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Technical Note

A technical note on challenge tests in human volunteers for multidimensional phenotyping

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ABSTRACT

Challenge tests are used to reveal the adaptability and resilience of humans to get insight in their health. When contrasting challenge tests are used, various forms of adaptability can be tested and this gives a multidimensional view on the health of these individuals. Here we introduce a methodology to describe the similarities and differences on how individuals are positioned in the health space based on the responses to different challenge tests. Results show that anabolic and catabolic challenges differentiate healthy subjects in rather different ways. Considering these results we hypothesize that combining challenge tests can improve the understanding of the underlying multidimensional phenotype.

1. Introduction

Challenge tests are applied to individuals to reveal information on their resilience to recover from a stressful situation. These tests match the rise of the new paradigm that organisms need to be adaptable and flexible to the changing environmental conditions in order to be considered healthy [1,2]. The objective of a challenge test is to position the individuals in the multivariate health space. Each type of challenge test provides a specific view on the health space and thus the individual positioning. The position of an individual depends on the response to the challenge. Some individuals will respond more extreme to a challenge while others are less affected. Such differentiation on the position of individuals in the health space using challenge test responses also allows the comparison of tests. The major aim of this work was to develop methodology to visualize the dissimilarities of various challenge tests on how they are able to differentiate healthy human volunteers. By repeating a challenge, it can be assessed whether this position is an invariant property of the individuals in the group. When this position is indeed an invariant property, the challenge uncovers a systematic feature that links directly to the subjects' phenotype. However, when the position of a repeat experiment is very different, it does not reflect an invariant property of these individuals. The latter can be caused by other biological processes such as a biorhythm that can confound the results, or a lack of systematic variation (noise). Although the effect of challenges can differ, they may result in a similar position of individuals due to their metabolic phenotype (sometimes called metabotype). We built on this property and compared the similarity of the individual position over several challenges. A-priori, we expected catabolic challenges in which food components are broken down into smaller units to release energy and anabolic challenges in which the smaller units are used to build new components the cells need to excite dichotomous physiological responses, and that the individual positions reflect a different set of phenotypic measures. With the rational that different challenges allow differentiating the individuals, six challenges were considered and the individual position was compared for similarity. Cross-correlations among 28 plasma amino acids profiled by a targeted LC–MS/MS approach in a time-resolved manner during the different challenges revealed a subset of metabolites comprising the branched chain amino acids (BCAA), phenylalanine and α-aminobutyrate as inherently co-regulated regardless of the challenge with BCAA showing the strongest correlation. BCAA comprise only around 20% of AA nitrogen in dietary protein but make up around 60% of the total plasma AA pool. Changes in plasma BCAA strongly associate with muscle metabolism [3] and comprise up to 40% of the essential amino acids in skeletal muscle [4]. The BCAA are also implicated in energy

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metabolism and insulin signaling [5]. More recently, it was shown that metabolism of BCAA in adipose tissue may play a more important role than anticipated with major changes in obese humans and diabetics [6,7]. With the focus on plasma BCAA we cover therefore important metabolites that relate overall metabolism in anabolic and catabolic states and probably reflect the responses in muscle and adipose tissues. However, the principles of our analysis may also be applied on other metabolites.

2. Methods

In a landmark normality study recently described, a group of 15 healthy young men preselected by homogeneity for BMI, age and other measures underwent a series of anabolic and catabolic challenges [8]. The challenges comprised a 36-h fasting (maximum 2.7 l of water), oral glucose tolerance test (OGTT), oral lipid tolerance test (OLTT), an exercise test (cycling ergometer), and two complex Meals. In total 50 samples were collected per individual, distributed over all the six challenges within 4 days. All details concerning the study design, sample collection, and measurement protocols and apparatus are found in Krug et al. [8].

2.1. Subjects

Volunteers were recruited into the human study center of the Else Kröner-Fresenius Center for Nutritional Medicine (Technische Universität München, Munich, Germany). After medical examination, 15 healthy, young and normal weight men were included into the study. They showed no metabolic abnormalities based on standard clinical chemistry, did not take any medication, and gave their written informed consent. The study protocol was approved by the ethical committee of the Technische Universität München (#2087/08) and corresponds with the Declaration of Helsinki.

