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Evaluation of the metabotype concept after intervention with oral glucose tolerance test and dietary fiber-enriched food: An *enable* study

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Abstract *Background and aims:* Evidence suggests that people react differently to the same diet due to inter-individual differences. However, few studies have investigated variation in response to dietary interventions based on individuals' baseline metabolic characteristics. This study aims to examine the differential reaction of metabotype subgroups to an OGTT and a dietary fiber intervention.
Methods and results: We assigned 356 healthy participants of an OGTT sub-study and a 12-week dietary fiber intervention sub-study within the *enable* cluster to three metabotype subgroups previously identified in the KORA F4 study population. To explore the association between plasma glucose level and metabotype subgroups, we used linear mixed models adjusted for age, sex, and physical activity. Individuals in different metabotype subgroups showed differential responses to OGTT. Compared to the healthy metabotype (metabotype 1), participants in intermediate metabotype (metabotype 2) and unfavorable metabotype (metabotype 3) had significantly higher plasma glucose concentrations at 120 min after glucose bolus ($\beta = 7.881$, $p = 0.005$; $\beta = 32.79$, $p < 0.001$, respectively). Additionally, the linear regression model showed that the Area under the curve (AUC) of plasma glucose concentrations was significantly different across the metabotype subgroups. The associations between metabotype subgroups and metabolic parameters among fiber intervention participants remained insignificant in the multivariate-adjusted linear model. However, the metabotype 3 had the highest mean reduction in insulin, cholesterol parameters (TC, LDLc, and non-HDLc), and systolic and diastolic blood pressure at the end of the intervention period.

Abbreviations: BMI, body mass index; HDLc, high-density lipoprotein cholesterol; non-HDLc, non-high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; TC, total cholesterol; hs-CRP, high-sensitive C-reactive protein; KORA, Cooperative Health Research in the Region of Augsburg.

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Conclusion: This study supports the use of the metabotype concept to identify metabolically similar subgroups and to develop targeted dietary interventions at the metabotype subgroup level for the primary prevention of diet-related diseases.

1. Introduction

There is growing evidence that people respond differently to the same diet due to inter-individual variations in genetic, epigenetic, microbiotic, and metabolic phenotypes [1–5]. To address this heterogeneity, the concept of personalized or precision nutrition has emerged [6]. Even though there is no clear definition of personalized nutrition, it is based on providing nutritional advice at the individual or subgroup level instead of general advice to the entire population [6,7]. Recent studies have shown that metabotyping can be a promising tool in the field of personalized nutrition [8–10]. Metabotyping is interpreted as grouping individuals into metabolically similar subgroups (called metabotypes) [8,11]. Metabotyping has proved to be effective in identifying subpopulations for developing targeted dietary advice [12,13]. In addition, metabotyping has also been used previously to identify differential responses to challenge tests and dietary interventions [3].

A challenge test helps investigate the individual's ability to maintain homeostasis when the diet is used as a perturbation agent [14]. Challenge tests, such the as oral glucose tolerance test (OGTT), have been used in clinical and nutritional studies to diagnose diabetes mellitus as well as to study the time-dependent variation of plasma glucose concentrations [15]. It is regarded as a gold standard to investigate the dynamic change in an individual's glucose homeostasis [16]. In the same way, intervention studies are regarded as the gold standard in nutritional studies for establishing a causal relationship between diet and health [17]. Many studies have identified metabolically similar groups based on the differential response to a challenge test [18–22] and dietary interventions [3,23,24]. However, the use of baseline metabolic phenotypes (based on baseline metabolic characteristics) for stratified evaluation of the effect of dietary interventions is limited [13]. To the best of our knowledge, no other study has used metabotypes identified in one study to investigate the response to challenge test or intervention in another independent study population.

In the current analysis, we aim to explore the differential metabolic reaction in study participants from two sub-studies within the *enable* cluster [25]. For this, we assigned the study population into three metabotype subgroups using a metabotype definition previously identified in the KORA F4 study [26] and investigated if participants in different metabotypes subgroup react differently to (i) an OGTT and (ii) dietary fiber supplementation. This approach would allow validating and developing the metabotype concept for broader use in primary disease prevention.

2. Method

2.1. Study population

Data included in this manuscript are from two sub-studies conducted within the *enable* cluster of nutrition research [25]. In the first sub-study, 365 healthy volunteers, including 205 adults aged 40–65 years ("middle agers") and 160 adults aged 75–85 years ("older adults"), were recruited in the *enable* human study centers in Freising and Nuremberg from February 2016 to February 2018. After a screening visit, eligible participants were invited for three consecutive visits 1, 2, and 3. Data for our current analysis are from the first and the second visits. During the first visit, baseline data were collected, including anthropometric and blood parameters, while during the second visit, an OGTT was performed.

From August 2017 to May 2018, the middle agers participants aged 40–65 from the OGTT sub-study were invited again to participate in a fiber intervention study called the Freising Fiber Acceptance study [27]. In a single-blinded (participant-blinded), randomized controlled trial, 108 study participants were assigned to an intervention or a placebo group in a 2:1 ratio. Both arms included the equal proportion of normal weight and elevated waist circumference individuals (>102 cm males and >88 cm females; representing high cardiometabolic risk). Participants of the intervention arm were provided with self-selected fiber-enriched foods for 12 weeks under free-living conditions to increase their fiber intake by 10 g per day, whereas the control group received self-selected complementary foods without fiber enrichment. The complimentary food accounted for roughly one-third of total caloric intake. A detailed description of food items along with fiber types and amounts are described in the recent paper by Brandl et al. [27]. The timeline and different visits of both sub-studies can be seen in supplementary figure S1.

