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Assessing individual metabolic responsiveness to a lipid challenge using a targeted metabolomic approach

Angela M. Zivkovic[1,2,3], **Michelle M. Wiest**[2], **UyenThao Nguyen**[2], **Malin L. Nording**[3], **Steven M. Watkins**[2], and **J. Bruce German**[1,4]

Angela M. Zivkovic: amzivkovic@ucdavis.edu; J. Bruce German: jbgerman@ucdavis.edu

[1] Department of Food Science and Technology, University of California, 1 Shields Avenue, Davis, CA 95616, USA [2] Lipomics Technologies, Inc., West Sacramento, CA 95691, USA [3] Department of Entomology, University of California, Davis, CA 95616, USA [4] Nestlé Research Centre, Lausanne, Switzerland

Abstract

The development of assessment techniques with immediate clinical applicability is a priority for resolving the growing epidemic in metabolic disease. Many imbalances in diet-dependent metabolism are not detectable in the fasted state. Resolving the high inter-individual variability in response to diet requires the development of techniques that can detect metabolic dysfunction at the level of the individual. The intra- and inter-individual variation in lipid metabolism in response to a standardized test meal was determined. Following an overnight fast on three different days, three healthy subjects consumed a test meal containing 40% of their daily calories. Plasma samples were collected at fasting, and 1, 3, 6, and 8 h after the test meal. Plasma fatty acid (FA) concentrations within separated lipid classes and lipoprotein fractions were measured at each time point. The intra-individual variation within each subject across three days was lower than the inter-individual differences among the three subjects for over 50% of metabolites in the triacylglycerol (TG), FA, and phosphatidylcholine (PC) lipid classes at 6 h, and for 25–50% of metabolites across lipid classes at 0, 1, 3, and 8 h. The consistency of response within individuals was visualized by principal component analysis (PCA) and confirmed by ANOVA. Three representative metabolites that discriminated among the three individuals in the apolipoprotein B (ApoB) fraction, TG16:1n7, TG18:2n6, and PC18:3n3, are discussed in detail. The postprandial responses of individuals were unique within metabolites that were individual discriminators (ID) of metabolic phenotype. This study shows that the targeted metabolomic measurement of individual metabolic phenotype in response to a specially formulated lipid challenge is possible even without lead-in periods, dietary and lifestyle control, or intervention over a 3-month period in healthy free-living individuals.

Keywords

Health assessment; Metabolic phenotype; Nutritional phenotype; Lipid metabolism; Postprandial; Response to challenge

1 Introduction

Research is increasingly documenting that humans are different from each other in their responses to diet (Ordovas et al. 2002; Ordovas and Shen 2008). The high degree of variability

Correspondence to: J. Bruce German, jbgerman@ucdavis.edu.

means that dietary and lifestyle solutions must ultimately be targeted to an individual's particular metabolic needs in order to be successful in the long term (German et al. 2003). Although the idea of personalized or individualized approaches for successful disease prevention has been widely recognized (Tegner et al. 2007; Watkins et al. 2001), the question of how to actually bring personalized health to practice is largely unanswered. New tools that can assess health with the same specificity and sensitivity as those that assess disease need to be developed (German et al. 2004; Lemay et al. 2007). In addition to analytical tools that can detect statistically smaller differences in a wider array of metabolites, and the informatic tools to interpret the data both statistically and biologically, new strategies of experimental design for nutritional trials also need to be developed.

One existing approach that accounts for high variability in metabolism in response to different diets and foods is the cross-over interventional study. The gold standard in nutrition research has long been the randomized, placebo-controlled intervention trial, in which individuals are randomized to either the treatment or control groups and the average responses of the two groups are compared. On the other hand, in cross-over studies individuals undergo both the treatment arm and the control arm in randomized order so that each individual serves as their own control, amplifying the statistical power of the study. In order to amplify the signal to noise ratio even further, researchers have combined the cross-over study design with the challenge approach, which examines the response of an individual over several hours following a meal (e.g. the postprandial state).

The importance of response to a challenge meal was first recognized in 1979 (Zilversmit 1979), when the hypothesis was proposed that triglyceride-rich lipoproteins in the postprandial state are acute mediators of atherogenesis. Since then, postprandial lipaemia—the prolonged circulation of lipids in response to a meal—has been found to be an independent predictor of risk for myocardial infarction (Stampfer et al. 1996) and has been found to be increased in patients with cardiovascular disease (Karpe 1997).

The vast majority of cross-over postprandial literature examines the effects of varying the relative fat content and FA composition of meals on postprandial lipaemia and endothelial function (Berry et al. 2007; Blackburn et al. 2003a, b; Cassader et al. 2001; Chong et al. 2007; Chung et al. 2004; Cohn et al. 1993; Fielding et al. 1996; Hennig et al. 2001; Higashi et al. 2001; Hyson et al. 2002; Nicolaiew et al. 1998; Paton et al. 2006; Potts et al. 1995; Rivellese et al. 2008; Sharrett et al. 2001; Siepi et al. 2002; Silveira et al. 1996; van Oostrom et al. 2003; Westphal et al. 2006). However, the challenge approach has been applied to investigate other aspects of responsiveness to meals including the effects of food components such as polyphenols (Papamichael et al. 2008), and lifestyle factors such as exercise (Silvestre et al. 2008; Weiss et al. 2008) and meal frequency (Murphy et al. 1996). Postprandial responsiveness has been used both in a long-term sense to investigate the effects of modulating chronic diet and lifestyle (Fuentes et al. 2008) and in an acute sense to investigate the immediate effects of varying meal composition on postprandial metabolism (Chong et al. 2007).

