TITLE: Metabotyping and its role in nutrition research

Short title: Metabotyping and personalised nutrition

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Abbreviations: BMI, body mass index; HDL-c, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; HSFAM, high-saturated fatty acid meal; IGF-1, insulin-like growth factor-1; IGF-BP3, insulin-like growth factor-binding protein 3; IGF-BP2, insulin-like growth factor-binding protein 2; MetS, metabolic syndrome; MMM, mixed Mediterranean-type meal; MMTT, mixed meal tolerance test; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; RCT, randomised controlled trial; TAG, triacylglycerol; TC, total cholesterol.

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Abstract

Personalised nutrition is at its simplest form the delivery of dietary advice at an individual level. Incorporating response to different diets has resulted in the concept of precision nutrition. Harnessing the metabolic phenotype to identify subgroups of individuals that respond differentially to dietary interventions is becoming a reality. More specifically, the classification of individuals in subgroups according to their metabolic profile is defined as metabotyping and this approach has been employed to successfully identify differential response to dietary interventions. Furthermore, the approach has been expanded to develop a framework for the delivery of targeted nutrition. This review examines the application of the metabotype approach in nutrition research with a focus on developing personalised nutrition. Application of metabotyping in longitudinal studies demonstrates that metabotypes can be associated with cardiometabolic risk factors and diet-related diseases while application in interventions can identify metabotypes with differential responses. In general, there is strong evidence that metabolic phenotyping is a promising strategy to identify groups at risk and to potentially improve health promotion at a population level. Future work should verify if targeted nutrition can change behaviours and have an impact on health outcomes.

Introduction

Poor diet quality is a major contributor to chronic diseases such as type 2 diabetes, cardiovascular diseases and various cancers[1,2]. Despite the well-known association between dietary patterns and diseases, interventions to change dietary habits have had a limited impact on wellbeing and public health outcomes[3,4]. In recent years, the diverse inter-individual responses to interventions have become apparent and support the need for the development of strategies that are based upon the delivery of advice to the individual[5-9]. Concomitant with this, different strategies have emerged for delivering advice taking personal characteristics into account. Furthermore, studies have demonstrated that personalisation of dietary advice is more effective in promoting improvements in the dietary habits of individuals compared to the general healthy eating advice[10-12].

Metabolomics is the study of small molecules in biological samples and is a powerful tool in the characterisation of individuals[13,14]. The set of metabolites in the human body, termed metabolome, is the product of metabolic reactions influenced by endogenous, lifestyle, and environmental factors[15,16]. Applications of metabolomics in nutrition research expanded in recent years and it has the potential to contribute to the delivery of personalised nutrition[17]. Metabotypes are defined as groups of similar individuals based on combinations of specific metabolites. Thus, individuals within a metabotype have similar metabolic profiles and those in different metabotypes have different profiles[17,18] (Figure 1). Metabotypes are often defined using cluster analysis, such as *k*-means analysis and hierarchical cluster analysis[18]. Application of metabotypes has identified differential response to interventions and have the potential of identifying optimal treatment strategies for individuals. For example, using serum metabolites Palau-Rodriguez *et al.*[19] identified two subgroups with different degrees of improvement in insulin resistance, total cholesterol (TC), low-density lipoprotein cholesterol (HDL-c) and uric acid following bariatric surgery. Importantly the metabolic changes in each cluster were independent of the baseline anthropometric/clinical parameters of the patients and the magnitude of weight loss. Another example identified metabotypes with different lipid responses to fenofibrate[20]. Similarly, in the field of nutrition science there are several examples of applications of metabotypes in healthy and subjects with chronic diseases for determining metabolically homogeneous subgroups with differential responses to dietary interventions[18]. However, the applications are not limited to intervention studies with the metabotyping approach being developed for the delivery of targeted nutrition[21,22]. Given the rapid growth of this area, the objective is to review the research conducted on metabotypes related to nutrition research and to identify gaps where further work is needed.

