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Clinical Metabolomics: The Trials of Investigating Metabolic Effects on Human Volunteers

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Presentation Outline



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1. Introduction

- Human Diversity

2. Implicit Strategies to Deal with Diversity in Clinical Trials

- Study design considerations
- The problems unique to clinical trials

3. Explicit Strategies to Deal with Diversity in Clinical Trials

- Options for the removal of unwanted variation in a data set

4. Example Study

- Detecting SUBTLE Metabolic Effects in Human Data

Human Diversity



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Humans



In-bred lab animals



1. Large diversity vs. less diversity
2. Many considerations in common
3. But also, many considerations unique
4. Animals: many factors can be controlled
5. Humans: little can be controlled

Strategies to Deal with Human Diversity in Clinical Trials



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Implicit and Explicit strategies:

- **Implicit strategies** are purely design driven. Identify factors that will be of influence and incorporate in the study design
- **Explicit strategies** will adopt a computational correction method that is predefined and uses knowledge about sample origin.
- Not mutually exclusive (usually used in combination)

Implicit Strategies: Study Design Considerations



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It is likely that **EVERYTHING** both genetic and environmental affects our metabolic profile.

Factors which should be taken into consideration in a well-designed study include (but are not limited to):

- 1) Gender
- 2) Age
- 3) BMI
- 4) Time of sample collection (diurnal variation)
- 5) Drugs (prescription and non-prescription incl. caffeine)
- 6) Diet (esp. vegetarian, alcohol, fish, certain vegetables)
- 7) Exercise

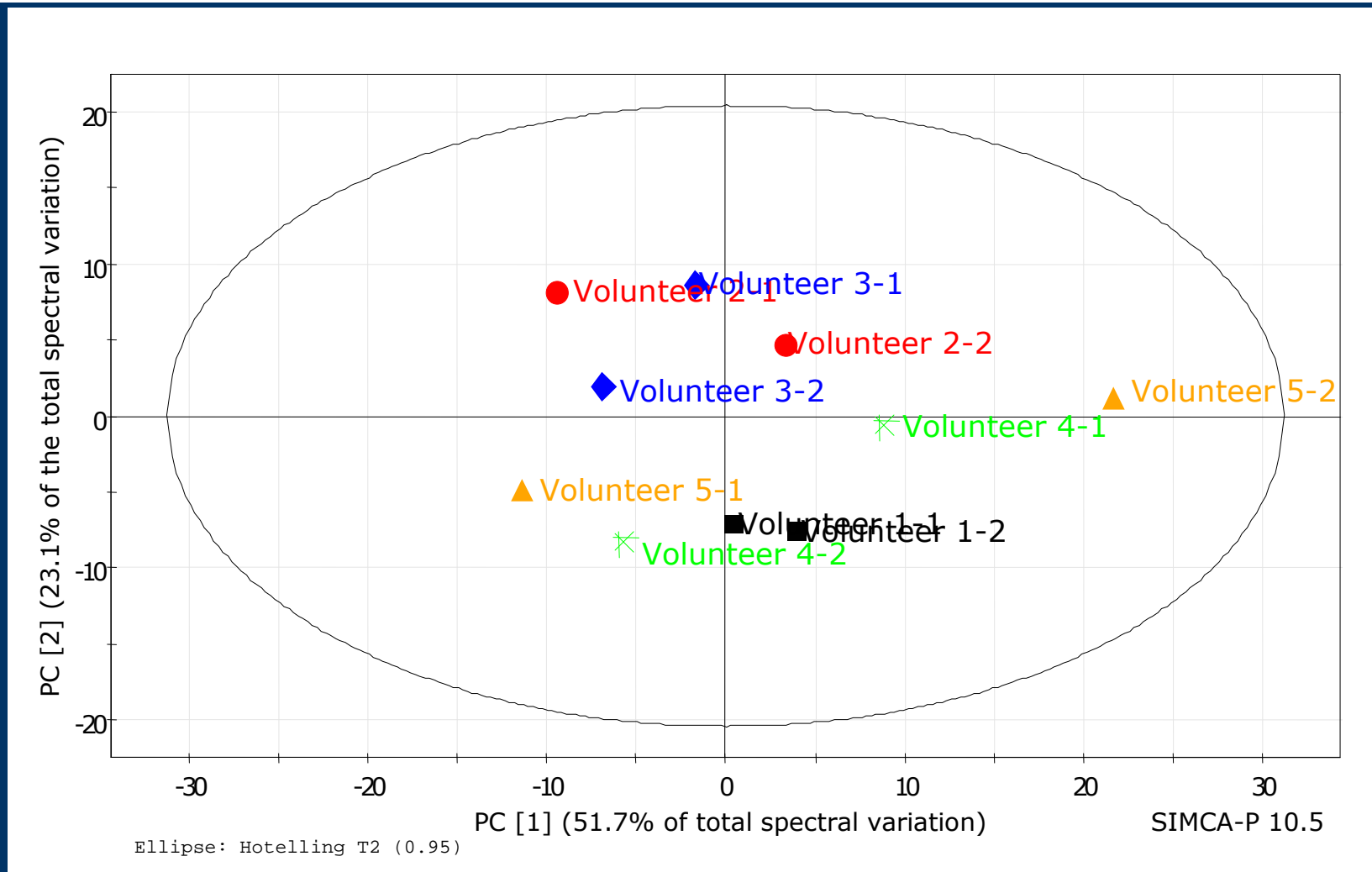
Problems with Implicit Strategies



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Even the Best Designed Human Trial
WILL contain 'Flaws'...

Problem 1: What is Healthy?