However, variability in response to diet remains high even with the postprandial cross-over design. For instance, Burge et al. recently found coefficients of variation for plasma TG concentrations and areas under the curve over 6 h in response to a lipid challenge as high as 98% (calculated from data provided in Burdge et al. 2003). This study included 6 similarly healthy, young, white, male subjects, indicating that even within a very narrowly defined group of individuals variation in response to a meal can be very high.

The question that remains to be addressed is whether the observed variation in response is a result of variation in the postprandial biology per se, or discrete and consistent differences among individuals. In other words, is the observed variability a function of true biological

differences between individuals or is it noise attributable to the daily variations in diet and lifestyle within each individual? Is the postprandial response itself an aspect of an individual's metabolic phenotype?

The current study was conducted to determine whether quantitative blood lipid response profiles following a standardized dietary challenge are consistent within healthy, free-living individuals when challenged at intervals of several weeks apart during which time the subjects' diets and lifestyles were explicitly not controlled. The key question addressed is whether the individual variation in metabolic response to the defined dietary challenge within each subject is significantly lower than the variation among the three subjects. Is it possible to distinguish unique metabolic phenotypes among healthy individuals who appear similar to each other by clinical assessment at fasting? Conversely, does biological noise (e.g. weekly and monthly fluctuations of normal metabolism related to changing dietary and other environmental conditions) mask true metabolic phenotype in response to the defined challenge?

A targeted metabolomic approach was undertaken to analyze the structural and energetic lipids comprehensively, including 38 FA within each of seven lipid classes that are distributed across several lipoprotein fractions, totaling 798 lipid metabolic endpoints measured at fasting and at four time points (1, 3, 6, and 8 h) after consumption of a test meal.

2 Materials and methods

2.1 Subjects

Three individuals, designated subject A, B, and C, were recruited from the University of California Davis campus. They were healthy adults aged 18–65 years with the following mean (±SEM) characteristics: age, 37 ± 16 years; weight, 65 ± 4 kg; body mass index (BMI), 21 ± 0.25 kg/m^2; fasting total cholesterol, 150 ± 46 mg/dl (3.89 ± 1.19 mmol/l); fasting TG, 26 ± 18 mg/dl (0.29 ± 0.2 mmol/l); fasting insulin, 9.3 ± 0.85 μIU/ml (64.6 ± 6 pmol/l); and fasting glucose, 86.9 ± 11 mg/dl (4.82 ± 0.6 mmol/l). For individual subject characteristics, refer to Table 1. Exclusion criteria were pregnancy or nursing, any existing medical condition/disease diagnosis, prescription medications, allergy to any of the ingredients in the dietary challenge, BMI > 25, anemia and/or conditions that would influence ability to donate blood safely, and recovery from an illness, injury, or infection in the previous two weeks. The Institutional Review Board of the University of California Davis approved the study protocol, and written informed consent was obtained from all subjects.

2.2 Study design

The subjects were tested on three days separated by several months (once in December, once in March, and once in April). The subjects' diets and other lifestyle factors (including exercise) were explicitly not controlled between test days in order to determine the true variability in lipid metabolism in healthy, free-living individuals maintaining their normal lifestyle. None of the subjects, however, undertook any major lifestyle changes during the course of the study, such as following a weight loss program or particular diet, starting or stopping exercise routines, and starting or stopping any prescription medications. A 24-h dietary recall was collected for the day prior to each dietary challenge. On the day of each study, the subjects arrived at the facility in the morning after an overnight (12-h) fast (no food or beverages except water after 8 pm). Subjects were weighed clothed without shoes, and a fasting blood sample was drawn by venipuncture. The subjects consumed the challenge meal within 20 min.

Postprandial blood draws were collected at 1, 3, 6, and 8 h using BD Vacutainer lavender-top EDTA tubes. Whole blood was centrifuged in a tabletop ultracentrifuge for 10 min at 4°C at 13,000 rpm within 15 min of collection. The plasma was immediately separated into 1.5-ml

aliquots, transported from the clinical facility to the analytical lab on ice, and immediately frozen at −70°C until analysis. Lipid and lipoprotein composition, and glucose, insulin, and lactate were measured at each time point.

2.3 Dietary challenge

The challenge meal was calculated to provide 40% of daily calories using each subject's BMI and activity level on the first of three study days to estimate daily caloric needs. The subjects' weights did not change throughout the study. The challenge meal was provided in the form of a blended beverage containing 1 cup (230 g) non-fat lactose-free milk, 1 cup (227 g) low-fat vanilla-flavored yogurt (1% fat), 30 g 100% whey chocolate-flavored protein powder, 118 g banana, and 22 g flax seed oil. The subjects also consumed 3 g borage oil, 3.6 g soy lecithin, and 3 g fish oil in capsule form with their blended beverage. The entire challenge meal provided approximately 790 calories with 36 g fat (24% by weight or 41% of calories), 38 g protein (25% by weight or 19% of calories), and 79 g carbohydrate (51% by weight or 40% of calories). The lipid composition of the meal was verified by quantitative lipid analysis and is shown in Table 2. The test meal was unique in that it consisted of a high proportion of polyunsaturated fatty acids (PUFA), at 68% of fat calories, and with saturated FA and monounsaturated fatty acids (MUFA) contributing 13 and 19% of fat calories, respectively. Additionally, α-linolenic acid (18:3n3) contributed 48% of total fat calories, which is an unusually high proportion of this FA for the typical American diet. Likewise, the inclusion of lecithin, borage oil, and fish oil provided high amounts of phospholipids, γ-linolenic acid (18:3n6), and the long-chain ω3 FA eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3), relative to the typical American diet.

