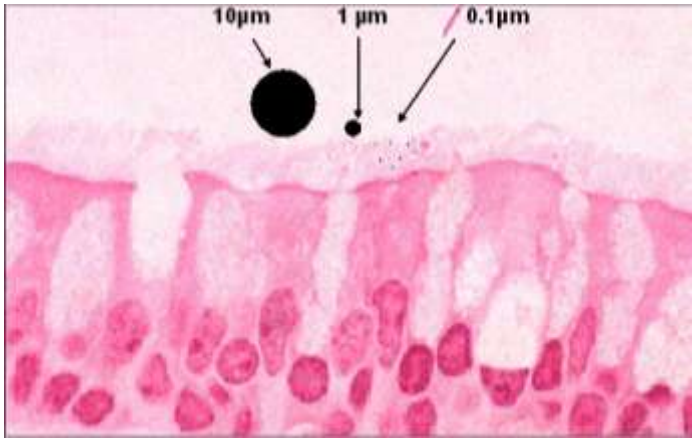


Air pollution / particulate matter

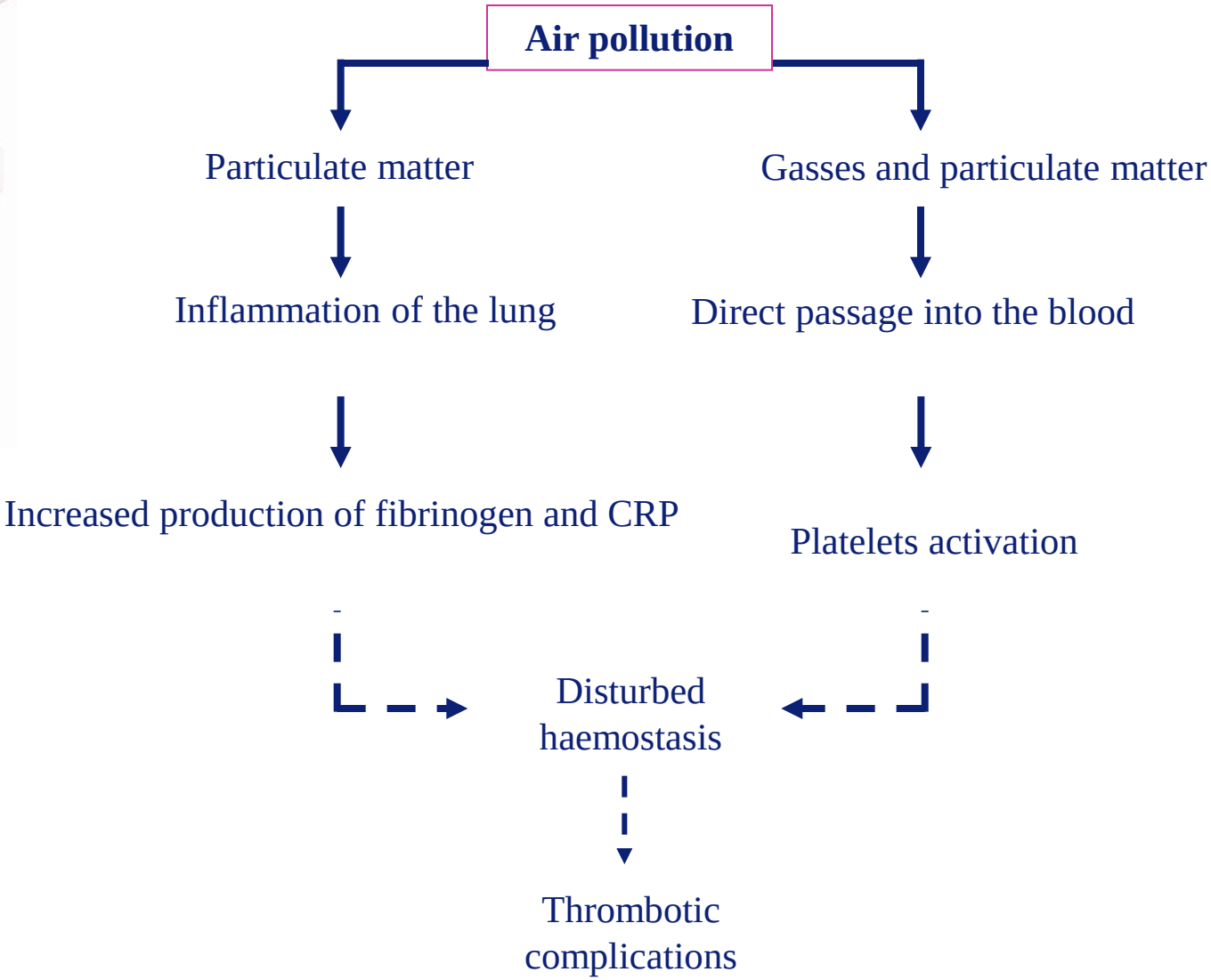
Particulate matter