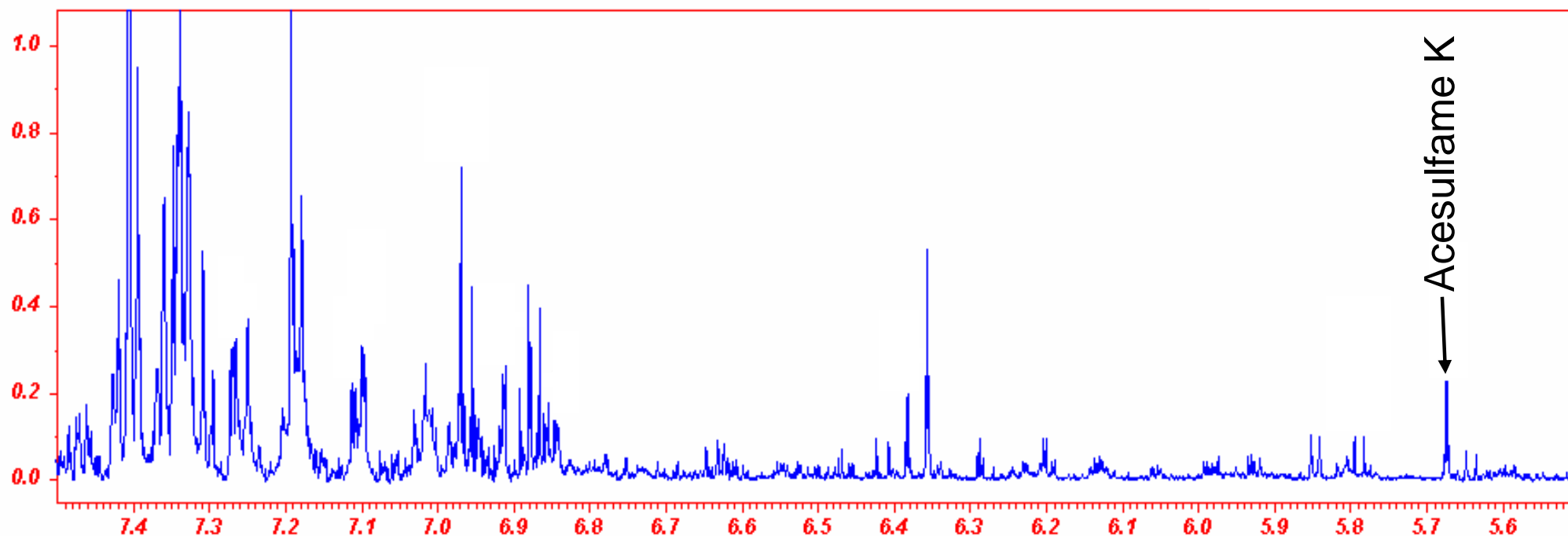


Volunteers may be unsuitable as study subjects because of health conditions they are unaware of.

Problem 2: Humans Lie ...



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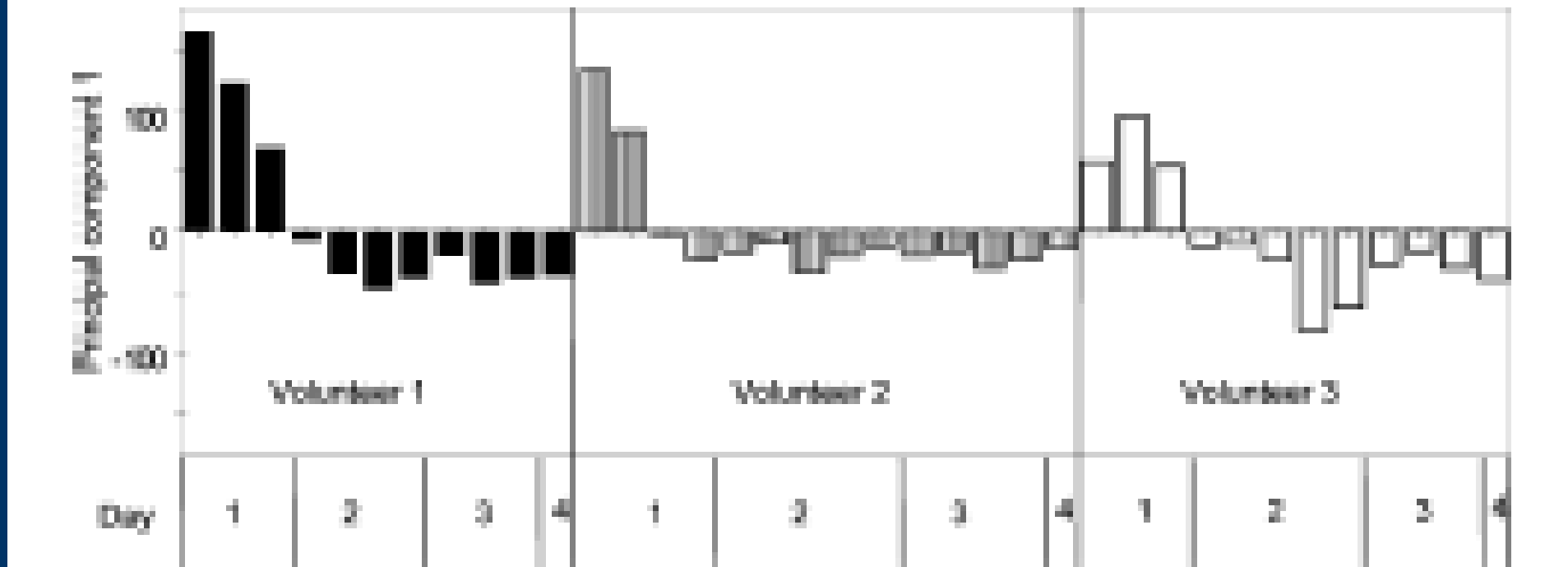
600 MHz ¹H NMR Spectrum of Human Urine Showing the
Detection of Sweetener in a Cola Light Drink

Note: Withdrawing financial reward for volunteers who are proven to have not followed study instructions is *allegedly unethical*....

