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A Scoping Review: Metabolomics Signatures Associated with Animal and Plant Protein Intake and Their Potential Relation with Cardiometabolic Risk

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Authors manuscript

Title: A scoping review: metabolomics signatures associated with animal and plant protein intake and their potential relation to cardiometabolic risk

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Abbreviations:

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AA: Amino acids, AAA: Aromatic amino acids, aMD: alternate Mediterranean Diet, AP: Animal proteins, BCAA: Branch-Chained Amino Acids, BMI: Body Mass Index, CVD: Cardiovascular diseases, DASH: Dietary Approach to Stop Hypertension, FIB: Food Intake Biomarker, HDI: Healthy Diet indicator, HEI: Healthy Eating Index, 2-HB: 2-hydroxybutyrate, 3-HIB: 3-hydroxyisobutyrate, MD: Mediterranean diet, NND: New Nordic Diet, PAGln: Phenylacetylglutamine, PP: Plant proteins, PP-to-AP ratio**:** plant to animal protein ratio, S_C: overall contribution score, S_D: study design contribution score, S_M: metabolite score, S_P: dietary contribution score TCA: Tricarboxylic acid, TMA: Trimethylamine, TMAO: Trimethylamine-N-oxide, T2D: Type 2 Diabetes

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ABSTRACT

The dietary shift from animal protein (AP) to plant protein (PP) sources is encouraged for both environmental and health reasons. For instance, PP are associated with lower cardiovascular and diabetes risks compared to AP, although the underlying mechanisms mostly remain unknown. Metabolomics is a valuable tool for globally and mechanistically characterizing the impact of AP and PP intake, given its unique ability to provide integrated signatures and specific biomarkers of metabolic effects through a comprehensive snapshot of metabolic status. This scoping review is aimed at gathering and analyzing the available metabolomics data associated with PP and AP-rich diets, and discussing the metabolic effects underlying these metabolomics signatures and their potential implication for cardiometabolic health.

We selected 24 human studies comparing the urine, plasma or serum metabolomes associated with diets with contrasted AP and PP intakes. Among the 439 metabolites reported in those studies as able to discriminate AP and PP-rich diets, 46 were considered to provide a robust level of evidence, according to a scoring system, especially amino acids (AA) and AA-related products. Branch-chained amino acids (BCAA), aromatic amino acids (AAA), glutamate, short-chain acylcarnitines and trimethylamine-N-oxide (TMAO), which are known to be related to an increased cardiometabolic risk, were associated with AP-rich diets, whereas glycine (rather related to a reduced risk) was associated with PP-rich diets. TCA cycle intermediates and products from gut microbiota AAA degradation were also often reported, but the direction of their associations differed across studies.

Overall, AP and PP-rich diets result in different metabolomics signatures, with several metabolites being plausible candidates to explain some of their differential associations with cardiometabolic risk. Additional studies specifically focusing on protein type, with rigorous intake control, are needed to better characterize the associated metabolic phenotypes and understand how they could mediate differential AP and PP effects on cardiometabolic risk.

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Teaser text:

The metabolomics signatures associated with animal and plant protein-rich diets were reviewed to better understand the metabolic effects of animal and plant protein and their impact on cardiometabolic health.

Keywords: amino acids; biomarkers; cardiovascular diseases; diabetes; dietary patterns; legumes; meat; metabolites; Mediterranean diet; nutrimetabolomics; vegetable proteins

Statement of significance: This work is the first review gathering and analyzing the available metabolomics data associated with diets with contrasted animal and plant protein intakes in humans and discussing the metabolic effects underlying these metabolomics signatures and their potential implication for cardiometabolic health.

INTRODUCTION

The transition towards more plant-based diets is encouraged with the perspective of moving towards more sustainable diets (1). The shift within protein sources is one key aspect of this transition and dietary guidelines tend to a lower intake of animal protein (AP) sources, more specifically processed and red meat, and a higher intake of plant protein (PP) sources such as whole grains, legumes, nuts and seeds, for improving both human (2–5) and planetary health (6–9). From the standpoint of human health, AP intake has been related with increased risk of several diseases. For instance, consumption of processed meat has been rated as carcinogenic to humans by the International Agency for Research on Cancer especially because of its link with colorectal cancer (10) while more recent evidence suggests low certainty and small effect size (11). AP intake has also been adversely associated with metabolic syndrome related-outcomes (12–18) and with higher risk (and PP with lower risk) or death from type 2 diabetes (T2D) and cardiovascular diseases (CVD)(19–25). Based on these

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recent and emerging evidence, the present review will specifically focus on the effects of AP and PP on cardiometabolic health.