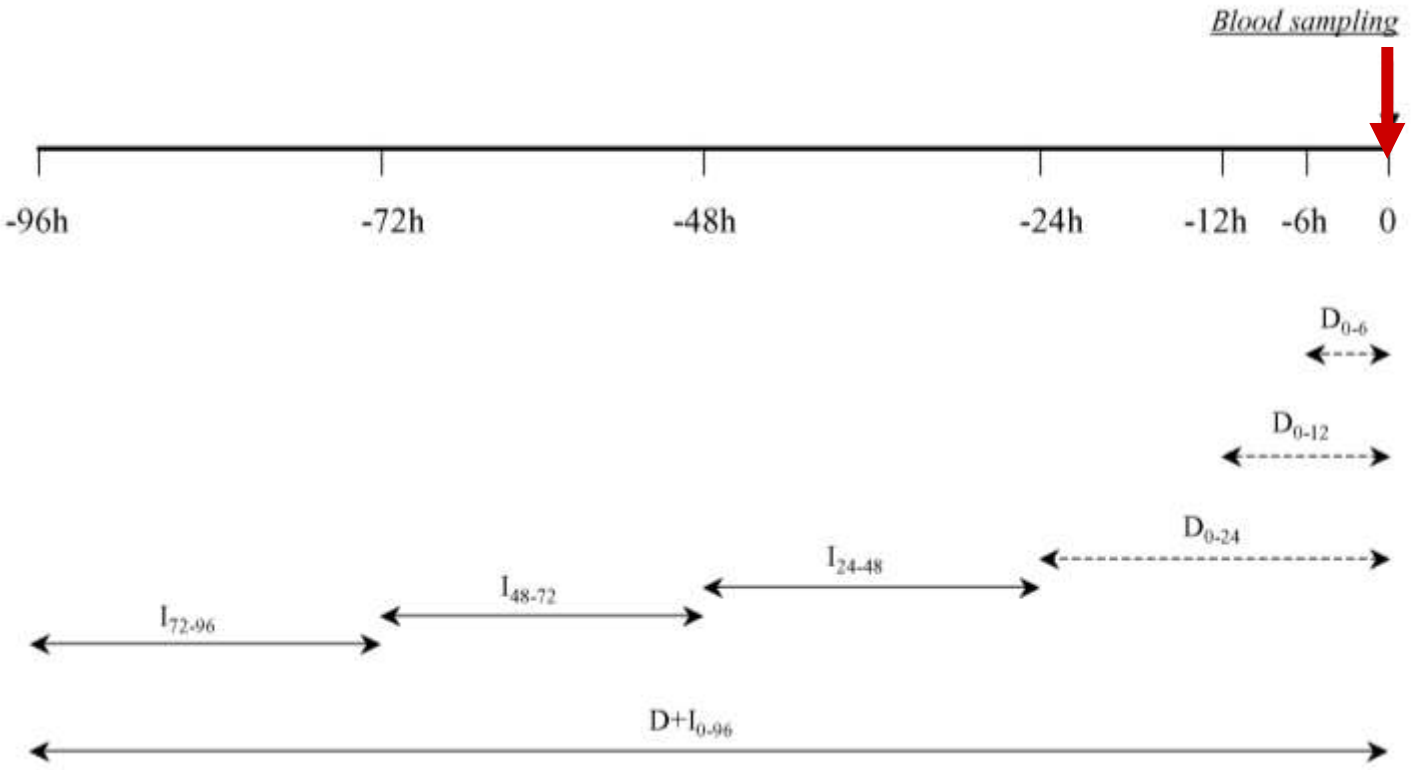


Indirect effects

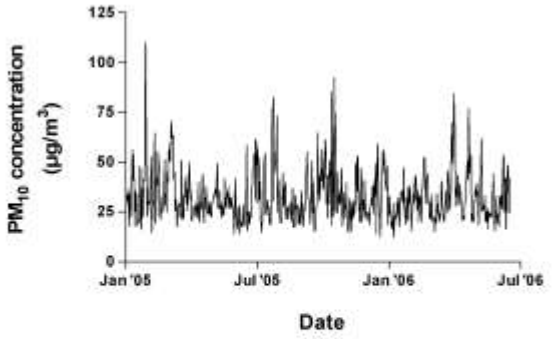
Direct effects