Problem 3: Imposing Dietary Restrictions on Volunteers



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In this case, prior to dietary intervention on day 4, the volunteers were asked to restrict their diet.*

* From Daykin et al (2005), J. Food Agric. Chem., 53: 1428-1434

Problems:

1. Large effect due to dietary restrictions
2. Dietary intervention is not studied in a normal, realistic scenario

Problem 4: Practical and Ethical Considerations



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Common Examples:

1. Some diseases cause incontinence

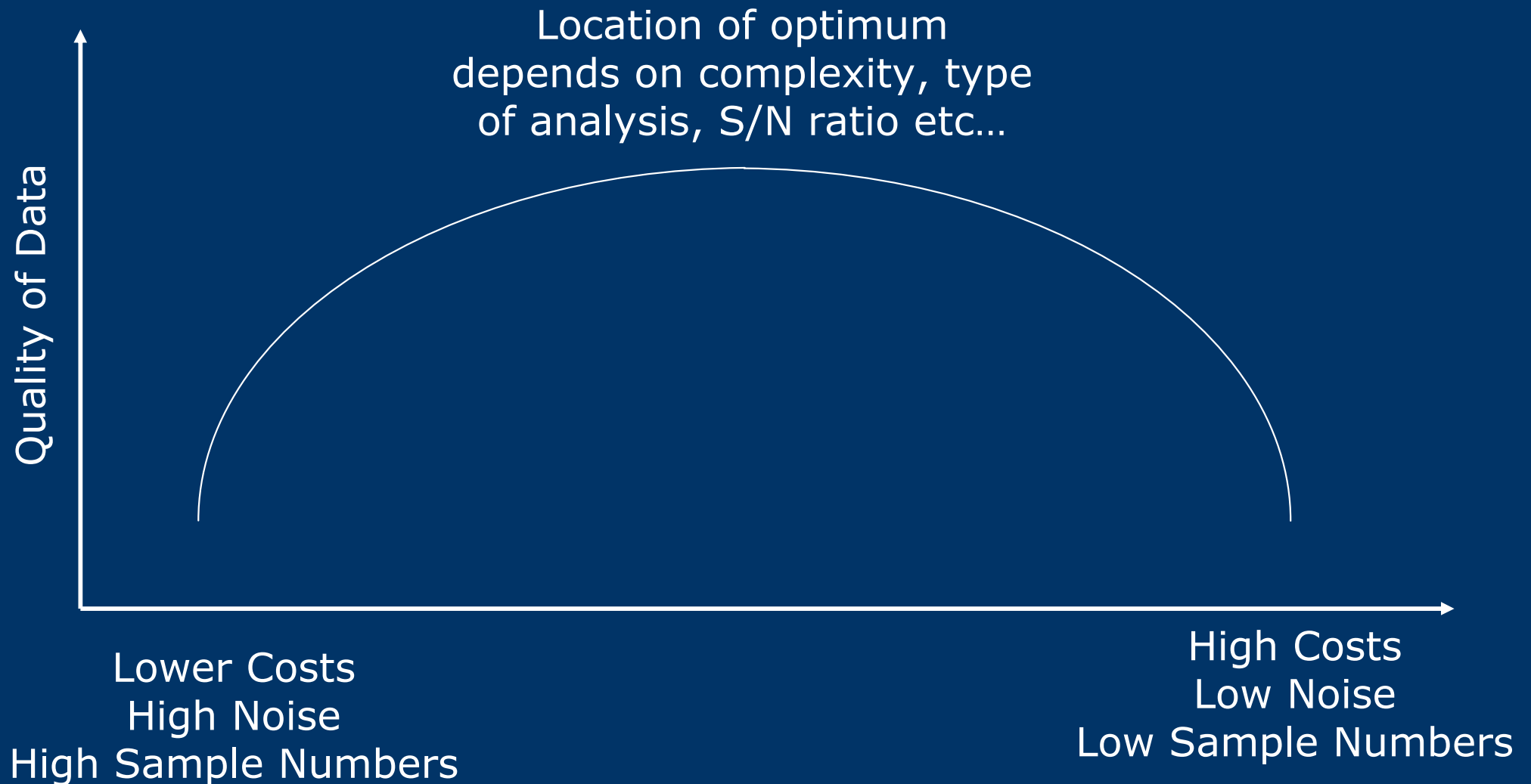
How do you request a first (morning) urine sample or mid-flow urine sample?

2. Not all 'standard' study criteria are permissible for all study groups

e.g. pregnant women can not be asked to fast



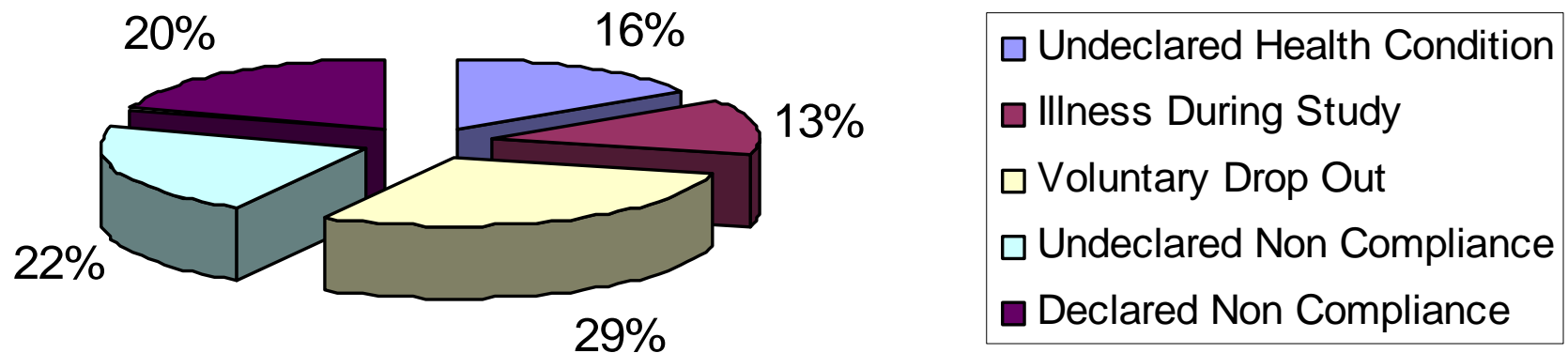
Problem 5: Optimum study group size



Problem 6: Commitment of Volunteers



Reasons Why Volunteers Drop Out of Studies*



Largest reason: Voluntary Drop Out.
Do not ask too much from your volunteers !