Participants included in both sub studies were non-smoking, community-dwelling Caucasians with body mass index (BMI) of 18.5–30.0 (–35.0) kg/m^2 and free from chronic diseases such as diabetes, hypertension, CVD, cancer, lung, liver, kidney, and other diseases. Individuals who were currently participating in another intervention study or had a blood transfusion or an unintended or intended weight loss of more than 5% in the last 3 months

were excluded. A detailed description of the inclusion and exclusion criteria can be found elsewhere [25,27].

All participants went through comprehensive phenotyping procedures where all measurements and sampling were done by trained professionals [25]. Blood samples from participants were collected after overnight fasting. Standardized questionnaires were used to assess sociodemographic characteristics, health status, lifestyle, and eating behavior. Written informed consent was obtained from all study participants before enrollment and the Good Clinical Practice guidelines were followed. The ethical committee of the Faculty of Medicine of the Technical University of Munich and Friedrich-Alexander- Universität Erlangen-Nürnberg, approved the OGTT study. Whereas the fiber intervention study was approved by the Ethical Committee of the Faculty of Medicine of the Technical University of Munich.

2.2. Parameters

2.2.1. Covariates

For the current analysis, we included the covariates age (years) as a continuous variable, sex (male/female), and physical activity (active/inactive) as a categorical variable. Physically active and very active participants were categorized as "active" whereas less active, almost inactive, and inactive participants were summarized as "inactive". All sociodemographic and lifestyle parameters were collected on the first visit of the OGTT study.

2.2.1.1. OGTT parameters After overnight fasting (12 h), baseline blood samples were collected. Next, a solution of 75 g of glucose in 300 ml of water was given to the participants. Blood samples were drawn at 30, 60, 90, 120, 180, and 240 min after glucose bolus, and glucose levels were determined using HemoCue Glucose 201+ (Ängelholm, Sweden).

2.2.1.2. Fiber parameters and outcome variables All participants were asked to follow their usual diet along with complementary foods and to record the intake daily for the whole study period of 12 weeks. The fiber intake was measured using diet diaries at baseline (visit 1), after four weeks (visit 2), and twelve weeks (visit 3). The energy content and macronutrient composition of diets were determined using OptiDiet Plus software (Version 5.1.2.046, GOE mbH, Linden, Germany) [27]. For the current analysis, we used the data only from the first and third visits.

In the fiber intervention sub-study**,** the metabolic parameters glucose, insulin, high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), total cholesterol (TC), and triglycerides were measured in a blood sample drawn at the fasting state at visits 1 and 3. Similarly, diastolic and systolic blood pressure were measured at all visits. All lipid parameters and insulin were analyzed in a certified lab (SynLab, Munich, Germany), and glucose concentrations were measured using HemoCue Glucose 201+ (Ängelholm, Sweden).

2.2.1.3. Metabotyping parameters We used five biochemical and anthropometric parameters (HDLc, non-HDLc, uric acid, fasting glucose, and Body mass index (BMI)) collected during the OGTT sub-study to assign study participants to the metabotype subgroups as described in detail in the statistical analysis section of this manuscript. The biochemical parameters HDLc, and uric acid were measured in blood serum at the first visit. BMI was measured at the first visit and was used as a continuous variable in kg/m^2. The fasting glucose values were measured by taking the baseline blood sample at the second visit before administering the oral glucose solution. We calculated non-high-density lipoprotein cholesterol (non-HDLc) by subtracting HDLc from TC. A detailed description of the measurement and handling of parameters has been provided previously [25]. As participants in the fiber intervention sub-study are a subpopulation of the OGTT sub-study, metabotype assignment to study participants was done only once.

2.3. Statistical analysis

2.3.1. Data preprocessing

Among 365 participants who received the OGTT, we excluded nine participants as they had missing glucose values at baseline or other time points. Similarly, out of 108 participants in the fiber intervention sub-study, we initially excluded 12 participants as 11 of them were not part of the OGTT sub-study, and one had missing information on blood parameters at visit 3. Since we aimed to examine if the people in different metabotype subgroups have a different reaction in metabolic parameters when intake of fiber increased, we were only interested in the intervention arm; thus, we excluded 29 participants from the placebo group. In summary, our final dataset included 356 participants from the OGTT sub-study, of which 67 participants also participated in the fiber intervention sub-study (Fig. 1).

The metabotypes used in this manuscript were identified previously in the population-based KORA F4 study [26] (submitted to a journal). Briefly, the K-means clustering method was used to identify three metabotype subgroups using five standard clinical parameters (HDLc, non-HDLc, uric acid, fasting glucose, and BMI) measured in serum samples of 3001 study participants. The "metabotype 3" was regarded as an unhealthy metabotype subgroup based on metabolic characteristics such as the highest median concentration of fasting glucose, uric acid, and BMI. In contrast "metabotype 1" showed favorable metabolic characteristics such as highest median HDLc and lowest median fasting glucose, uric acid, BMI, and non-HDLc and was regarded as a healthy metabotype. The metabolic characteristics of, "metabotype 2" was in between two metabotype subgroups and was regarded as an intermediate metabotype subgroup. In the current analysis, we assigned the study participants to these metabotype subgroups by minimizing the Euclidean distance of the z-standardized five clustering parameters (HDLc, non-HDLc, uric acid, fasting glucose, and BMI) to the respective z-standardized cluster centers of these parameters.