2.4 Compositional lipid analysis

The plasma samples were analyzed by Lipomics Technologies, Inc. (West Sacramento, CA) as whole plasma, and as an ApoB and an apolipoprotein A (ApoA) fraction. The ApoA and ApoB fractions were separated by precipitation according to the Tung-B method (phosphotungstate/Mg^{2+}) described by Demacker et al. (1997). Briefly, 200 μl of plasma were mixed with 500 μl of precipitation reagent (final concentrations, 1.1 μmol of phosphotungstic acid and 50 mmol of $MgCl_2$ per ml of plasma). After incubation, the supernatant fraction was aspirated with a Pasteur pipette to collect the ApoA fraction, and the pellets were taken as the ApoB fraction.

Compositional lipid analysis was performed according to the method described by Watkins et al. (2002). Briefly, the lipids from plasma (200 μl) were extracted using a modified Folch extraction in chloroform:methanol (2:1 v/v) (Folch et al. 1957). Extracted lipids were separated by preparative HPLC into eight lipid classes—FA, diacylglycerol (DG), TG, free cholesterol, cholesterol ester (CE), lysophosphatidylcholine, phosphatidylcholine (PC), and phosphatidyl-ethanolamine (PE). FA from all lipid classes except free cholesterol were trans-esterified in methanolic HCl. The resulting FA methyl esters were extracted and analyzed by gas chromatography using an Agilent 6890 gas chromatograph (Palo Alto, CA) equipped with a 30-m HP-88 capillary column and a flame-ionization detector. The following FA were measured: 14:0, 15:0, 16:0, 18:0, 20:0, 22:0, 24:0, 14:1n5, 16:1n7, t16:1n7, 18:1n9, t18:1n9, 18:1n7, 18:2n6, t18:2n6, 18:3n6, 18:3n3, 18:4n3, 20:1n9, 20:2n6, 20:3n9, 20:3n6, 20:4n6, 20:3n3, 20:4n3, 20:5n3, 22:1n9, 22:2n6, 22:4n6, 22:5n3, 22:6n3, 24:1n9, and 24:6n3, and the plasmalogen derivatives of 16:0, 18:0, 18:1n9, and 18:1n7.

2.5 Statistical analysis

The data were analyzed as both quantitative (nmol FA/g plasma for FA, and nmol lipid class/g plasma for total lipid classes) and mol% (mol FA as a percentage of mol total FA in the lipid class), and visualization tasks were performed using JMP software (SAS Institute, Inc., Cary,

NC). Separate one-way ANOVA was used to detect significant differences among subjects in FA and lipid class concentrations, as well as to detect intra-individual differences across days. All pair-wise comparisons among subjects were conducted with Tukey 95% simultaneous confidence intervals with an individual confidence level = 97.80%.

2.6 Multivariate analysis

PCA was used to overview clustering among the samples based on the multivariate data expressed as plasma mol% FA in each lipid class, and to visualize the intra- and inter-individual variation in response to the challenge meal. Information from the complete data set was reduced to a few principal components displayed as a set of scores describing maximum variation within the data. The software used for PCA was Simca-P 11.5 (Umetrics, Umeå, Sweden). Before modeling, the variables minus the 6 out of 38 lowest-abundance metabolites were mean centered, scaled to unit variance, and log transformed.

Individual Discriminators (ID) were defined as metabolites that differentiated individuals from each other by way of having an intra-individual variation that was lower than the inter-individual variation, as assessed by one-way ANOVA. The percentage of ID out of all FA measured within each lipid class was calculated and presented for each time point.

3 Results

A targeted metabolomic approach was used to quantify a total of 38 FAs present within each of seven lipid classes (TG, DG, CE, lysophosphatidylcholine, PC, PE, and FA) within whole plasma and two lipoprotein fractions (ApoB, and ApoA), for a total of 798 individual metabolites, at five time points (0, 1, 3, 6, and 8 h) on three days (once each in December, March, and April) in three individuals. The total number of discrete metabolite measurements per individual was 11,970, and the total number of measurements in the study was over 35,000. Many of the key metabolites known to be important indicators of flux through specific key lipid metabolic pathways had low variability within individuals across the three days.