*Numbers presented are based on observations over several studies in our lab

Explicit Strategies: Removing Interperson Variation Computationally



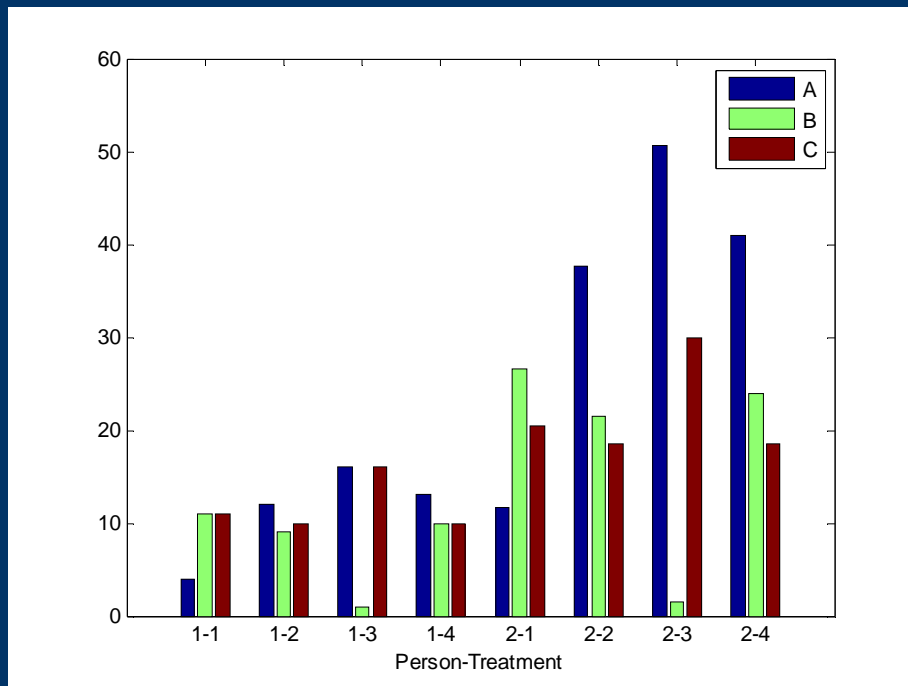
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1. In most cases, application of standard tools such as 'discriminant analysis' will filter out unwanted variation
2. Where metabolic effects under study are much smaller than inter-individual effects (e.g. nutritional studies), additional tools are required
3. It is advisable to keep methods as simple as possible. Pre-processing is part of the model: it adds to model complexity and has to be included in all validation steps

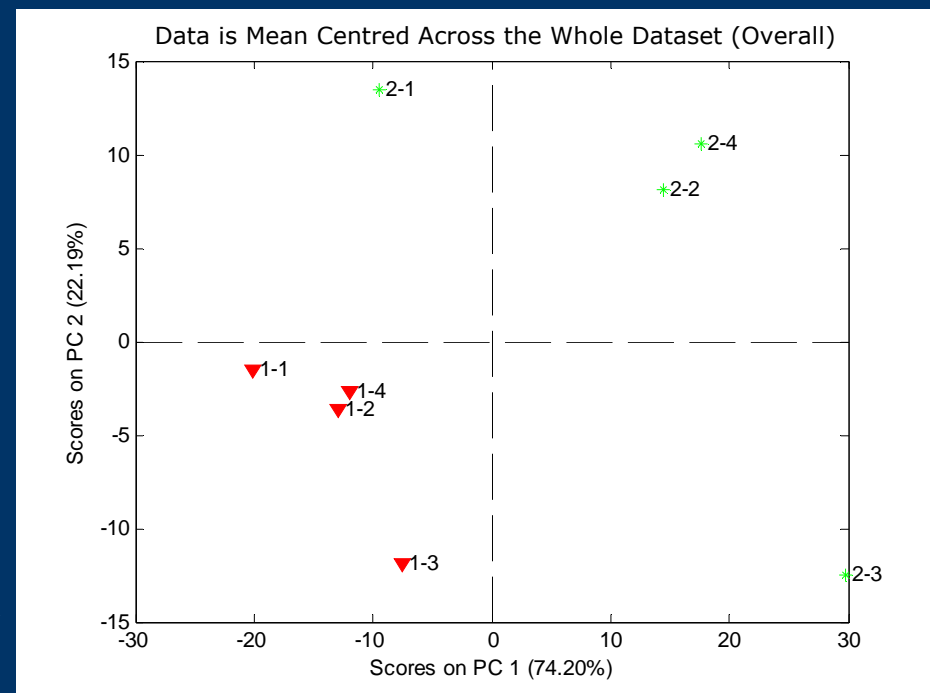
Explicit Strategies: Artificial (Illustration) Dataset



