

1 **TITLE: Metabotyping and its role in nutrition research**

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3 **Short title:** Metabotyping and personalised nutrition

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14 **Abbreviations:** BMI, body mass index; HDL-c, low-density lipoprotein cholesterol; HOMA-IR,
15 homeostatic model assessment for insulin resistance; HSFAM, high-saturated fatty acid meal; IGF-
16 1, insulin-like growth factor-1; IGF-BP3, insulin-like growth factor-binding protein 3; IGF-BP2,
17 insulin-like growth factor-binding protein 2; MetS, metabolic syndrome; MMM, mixed
18 Mediterranean-type meal; MMTT, mixed meal tolerance test; OGTT, oral glucose tolerance test;
19 OLTT, oral lipid tolerance test; RCT, randomised controlled trial; TAG, triacylglycerol; TC, total
20 cholesterol.

21

22 **Key words:** Cluster analysis: Metabotypes: Personalised nutrition: Targeted nutrition

23 Abstract

24

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

39 Introduction

40

41 Poor diet quality is a major contributor to chronic diseases such as type 2 diabetes,
42 cardiovascular diseases and various cancers^(1,2). Despite the well-known association between dietary
43 patterns and diseases, interventions to change dietary habits have had a limited impact on wellbeing
44 and public health outcomes^(3,4). In recent years, the diverse inter-individual responses to
45 interventions have become apparent and support the need for the development of strategies that are
46 based upon the delivery of advice to the individual⁽⁵⁻⁹⁾. Concomitant with this, different strategies
47 have emerged for delivering advice taking personal characteristics into account. Furthermore,
48 studies have demonstrated that personalisation of dietary advice is more effective in promoting
49 improvements in the dietary habits of individuals compared to the general healthy eating advice⁽¹⁰⁻
50 ¹²⁾.

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

73

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

76

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

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

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

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

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

122

123 **Metabolic phenotyping to investigate differential responses to dietary challenges and** 124 **interventions**

125

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

134 Krishnan *et al.*⁽²⁸⁾ investigated the differential responses of metabotypes to dietary
135 challenges. The authors used low and high glycaemic index meals in a crossover randomised trial
136 with healthy overweight women (n = 24, 20 to 50 years old) to identify response patterns that could
137 provide insight into early subclinical glycaemic disruption. By using blood glucose, insulin, and
138 leptin responses to the challenges, individuals were clustered into three metabotypes. While the
139 most populated metabotype presented little deviation from the expected response to the dietary
140 challenges, the two minor metabotypes were suggestive one of sub-clinical insulin resistance and
141 the other of hyperleptinemia. In the Metabolic Challenge (MECHE) Study, healthy subjects (n =
142 214, 18 to 60 years old) were randomised to one of three groups to receive oral glucose tolerance

143 tests (OGTTs) and/or oral lipid tolerance tests (OLTTs) and four metabolotypes were identified based
144 on their blood glucose response curves to the OGTT (n=116)⁽²⁹⁾. The cluster with the most adverse
145 metabolic profile at baseline presented a reduced β -cell function and differential responses to
146 insulin and c-peptide during OGTT and OLTT, as well as to glucose and TAG during the OLTT,
147 which characterises this metabolotype as at risk. The postprandial metabolic responses to different
148 kinds of bread - refined rye bread, whole-meal rye bread, and a control refined wheat bread - were
149 investigated in a crossover randomised controlled trial (RCT) with healthy postmenopausal women
150 (n = 19, 61 \pm 4.8 years)⁽³⁰⁾. The clustering of the fasting metabolic profile identified two distinct
151 metabolotypes. Women with higher fasting concentrations of leucine and isoleucine and lower fasting
152 concentrations of sphingomyelins and phosphatidylcholines had higher insulin responses despite
153 similar glucose concentrations after all kinds of bread, suggesting higher insulin resistance. In a
154 recent study with data from the NutriTech project, the response to the intervention was only evident
155 following the classification of the individuals into metabolotypes⁽²⁶⁾. Healthy subjects (n = 72, 59 to
156 64 years old) were enrolled to a mixed meal tolerance test (MMTT) before and after 12 weeks
157 targeting moderate weight loss (basal BMI 29.7 \pm 2.7 kg/m²). The intervention group (n = 40)
158 consumed a diet that reduced caloric intake by 20%, whereas subjects in the control group (n = 32)
159 consumed an average European diet matched to their energy expenditure to maintain body weight.
160 Two metabolotypes were reported based on the plasma concentration of metabolites (markers of
161 lipolysis, fatty acid β -oxidation, and ketogenesis) during the mixed meal challenge test. Before the
162 intervention, individuals from metabolotype B (n = 36) showed slower glucose clearance, increased
163 visceral fat volume, higher hepatic lipid concentrations, and a less healthy dietary pattern according
164 to the urinary metabolomic profile when compared to individuals from metabolotype A. Following
165 the weight loss (~5.6 kg), only the individuals from metabolotype B showed positive changes in the
166 glycaemic response to the MMTT. Since the metabolite differences found between metabolotypes A
167 and B are all closely associated with insulin signalling, the metabolotype B was considered to be
168 prediabetic with a modestly impaired insulin action. Collectively, all these studies clearly
169 demonstrate that the use of a metabolotype approach in conjunction with meal challenges has the
170 ability to characterise individuals into meaningful subgroups which could receive targeted nutrition
171 advice to lower the individual disease risk⁽³⁰⁾.