The outliers of the outcome parameters from both OGTT as well as fiber intervention sub-studies were identified

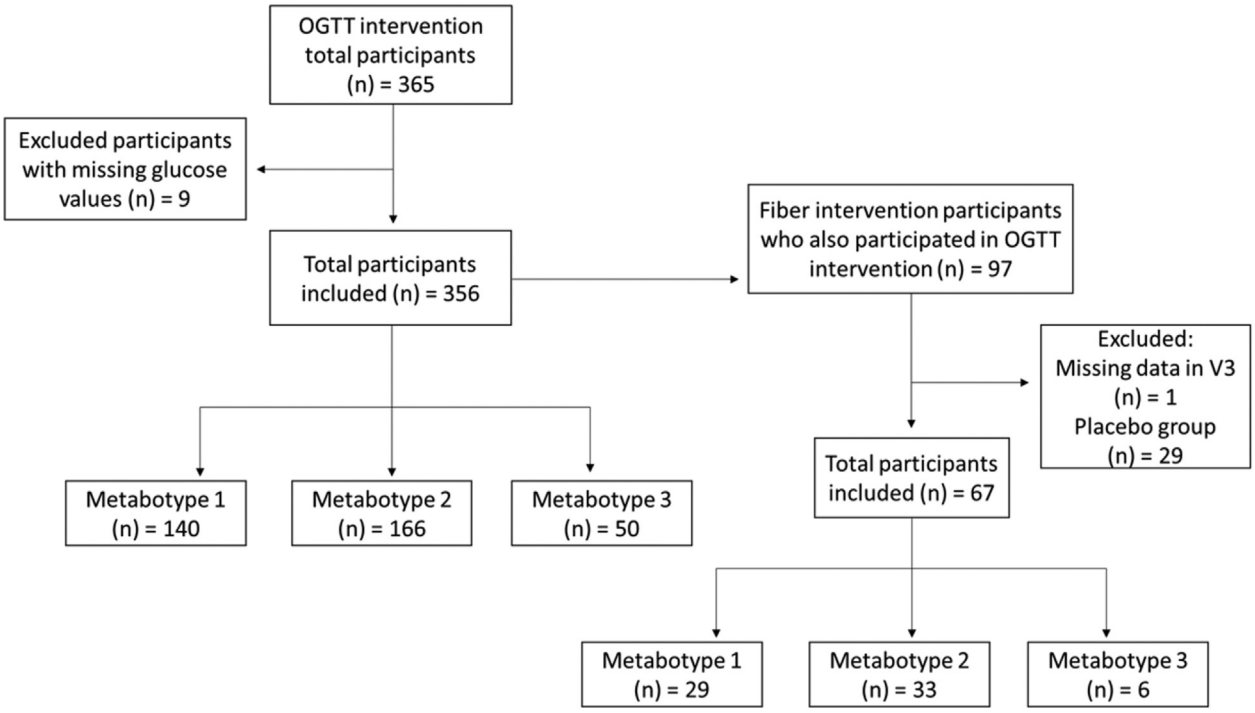

Figure 1 Study flow diagram.

using the 'boxplot' function in R. The identified outliers were converted into missing values and were imputed along with missing data originally present in the datasets. The Multivariate Imputation by Chained Equations 'mice' package [28] in R was used to impute datasets which generated five complete data sets with ten iterations each.

2.3.2. Descriptive statistics

We described the baseline characteristics of both study populations in total and stratified by metabotypes. Mean and standard deviation (SD) were used for continuous variables and absolute frequency and percentage for categorical variables. We analyzed the differences in the distribution between metabotypes in the OGTT study population using the non-parametric Kruskal-Wallis-Test for continuous variables and Pearson's chi-square test for categorical variables. In the case of the fiber intervention study population, we did not analyze the difference as only six participants were assigned to metabotype subgroup 3.

2.3.3. Regression

In the framework of the oral glucose challenge, we used two different models to assess the effect of the metabotype on the change of plasma glucose values. In the first model, we used a linear mixed model where the outcome variable was glucose values and fixed effects were metabotype and time of measurement. The model was further adjusted for age, sex, and physical activity. In the second model, we used a linear regression model where the outcome variable was the baseline adjusted standardized Area under the curve (AUC) of plasma glucose values and the main effect was metabotypes. Similar to the mixed model, this model was also adjusted for age, sex, and physical activity. To calculate the AUC of every individual, first we subtracted the baseline glucose value from every repeated glucose measurement. Then we used the 'AUC' function from the 'metrumrg' package in R to calculate the AUC. After that, we standardized the each AUC by subtracting it from the mean and then dividing it by the standard deviation. As a sub-analysis, we stratified the study population into middle agers (40–65 years) and older adults (75–85 years) and repeated both models with the same outcome and adjustment variables.

Regarding the fiber intervention sub-study, we used linear regression models to investigate the association between metabotype and metabolic parameters when the intake of dietary fiber increased. For this purpose, we analyzed ten different models with the outcome variables assessing the change in metabolic parameters, namely Δ glucose, Δ insulin, Δ TC, Δ LDLc, Δ HDL cholesterol, Δ Non-HDL cholesterol, Δ triglycerides, Δ hs-CRP, Δ systolic blood pressure, and Δ diastolic blood pressure, where Δ represents the difference of the value at visit 3 (after 12 weeks of intervention) to visit 1 (before the start of the intervention). The main variables of interest in the models were changes in fiber intake (continuous variable, difference between intake at visit 3 and visit1) and metabotype (where metabotype 1 was regarded as the reference category). The models were adjusted for age, sex, and physical activity. Due to the low number of subjects in the metabotype subgroups, we reported the confidence

intervals instead of the p-values to show the uncertainty of estimates.

The regression models were performed in all five imputed datasets and the final results were pooled using the 'testEstimates' function for linear mixed models and the 'pool' function for linear regression models. All statistical analyses were done using the statistical software R version 4.0.3 and RStudio Version 1.1.423.

3. Results

Table 1 presents the baseline characteristics of study participants undergoing the OGTT stratified by three metabotype subgroups. 56% of participants were middle agers with a mean age of 52.5 ± 7.0 years, and 44% were older adults with a mean age of 78.1 ± 2.7 years. The proportion of male and female participants included in the study was almost equal. Most of the participants were physically active (66.6%) and had a mean BMI of 26.5 ± 4.0 kg/m^2.