The contrasting effects of AP and PP on cardiometabolic health could be mediated by different factors, such as the “protein package” (i.e. the nutrients and compounds present in protein-rich foodstuff and associated to protein intake) and the overall diet quality associated to AP and PP food sources intake (26–30). The hypothesis that differences in intrinsic amino acid (AA) composition between PP and AP could contribute to their differential effects on cardiometabolic health has also been proposed (29,31). Indeed, AP have a higher content in essential AA such as branched-chain AA (BCAA) and aromatic AA (AAA), while PP have a higher content in non-essential AA such as arginine and glutamate (see **Figure 1**, (32)). Some of these AA were reported as being strongly associated with cardiometabolic health (33–36).

In recent years, metabolomics has developed significantly in the field of nutritional sciences in which its application is now often referred to as nutrimetabolomics (37). One active orientation in this field is the search for food intake biomarkers (FIB) (38,39), which are promising tools to evaluate the exposure to food intake by alleviating the usual caveats of dietary assessment obtained from self-reported questionnaires (40). The nutrimetabolomics research also aims at untangling the physiological effects of dietary patterns, foods and nutrient consumption through the discovery of effect biomarkers revealing the impact of a specific nutritional effector on a given physiological function (effect biomarkers).

Although several hypotheses have been proposed, the mechanistic bases for the effects of AP and PP on cardiometabolic health are still widely unknown. A metabolomics-based strategy could therefore point to novel hypotheses leading to better understanding of the molecular processes involved. Interestingly, although the association of AP and PP intake with cardiometabolic health has been extensively discussed (29,31,41), only one cross-sectional study has specifically investigated the differences in the plasma metabolome associated with AP and PP intake (42), highlighting the gap in knowledge between metabolomics signatures and protein source consumption in the context of cardiometabolic risk. Thus, this review aims

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at gathering and analyzing the available data on the metabolomics signatures associated with PP and AP intake to better understand their metabolic effects, and more specifically their impact on cardiometabolic health.

CURRENT STATUS OF KNOWLEDGE

STUDIES AND METABOLITES SELECTION USING A DEDICATED SCORING SYSTEM

Given that very few data on the metabolites associated with AP and PP intake are available in pre-clinical models, we focused our analysis on human studies (both observational and interventional) in which a metabolomics approach was applied to plasma, serum or urine samples. The evidence strictly specific to AP and PP metabolomics signatures is scarce (42), but there is more evidence available regarding the metabolites associated with broader dietary changes. After an extensive review of the literature, we retained a subset of studies in which we were able to determine that protein type was an important factor differentiating the diets under study (**Figure 2**).

Overall, 3 types of dietary comparisons were identified. Firstly, the comparison of omnivores with vegetarians and vegans is likely to reflect a strong contrast in AP and PP intakes. For instance, in Western countries, the proportion of AP in total protein intake is around 70% in omnivores (12,27,43–45), against 30% in vegetarians (46–48). Secondly, although, little quantitative data is available regarding AP and PP intake differences associated to healthy dietary patterns such as the Mediterranean diet (MD), Healthy Eating Index (HEI) or Dietary Approach to Stop Hypertension (DASH), the metabolites associated with these dietary patterns are likely to be associated with AP or PP intake as those dietary patterns contain a higher plant-based fraction (see **Supplemental Table S1** for detailed description of the dietary pattern

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adherence scores reported across selected studies). For example, the MD is likely to be associated to a higher PP intake than a typical western diet as it includes a high consumption of legumes, nuts and whole grains and a relatively low proportion of AP, corresponding to a moderate consumption of fish, poultry and dairy foods and a low consumption of red meat products (49). Thirdly, the comparison of metabolites associated with dietary patterns constructed a posteriori by cluster analyses can be of relevance when these patterns are closely related to AP or PP food groups, though the actual AP and PP intake differences might be difficult to quantify.

Overall, 24 references met the above criteria and were considered in this review: 16 observational studies (42,50–64) and 8 clinical trials (65–72) (**Table 1, Table 2**). Regarding the analytical approach used, 16 references were MS-based (42,51,52,55–60,62,63,66–68,71,72) and 8 were NMR-based (50,53,54,61,64,65,69,70). Among the 8 clinical trials, 3 compared omnivore vs vegetarian diets or meals (65,70,72), 2 investigated the effects of the MD (69,71) and the remaining 3 measured the effects of the New Nordic Diet (NND) (rich in typical northern vegetables, whole grains, legumes and fish) (66–68).

All observational studies had a cross-sectional design and the inter-group differences across the metabolome are rather expected to result from a long-term exposure to the diets compared. One study directly compared AP and PP intake (42), 6 compared omnivores with vegetarians and vegans (50–54,64), 6 compared groups according to adherence to defined dietary patterns (55–60) and 3 according a posteriori (data-driven) dietary patterns (61–63). While most studies were conducted on healthy participants, 8 of them included participants with cardiometabolic risk factors (42,58,66–69,71,72).