3.1 Variation among individuals was greater than within individuals

The data were first examined with PCA to determine whether the plasma lipid metabolome was visually distinguishable among the three individuals, meaning that the measurements for each individual across days and time points did not overlap with those of the other individuals. The first two components of the model (PC1 and PC2) explained 37% of the variance ($R2 = 0.371$, and $Q2 = 0.261$). When examining fasting clinical measurements only (as shown in Table 1), the individuals were indistinguishable, meaning that their fasting glucose, insulin, cholesterol, and TG concentrations were equally within the clinically accepted normal ranges. However, with multivariate analysis of the plasma lipid metabolome in response to a standardized dietary challenge, it was possible to identify distinct groups in the score plot (Fig. 1), clearly separating the individuals from each other. Inter-individual variation dominated, confirming that the inter-individual variation among the three individuals was higher than the intra-individual variation within each individual across the three days. The score plot clearly shows each individual's postprandial time points (from all three days) grouped together in a distinct, non-overlapping cluster relative to those of the other two individuals. If the intra-individual variation had been higher than the inter-individual variation, clustering of the three days would be expected rather than of the three individuals, with significant overlap among subjects.

The percentage of ID was then calculated in order to determine how many of the metabolites within each lipid class behaved consistently within individuals (i.e. had a significantly lower intra- than inter-individual variation). At baseline, the TG lipid class was composed of over

50% ID, with PC at just over 40%, and CE, PE, and FA around 25% ID (Fig. 2). At 1 h the % ID across lipid classes decreased. At 3 h, CE became the lipid class with the highest proportion of ID, with PC also increasing in the percentage of ID, whereas FA became the lipid class with the lowest proportion of ID. The 6-h time point had the highest signal across lipid classes, with all lipid classes having over 25% ID, and FA, TG, and PC having over 50% ID. Finally, at 8 h PC and TG had the highest proportion of ID, whereas the DG lipid class contained a large amount of variability indicated by no metabolites that were significant ID. These data show that several lipid classes have a high abundance of metabolites that are capable of discriminating between individuals despite the explicit lack of dietary control over the months of the experiment. This suggests that although some metabolites better reflect short-term changes in dietary lipid composition and/or inconsistent metabolic response to the same challenge, a significant proportion of lipids measured are consistent markers of metabolic response and, therefore, are reflective of nutritional phenotype. The 6-h time point was particularly informative and had the highest proportion of ID across lipid classes.

3.2 Response curves were unique to each individual

The data were then examined with statistical approaches one metabolite at a time. Three of the metabolites that were determined to be ID by ANOVA, and also by PCA to be influential in distinguishing the individuals (e.g. they were located in the periphery of the PCA loading plot), were chosen as representative metabolites that were important determinants of the inter-individual variation. Within the TG lipid class, most metabolite response curves were almost identical on each of the three days for each individual. Figure 3 shows the postprandial response curves of each of the three individuals of a key TG metabolite in the ApoB fraction—linoleic acid (18:2n6). The graphs clearly show that the intra-individual variation for each individual at each time point was low, whereas the differences among individuals were significant. The postprandial response curves were unique to each individual and were reproducible over the three days. The *P*-values of the ANOVA for each time point were: 0.0015, 0.0051, 0.0004, <0.0001, and <0.0001 for 0, 1, 3, 6, and 8 h, respectively, confirming that the differences among subjects were significant at each time point. At all time points except 3 h, the intra-individual variation was comparable to the variation observed for the fasted condition. Even at 3 h, the point of maximal signal (i.e. the postprandial lipid peak) and hence maximal variation, the intra-individual variability was sufficiently small to be statistically powerful in discriminating among individuals.

The response curves for all three individuals for another key metabolite—palmitoleic acid (16:1n7)—in the ApoB fraction of the TG lipid class are shown in Fig. 4. Again, the individual response curves for this metabolite clearly varied among individuals, yet the intra-individual variation was low. The patterns mirrored those of linoleic acid shown in Fig. 2—in subject A, the metabolite reached a peak concentration at 3 h and returned to baseline by 6 h; in subject B, the metabolite reached a similar high peak concentration at 3 h, but was only partially cleared by 8 h; and in subject C, the much lower metabolite peak occurred at 1 h and returned to baseline by 6 h. Again, ANOVA confirmed these findings and the *P*-values for each time point were 0.0036, 0.0043, 0.0002, <0.0001, and 0.0001 for 0, 1, 3, 6, and 8 h, respectively.

The observation of high inter-individual differences yet low intra-individual differences in metabolic response was not unique to the TG lipid class. Results for α-linolenic acid (18:3n3) in the PC lipid class in the ApoB fraction are shown in Fig. 5. Again, the pattern of response differed among the three individuals, yet was consistent within individuals. *P*-values from ANOVA again confirmed that the differences were significant for each time point, and were 0.0032, 0.0093, <0.0001, 0.0002, and 0.0026 at 0, 1, 3, 6, and 8 h, respectively.

4 Discussion

The null hypothesis of this study was that fluctuations within individuals would be sufficiently large as to preclude statistical attempts to identify individual postprandial responses as a characteristic of an individual's metabolic phenotype. In opposition to the null hypothesis, multiple metabolic output variables related to variations in overall metabolic health were statistically different, and three representative metabolites were shown. Whereas the variation within individuals across days was low, the differences among subjects were significant. The results show that the examination of postprandial response to a standardized dietary challenge can indeed reveal aspects of diet-dependent metabolic phenotype within individuals, even without specific lead-in periods and dietary monitoring in the days and weeks prior to assessment.