Out of 356 participants, 39% were assigned to metabotype 1, 47% to metabotype 2, and 14% to metabotype 3. Participants in the metabotype 3 subgroup had the highest mean age (65.5 ± 12.6 years), highest BMI (30.6 ± 3.2 kg/m^2), and were more often males (80%). In contrast, participants in metabotype 1 had the lowest mean BMI (23.52 ± 3.19 kg/m^2), were more physically active (79%) and were more often females (74%). The distribution of metabolic parameters across different metabotype subgroups were in accordance with the original metabotype definition, where individuals in metabotype 3 subgroup had the highest mean value of uric acid (7.12 ± 1.44 mg/dl), glucose (110.52 ± 9.58 mg/dl), and had the lowest mean concentration of HDLc (49.64 ± 11.19 mg/dl). Similarly, the participants in metabotype 1 had the lowest mean concentration of metabolic parameters like non-HDLc, uric acid, and glucose.

Fig. 2 shows the distribution of glucose values at different time points across the three metabotype subgroups and stratified by the middle agers and older adults. Metabotype 3 had the highest response to the oral glucose bolus concentration at all measured time points with peak glucose value reaching at 60 min. Metabotype 2 had the intermediate response whereas metabotype 1 had the least response to the oral glucose bolus. However, except at 60 and 90 min, both metabotype 1 and 2 had almost similar glucose values. In the case of older adults, metabotype 2 and 3 had similar glucose at all measured time points.

We saw similar results in the linear mixed model, which examined the association between plasma glucose values and different metabotype subgroups when adjusted for time, sex, age, and physical activity (Table 2). Compared to metabotype 1, which was regarded as the reference category, both metabotypes 2 and 3 were significantly

Table 1 Baseline characteristics of the total study population and by three metabotype subgroups.

	Total (N = 356)	Metabotype 1 (N = 140)	Metabotype 2 (N = 166)	Metabotype 3 (N = 50)	P value
Age (years)					
Mean (SD)	63.75 (13.85)	64.16 (14.48)	62.87 (13.67)	65.54 (12.64)	
Middle agers (40–65 years)	52.52 (6.95)	51.26 (7.11)	52.74 (6.75)	55.04 (6.75)	0.447
Older adults (75–85 years)	78.09 (2.72)	78.02 (2.75)	78.24 (2.68)	77.86 (2.78)	0.049
Missing	1 (0.3%)	1 (0.7%)	0 (0%)	0 (0%)	0.824
Age category					0.318
Middle agers (40–65 years)	199 (56.05%)	72 (51.79%)	100 (60.24%)	27 (54.00%)	
Older adults (75–85 years)	156 (43.94%)	67 (48.20%)	66 (39.75%)	23 (46.00%)	
Missing	1 (0.3%)	1 (0.7%)	0 (0%)	0 (0%)	
Sex					<0.001
Male	177 (49.71%)	37 (26.42%)	100 (60.24%)	40 (80.00%)	
Female	179 (50.28%)	103 (73.57%)	66 (39.75%)	10 (20.00%)	
Smoking					0.516
Never smokers	196 (55.06%)	77 (55.00%)	95 (57.23%)	24 (48.00%)	
Ex-smokers	160 (44.94%)	63 (45.00%)	71 (42.77%)	26 (52.00%)	
Physical activity					<0.001
Active	237 (66.57%)	110 (78.57%)	100 (60.24%)	27 (54.00%)	
Inactive	119 (33.42%)	30 (21.43%)	66 (39.75%)	23 (46.00%)	
BMI (kg/m^2)					<0.001
Mean (SD)	26.50 (4.03)	23.52 (3.19)	27.78 (3.01)	30.63 (3.15)	
HDL (mg/dl)					<0.001
Mean (SD)	61.67 (16.82)	75.46 (15.47)	53.68 (10.01)	49.64 (11.19)	
Non − HDL (mg/dl)					<0.001
Mean (SD)	160.72 (39.39)	144.59 (36.77)	177.56 (34.93)	149.98 (38.45)	
Uric acid (mg/dl)					<0.001
Mean (SD)	5.71 (1.41)	4.63 (0.90)	6.19 (1.08)	7.12 (1.44)	
Glucose (mg/dl)					<0.001
Mean (SD)	94.23 (10.86)	90.96 (8.59)	92.08 (8.40)	110.52 (9.58)	

Mean (SD) for continuous variables and n (column %) for categorical variables; P values are from the Kruskal-Wallis-Test for continuous variables and Pearson's chi-squared test for categorical variables.