To account for the differences in study design and for the disparities expected in AP and PP intakes, we built a scoring system that balances the potential contribution of each study to improve our ability to identify metabolites associated to AP and PP-rich diets. Briefly, for each study, we applied a study design contribution score (S_D) based on the levels of evidence defined by the Oxford Centre for Evidence-Based Medicine (73) and a dietary contribution

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score (S_P) based on the expected differences in protein type intake and available dietary data (**Figure 2**). The highest S_P values were attributed to studies investigating directly the association of the metabolome with AP and PP intake, followed by studies comparing either vegetarians or vegans with omnivores, different adherence levels to healthy dietary patterns, and finally a posteriori (data driven) dietary patterns. Then, for each study, an overall contribution score (S_C) was calculated by averaging S_D and S_P, which ranged from 0.33 to 0.84 across studies (**Supplemental Table S2 and S3**). For each selected study, we retrieved the discriminating metabolites (see **Supplemental text** for additional explanations). Then, we summed the S_C of each study reporting a specific metabolite to obtain an overall metabolite score (S_M). For a given metabolite, a high S_M can be the result of its presence in numerous studies with a low S_C or its presence in only a few studies but with a high S_C. We finally distinguished a short-list of metabolites being associated with AP and PP-rich diets with a higher level of evidence among those having the highest S_M values, by applying a S_M cut-off value of 1.51 which represent 3-fold of 60% of the maximum S_C attributed (0.84), so as to highlight the metabolites cited in at least 3 studies of relatively good relevance.

OVERALL HIGH HETEROGENEITY IN THE REPORTED METABOLITES BUT CONVERGENCE ON AA METABOLISM

Across the selected studies, 439 metabolites were reported as discriminant between diets with varying AP and PP contents, with a mean S_M of 0.89. We summed the S_M values of all metabolites within one metabolite class to estimate its overall contribution to the global metabolomics signature obtained (**Figure 3**). Metabolites related to protein and AA metabolism had the highest contribution (high number of metabolites and high mean S_M), followed by phosphatidylcholines (high number of metabolites), and then by metabolites of energy and carbohydrate metabolism and acylcarnitines (moderately high number of metabolites and a

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moderately high mean S_M). Taken together, the different lipid classes contributed to a high proportion (45%) to the overall metabolomics signature.

Moreover, only 46 of these 439 discriminant metabolites were judged to have a sufficient level of evidence, using a S_M cut-off value of 1.51 that ensures concordance of metabolite detection across studies of good relevance (see **Table 3**). Among them, 14 (30%) were AA, which highlights their important role in the metabolomics signatures associated with AP or PP-rich diets. This short-list also contained other important endogenous metabolites such as tricarboxylic acid (TCA) cycle intermediates or creatine, creatinine and choline, mostly provided by the dietary intake of animal products. This short-list contained few lipids although they were very abundant previously, probably because of the high variability of this particular family that may explain the lack of convergence between studies. Moreover, the high contribution of AA-related molecules to metabolomics signatures can be interpreted in terms of biomarkers of exposure and/or effects. Indeed, such metabolomics signatures could reflect the difference in the intrinsic AA composition of AP and PP (Figure 1), which intakes were contrasted in the diets compared, but are probably not simple protein FIB, since circulating AA levels are usually not considered to directly reflect AA dietary intake (48). They more likely constitute effects biomarkers, either of overall dietary patterns with contrasted protein sources or more specific to protein type intake as differences in AA intake could lead to modifications in protein and AA metabolism that in turn become visible in the metabolome.

REPLICATION OF ALREADY KNOWN FOOD INTAKE BIOMARKERS

Across the selected studies, many identified metabolites were already known as potential FIB. For instance, several metabolites known for their association with animal products and meat consumption were found associated with high AP diets.

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Creatine and its degradation product creatinine have been proposed as total meat FIB, creatine being more reliable than creatinine which excretion is closely related to individual muscle mass (74). Both metabolites had amongst the highest S_M values (4.11 and 4.24 respectively) and were often associated with high AP diets (42,51,53,54,57,64,65,69,70). Likewise, 3-methylhistidine (S_M=2,06) has been proposed as a total meat and poultry FIB (74) and was associated with diets with a higher proportion of AP (50,69,72).

Metabolites associated with plant food intake were also reported to be associated with PP-rich diets. For instance, hippurate (S_M=2,39), a metabolite formed through endogenous glycine conjugation of benzoic acid but also through gut microbiota degradation of dietary polyphenols (75,76), was positively associated with diets with a high proportion of PP (50,59,66), to one exception in a MD intervention study (69).