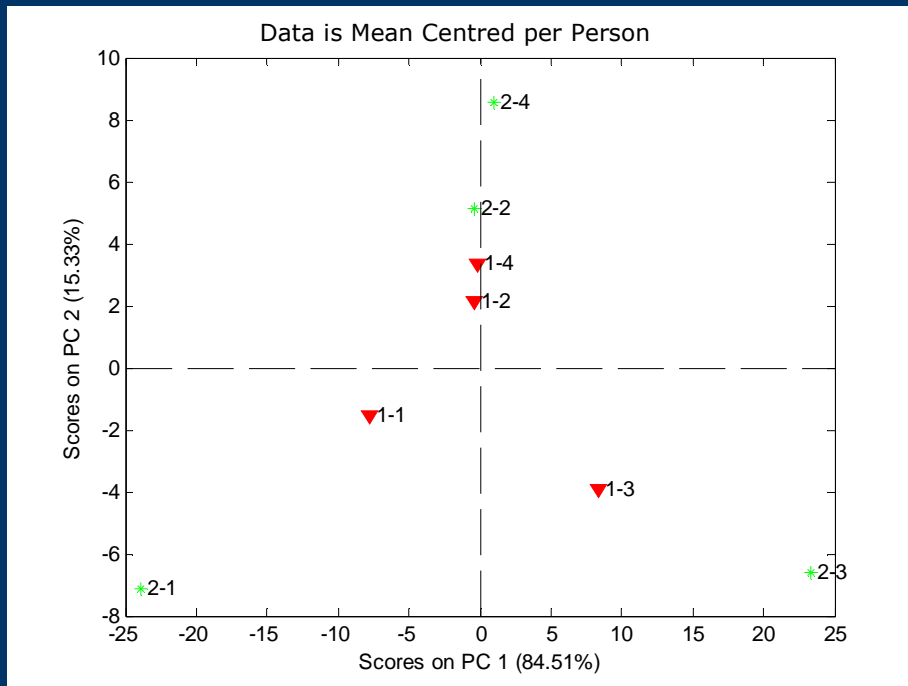
3 Metabolites, 2 volunteers, 4 treatments



PCA

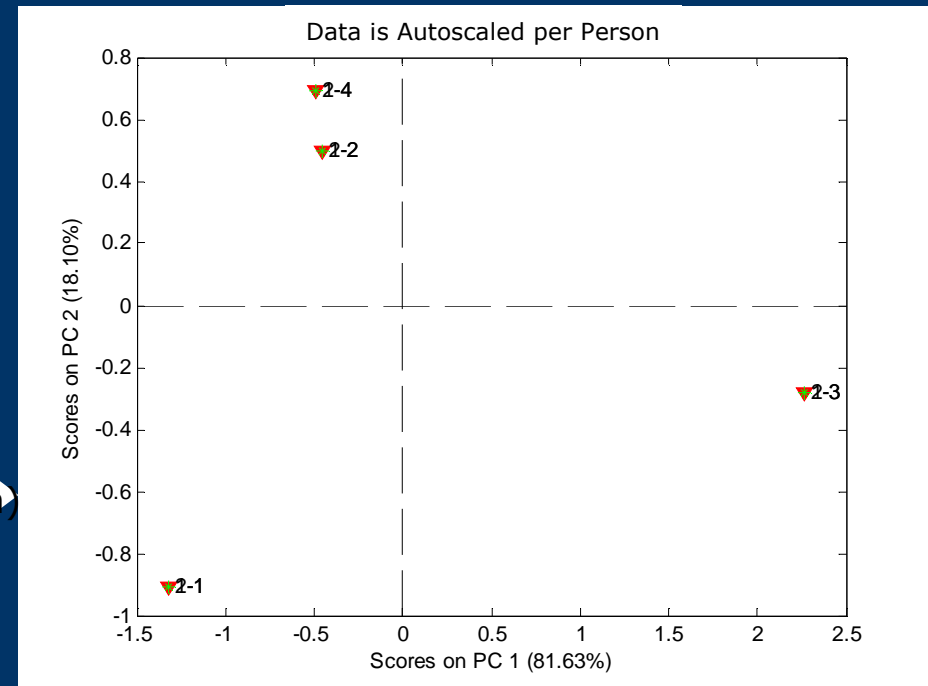


Explicit Strategies: Correction per person before PCA



$-\text{Avg}_{(\text{Person})}$

$/\text{Std}_{(\text{Person})}$





The most appropriate method depends on research question:

Do you want everybody to be the same?

- Use Mean Centring to remove a personal 'base level'
- Use Auto Scaling to also remove personal sensitivities to treatments
- Express data as difference to or fraction of t_n measurement to enhance dynamic effects
- Normalise to a certain metabolite or external measurement (food intake, total body fluid volume produced, physiological data ...)



The Effect of Potential Functional Food Ingredients (Anti-Oxidant Vitamins) on Recovery After Exercise