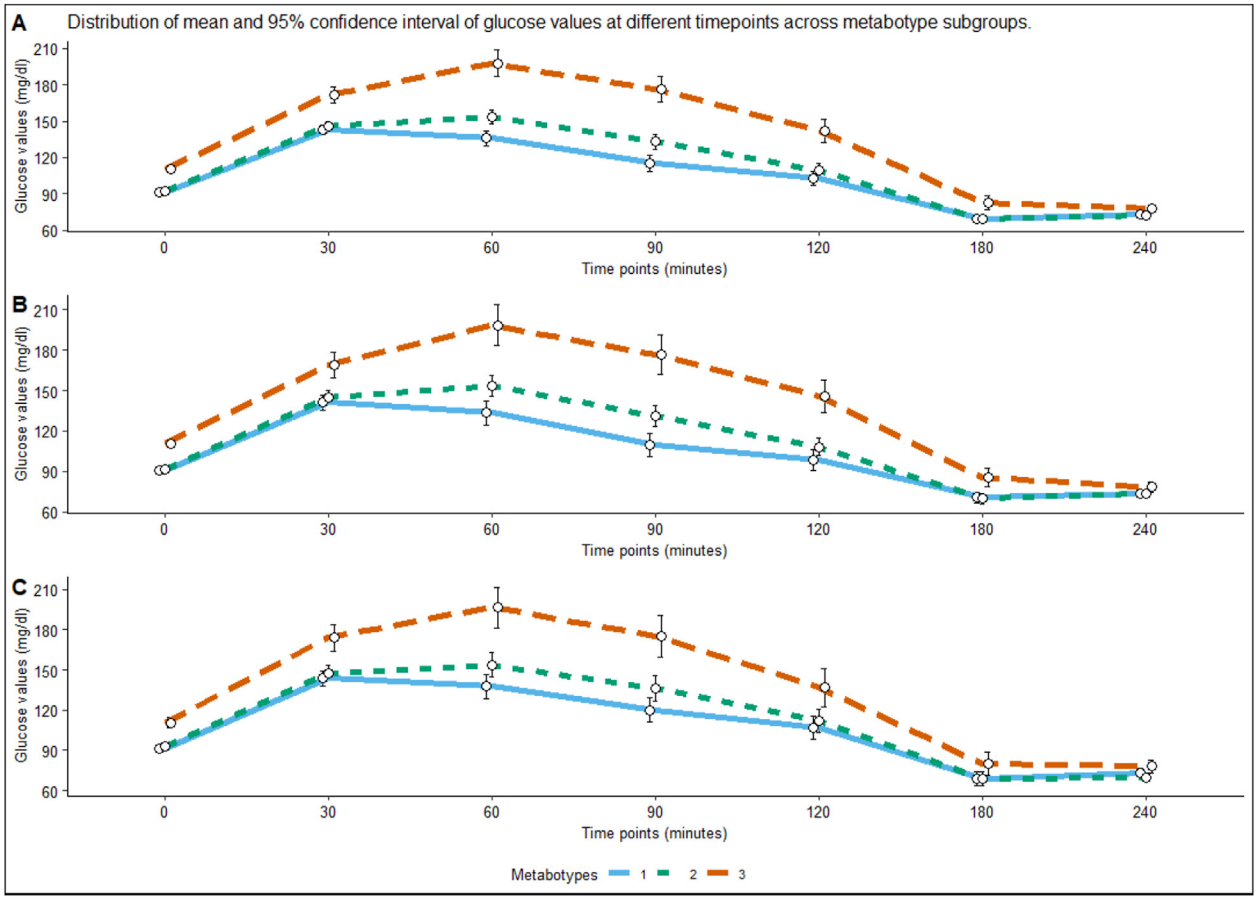

Figure 2 Plasma glucose values at different time points after oral glucose bolus (OGTT) across three metabotype subgroups in total population (A) and stratified by middle-agers (40–65 years) (B) and older adults (75–85 years) (C). The mean and confidence interval is from the original dataset and is not adjusted for covariates.

associated with plasma glucose values. When we stratified the models by age group, in the middle agers, only metabotype 3 had a significant effect. However, in older adults a significant effect of both metabotype groups 2 and 3 could be identified. Using a linear regression model, which examined the association between standardized AUC of plasma glucose values and metabotype groups adjusted for sex, age, and physical activity (Table 3), we confirmed the results of the mixed model. Similar to the linear mixed model, compared to the reference category (metabotype 1), metabotypes 2 and 3 were significantly associated with the standardized AUC. When stratifying the analysis by age groups only metabotype 3 had a significant effect.

The characteristics of participants in the fiber intervention study are presented in Table 4. The mean age of the study population was 53.3 ± 6.7 years. Almost 57% of participants were physically active with a mean BMI of 27.3 ± 4.2. Among all 67 participants, 29 participants (43.2%) were assigned to metabotype 1, 32 participants (47.7%) to metabotype 2, and only 6 participants (8.9%) to metabotype 3. Mean daily fiber consumption among all 67 participants at visit 1 was 22.2 ± 7.8 g/d. At the end of the intervention phase (after 12 weeks), the average intake of fiber was 36.0 ± 8.8 g/d with a mean increase of 13.8 ± 9.6 g/d. The highest increase in fiber intake (17.6 ± 12 g/d) was seen in metabotype 3 participants resulting in mean consumption of 37.8 g per day.

After 12 weeks, subjects in metabotype 3 still had the highest level of glucose, insulin, hs-CRP, and systolic blood pressure and the lowest HDLc concentration (Fig. 3). However, when looking at the difference between visit 1 and visit 3, the six subjects attributed to metabotype 3 showed the highest mean decrease in insulin, cholesterol parameters (TC, LDLc, and non-LDLc) as well as systolic and diastolic blood pressure values (Table 5).

Table 6 shows the results of the linear regression models that examined the association of metabolic parameters and metabotype subgroups when fiber intake was increased. No statistically significant association between change in outcome variables (metabolic parameters) and change in fiber intake was obtained. Regarding the metabotype subgroups, we could identify the significant association between metabotype 2 and Δ HDL only.

4. Discussion

In our analysis, we investigated the differential response of subjects attributed to three metabotype subgroups to an OGTT and a dietary fiber intervention. Compared to other

Table 2 Association between metabotypes subgroups and change in plasma glucose concentrations after OGTT in the total sample and stratified by age groups.

	Estimate	CI [Lower, Upper]	P value
All participants			
Metabotype 1	Reference category		
Metabotype 2	7.88	[2.43, 13.33]	**0.005**
Metabotype 3	32.79	[24.90, 40.68]	**< 0.001**
Baseline glucose value	Reference category		
Glucose value at 30 min	53.74	[50.66, 56.83]	**< 0.001**
Glucose value at 60 min	58.28	[55.19, 61.38]	**< 0.001**
Glucose value at 90 min	37.01	[33.91, 40.11]	**< 0.001**
Glucose value at 120 min	16.03	[12.95, 19.13]	**< 0.001**
Middle agers (40–65 years)			
Metabotype 1	Reference category		
Metabotype 2	6.67	[-0.76, 14.11]	0.078
Metabotype 3	31.74	[21.18, 42.32]	**< 0.001**
Baseline glucose value	Reference category		
Glucose value at 30 min	53.38	[49.15, 57.61]	**< 0.001**
Glucose value at 60 min	58.49	[54.25, 62.73]	**< 0.001**
Glucose value at 90 min	34.81	[30.58, 39.05]	**< 0.001**
Glucose value at 120 min	14.67	[10.44, 18.91]	**< 0.001**
Older adults (75–85 years)			
Metabotype 1	Reference category		
Metabotype 2	8.81	[0.55, 17.08]	**0.037**
Metabotype 3	33.41	[21.12, 45.71]	**< 0.001**
Baseline glucose value	Reference category		
Glucose value at 30 min	54.20	[49.71, 58.71]	**< 0.001**
Glucose value at 60 min	58.02	[53.49, 62.55]	**< 0.001**
Glucose value at 90 min	39.81	[35.25, 44.38]	**< 0.001**
Glucose value at 120 min	17.77	[13.24, 22.30]	**< 0.001**