EFFECT OF AP AND PP-RICH DIETS ON METABOLISM

Association between metabolomics signatures and cardiometabolic risk

Several studies included in this review conducted mediation analyses between metabolomics signatures derived from diets with different proportions of AP and PP and cardiometabolic risk or risk factors. In an analysis of the PREDIMED baseline data, adherence to MD and the related metabolomics signature (60 metabolites) were associated with a lower CVD incidence (58). Furthermore, the association between the metabolomics signature and CVD risk remained significant when adjusting for MD adherence. In addition, the association between MD score and CVD was 37% mediated by the metabolomics signature, in accordance with another analysis in which the metabolomics signature derived from MD adherence also mediated 37% of the negative association between MD adherence and HOMA-IR (57).

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These mediation analyses suggest that some of the discriminating metabolites between diets with contrasted AP and PP levels may contribute to explain the differential effects of these diets on cardiometabolic risk, by acting as some of the causal effectors.

BCAA, AAA and acylcarnitines

BCAA (leucine, isoleucine and valine) and AAA (such as tyrosine and phenylalanine) are present in high quantities in AP (Figure 1). In association with certain short-chain acylcarnitines, they have been consistently reported together in the metabolic signatures associated with several metabolic diseases and are discussed together.

All BCAA and AAA were in the short-list of metabolites consistently identified by relevant studies (with high S_M values ranging between 4,83 and 2,05, Table 3) and were most consistently associated with high-AP diets, this association being repeated in observational and at least one interventional study for each metabolite (with the exception of tyrosine, reported only in observational studies). They were positively associated with omnivore diets (50–53,64,70), with the Average Danish Diet compared to the NND (67), and negatively associated with PP-to-AP ratio and PP intake (42) and with MD adherence (57,71). In a dietary cluster analysis, the 3 BCAA, tyrosine and phenylalanine were also associated with the Western dietary pattern but not the vegetarian one (62). Conversely, an association with PP-rich diets was observed in only 2 cases: phenylalanine was positively associated with the PP-to-AP ratio (42) and leucine was positively associated with the 2 MD intervention groups of the PREDIMED study (69).

Short-chain acylcarnitines, especially C3 and C5 acylcarnitines, are byproducts of BCAA mitochondrial oxidation (77). It is therefore not surprising that short-chain acylcarnitines have been reported to be associated with high-AP diets. This was the case in particular for acylcarnitine C4 and C2 (present in the short-list of Table 3 with high S_M values of 2.44 and 2.18, respectively), which were consistently reported in both observational and interventional

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studies (42,51,58,61,62,65,68,71). In additional analyses, urine acylcarnitine C2 was positively associated with meat intake, further confirmed by a comparison across meat intake percentile groups (61). C3-acylcarnitine (S_M=1.29) was positively associated with AP-rich diets in a cross-sectional study comparing vegans and non-vegans (51) and an MD randomized trial (71). Finally C5-acycarnitine (S_M=0.90) was also associated with AP-rich diets, since it was associated with a western diet dietary cluster (62) and found at higher levels in habitual non-vegans (51).

BCAA, AAA and short-chain acylcarnitines were associated with several cardiometabolic risk factors. In the Fenland study, adherence to MD was inversely associated with HOMA-IR, and the integration of acylcarnitines in the prediction model resulted in an 11% attenuation of the association, suggesting their implication in the initiation and progression of insulin resistance (57). Further, in the metabolomic profiling of Asian Indians living in the USA (62), one of the identified clusters of metabolites included BCAA, phenylalanine, and tyrosine, C2 and C5 acylcarnitines and was positively associated with high animal product intake dietary pattern and several cardiometabolic risk markers (higher fasting insulin, higher HOMA-IR, total cholesterol, triglyceride and fat liver levels).

Outside the studies included in this review, there is wealth of examples of associations between metabolic disorders and this group of metabolites. For instance, a PCA component including BCAA, C3 and C5 acylcarnitines, phenylalanine, tyrosine, methionine and glutamate/glutamine was positively associated with obese compared to lean subjects and had a significant linear relationship with HOMA-IR, even after adjusting for the lean versus obese status (36). Since then, elevated plasma BCAA, phenylalanine and tyrosine have been often reported in cardiometabolic related conditions (78–81) and numerous cohort studies (78–80,82–92). Those metabolites also seem to have a predictive value for cardiometabolic disease risk. BCAA, tyrosine, phenylalanine were predictive of higher T2D and CVD risk in prospective cohorts (93–98). Regarding acylcarnitines, while several studies showed an

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association with CVD (96,99–101), only C5 and C16-acylcarnitines were reported so far as positively associated with increased risk of T2D (94).