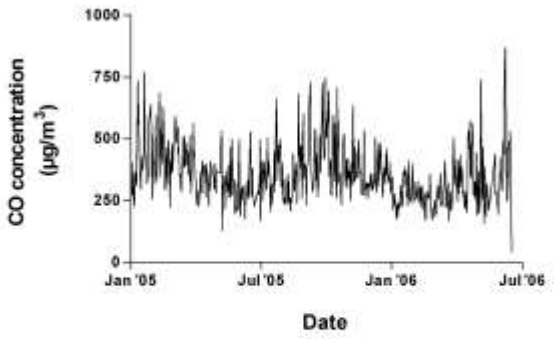


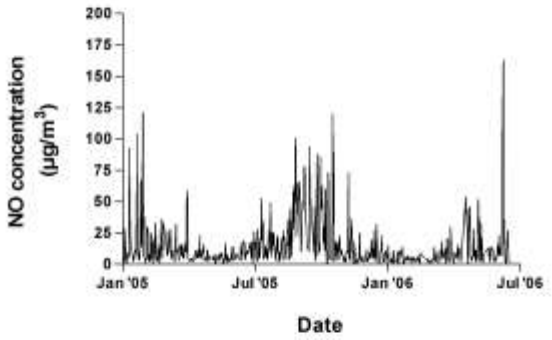
Time-lags of estimated exposure to air pollution before blood sampling