Estimate and P values are obtained by calculating means of estimates and p values of linear mixed models over all five imputed datasets. All models were adjusted for age, sex, and physical activity. Significant P values (<0.05) are represented in bold.

subgroups, the subjects in the unfavorable metabotype subgroup showed a significantly higher plasma glucose response to OGTT. When a sub-population of the OGTT sub-study was subjected to a 12-week fiber intervention sub-study, no statistically significant effects of fiber intervention and metabotype subgroups were seen in metabolic parameters when adjusted for age, sex, and physical activities. However, the participants in unfavorable metabotype had the highest mean decrease in insulin, lipid parameters (TC, LDLc, and Non-LDLc), and blood pressure parameters.

We assigned participants in our current analysis to known metabotypes developed in the population-based KORA F4 study by minimizing the Euclidean distances. The metabolic characteristics were in line with the original metabotype definition. This result demonstrates that metabotypes used in the current study are transferable i.e. individuals in an independent cohort could be assigned easily to existing metabotypes without grouping separately by cluster analysis [29].

Previous studies have used challenge tests to identify metabolically similar subgroups based on the metabolic response after mixed meal tolerance test [20], fructose meal challenge [22], and paired meal challenge tests with high and low glycemic index meals [18]. Similarly, Morris et al. [19] identified four different metabotype subgroups based on the glucose response to OGTT. In contrast, in this study, we used predefined metabotypes to investigate the differential response to OGTT. However, our results are in accordance with the results from Morris et al. [19] where the most unfavorable metabotype subgroup showed unfavorable characteristics like highest BMI and age, highest glucose peak, and highest glucose values at all measured time points including baseline and 120 minutes. Studies have shown that glucose pattern and time to peak after OGTT are predictors of cardiometabolic diseases. Lin et al. [30] found that a longer time to peak glucose during the OGTT was associated with a higher Framingham 10-year risk score and a higher prevalence of type 2 diabetes (T2D) among individuals with impaired fasting glucose. Thus, the highest response to OGTT shown by the unfavorable subgroup in our current analysis proves that metabotyping can be used to identify high-risk subpopulations.

Similar to previous studies [31,32], we also found a decrease in insulin, TC, LDLc, Non-HDLc, and triglyceride from visit 1 to visit 3 in the total study population when dietary fiber intake was increased. The highest decrease was observed in the unfavorable metabotype subgroup, metabotype 3. However, in the linear regression model, we did not obtain statistically significant results for both, fiber intake and metabotype subgroups, for most of the metabolic parameters. This is likely due to the very limited sample size in the fiber intervention study, with only six participants in the metabotype 3 subgroup. Due to this reason, we also could not investigate the interaction

Table 3 Association between metabotypes subgroups and standardized Area under the curve (AUC) of plasma glucose concentrations after OGTT in the total sample and stratified by age groups.

	Estimate	CI [Lower, Upper]	P value
All participants			
Metabotype 1	Reference category		
Metabotype 2	0.31	[0.07, 0.54]	**0.008**
Metabotype 3	0.74	[0.41, 1.08]	**< 0.001**
Middle agers (40–65 years)			
Metabotype 1	Reference category		
Metabotype 2	0.27	[-0.05, 0.60]	0.103
Metabotype 3	0.70	[0.22, 1.17]	**0.004**
Older adults (75–85 years)			
Metabotype 1	Reference category		
Metabotype 2	0.32	[-0.004, 0.65]	0.052
Metabotype 3	0.76	[0.27, 1.25]	**< 0.001**

The standardized AUC is calculated using the trapezoid function and standardizing the outcome. The Estimate and P values are obtained by calculating means of estimates and p values of the linear regression model over all five imputed datasets. All models were adjusted for age, sex, and physical activity. Significant P values (<0.05) are represented in bold.

between fiber and metabotype subgroups. However, rather large negative estimates for fiber and metabotype effects in regression models with outcome variables LDLc, HDLc, non-HDLc, and TC suggest that with a larger sample size, significant results could have been obtained.

Other studies have also used the metabotyping concept to examine the effect of the dietary intervention [23,24,33]. An intervention study by O'Sullivan et al. [23] found no significant effect of Vitamin D supplementation on metabolic parameters. However, when the population was stratified into five clusters, one of the clusters characterized by lower serum 25(OH)D showed a significant decrease in insulin, homeostatic model assessment scores, and C-reactive protein. Likewise, in our previous analyses of the KORA FF4 cohort, we found that a significant association between dietary pattern and T2D detected in all participants remained significant only in the unfavorable subgroups when the analysis was stratified by metabotype [34]. Together, these studies, along with our results, demonstrate that the metabotyping concept can be used to identify metabolically similar subpopulations, which may benefit from a targeted dietary intervention.