S. Coolen¹, J. van Duynhoven¹, F. Wulfert², C.A. Daykin³

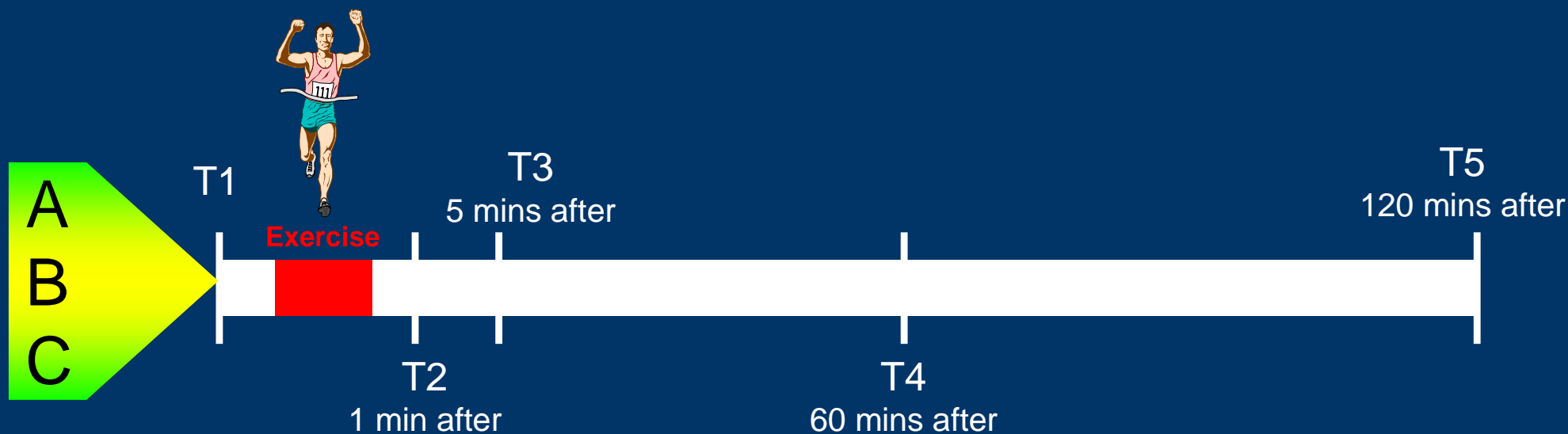
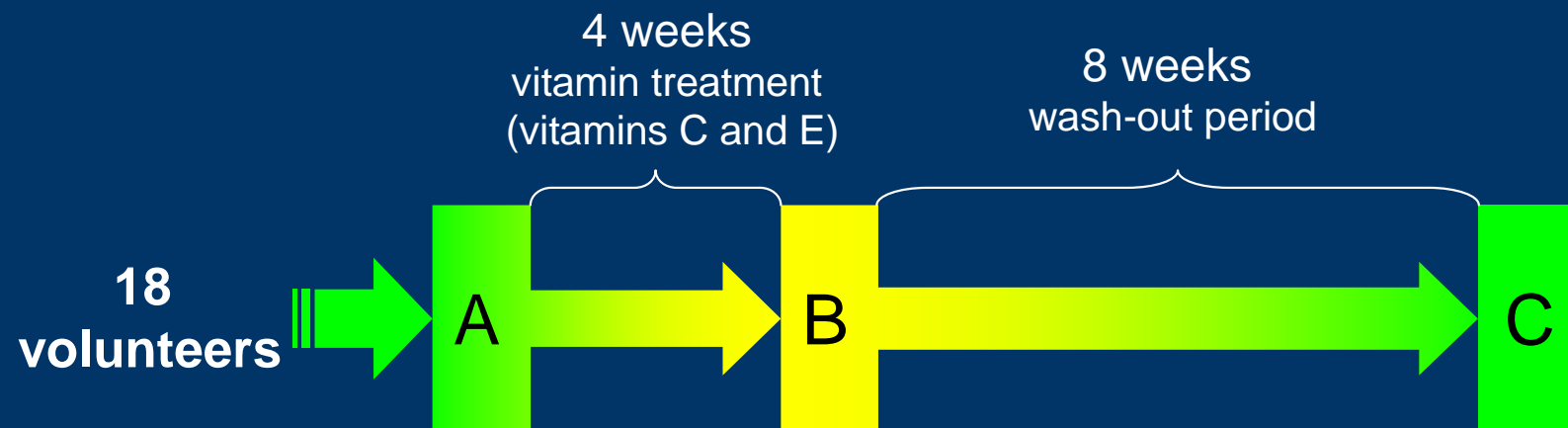
¹ Unilever Research and Development, Vlaardingen, The Netherlands

² Division of Food Sciences, School of Biosciences, University of Nottingham, UK

³ Centre for Analytical Biosciences, School of Pharmacy, University of Nottingham, UK