It is well documented that chronic diet affects the levels of lipids and individual FA in the plasma. CE are indicative of dietary fat intake over the past 2 weeks (Katan et al. 1997). The intake of olive oil is correlated with levels of 18:1, dairy is correlated with levels of 15:0 and 17:0, and levels of long chain ω3 FA are indicative of fish intake (Fusconi et al. 2003). The FA profiles of children who are vegetarian, lacto-ovo-vegetarian, or semi-vegetarian (Krajcovicova-Kudlackova et al. 1997a), as well as adult vegetarians (Krajcovicova-Kudlackova et al. 1997b), have higher levels of 18:2n6 and 18:3n3, as well as lower levels of 16:1n7, 20:4n6, and 22:6n3. The consumption of a Mediterranean diet results in higher levels of MUFA and ω3 FA, as well as lower levels of PUFA and ω6 FA (Urquiaga et al. 2004). High pasta and low red meat consumption are correlated with lower levels of saturated FA and higher levels of MUFA (Scaglioni et al. 2004). However, in this study we examined the effects of a particular challenge on the changes in lipid metabolites rather than exploring the differences at fasting between individuals. Although we found that up to 50% of metabolites measured within lipid classes such as TG were able to distinguish among individuals and were thus considered to be discriminators of metabolic phenotype (e.g. were ID), this means that the other 50% of metabolites were, in fact, likely to be more dependent on chronic diet. However, since this study was not designed to distinguish those metabolites that are reflective of chronic diet from those that are not, we did not speculate about the nature of variation in the metabolites that were not found to be ID.

The time points in this study were chosen based on previous studies of postprandial lipid metabolism that are available in the literature (Cohn et al. 1993; Cohn et al. 1989; Li et al. 2003; Lichtenstein et al. 1993; Schaefer et al. 2001); however, the majority of such studies were designed to quantify TG and total cholesterol concentrations. The time points that best reflect the appearance and disappearance of specific FA and, indeed, specific lipid classes and lipoprotein particles may be different. Future studies examining the specific formulation of dietary challenges can now proceed in order to better detect changes in specific metabolic pathways, metabolic disorders, and nutritional phenotypes of interest.

The quantitative analysis of plasma lipid biomarkers in response to a standardized dietary pathway probe has shown to be a powerful tool for the clinical assessment of lipid metabolic status. Not all, but certainly many, of the predominant metabolic diseases associated with Western diets are lipid-related, regardless of whether dietary lipids in and of themselves are the primary cause of the disturbance in metabolism or whether it is other aspects of dietary composition that cause the lipid dysregulation. For example, in certain individuals with non-alcoholic steatohepatitis, the dietary component that causes liver lipid accumulation and its subsequent liver dysfunction is an excess of simple carbohydrates leading to increased de novo lipogenesis (Diraison et al. 2003). A surprisingly high proportion of chronic disease leads to disturbances in lipid metabolism, which ultimately cause the most deleterious health effects associated with a particular disease. For instance, in end-stage renal disease, although up to

20% of patients may need kidney transplant over 5 years, the most immediate health concern is actually the high mortality rate (up to 45% of patients) associated with cardiovascular disease and diabetes, both of which are strongly associated with dyslipidemia (Keith et al. 2004).

The results of the present study are not consistent with a model of normal humans exhibiting a common “normal” postprandial response to a lipid load. In contrast, this study found that the postprandial responses of three healthy individuals were unique and distinguishable from each other, with metabolites that were able to act as ID, or discriminators of individual metabolic phenotype. This was apparent from the unbiased and unsupervised multivariate analysis of the entire dataset through PCA, which clearly illustrated a high degree of clustering within each subject, indicating that intra-individual variation across days was low, whereas inter-individual variation among the three subjects was significant. The statistical analysis of one metabolite at a time applied to three representative metabolites confirmed these findings. Despite being equally normal by metabolic criteria as assessed clinically at fasting, the three individuals nonetheless had distinct postprandial responses to the standardized challenge.

One potential shortcoming of this study is the sample size ($n = 3$). If our aim had been to discover the average human response to this particular nutritional challenge then we should indeed have included a larger number of subjects that is adequately powered to detect the average response. However, in this case, our aim was not to determine the average population response. In fact, we were specifically interested in the unique response of each of the individual subjects to the defined challenge. For this reason we studied the three subjects three times. Future studies will need to determine the optimal number of times that an individual must be studied in order to be confident of their metabolic phenotype in response to a particular challenge, however, from this study we can conclude that three times was adequate in this group of individuals. The responses of individuals to different challenges must also be determined. For example, this particular challenge meal was high in PUFA relative to the typical Western diet, and was delivered in a liquid form. The influence of solid versus liquid meals, variations in FA composition and macronutrient composition, and a vast number of other dietary factors remain to be explored to determine the array of individual responses to meals and how these contribute to the assessment of individual metabolic and nutritional phenotype.

5 Conclusions

The key question addressed in this study was whether the intra-individual variation in the postprandial response to a standard challenge is lower than the inter-individual variation if administered to the same free-living individuals months apart. If so, the process of developing a standardized challenge could become part of normal human assessment as a means to define a discrete postprandial metabolic signal indicative of an individual’s nutritional and metabolic phenotype. Diet has long been thought of as a confounding variable in health assessment, with true metabolic signatures or signals being disguised by temporal fluctuations in diet and lifestyle patterns. In contrast to this long-held belief, the current study found that intra-individual variation in lipid response to a constant challenge was highly reproducible in normal individuals despite an explicit lack of dietary and lifestyle control during the course of the study. This study is a first-step application of standardizing dietary challenges based on comprehensive lipid metabolite measurements taken through the postprandial state. The significance of reproducible and measurable metabolic phenotypes in healthy people is that the data can be used to predict a pattern that may lead to disease, thus making possible early, targeted, and personalized intervention *before* overt disease symptoms appear.