Metabolic phenotyping of longitudinal data to examine associations with cardiometabolic risk factors and diet-related diseases

Longitudinal studies are important tools in the epidemiological setting to investigate the aetiology of a disorder and indicate risk factors or population groups that may be targeted as part of prevention strategies. In fact, within the metabolic phenotype approach, longitudinal studies offer the possibility to study subgroups of individuals (metabotypes) over a period of time and the potential to identify those at higher risk of disease development. A summary of studies examining longitudinal associations of metabotypes to cardiometabolic risk factors and diet-related diseases is presented in Table 1.

In order to identify risk profiles for the emergence of metabolic syndrome (MetS), Ventura *et al.*[23] assessed a nonclinical sample of healthy non-Hispanic white girls (n = 154) in a retrospective analysis with follow-up performed every two years from five to 13 years old. Six risk factors for MetS (waist circumference, systolic blood pressure, diastolic blood pressure, HDL-c, triacylglycerol (TAG), and blood glucose) were used in cluster analysis to determine metabotypes at age 13. At age five, the higher MetS risk group had the highest body mass index (BMI) relative to the other groups. Across childhood, both the higher MetS risk and the hypertension risk groups had significantly greater increases in weight and fat mass, while the higher MetS risk group had the highest daily sweetened beverage intake. Findings from this study support the role of metabotypes for identifying people at higher risk who could be targeted by clinicians as part of preventive healthcare.

Application of metabotypes to baseline data in longitudinal studies can be very useful in defining at-risk groups which could be targeted for prevention of undesirable health outcomes. The European Childhood Obesity Project (CHOP), using a Bayesian agglomerative clustering method on 21 plasma amino acids and 146 polar lipids, classified healthy infants (n = 154) of six months of age into 20 metabotypes in order to predict later obesity risk[24]. Only the four biggest clusters ($n \geq 14$) were analysed and at the baseline cluster 3 had the lowest weight, height, insulin-like growth factor-1 (IGF-1) free, and insulin-like growth factor-binding protein 3 (IGF-BP3), and the highest insulin-like growth factor-binding protein 2 (IGF-BP2). The BMI z-score at six years of age tended to differ (unadjusted $p = 0.07$) among clusters, with cluster 3 presenting the highest median and largest proportion of overweight/obese children. These results support the concept that even very young individuals can be clustered according to their inter-individual differences so that the clusters provide insight into later development and health and opportunities for developing more targeted and personalised intervention strategies.

Another notable example employing metabotypes in a prospective cohort is the KORA F4 Study in which 1,729 adults aged 32 to 77 years were clustered based on BMI and 33 biochemical markers[25]. For each of the three metabotypes identified, the current disease prevalence and the incidence in the follow-up cohort seven years later was determined. The "high-risk" cluster showed the most unfavourable biomarker profile with the highest BMI and prevalence of cardiometabolic diseases at the baseline as well as the highest incidence of hypertension, type 2 diabetes, hyperuricemia/gout, dyslipidaemia, all metabolic, and all cardiovascular diseases together. This study provides strong evidence that metabotyping is a robust approach for identifying groups of individuals that could be targeted for prevention strategies.

Overall, the derivation of metabotypes in longitudinal studies to predict cardiometabolic risk factors and diet-related diseases is nascent. However, replication of the metabotypes in other populations is a necessary next step. Notwithstanding this, the presented studies make a strong case for the metabotype approach and highlight its potential in identifying groups that could benefit from targeted dietary advice.

Metabolic phenotyping to investigate differential responses to dietary challenges and interventions

Differential responses to dietary interventions are becoming increasingly recognised. Concomitantly, metabolic phenotyping has emerged as a useful tool to examine responses to interventions. In the context of nutrition, health can be defined as the ability of an organism to adapt to challenges[26]. Challenge tests investigate the disturbance and restoration of homeostasis of an individual using a dietary challenge as a physiological stressor[27]. In combination with metabolomics, dietary challenges have been used to identify groups of subjects with distinct metabolic phenotypes/metabotypes and unique responses. Table 2 illustrates studies which focus specifically on differential responses of metabotypes to dietary challenges and intervention studies.