2.2. Analysis of plasma amino acids

The quantification of amino acids in plasma samples was performed using a targeted LC–MS/MS approach with iTRAQ (r) (AA45/32TM Phys REAG Kit, Applied Biosystems, USA) as previously described [9]. The preparation of samples was done according to the manufacturer's instructions.

2.3. Statistical analysis

The response data of each challenge for all individuals in the study were collected in a three-way array. The time profiles of all BCAA for each challenge test are stored in a three-way data array with modes $I(i = 1…I)$ for individuals, $M(m = 1…M)$ for amino acids and $T(t = 1…T)$ for time. Note that for the various challenges the T mode varies. For the approach used in this paper, also the M mode of metabolites is allowed to vary although in this application this is not the case. A thorough introduction to multi-way models is given by Smilde, Bro, and Geladi [10]. Adopting their notation, a general three way matrix is denoted as:

$\underline{X}$

with dimension $(I \times M \times T)$, where each element is indicated as

x_{imt}.

In this multi-way array the 15 volunteers form the individual mode, the three BCAA form the metabolite mode, and the different time points form the time mode. Each element of the three-way array represents the levels of a certain metabolite from an individual at a certain time point. The length of the time mode is different for each challenge for example, the OGTT has 8 time points (over 240 min) while the Meal2 challenge only has 4 time points (over 180 min). The individuals in each three-way array are the same. This is also true for the 3 BCAA in the metabolite mode although this is not necessary for the approach applied in this paper.

Before the analysis each three-way array is properly preprocessed to remove overall scale differences over the different metabolites and the different time points. The data is preprocessed by centering over the individual mode I such that every tube (all elements for the same metabolite m and time t) has a zero mean. The M and T modes are then scaled per slab (all elements for a specific metabolite m and all elements for a specific time point t respectively) such that the sum of squares for each slab, after scaling, equals the number of elements. Since the scaling is performed over two modes, the procedure needs to be repeated until convergence [10,11]. The slab scaling is repeated four times, and convergence was confirmed.

As the interest is in differentiating the individuals based on their challenge test response, each three-way array is decomposed using a Tucker3 multi-way model to obtain the positions of individuals, and which metabolites and time points contribute most to this position. The decomposition using the Tucker3 model for 2 components for each mode is as follows.

$$x_{imt} = \sum_{p=1}^{2}\sum_{q=1}^{2}\sum_{r=1}^{2} a_{ip} b_{mq} c_{tr} g_{pqr} + e_{imt}$$

Here the parameters a, b and c are describing the I, M and T modes respectively and e contains the residuals.

The decomposition is performed for each three-way array leaving us with 6 sets of individual positions. Subscripts are added to designate the challenge, such that A_j contains the positioning of individuals after the jth challenge. Next, the challenges are compared using the RV matrix correlation coefficient.

$$RV_{jj'} = \frac{||A_j^T A_{j'}||^2}{\sqrt{||A_j^T A_j||^2 * ||A_{j'}^T A_{j'}||^2}}$$

Since all individual Tucker3 scores are defined to be orthonormal, the RV values can be transformed into Euclidean distances. If the position of two challenges is similar the RV coefficient is close to 1, while unrelated positions will have an RV coefficient close to 0.

A pair wise comparison of all individual positions of the 6 challenges leaves a 6 by 6 RV correlation matrix. We use a classical multidimensional scaling approach to obtain a clear visual interpretation of the RV correlation matrix. In this schematic representation the challenge tests are located close to other challenge tests that differentiate the individuals in a similar way while they are far away from challenge tests that lead to completely different individual positions.

3. Results and discussion

Cross-correlations from 200 plasma metabolites profiled in a time-resolved manner during the different challenges revealed a subset of metabolites comprising the branched chain amino acids (BCAA), phenylalanine and α-aminobutyrate as inherently co-regulated regardless of the challenge with BCAA showing the strongest correlations. Fig. 1 shows their correlations.

In this application we focus on similarities and dissimilarities of the individual position resulting from a challenge. Based on the correlation results shown in Fig. 1 we selected the BCAA group as the metabolite set for the analyses. In total six configurations are obtained from the six challenges considered. The percentages of variation explained by the Tucker3 model for the different challenges range from 65% (Meal) to 87% (OGTT). The loadings of the individual modes A_j are used for assessing the individual inter-challenge correlation.