Several explanations have been discussed to explain the potential causal link of this group of metabolites with cardiometabolic disorders. There is supporting evidence that BCAA overload promotes mitochondrial dysfunction by acylcarnitine accumulation (102). One possible mechanism involves the metabolite 3-hydroxyisobutyrate (3-HIB), a byproduct of valine oxidation that occurs in the mitochondria, but which is also able to leak into the blood circulation (77). In rodents, 3-HIB has been shown to favors fatty acid accumulation in the muscle (103) by leading to acylcarnitine accumulation inside the mitochondria and promoting mitochondrial dysfunction, thought to promote insulin resistance (102). In humans, plasma 3-HIB was significantly associated with a higher risk of T2D, even after adjustment for plasma BCAA and obesity (104). Interestingly, 3-HIB (S_M=0,58) was positively associated with habitual meat consumption in the present review (53).

The results regarding 2-hydroxybutyrate (2-HB) also strengthen the hypothesis of increased fatty acid oxidation in association with AP intake. In the present review, 2-HB (S_M=0.94) was associated with AP-rich diets in 2 cross-sectional studies (63,91). On the other hand, 2-HB has been associated with a higher risk of insulin resistance and T2D in prospective cohorts (105,106). In addition, in an untargeted metabolomics analysis, 2-HB proved to be very discriminant and able to distinguish insulin resistant individuals from insulin sensitive ones (107). Given that the production of 2-HB from α-ketobutyrate (a byproduct of methionine and threonine) requires the oxidation of NADH to NAD+, a higher concentration of 2-HB could reflect a higher fatty acid oxidation, known to increase the NADH/NAD+ ratio (105,107). Another explanation for the high 2-HB levels could relate to an increase α-ketobutyrate production during the conversion of cystathionine to cysteine, in order to fuel glutathione synthesis under high oxidative stress conditions (107).

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In conclusion, the consumption of diets richer in AP is associated with higher plasma BCAA, AAA and short-chain acylcarnitines. Higher levels of those metabolites have been observed in individuals with higher cardiometabolic risk factors such as insulin resistance. The causal role of these metabolites in the onset of insulin resistance is not fully established but there is growing evidence of a mechanism involving an overload of mitochondrial beta-oxidation capacity induced by high BCAA circulating levels and concomitant acylcarnitine mitochondrial accumulation. Therefore, BCAA, phenylalanine, tyrosine and short-chain acylcarnitines are good candidates to explain the association between AP intake and increased cardiometabolic risk.

Glycine, glutamine and glutamate

Non-essential AA glycine, glutamine and glutamate, which were also in the short-list of metabolites consistently identified by relevant studies (with high S_M values ranging between 4,03 and 2,41, Table 3), are known for their implication in cardiometabolic health.

Glutamate (S_M=2.41) was positively associated to AP-rich diets in 4 cross-sectional studies: it was negatively associated with MD adherence (57,58), positively associated with a dietary pattern high in meat and low in vegetable (63) and with omnivores compared to vegetarians (52). However, fish-eaters and vegetarians had higher plasma glutamate than omnivores in the EPIC Oxford cohort (51), and glutamate was positively associated with vegetarian meals and negatively with vegan meals and omnivore meals in one interventional study (70), a finding that cannot be interpreted in terms of association with AP and PP meal intake.

Plasma or serum glutamine (S_M=2.50) was positively associated with PP-rich diets in 3 cross-sectional studies comparing habitual omnivores and vegetarians or vegans (51,53,64). This was consistent with its lower presence in the urine of high meat eaters in a 2-week clinical trial (65).

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Glycine (S_M=4.03) was found very consistently to be positively associated with PP-rich diets both in observational and interventional studies. Plasma or serum glycine was positively associated with habitual vegetarianism and veganism (50,51,53,54) and with the NND intervention group in a Danish clinical trial (67). In urine, it was further associated with the MD intervention groups in the PREDIMED study (69) and with a dietary pattern with a high proportion of plant-foods (61). Additional findings, which were not obtained with a metabolomics approach, also reported a positive association between plasma glycine and PP sources intake (48,108–110), despite glycine intake usually being lower in diets richer in PP.

Consistent with these findings, an analysis of the EPIC-Potsdam cohort found a negative association between red meat consumption and plasma glycine (111). The association between red meat consumption and T2D risk was 30% mediated by plasma glycine, suggesting that glycine could be a potential mediator of the differential effect of AP and PP on cardiometabolic health.