Krishnan *et al.*[28] investigated the differential responses of metabotypes to dietary challenges. The authors used low and high glycaemic index meals in a crossover randomised trial with healthy overweight women (n = 24, 20 to 50 years old) to identify response patterns that could provide insight into early subclinical glycaemic disruption. By using blood glucose, insulin, and leptin responses to the challenges, individuals were clustered into three metabotypes. While the most populated metabotype presented little deviation from the expected response to the dietary challenges, the two minor metabotypes were suggestive one of sub-clinical insulin resistance and the other of hyperleptinemia. In the Metabolic Challenge (MECHE) Study, healthy subjects (n = 214, 18 to 60 years old) were randomised to one of three groups to receive oral glucose tolerance

tests (OGTTs) and/or oral lipid tolerance tests (OLTTs) and four metabotypes were identified based on their blood glucose response curves to the OGTT (n=116)[29]. The cluster with the most adverse metabolic profile at baseline presented a reduced β-cell function and differential responses to insulin and c-peptide during OGTT and OLTT, as well as to glucose and TAG during the OLTT, which characterises this metabotype as at risk. The postprandial metabolic responses to different kinds of bread - refined rye bread, whole-meal rye bread, and a control refined wheat bread - were investigated in a crossover randomised controlled trial (RCT) with healthy postmenopausal women (n = 19, 61 ± 4.8 years)[30]. The clustering of the fasting metabolic profile identified two distinct metabotypes. Women with higher fasting concentrations of leucine and isoleucine and lower fasting concentrations of sphingomyelins and phosphatidylcholines had higher insulin responses despite similar glucose concentrations after all kinds of bread, suggesting higher insulin resistance. In a recent study with data from the NutriTech project, the response to the intervention was only evident following the classification of the individuals into metabotypes[26]. Healthy subjects (n = 72, 59 to 64 years old) were enrolled to a mixed meal tolerance test (MMTT) before and after 12 weeks targeting moderate weight loss (basal BMI 29.7 ± 2.7 kg/m^2). The intervention group (n = 40) consumed a diet that reduced caloric intake by 20%, whereas subjects in the control group (n = 32) consumed an average European diet matched to their energy expenditure to maintain body weight. Two metabotypes were reported based on the plasma concentration of metabolites (markers of lipolysis, fatty acid β-oxidation, and ketogenesis) during the mixed meal challenge test. Before the intervention, individuals from metabotype B (n = 36) showed slower glucose clearance, increased visceral fat volume, higher hepatic lipid concentrations, and a less healthy dietary pattern according to the urinary metabolomic profile when compared to individuals from metabotype A. Following the weight loss (~5.6 kg), only the individuals from metabotype B showed positive changes in the glycaemic response to the MMTT. Since the metabolite differences found between metabotypes A and B are all closely associated with insulin signalling, the metabotype B was considered to be prediabetic with a modestly impaired insulin action. Collectively, all these studies clearly demonstrate that the use of a metabotype approach in conjunction with meal challenges has the ability to characterise individuals into meaningful subgroups which could receive targeted nutrition advice to lower the individual disease risk [30].

In contrast to other studies that used the responses to challenges to form clusters, Lacroix *et al.*[31] used only fasting metabolic data in a crossover RCT designed to evaluate the metabolic and vascular effect of a high-saturated fatty acid meal (HSFAM) and a mixed Mediterranean-type meal (MMM). Age, BMI, glycaemic and lipid parameters were used to cluster healthy men (n = 28, 18 to 50 years old) into two metabotypes at baseline. Compared to the healthiest group, the less healthy group showed significantly higher BMI, insulin, and homeostatic model assessment for insulin

resistance (HOMA-IR), in addition to less favourable lipid profile and a lower intake of fruit and vegetables (dietary pattern score = 5.1 ± 1.7 *vs* 3.9 ± 1.4). Following the meal challenges, the less healthy group experienced a greater significant increase in triacylglycerols with MMM and endothelial dysfunction with HSFAM, in comparison to the healthier group. The MMM did not significantly alter postprandial endothelial function in both groups. The authors concluded that the less healthy group would benefit even more from consuming meals representative of a Mediterranean-type diet given its nondeleterious endothelial properties, indicating the potential of cluster techniques to individualise dietary advice.