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Abbreviations

FA	Fatty acid
TG	Triacylglycerol
ApoB	Apolipoprotein B
ApoA	Apolipoprotein A
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
CE	Cholesterol ester
DG	Diacylglycerol
PCA	Principal component analysis
ID	Individual discriminators
BMI	Body mass index
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid

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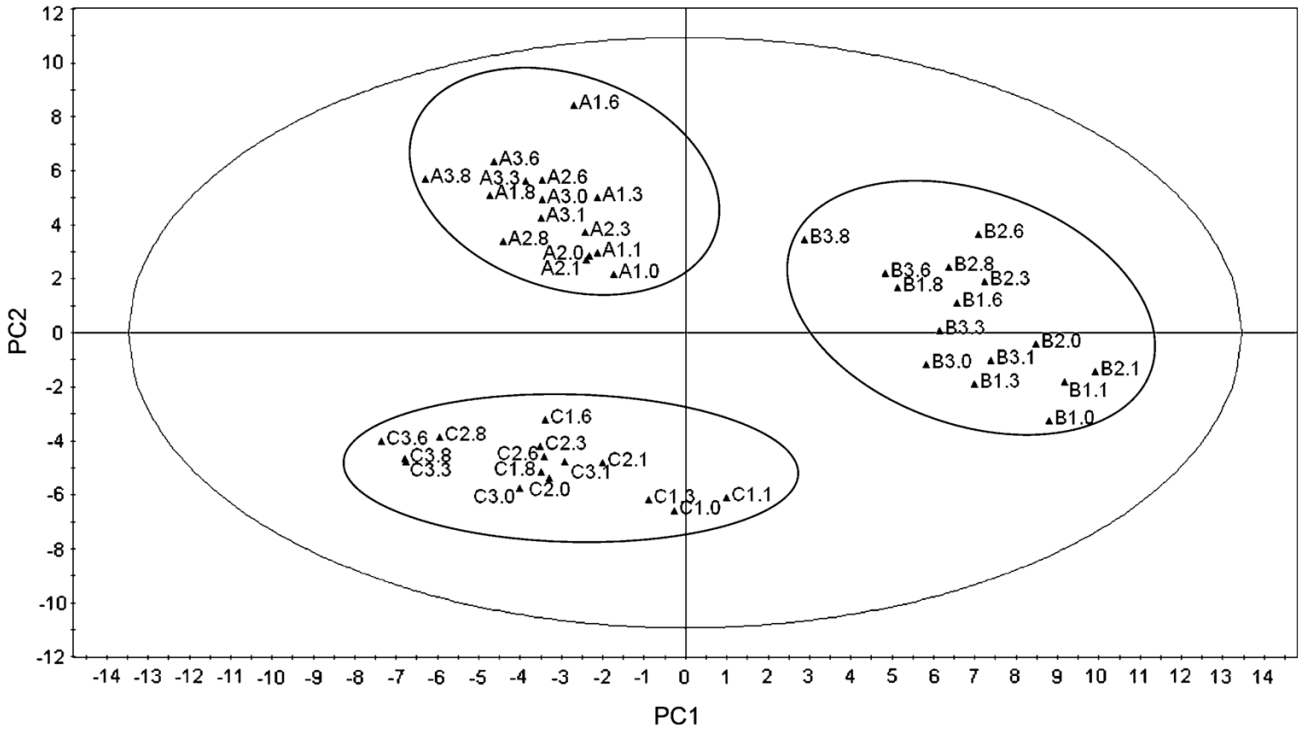

Fig. 1.

Score plot of principal component analysis (PCA) of plasma lipid metabolome. PC1, principal component 1; PC2, principal component 2. Each point/triangle represents a specific time point (0, 1, 3, 6, and 8 h) on a specific day (1, 2, 3) for each subject (A, B, C) such that A2.0 is the 0 h time point on day 2 for subject A, B3.6 is the 6 h time point on day 3 for subject B, C2.1 is the 1 h time point on day 2 for subject C, and so on