Indeed, a meta-analysis of 47 case-control studies showed decreased serum glycine and glutamine and increased glutamate in patients with obesity and metabolic syndrome compared to healthy controls (112), in accordance with several cross-sectional analyses (78,79). On the other hand glutamate was rather positively associated to cardiometabolic risk factors (78,80,113). A consistent set of studies also showed that the circulating levels of these AA are predictive of cardiometabolic disease risks. Plasma glycine and glutamine were negatively associated with T2D risk while plasma glutamate was associated with higher risk in two meta-analyses of prospective cohorts (94,101). Glutamate was also associated with a higher risk of CVD in several prospective cohorts (114–116), while glutamine-to-glutamate ratio and glycine were linked to a lower risk (114,117,118). Interestingly, one of the studies selected in this review demonstrated both a negative association between plasma glutamate and MD and a positive association with CVD risk (58).

Several mechanisms have been discussed to explain the positive effects of glycine and glutamine on cardiometabolic health, including their implication in glutathione synthesis for the

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regulation of oxidative stress (119,120). In addition, in endothelial cells, the production of ammonia from glutamine to glutamate leads to increased heme degradation and byproduct production, i.e. the bile pigments biliverdin and bilirubin, and carbon monoxide (CO), known to be involved in oxidative stress and inflammatory regulation (119). Glutamine could also be indirectly implicated in endothelial cell function and nitric oxide synthesis, a regulator of blood flow and pressure (121). In the intestine, glutamine is converted into glutamate and citrulline, the latter being exported into the circulation and further converted by the kidney into arginine, the substrate for nitric oxide synthase (119,122). Citrulline (S_M=1.74) was indeed a discriminant metabolite across 3 studies included in this review, with a positive association with high-PP diets in a cross-sectional study comparing omnivores with vegetarians and vegans and in a MD randomized trial (51,71) and a negative association with MD adherence level in another cross-sectional study (57). Other potential explanatory mechanisms include the anti-inflammatory effect of glutamine given its capacity to stimulate macrophage differentiation into the M2 anti-inflammatory type (123).

In contrast, although the mechanisms by which glutamate could contribute to an increased cardiometabolic risk are not well known, its toxicity to the β-cells in a high glucose context has been proposed as a possible explanation to the increased T2D risk observed (124,125).

To conclude, there is strong evidence that glycine is associated with PP-rich diets and decreased cardiometabolic risk. Glutamine and glutamate seem to be associated with PP and AP-rich diets, respectively, although with a less conclusive level of evidence than for glycine. Their associations with cardiometabolic health are contrasted, the positive association of glutamine being backed-up by more mechanistic explanations than the negative one for glutamate.

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TCA cycle intermediates

In our analyses, several TCA cycle intermediates, such as citrate (S_M=3.09), isocitrate (S_M=1.51), succinate (S_M=1.99), malate (S_M=1.15) and cis-aconitate (S_M=1.10), were repeatedly associated with diets with different proportions of AP and PP (Table 3). However, there was no clear trend regarding the direction of those associations. For instance, malate was positively associated with PP intake and the PP-to-AP ratio (42), but it was negatively associated with MD, aHEI and DASH score (59) in two observational studies. Glycolysis end products such as lactate (S_M=1.55) and pyruvate (S_M=0.84) were reported to be either associated to vegetarian meal consumption or to the average Danish diet in another nutritional intervention (67,70).

The TCA cycle is essential in energy metabolism, and the modulation of its related metabolites is indicative of effects on the central metabolism. However, further investigations are needed to clarify the direction of the association with either AP or PP-rich diets. In addition, there is not yet enough data to understand whether and how these metabolites are linked to cardiometabolic health and if they could be meaningful in the explanation of the physiological effects of AP and PP intake.

EFFECTS OF AP AND PP-RICH DIETS ON GUT MICROBIOTA METABOLISM

Amongst the studies included in this review, the three that specifically focused on gut microbiota showed differences in activity and composition between vegan, vegetarian and omnivore diets (54), and according to MD adherence (52,71).

Plasma and urine metabolomics data also suggested that AP and PP-rich diets induce a differential activity of gut microbiota, with several gut-derived metabolites being involved (42,50,61,65,66,68,69,71).

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TMAO

Trimethylamine-N-oxide (TMAO) comes from two different sources. TMAO is produced by the liver's conversion of trimethylamine (TMA), which itself results from the degradation by the gut microbiome of several dietary precursors such as choline, phosphatidylcholine, betaine, carnitine, dimethylglycine and ergothioneine (126). Plasma TMAO is also increased by the consumption of fish (in which it is naturally present) and has therefore been proposed as a short-term FIB of this food category (74).