Application of the metabotype approach has also encompassed dietary interventions that did not involve meal challenges. Wang *et al.*[32] in a controlled crossover study with healthy subjects (n = 23, 36 to 69 years old) identified groups of individuals with differing plasma carotenoids response to carotenoid-rich beverages. Following three weeks of daily intake of watermelon juice (20 mg lycopene, 2.5 mg β-carotene, n=23; 40 mg lycopene, 5 mg β-carotene, n=12) or tomato juice (18 mg lycopene, 0.6 mg β-carotene, n=10), cluster analysis applied to weekly carotenoid responses identified groups of individuals with differential responses. This, in turn, was used to classify individuals as strong responders or weak responders to the carotenoid intake. These findings demonstrate that subgroups of individuals can have differential responses to interventions which could be harnessed in the future to give more precise dietary advice. With respect to employing a metabotype approach for dietary interventions in clinical populations or disease risk factors, two studies are noteworthy. In a sample of high-risk cardiovascular subjects (n = 57, ≥55 years old) a four-week crossover RCT identified differential responsiveness to red wine polyphenol[33]. At baseline, fasting blood and urinary metabolites and anthropometric parameters were used to cluster individuals in four metabotypes, including a higher risk cluster and a healthier cluster. Following 28 days of dealcoholized red wine intake (polyphenol content = 733 equivalents of gallic acid/day), concentrations of urinary 4-hydroxyphenylacetate significantly increased in the healthier cluster compared to the higher risk cluster, indicating a differential response in this cluster. In a double-blind four-weeks RCT with healthy subjects (n = 135, 18 to 63 years), the effect of vitamin D supplementation (15 mg vitamin D_3 per day) to improve markers of the metabolic syndrome was only visible after the classification of the sample into metabotypes[34]. The vitamin D supplementation significantly increased the serum 25-hydroxyvitamin D in comparison to the placebo group, but there was no effect of supplementation on the measured markers of the metabolic syndrome. Based on 13 fasting blood biomarkers, one cluster characterised by low concentrations of vitamin D and higher concentrations of adipokines showed a significant decrease in insulin, HOMA-IR scores, and c-reactive protein and inverse relationship between the change in serum vitamin D and glucose. Collectively, these examples clearly present how comprehensive

phenotyping may identify subgroups of individuals that can benefit from specific dietary interventions.

The metabotype approach represents a tool through which we can start to understand individual responses to interventions. The ultimate goal will be to harness this information to deliver personalised nutrition.

Harnessing the metabotype approach to deliver targeted nutrition

To the best of our knowledge, there are only two published examples of a framework for the delivery of personalised nutrition using a metabotype approach (Table 3).

In 2015, O'Donovan *et al.*[22] proposed a framework based on metabotyping using four commonly measured fasting markers of metabolic health (TAG, TC, HDL-c, and glucose). Application of the approach in 875 adults resulted in 3 metabotypes. Individuals in cluster 1 (n = 274) had high TC concentrations, individuals in cluster 2 (n = 423) had adequate concentrations of all four biomarkers, and individuals in cluster 3 (n = 178) had the most unfavourable metabolic profile with high concentrations of TAG, TC and glucose and the lowest concentration of HDL-c. Targeted dietary advice was developed for each metabotype incorporating characteristics of the metabotype and personal traits. In order to test the reliability of the approach to deliver personalised dietary advice, the targeted approach was compared with an individual-based approach manually compiled and delivered by a dietician for a random sample of participants (n = 99). An excellent agreement of 89% (range 20 - 100%) was found between the methods, considering the dietary advice given with the targeted approach in relation to those given with the individual-based approach. The most important strength of this study is the fact that for clustering individuals only four biomarkers of metabolic health routinely measured were used. Furthermore, the approach generated a limited number of decision trees with simple and clear messages which allow the automation of the delivery of personalised dietary advice to individuals who are not high priority dietetic patients or where the access to a dietician is limited. All these features make the proposed framework easily transferable to a clinical or primary care setting.