Most of the studies so far have investigated the variation in response using either challenge tests or dietary interventions. Furthermore, they have identified metabolically similar subgroups using the same study population. To our knowledge, this is the first study using previously identified metabotypes (i.e., defined in an independent study) to investigate the differential response to both, an OGTT and a dietary fiber intervention. Moreover, metabotypes used in our study are based on only five routinely measured parameters, which makes it cost-effective and easy to reproduce in another study population. The use of OGTT, regarded as the gold standard to measure plasma glucose reaction after glucose bolus, underlines our findings. Another strength includes the use of a real-world setting to conduct the fiber intervention study where fiber-enriched foods were provided using everyday foods. Participants were allowed to select the referred foods themselves, which might have increased their adherence to the intervention. However, as only healthy individuals were included in the study, there is also a possibility that the participants were health-conscious and motivated to improve their health behaviors. As healthy individuals tend to have a better capacity to maintain homeostasis [35] the intervention of 12 weeks might have been too short to detect any effect of fiber intake. Also, due to the inclusion of healthy subjects, only

Table 4 Baseline characteristics and metabolic parameters in participants of the fiber intervention study in the total sample and by three metabotype subgroups.

	Total (N = 67)	Metabotype 1 (N = 29)	Metabotype 2 (N = 32)	Metabotype 3 (N = 6)
Age (years)	53.30 (6.72)	52.20 (7.38)	53.70 (5.84)	**56.83 (7.52)**
Sex				
Male	33 (49.30%)	7 (24.10%)	**22 (68.80%)**	4 (66.67%)
Female	34 (50.70%)	**21 (75.90%)**	10 (31.20%)	2 (33.33%)
BMI (kg/m2)	27.30 (4.21)	25.10 (4.43)	28.60 (3.14)	**30.82 (2.91)**
Smoking				
Never smoker	32 (47.80%)	14 (48.30%)	**16 (50.00%)**	2 (33.33%)
Ex-smoker	35 (52.20%)	15 (51.70%)	**16 (50.00%)**	4 (66.67%)
Physical activity				
Active	38 (56.70%)	**18 (62.10%)**	17 (53.10%)	3 (50.00%)
Inactive	29 (43.30%)	11 (37.90%)	**15 (46.90%)**	3 (50.00%)
Visit 1 Fiber (g/d)	22.20 (7.86)	**22.70 (10.10)**	22.30 (6.02)	20.20 (4.63)
Missing	1 (1.50%)	1 (3.40%)	0 (0.00%)	0 (0.00%)
Visit 3 Fiber (g/d)	36.00 (8.77)	35.40 (9.33)	36.20 (7.83)	**37.79 (11.70)**
Missing	2 (3.00%)	0 (0.00%)	2 (6.20%)	0 (0.00%)
Fiber change (visit 3- visit 1)	13.80 (9.58)	12.90 (10.40)	13.90 (8.33)	**17.60 (12.00)**
Missing	3 (4.50%)	1 (3.40%)	2 (6.20%)	0 (0.00%)

Continues values are presented as mean (SD) and categorical variables are presented as n (column %). The highest values are represented in bold.