TMAO was in the short-list of metabolites consistently identified by relevant studies (with a high S_M value of 3,79, Table 3) and was associated to AP-rich diets in both observational and interventional studies. Urinary TMAO was hence positively associated with omnivore diet (50), a dietary pattern with a high meat intake (61), the control group of the PREDIMED trial (69) and the high meat group of another nutritional intervention (65). The only exception was observed in the New Nordic trial where higher TMAO levels were found for the diet with a higher proportion of PP, but probably because this diet was also richer in fish (66,68). In addition, similar findings were obtained in an interesting 16-week cross-over randomized trial where participants consuming 2 plant-based alternatives per day instead of 2 serving of meat, while maintaining similar intake for all food categories, had lower serum TMAO levels overall (127). Similar results were found in another well-controlled trial, where a red meat diet led to higher plasma TMAO levels than a white meat diet and a non-meat diet richer in PP (128).

The differences in TMAO concentration between dietary protein sources could result from a higher intake of TMAO precursors with AP-rich diets, but also depend on other factors, such as gut microbiota composition, hepatic enzyme activity converting TMA to TMAO, and TMAO renal excretion (129). The higher TMAO levels associated with AP-rich diets could also indicate higher cardiometabolic risk associated with this type of protein sources, without it necessarily being implicated causally.

TMAO plasma levels have indeed been positively associated with all-cause mortality, major adverse cardio- and cerebro-vascular events (130–132) and T2D (133). In addition, TMAO has

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also been linked to several risk factors such as hypertension (134), elevated BMI (135) and systemic inflammation, as assessed by C-reactive protein (136).

Research is still active to understand the mechanistic bases of these associations and whether they are causal (126,129). In addition, these findings still need to be reconciled with the association of TMAO with fish intake, considered beneficial to cardiometabolic health (137), in particular cardiovascular health (138). Despite these limitations, these recent findings suggest that TMAO could be a promising biomarker of cardiometabolic risk. Therefore, since we found in this review that TMAO is associated with AP-rich diets, it can be considered as a potential mediator or biomarker of the higher cardiometabolic risk associated with higher consumption of this protein type.

Products of AAA gut microbiota degradation

Although most proteins are digested and absorbed in the small intestine, a small fraction can reach the colon and be fermented by the microbiota. Across the selected studies, several microbiota metabolites derived from AAA degradation have been reported as discriminant of AP and PP-rich diets.

Phenylacetylglutamine (PAGln), a product of phenylalanine, was in the short-list of the metabolites consistently identified by relevant studies (S_M=2.43). It was inversely associated in cross-sectional studies with the PP-to-AP ratio (42) but positively associated with a dietary cluster with a low meat intake (61), where secondary analyses reported a positive association between its urinary concentration and vegetable intake. Results are conflicting regarding PAGln association in MD randomized trials as it was reported to be either positive (69) or negative (71).

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Although no conclusion can be drawn regarding the direction of its association with AP or PP-rich diets, this metabolite is worth mentioning as emerging evidence suggests its association with increased CVD risk (139,140).

Tryptophan can also be catabolized by specific bacteria of the gut into indole propionic acid, indole lactic acid and indole acetic acid (145). These 3 tryptophan-bacteria derived byproducts were reported across the articles selected in this review, but the direction of the association with either AP or PP-rich diets was not clear. Plasma indole-3-propionate (S_M=1,41) was associated with high PP intake (42) and to adherence to MD in an 8-week dietary intervention (71). In the same trial, oxindole-3-acetic acid (S_M=0,71) was also positively associated with MD adherence. On the other hand, urinary indole-3-acetic acid glucuronide (S_M=0,71) (66) and plasma indole-3-lactic acid (S_M=0,71) (68) were positively associated with the Average Danish Diet that predominantly includes AP sources.

Interestingly, these indole derivatives are thought to exert a positive effect on the intestinal physiology (improved intestinal immune response, regulation of the intestinal barrier and gastrointestinal motility and stimulation of glucagon-like-peptide 1 secretion), but also on the host, by promoting direct anti-inflammatory effects. The evidences regarding these mechanisms, reviewed extensively recently (141,142), are mostly derived from in vitro and animal studies and additional research is needed to confirm these findings in human studies.

Indoxyl sulfate (S_M=1,41) , another metabolite of tryptophan, and p-cresol (S_M=2,08) derived from tyrosine (143) were generally associated with AP-rich diets across the selected studies. Plasma indoxyl sulfate was negatively correlated to PP intake (42) and adherence to the MD, as was p-cresol (71). However, p-cresol was also positively associated with the MD intervention groups of the PREDIMED study (69). Both are well known uremic toxins with harmful effects in chronic kidney disease (144).