Publication Submitted to Journal of Vascular Research

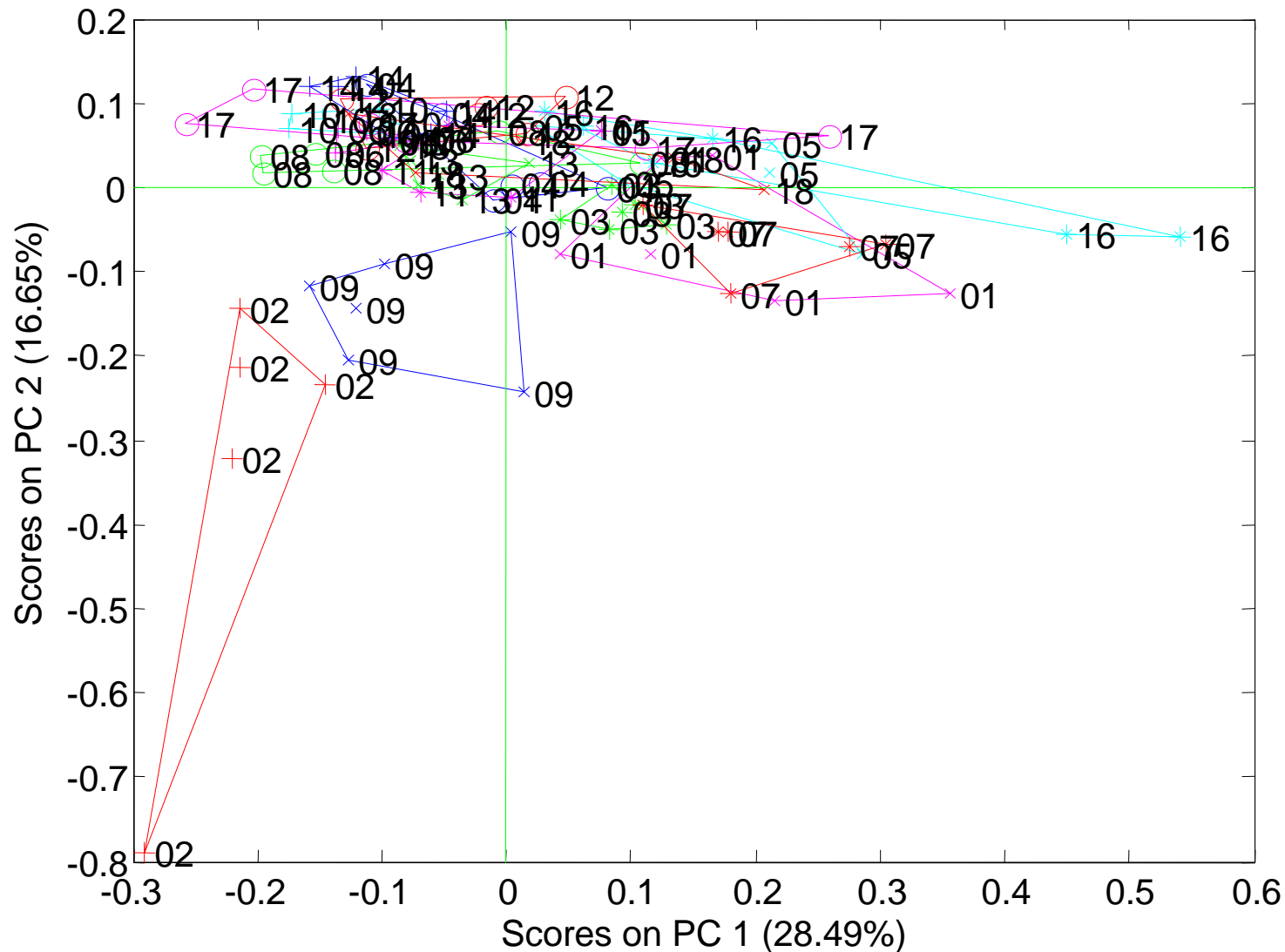
Example Study: Study Design



Example Study:

Major Variation in Data due to Inter-Individual Variation

Correction for Effect Required

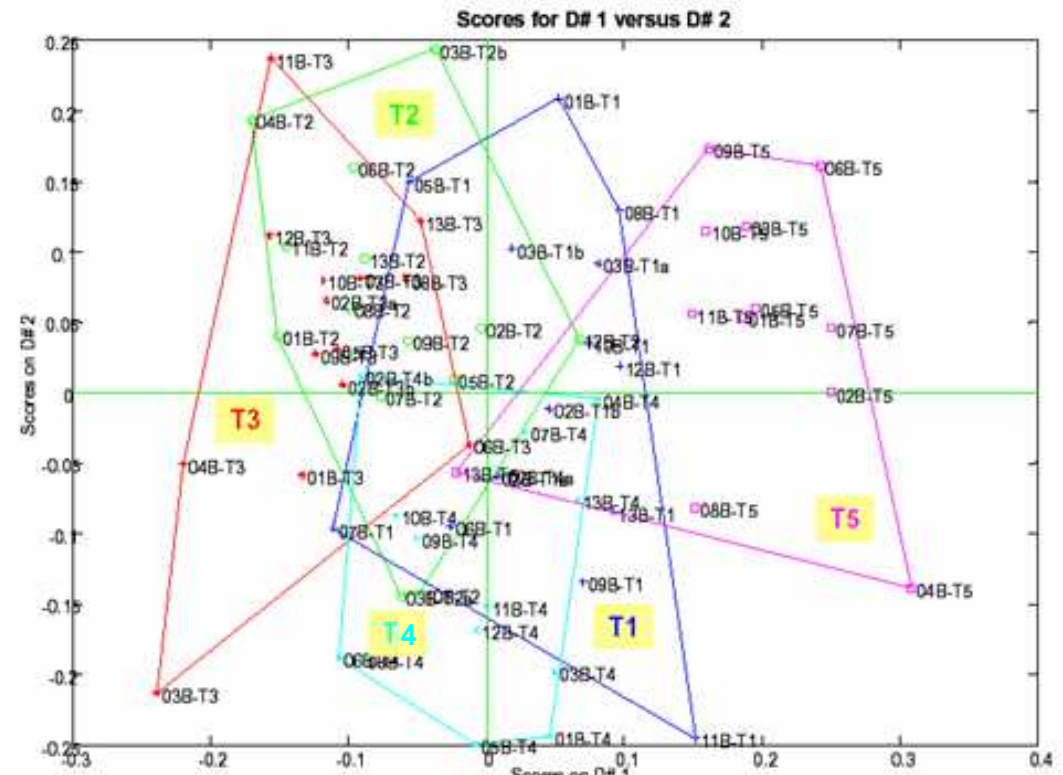
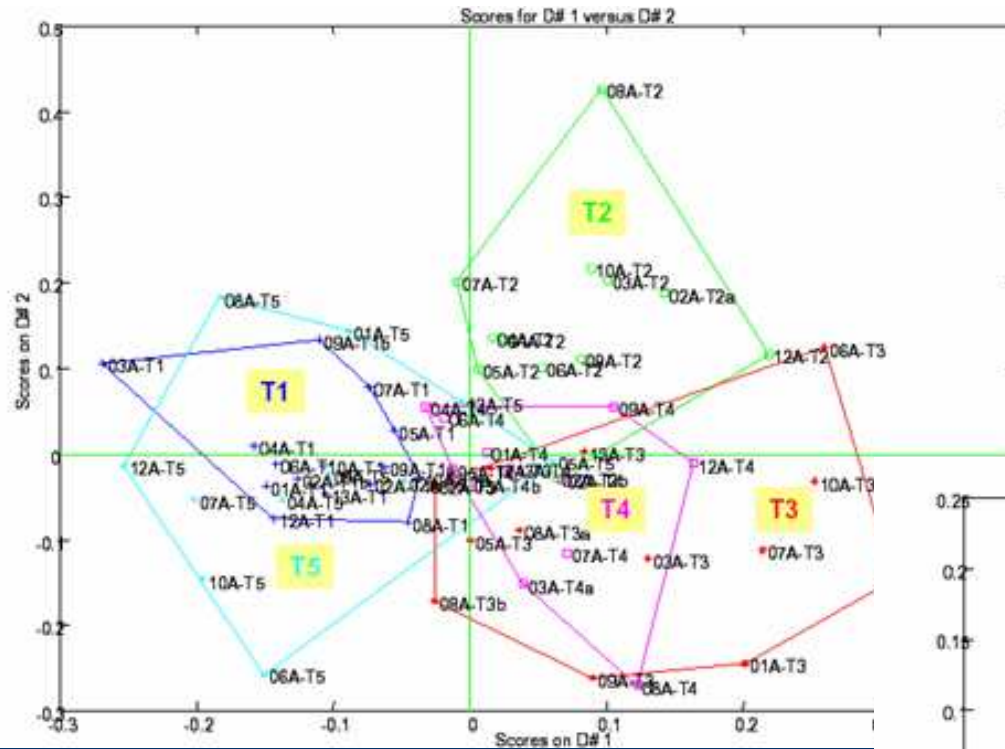