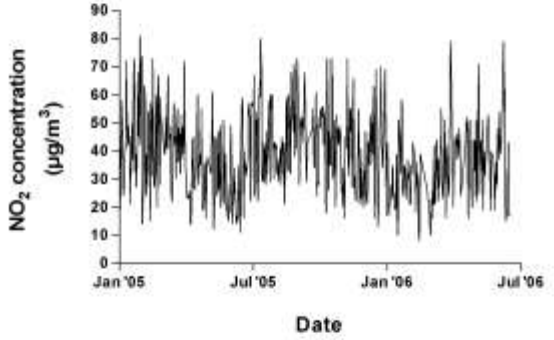


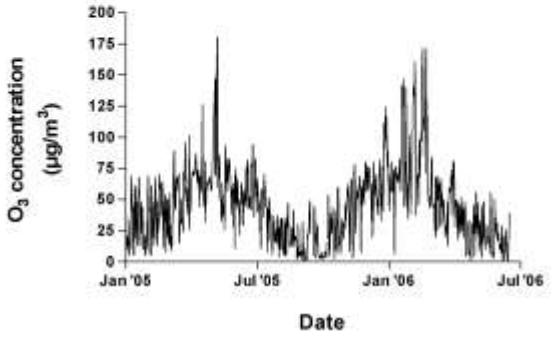
Variation in air pollution



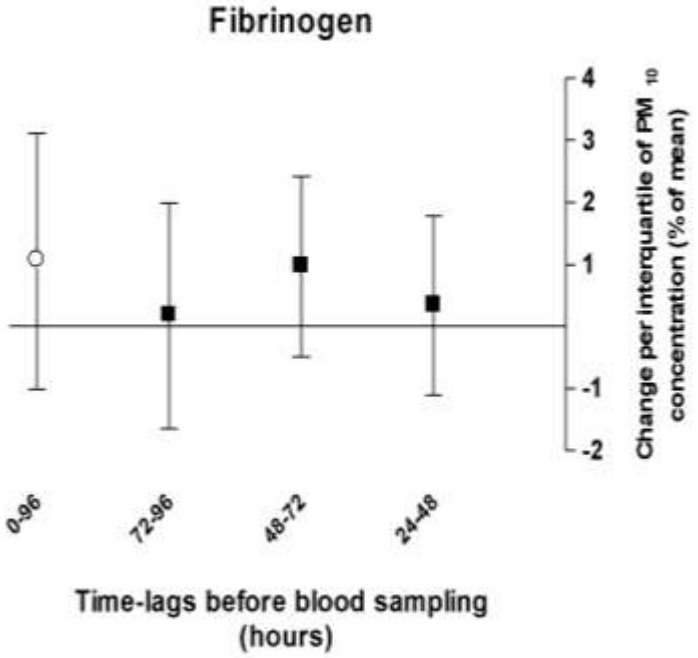


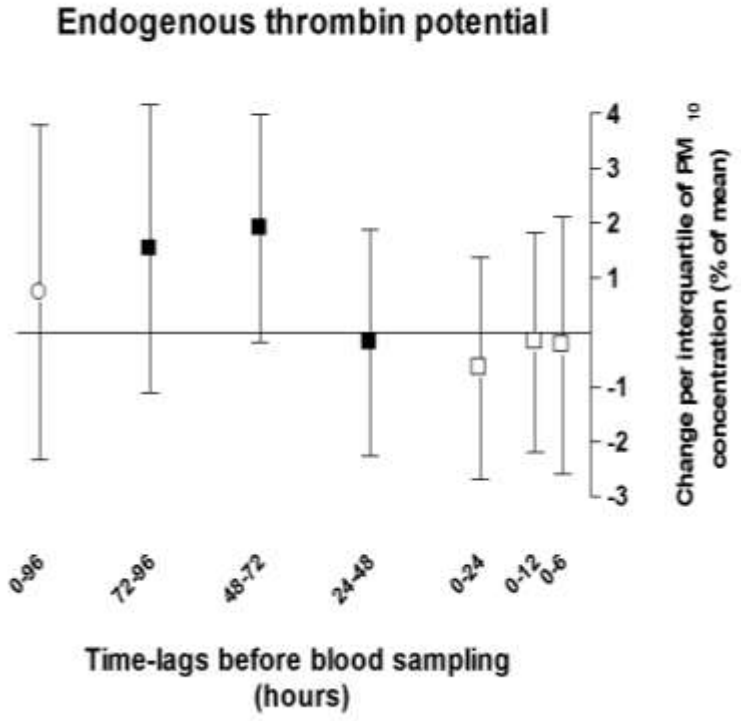






Effects of particulate matter





Conclusions

- The within-subject variation is much smaller than the between-subject variation
- For most assays, doing triplicate measurements gives a good estimate of the habitual level (±10%)
- Levels of fibrinogen and thrombin generation showed a strong component of seasonal variation with higher levels during the summer and autumn
- Air pollution may explain part of the biological variation

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Recommendations

- Repeated measurements
  - $SD^2_{total} = SD^2_{between} + (\underbrace{SD^2_{within}}_{X} + \underbrace{SD^2_{analytical}}_{Y})$
- Analytical specifications
  - Diagnosis
    - $SD_{(assay)} <= 0.58 * SD_{(total)}$
    - Assay variation adds a max. of 12% variability to the total test variability

    (*i.e.* assay adds only 12% “noise” to the true biological “signal”)
  - Monitoring
    - $SD_{(assay)} <= 0.50 * SD_{(intra)}$
    - Assay variation adds a max. of 10% variability to the total test variability

Introduction

- Knowing biological variation can help to determine:
  - What is the true habitual level in an individual
  - Quality specifications:
    - Clinical laboratories: Diagnosis
    - Population-based studies: Monitoring