Development of this approach for a more diverse population was achieved in proof of concept format with data from seven European countries[35]. Twenty-seven fasting metabolic markers measured in finger-prick blood samples, including cholesterol, individual fatty acids and carotenoids, were clustered into three metabotypes. Individuals in cluster 1 (n = 326) had the highest TC and circulating trans-fatty acids and the lowest omega-3 index and was therefore considered the metabolically unhealthy cluster. Cluster 2 (n = 433) was labelled the healthy group as individuals in this metabotype had the highest average omega-3 index and total carotenoid

concentrations and the lowest total saturated fatty acids. Individuals in cluster 3 (n = 595) had the lowest average TC and highest levels of stearic fatty acid. Decision trees with targeted dietary advice were developed on the metabolic markers (total cholesterol, total saturated fatty acids, omega-3 index, and carotenoids), demographics, and five key nutrients (salt, iron, calcium, folate, and fibre). The targeted approach was compared to the messages delivered by nutritionists as part of the Food4Me study (n = 180) to participants receiving personalised dietary advice. An average match of 82% at the level of delivery of the same dietary message was found and the agreement was also good by cluster, with an average match of 83% for cluster 1, 74% for cluster 2 and 88% for cluster 3. These results, obtained in a European population from seven countries with diverse cultures and dietary intakes, confirm the metabotype approach as a robust approach to the delivery of targeted dietary advice and its applicability in different populations.

Conclusions and future directions

While metabotyping emerged initially to distinguish individuals with and without diet-related diseases, it has rapidly developed to identify those at metabolic risk and interrogate response to dietary interventions. With a heightened interested in inter-individual variation in response to interventions, the approach presents an unbiased method of identifying differential responses. The ultimate goals will be to harness the approach for the delivery of personalised nutrition. However, further work is needed in understanding the biological mechanisms underlying the differential responses. We need detailed studies examining the underlying biology responsible for the different metabotypes and deciphering the role of genetics and the microbiome will be important future steps. Building this evidence base will be important for the further development of the metabotype concepts.

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The framework comprising the metabotypes and decision trees represents a model for the delivery of personalised nutrition. However, there is a paucity of data demonstrating the impact of such approach on metabolic health parameters. Future studies are warranted to demonstrate that the approach is effective in changing behaviours and health outcomes.

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Conflict of Interest

None

Authorship

EH and LB contributed to the conception and design of the review, EH drafted the manuscript, and EH and LB edited the manuscript.

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Table 1. Summary of studies examining longitudinal associations of metabotypes to cardiometabolic risk factors and diet-related diseases.