172 In contrast to other studies that used the responses to challenges to form clusters, Lacroix
173 *et al.*⁽³¹⁾ used only fasting metabolic data in a crossover RCT designed to evaluate the metabolic and
174 vascular effect of a high-saturated fatty acid meal (HSFAM) and a mixed Mediterranean-type meal
175 (MMM). Age, BMI, glycaemic and lipid parameters were used to cluster healthy men (n = 28, 18 to
176 50 years old) into two metabolotypes at baseline. Compared to the healthiest group, the less healthy
177 group showed significantly higher BMI, insulin, and homeostatic model assessment for insulin

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

186 Application of the metatype approach has also encompassed dietary interventions that
187 did not involve meal challenges. Wang *et al.*⁽³²⁾ in a controlled crossover study with healthy
188 subjects (n = 23, 36 to 69 years old) identified groups of individuals with differing plasma
189 carotenoids response to carotenoid-rich beverages. Following three weeks of daily intake of
190 watermelon juice (20 mg lycopene, 2.5 mg β -carotene, n=23; 40 mg lycopene, 5 mg β -carotene,
191 n=12) or tomato juice (18 mg lycopene, 0.6 mg β -carotene, n=10), cluster analysis applied to
192 weekly carotenoid responses identified groups of individuals with differential responses. This, **in**
193 **turn**, was used to classify individuals as strong responders or weak responders to the carotenoid
194 intake. These findings demonstrate that subgroups of individuals can have differential responses to
195 interventions which could be harnessed in the future to give more precise dietary advice. With
196 respect to employing a metatype approach for dietary interventions in clinical populations or
197 disease risk factors, two studies are noteworthy. In a sample of high-risk cardiovascular subjects (n
198 = 57, ≥ 55 years old) a four-week crossover RCT identified differential responsiveness to red wine
199 polyphenol⁽³³⁾. At baseline, fasting blood and urinary metabolites and anthropometric parameters
200 were used to cluster individuals in four metatypes, including a higher risk cluster and a healthier
201 cluster. Following 28 days of dealcoholized red wine intake (polyphenol content = 733 equivalents
202 of gallic acid/day), concentrations of urinary 4-hydroxyphenylacetate significantly increased in the
203 healthier cluster compared to the higher risk cluster, indicating a differential response in this cluster.
204 In a double-blind four-weeks RCT with healthy subjects (n = 135, 18 to 63 years), the effect of
205 vitamin D supplementation (15 mg vitamin D₃ per day) to improve markers of the metabolic
206 syndrome was only visible after the classification of the sample into metatypes⁽³⁴⁾. The vitamin D
207 supplementation significantly increased the serum 25-hydroxyvitamin D in comparison to the
208 placebo group, but there was no effect of supplementation on the measured markers of the
209 metabolic syndrome. Based on 13 fasting blood biomarkers, one cluster characterised by low
210 concentrations of vitamin D and higher concentrations of adipokines showed a significant decrease
211 in insulin, HOMA-IR scores, and c-reactive protein and inverse relationship between the change in
212 serum vitamin D and glucose. Collectively, these examples clearly present how comprehensive

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

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

218 219 **Harnessing the metabotype approach to deliver targeted nutrition**

220
221 To the best of our knowledge, there are only two published examples of a framework for
222 the delivery of personalised nutrition using a metabotype approach (Table 3).

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

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

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

259

260 **Conclusions and future directions**

261

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

272

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

277

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284

285 **Conflict of Interest**

286

287 None

288

289 **Authorship**

290

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

292 EH and LB edited the manuscript.

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387 561-569.

Table 1. Summary of studies examining longitudinal associations of metabolotypes 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 <i>et al.</i> ⁽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 metabolotypes. 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 <i>et al.</i> ⁽²⁴⁾	Identify predictive metabolotypes 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 metabolotypes. 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 <i>et al.</i> ⁽²⁵⁾	Define metabolotypes 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 <i>k</i> -means cluster analysis.	Three metabolotypes. 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

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

Author	Objective	Study design	Study sample	Dietary challenge(s)	Intervention	Variables and method for clustering	Main findings
Fiamoncini <i>et al.</i> ⁽²⁶⁾	Investigate the metabolic response of metabolotypes 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 metabolotypes. At baseline, metabolotype B had slower glucose clearance, increased intra-abdominal adipose tissue mass, higher hepatic lipid concentrations, and less healthy dietary pattern than metabolotype A. Following the weight loss (~5.6 kg), only metabolotype B showed positive changes in the glycaemic response to the MMTT, with improvements in metabolites of amino acid, acylcarnitines, and biochemical parameters.
Krishnan <i>et al.</i> ⁽²⁸⁾	Identify metabolotypes 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 metabolotypes. The two minor groups were one suggestive of sub-clinical insulin resistance and the other of hyperleptinemia.
Morris <i>et al.</i> ⁽²⁹⁾	Identify metabolotypes 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 metabolotypes. Cluster 1 was at risk with the highest BMI, TAG, hsCRP, c-peptide, insulin, and HOMA- IR and the lowest VO ₂ max. 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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>et al.</i> ⁽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 <i>k</i> -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 <i>et al.</i> ⁽³³⁾	Investigate urinary changes in metabolotypes 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 <i>k</i> -means cluster analysis.	Four metabolotypes. 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 <i>et al.</i> ⁽³⁴⁾	Identify metabolotypes 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 <i>Lactobacillus salivarius</i> , 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 <i>k</i> -means cluster analysis.	Five metabolotypes. 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 metabolotypes 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 <i>et al.</i> ⁽²²⁾	875 subjects aged 18-90 years in the Irish National Nutrition Survey in Ireland	Fasting TAG, TC, HDL-c, and glucose clustered by <i>k</i> -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 <i>et al.</i> ⁽³⁵⁾	1354 subjects ≥18 years in the Food4Me Study in 7 European countries	27 fasting metabolic markers (TC, fatty acids, and carotenoids) clustered by <i>k</i> -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.