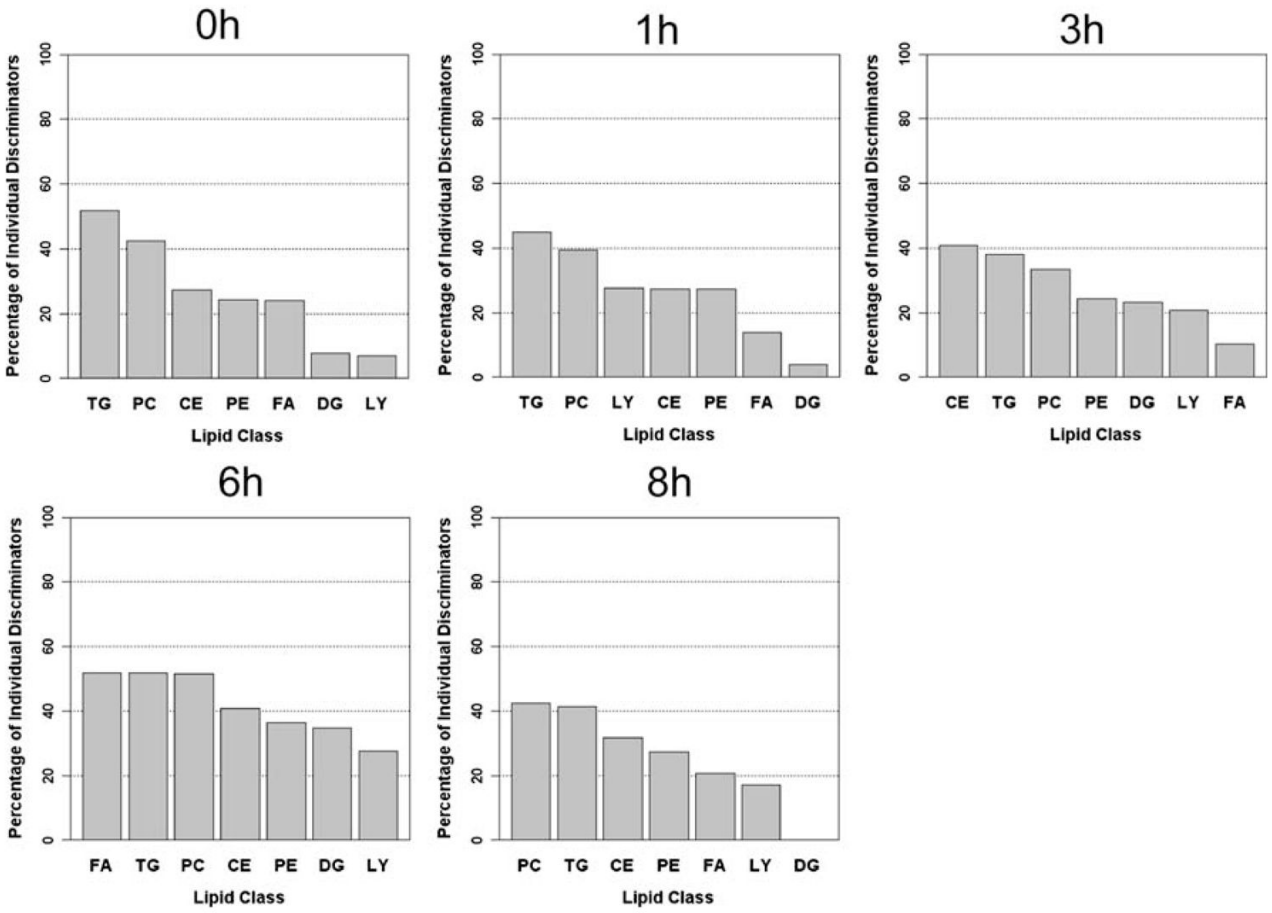

Fig. 2.

Percentage of individual discriminators (ID) in each lipid class. The percentage of ID (metabolites for which the intra-individual variation across three days was lower than the inter-individual variation among subjects as assessed by one-way ANOVA) is shown for each lipid class in descending order for each time point. *TG* triacylglycerol, *CE* cholesterol ester, *DG* diacylglycerol, *FA* free fatty acids, *LY* lysophosphatidylcholine, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine

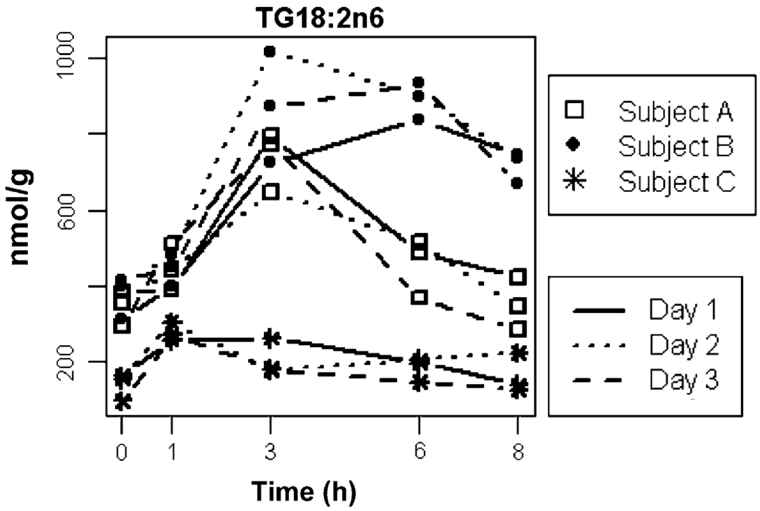

Fig. 3.