Author	Objective	Study design	Study sample	Follow-up period	Variables and method for clustering	Main findings
Ventura *et al.*[23]	Describe risk profiles for metabolic syndrome during adolescence.	Retrospective longitudinal study	154 nonclinical white girls at 13-year-old in the USA	Every 2 years by 8 years	6 risk factors for metabolic syndrome (waist circumference, SBP, DBP, and fasting HDL-c, TAG, and glucose) clustered by mixture model.	Four metabotypes. At age 13, the higher metabolic syndrome risk group and the hypertension risk group had more family history of type 2 diabetes and obesity. Across childhood, the higher metabolic syndrome risk group and the hypertension risk group had greater increases in BMI and fat mass, as well as the former had the higher intake of sweetened beverages; a dyslipidaemia risk group had the lowest physical activity.
Kirchberg *et al.*[24]	Identify predictive metabotypes for childhood obesity.	Prospective longitudinal study	154 healthy, singleton, term, and breastfed infants aged 6-months in the Childhood Obesity Project (CHOP) trial in Europe	6 years	21 fasting plasma amino acids, sum of hexoses and 146 polar lipids (free carnitine, 40 acylcarnitines, 11 lyso PCs, 91 PCs, and 14 sphingomyelins) clustered by Bayesian agglomerative method.	Twenty metabotypes. Only the four biggest clusters ($n \geq 14$) were analysed and at 6 months of age cluster 3 had the lowest weight, height, IGF-1 free, and IGF-BP3, and the highest IGF-BP2. The BMI z-score at 6 years of age tended to differ (unadjusted $p = 0.07$) among clusters, with cluster 3 presenting the highest median and large proportion of overweight/obese children.
Riedl *et al.*[25]	Define metabotypes of diet-related diseases.	Prospective longitudinal study	1729 adults aged 32-77 years in the population-based KORA F4 study in Germany.	7 years	BMI and 33 fasting biochemical parameters clustered by *k*-means cluster analysis.	Three metabotypes. At the baseline, cluster 3 showed the most unfavourable marker profile with the highest prevalence of cardiometabolic diseases. After the follow-up, disease incidence was higher in cluster 3 compared to clusters 2 and 1, respectively, for hypertension (41.2%, 25.3%, 18.2%), type 2 diabetes (28.3%, 5.1%, 2.0%), hyperuricemia/gout (10.8%, 2.3%, 0.7%), dyslipidaemia (19.2%, 18.3%, 5.6%), all metabolic (54.5%, 36.8%, 19.7%), and all cardiovascular (6.3%, 5.5%, 2.3%) diseases together.

SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; TAG, triacylglycerol; BMI, body mass index; PCs, phosphatidylcholines; IGF-1, insulin-like growth factor 1; IGF-BP3, insulin-like growth factor-binding protein 3; IGF-BP2, insulin-like growth factor-binding protein 2.

Table 2. Summary of studies investigating differential responses of metabotypes to meal challenges and dietary interventions.

Author	Objective	Study design	Study sample	Dietary challenge(s)	Intervention	Variables and method for clustering	Main findings
Fiamoncini *et al.*[26]	Investigate the metabolic response of metabotypes to an MMTT before and after weight loss.	Metabolic challenge before and after a 12 weeks RCT	70 healthy subjects (based on fasting glucose, insulin, and blood pressure) aged 59-64 years in NutriTech Study in Europe	Mixed-meal tolerance test (400 ml of high-calorie drink with 33% carbohydrates, 59 lipids, and 8% protein).	Control group: European diet for weight stability. Intervention group: supervised diet for weight loss	Response concentrations of plasma markers of lipolysis, fatty acid β-oxidation, and ketogenesis clustered by HCA.	Two metabotypes. At baseline, metabotype B had slower glucose clearance, increased intra-abdominal adipose tissue mass, higher hepatic lipid concentrations, and less healthy dietary pattern than metabotype A. Following the weight loss (~5.6 kg), only metabotype B showed positive changes in the glycaemic response to the MMTT, with improvements in metabolites of amino acid, acylcarnitines, and biochemical parameters.
Krishnan *et al.*[28]	Identify metabotypes of response to meals with different GI.	Metabolic challenge in a crossover randomised trial	24 healthy pre-menopausal women aged 20-50 years in the USA	High GI and low GI meals preceded by a 3-days run-in diet matching the GI of the tested meal.	Not tested	Response concentrations of blood glucose, insulin, and leptin clustered by PCA.	Three metabotypes. The two minor groups were one suggestive of sub-clinical insulin resistance and the other of hyperleptinemia.
Morris *et al.*[29]	Identify metabotypes of response to an OGTT.	Metabolic challenge in a randomised trial	116 healthy subjects aged 18-60 years in the Metabolic Challenge (MECHE) Study in Ireland	75g OGTT or an OLTT (54g of lipids and 12g of carbohydrates)	Not tested	Response curves of blood glucose to OGTT clustered by mixed-model	Four metabotypes. Cluster 1 was at risk with the highest BMI, TAG, hsCRP, c-peptide, insulin, and HOMA- IR and the lowest VO_2max. Cluster 1 had a reduced β-cell function and differential responses to insulin and c-peptide during OGTT and to insulin, glucose, and TAG during OLTT.