Example Study: The Exercise Metabolome at Appointments A and B



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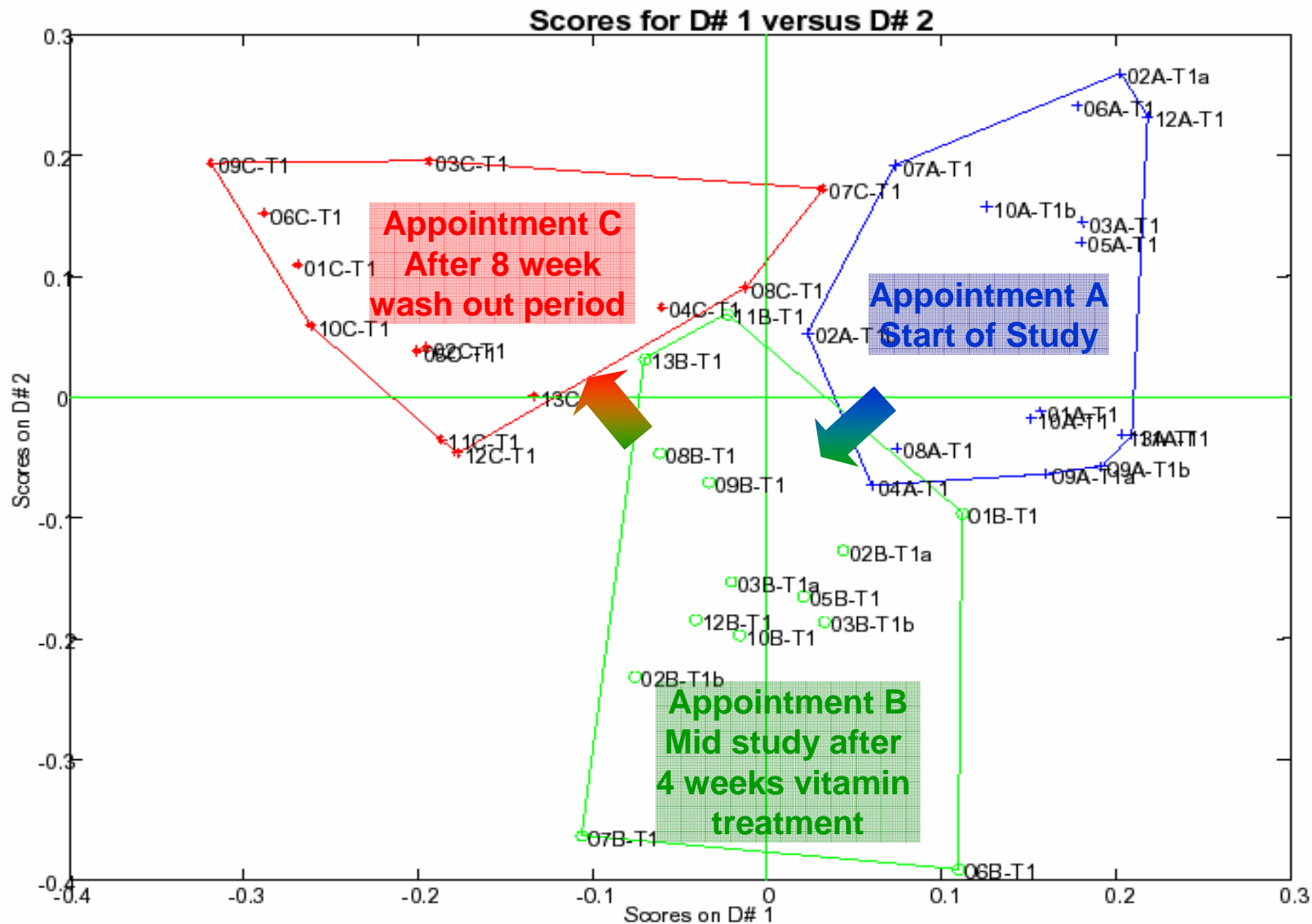
Exercise Effect At Start of Study
(Appointment A)



Exercise Effect After 4 Weeks
Vitamin C and E Treatment
(Appointment B)

Appointment C
profile similar to A except that
recovery complete by T4

Example Study: The Anti-Oxidant (and Cheating!) Metabolome at Time-Point T3



Conclusions



1. Design of human trials is fraught with problems
2. Minimising effects by good study design helps but some 'rogue' samples will always slip through the net
3. Data can be further noise-reduced with simple data pre-processing strategies
4. After implementing these steps, metabolomics can even be used to distinguish SUBTLE metabolic effects
5. This is essential in a changing health care market, where interests increasingly focus on prevention rather than cure of disease

Acknowledgements



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