Postprandial response curves of 18:2n6 in TG lipid class. The concentrations of 18:2n6TG at each time point are shown in nmol FA/g. The lines connecting the time point measurements represent the 3 different days on which measurements were made

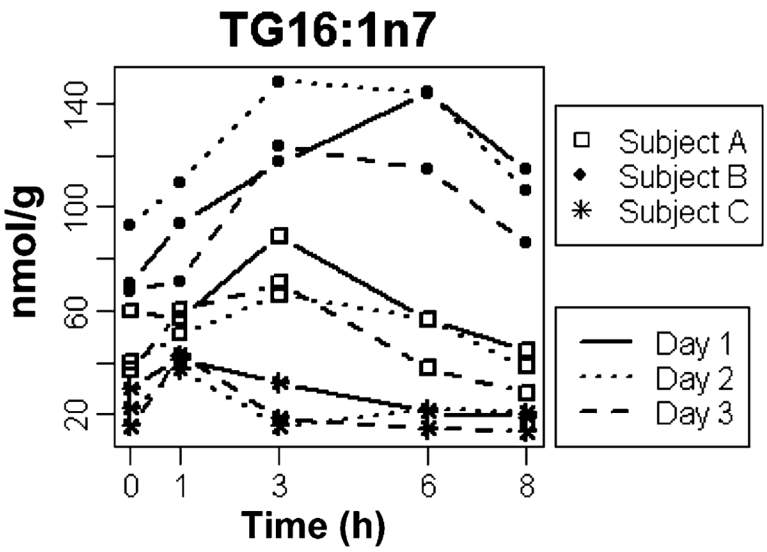

Fig. 4.

Postprandial variation in 16:1n7 in TG lipid class. The concentrations of 16:1n7TG at each time point are shown in nmol FA/g. The lines connecting the time point measurements represent the three different days on which measurements were made

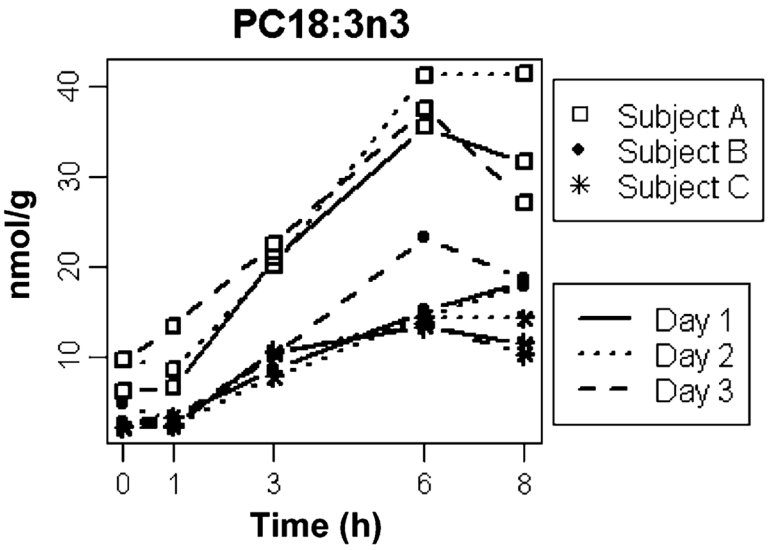

Fig. 5.

Postprandial variation in 18:3n3 in phosphatidylcholine (PC) lipid class. The concentrations of 18:3n3PC at each time point are shown in nmol FA/g. The lines connecting the time point measurements represent the three different days on which measurements were made

Table 1

Subject characteristics

Characteristics	Subject			Normal range
	A	B	C	
Age	30	55	27	
Weight (kg)	63	62	70	
Height (m)	1.73	1.7	1.83	
BMI (kg/m^2)	21	21	21	18.5–24.9
Gender (M/F)	F	M	M	
Fasting TC[a], mg/dl (mmol/l)	140 (3.6)	200 (5.2)	109 (2.80)	≤200 (5.2)
Fasting TG, mg/dl (mmol/l)	67 (0.75)	124 (1.4)	34 (0.38)	≤150 (1.7)
Fasting insulin, μIU/ml (pmol/l)	8.4 (58.3)	9.4 (65.2)	10.1 (70.1)	6.0–27.0 (41.7–187.5)
Fasting glucose, mg/dl (mmol/l)	76.4 (4.24)	99.1 (5.5)	85.2 (4.73)	60–109 (3.33–6.05)

TC total cholesterol

Table 2

Test meal lipid composition

FA	mg	FA	mg	FA	mg	FA	mg
14:0	568.2	18:1n9	5,342.5	20:2n6	51.9	22:6n3	731.8
14:1n5	58.3	18:1n7	272.6	20:3n6	19.0	24:0	37.2
15:0	47.8	t18:2n6	0.0	20:4n6	87.7	24:1n9	51.2
dm16:0	6.5	18:2n6	5,885.0	20:3n3	0.0	24:6n3	0.0
16:0	3,543.3	18:3n6	681.0	20:4n3	66.1	Total	32,900.0
t16:1n7	0.0	18:3n3	13,550.5	20:5n3	1,088.3	SFA	4,307.4
16:1n7	120.2	18:4n3	94.6	22:0	55.0	MUFA	6,135.1
dm18:0	3.1	20:0	56.0	22:1n9	67.8	PUFA	22,447.2
dm18:1n9	0.0	20:1n15	0.0	22:2n6	4.5	n3	15,648.7
dm18:1n7	0.7	20:1n12	0.0	22:4n6	53.7	n6	6,797.2
18:0	0.0	20:1n9	222.4	22:5n6	14.4	n7	392.8
t18:1n9	0.0	20:3n9	1.4	22:5n3	117.3	n9	5,685.4

Lipid composition is shown as mg fatty acid (FA) per test meal (including the blended beverage containing the flax seed oil, and the fish oil, borage oil, and lecithin capsules)

dm plasmalogen, *t trans* double bond, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n3* ω3 fatty acids, *n6* ω6 fatty acids, *n7* ω7 fatty acids, *n9* ω9 fatty acids