Table 2. *Continued*

Author	Objective	Study design	Study sample	Dietary challenge(s)	Intervention	Variables and method for clustering	Main findings
Moazzami *et al.*[30]	Investigate the metabolic response of metabotypes to different types of bread.	Metabolic challenge in a crossover RCT	19 healthy post-menopausal women (61 ± 4.8 years) in Finland	Refined wheat, whole-meal rye, and refined rye breads, providing 50g of carbohydrate.	Not tested	189 fasting metabolites (21 amino acids, 17 biogenic amines, 47 acylcarnitines, 38 PCs, 39 acyl-alkyl PCs, 14 lyso PCs, 15 sphingomyelins, and 1 hexose) clustered by O-PLS, HCA, and PCA.	Two metabotypes. Subgroup B, with the lower fasting concentrations of sphingomyelins and diacyl-PCs and the higher concentrations of BCAA had the higher insulin responses to all kinds of bread, despite similar glucose response to metabotype A, suggesting higher insulin resistance.
Lacroix *et al.*[31]	Evaluate the endothelial and metabolic response of metabotypes to complete meals.	Metabolic challenge in a crossover RCT	28 healthy men aged 18-50 years in Canada	High-saturated fatty acid meal (HSFAM) and mixed Mediterranean-type meal (MMM).	Not tested	Age, BMI, HOMA-IR, and fasting glucose, insulin, TC, LDL-c, HDL-c, and TAG clustered by HCA.	Two metabotypes. Group 1 had a higher BMI, HOMA-IR, and fasting insulin, TC, non HDL-c, TAG, and TAG:HDL-c, and a lower intake of fruits and vegetables. Following the MMM, the healthiest group (Group 2) had a lower increase in TAG, with no difference in postprandial endothelial function. The HSFAM induced postprandial endothelial dysfunction only in Group 1.
Wang *et al.*[32]	Identify metabotypes of response to dietary carotenoids	Crossover 3 weeks trial	23 healthy subjects aged 36-69 years in the USA	Not tested	Watermelon juice (20.1 mg/d lycopene + 2.5 mg/d carotene) and a second watermelon juice (40.2 mg/d lycopene + 5.0 mg/d carotene) or tomato juice (18.4 mg/d lycopene + 0.6 mg/d carotene)	Temporal response concentrations of plasma carotenoids (β-carotene, lycopene, phytoene, and phytofluene) clustered by *k*-means cluster analysis.	Five metabotypes per carotenoid per intervention type. Strong or weak responders to each carotenoid were identified. Responses were associated with genetic variants of carotenoid-metabolising enzyme.