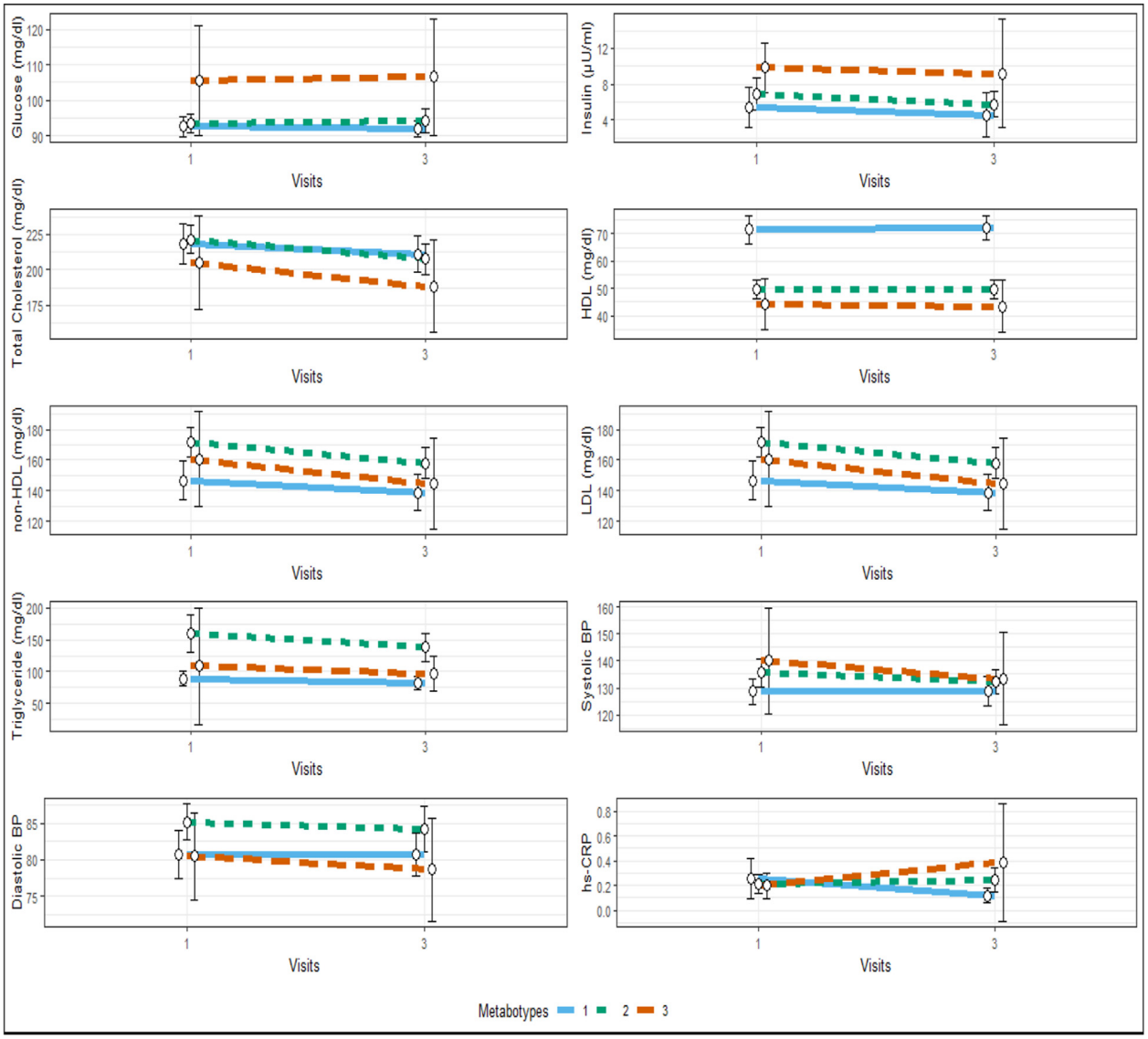

Figure. 3 Distribution of mean and 95% confidence interval of different outcome parameters at visit 1 and visit 3 (after 3 weeks of fiber intervention) across three metabotype subgroups. The mean and confidence interval is from the original dataset and is not adjusted for covariates.

Table 5 Mean change in metabolic parameters after 12 weeks of fiber intervention across three metabotype subgroups.

Parameters	Total (N = 67)	Metabotype 1 (N = 29) Mean (SD)	Metabotype 2 (N = 32)	Metabotype 3 (N = 6)	P value
Δ Glucose	0.02 (7.33)	**−0.51 (6.81)**	0.33 (7.82)	1.00 (8.24)	0.76
Δ Insulin	−0.94 (2.17)	−0.83 (1.92)	−1.00 (2.22)	**−1.19 (3.28)**	0.77
Δ TC	−11.31 (19.36)	−7.27 (22.47)	−13.93 (16.36)	**−16.83 (16.94)**	0.15
Δ LDLc	−2.21 (16.27)	1.48 (16.74)	−4.59 (15.89)	**−7.33 (14.51)**	0.20
Δ HDLc	0.07(7.11)	0.28 (8.72)	0.06 (5.44)	**−0.83 (7.54)**	0.86
Δ Non-HDLc	−11.46 (16.95)	−7.72 (18.22)	−14.00 (16.04)	**−16.00 (13.88)**	0.10
Δ Triglyceride	−10.88 (29.34)	−4.86 (22.99)	**−16.75 (32.55)**	−8.66 (36.64)	0.23
Δ Systolic BP	−1.64 (8.41)	0.07 (7.49)	- 2.84 (8.80)	**−3.53 (10.42)**	0.34
Δ Diastolic BP	−0.70 (6.79)	−0.06 (5.76)	−1.06 (7.23)	**−1.83 (9.68)**	0.88
Δ hs-CRP	−0.01 (0.06)	**−0.02 (0.05)**	−0.01 (0.27)	0.01 (0.06)	0.53

The mean (SD) were obtained from the means of mean and SD calculated by subtracting values at visit1 from values at visit 3 done overall five imputed datasets. P values are the means of P values from the Kruskal-Wallis-Test done overall five imputed datasets. The values in the table are not adjusted. The highest reduction in metabolic parameters is marked in bold.

Table 6 Association between metabotypes subgroups and change in metabolic parameters among 12-week fiber intervention participants.

Models		Intervention effect		Metabotype effect (versus metabotype 1)			
		Δ Fiber intake		Metabotype 2		Metabotype 3	
		Estimate	CI [Lower, Upper]	Estimate	CI [Lower, Upper]	Estimate	CI [Lower, Upper]
1	Δ Glucose	0.06	[-0.15, 0.27]	0.16	[-4.21, 4.53]	1.27	[-5.99, 8.54]
2	Δ Insulin	−0.01	[-0.07, 0.05]	−0.51	[-1.78, 0.76]	−0.45	[-2.58, 1.68]
3	Δ TC	−0.39	[-1.01, 0.22]	−5.11	[-16.63, 6.41]	−5.38	[-24.54, 7.30]
4	Δ LDLc	−0.34	[-0.85, 0.16]	−6.20	[-15.78, 3.37]	−8.61	[-24.54, 7.30]
5	Δ HDLc	−0.06	[-0.29, 0.17]	−4.95	**[-9.82, -0.09]**	−6.82	[-14.36, 0.71]
6	Δ non-HDLc	−0.31	[-0.82, 0.19]	−5.46	[-15.59, 4.66]	−5.70	[-22.54, 11.13]
7	Δ Triglyceride	0.18	[-0.69, 1.04]	−13.02	[-30.62, 4.57]	−4.21	[-35.53, 27.10]
8	Δ Systolic BP	0.11	[-0.12, 0.36]	−4.98	[-9.97, 0.02]	−6.17	[-14.48, 2.14]
9	Δ Diastolic BP	0.15	[-0.05, 0.36]	−1.05	[-5.12, 3.02]	−2.24	[-9.02, 4.53]
10	Δ hs-CRP	0.001	[-0.001, 0.002]	0.02	[-0.01, 0.06]	0.04	[-0.02, 0.11]

Δ represent the difference of parameters value at follow-up (visit 3) to the baseline value (visit 1). Estimate and 95% Confidence interval (CI) were obtained from means of estimates and confidence intervals from the linear regression models done over all five imputed datasets. All models were adjusted for age, sex, and physical activity. The significant value is marked in bold.

six participants were eventually attributed to metabotype 3. In addition, the use of strict inclusion criteria has made the results less transferable to the general population. Therefore, the use of a large population-based study sample with a longer intervention period might help to detect the true effects of a fiber intervention in the different metabotype subgroups [32].

In conclusion, we showed that participants in different metabotype subgroups have a differential response to OGTT. Findings from this study support the use of the metabotyping concept to explore the inter-individual variation to diet. The successful replication of metabotypes identified in a different study population further enhances the validity of the metabotype concept applied in this study. The development of targeted dietary advice to subjects in metabotype subgroups may help to deliver personalized or stratified nutritional advice to prevent cardiometabolic diseases in the future.

Declaration of competing interest

The authors of this manuscript declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2022.06.007.

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