Table 2. *Continued*

Author	Objective	Study design	Study sample	Dietary challenge(s)	Intervention	Variables for clustering	Main findings
Vázquez-Fresno *et al.*[33]	Investigate urinary changes in metabotypes following red wine polyphenol intake.	Crossover 4 weeks RCT	57 high-risk subjects aged ≥55 years in Spain	Not tested	Red wine polyphenol intake (733 equivalents of gallic acid/day) in the form of dealcoholized wine.	67 fasting blood and urinary markers and 2 anthropometric parameters (BMI and waist-to-hip ratio) clustered by *k*-means cluster analysis.	Four metabotypes. Following the intervention, 4-hydroxyphenylacetate concentrations significantly increased in the healthier cluster compared to the higher risk cluster, while glucose was higher in higher risk cluster compared to the healthier cluster; tartrate was higher for both clusters.
O'Sullivan *et al.*[34]	Identify metabotypes of response to vitamin D supplementation in terms of the metabolic syndrome.	Double-blind 4 weeks RCT	135 healthy subjects aged 18-63 years in Ireland	Not tested	Group 1: 15 μg vitamin D_3 + 10^9 CFU *Lactobacillus salivarius*, group 2: vitamin D + placebo probiotic, group 3: placebo vitamin D + probiotic, and group 4: placebo vitamin D + placebo probiotic.	13 fasting blood markers of the metabolic syndrome (leptin, resistin, adiponectin, IL-6, hsCRP, TNF-α, insulin, C-peptide, TC, TAG, NEFA, glucose, HOMA-IR) and 25(OH)D concentrations clustered by *k*-means cluster analysis.	Five metabotypes. Cluster 5, with lower serum 25(OH)D and higher concentrations of adipokines at baseline, showed significant improvements in insulin, HOMA-IR, and hsCRP, as well as an inverse correlation between changes in serum 25(OH)D and glucose concentrations.

MMTT, mixed-meal tolerance test; RCT, randomised controlled trial; HCA, hierarchical cluster analysis; GI, glycaemic index; PCA, principal component analysis; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; BMI, body mass index; TAG, triacylglycerol; hsCRP, high sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; PCs, phosphatidylcholines; BCAA, branched-chain amino acids; O-PLS, orthogonal partial least squares, TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TAG, triacylglycerol; CFU, colony-forming units; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha; NEFA, non-esterified fatty acid; 25(OH)D, 25-hydroxyvitamin D.

Table 3. Summary of studies developing targeted dietary advice solutions for metabotypes through the decision tree approach.

Author	Study sample	Variables and method for clustering	Clusters' biomarker characterisation	Design of decision trees	Validation of decision trees	Main findings
O'Donovan *et al.*[22]	875 subjects aged 18-90 years in the Irish National Nutrition Survey in Ireland	Fasting TAG, TC, HDL-c, and glucose clustered by *k*-means cluster analysis.	Cluster 1 (n = 274) had high TC, cluster 2 (n = 423) had adequate concentrations of all biomarkers, and cluster 3 (n = 178) had high TAG, TC, and glucose.	One decision tree by cluster. Dietary advice was based on the biochemical cluster's characteristics and branches for BMI, waist circumference, and blood pressure.	Comparison with individual-based approach manually compiled and delivered by a dietician (n = 99).	Three decision trees with 12 possible messages each, which are the combination of 20 possible advice. An average agreement of 89% (range 20 - 100%) was found between the targeted advice and the individual-based approach with 69% of the participants presenting an agreement of 100%.
O'Donovan *et al.*[35]	1354 subjects ≥18 years in the Food4Me Study in 7 European countries	27 fasting metabolic markers (TC, fatty acids, and carotenoids) clustered by *k*-means cluster analysis.	Cluster 1 (n = 326) had the highest TC and trans-fatty acids and the lowest omega-3 index, cluster 2 (n = 433) had the highest omega-3 index and total carotenoid and the lowest total saturated fat, and cluster 3 (n = 595) had the lowest TC and highest stearic acid.	Two decision trees by cluster. The first was based on biomarkers (TC, total saturated fat, omega-3 index, and carotenoids) with branches for TC, BMI, and waist circumference. The second was based on the individual intakes of five nutrients (salt, iron, calcium, folate, and fibre).	Comparison with personalised dietary advice based on phenotypic features and delivered by nutritionists (n = 180)	A wide set of messages raised from the combination of two decision trees and ranged from 2 to 6 per participant. An average agreement of 82% was found between the targeted advice and the individual-based approach, with an average agreement of 83, 74, and 88% for clusters 1, 2, and 3, respectively.

TAG, triacylglycerol; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; BMI, body mass index.

Fig. 1. An overview of the concept of metabotyping for the delivery of personalised nutrition. Intrinsic and extrinsic factors influence the metabolic phenotype of individuals. Groups of individuals with similar metabolic phenotypes are termed metabotypes.