Together, these findings highlight the possible implication of these microbial AAA degradation products on cardiometabolic health, with either an increase in CVD risk for PAGIn or beneficial

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effects for indole derivatives. Because they were reported as discriminant by several studies included in this review, they are likely to be associated with diets with contrasted protein type intake but the direction of those associations needs to be clarified.

LIMITS

Several limitations should be mentioned regarding this review. To the best of our knowledge, only one cross-sectional study specifically investigated the metabolomics signature associated to the nature (AP or PP) of the protein intake (42). To reduce this knowledge gap, we also considered both interventional and observational studies in which AP and PP intakes were likely to differ between groups, even if the magnitude of these differences were often not precisely quantified. With the implementation of a dedicated scoring system for weighting each study's contribution according to the extent of the expected differences in protein intake between its groups and the level of evidence expected from its study design, we were able to account for these between-studies differences in our global analysis. However, we could not fully compensate for the initial lack of dietary data, and the design quality was only roughly estimated since no validated tool is available to assess all those criteria on the same scale.

Overall, the metabolites reported here were discussed in relation to AP or PP intake, but this interpretation remains subject to caution because of several additional reasons. Firstly, AP and PP are not consumed alone but as whole foods within diets: the "whole food package" or "protein package" not only contains dietary proteins but also other nutrients and compounds, such as fiber, vitamins, unsaturated or saturated fats, that may contribute to the physiological effects observed (145). Secondly, the consumption of AP and PP sources is closely associated with overall diet quality. For instance, fish consumption is associated with a healthier dietary pattern, with higher vegetable intake and lower meat and alcohol intake (26). PP itself is

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associated with nutrient adequacy and diet quality (27,28), and the nutrient clusters associated with AP and PP could be important mediators of long term health effects (29,30). Consequently, findings obtained in a specific dietary context cannot be readily generalized to other populations with a different dietary environment.

It should also be kept in mind that analyses grouping all AP or PP sources together are not representative of the large variability of their metabolic effects that differ according to the specific sources, especially within AP sources. A set of meta-analyses investigating the effects of food group intakes on health outcomes has shown an increased risk of all-cause mortality, CVD, hypertension, T2D and overweight associated with red meat and processed meat while fish and dairy intake were associated with decreased risks (146–150).

On the other hand, to date, there is no available analytical tool able to describe the entire human metabolome. Therefore, our understanding of the modulation of the metabolome remains limited to the detected and annotated molecules.

MAIN FINDINGS AND PERSPECTIVES

Overall, the consumption of AP and PP-rich diets have been associated with distinct metabolites, confirming the relevance of nutrimetabolomics in exploring the complex interactions between dietary protein sources and health outcomes.

Despite considerable heterogeneity in the metabolites identified and the level of evidence, several of them were good candidates to explain the effects of AP and PP intake on cardiometabolic health (**Figure 4**). Plasma BCAA, AAA and short-chain acylcarnitines were positively associated with AP-rich diets: they are of particular interest because they have often been reported together and have been repeatedly associated with cardiometabolic risk factors in multiple design settings. This metabolic signature might be an early marker of muscle

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lipotoxicity, and partially explain the positive association between AP intake and higher cardiometabolic risk. AP-rich diets also induce higher TMAO urinary levels, which have been linked to higher cardiovascular risks, although causality remains to be established. In contrast, circulating levels of glycine and glutamine were positively associated with PP-rich diets, and both have been proposed to exert beneficial effects on cardiometabolic health. On the other hand, glutamate has been associated with AP-rich diets and increased cardiometabolic risk.

To overcome the limits discussed previously regarding the difficulty of interpreting differences in diet in terms of variations of protein source in the studies published, additional studies with a specific focus on protein type, detailed dietary data and strict intake control are needed: both observational studies including a large number of participants and closely describing the protein and AA intake, and randomized, controlled trials investigating the specific effects of AP and PP intake on plasma and urinary metabolomes. Such detailed studies are required to better characterize the metabolites associated to PA and PP intake, and to understand how they can impact cardiometabolic risk.

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FIGURES

Figure 1: Amino acid compositions of the most common animal protein and plant protein sources, expressed as a percentage of egg amino acid content.

Values for each category of protein sources were calculated as the average amino acid content of 3 or 4 common foods within the category as follows, based on USDA database (32): Cereals: white wheat flour, white rice, corn flour. Pulses: Red kidney beans, lentils, tofu, chickpeas. Nuts: almonds, walnuts, peanuts. Dairy: whole milk (cow), plain yogurt (cow), Gruyere cheese. Terrestrial meat: beef, pork, chicken. Fish: cod, salmon, trout. Egg: only the values corresponding to whole hen egg were used. Data are presented as a percentage of egg amino acid content per 100g of total amino acid.

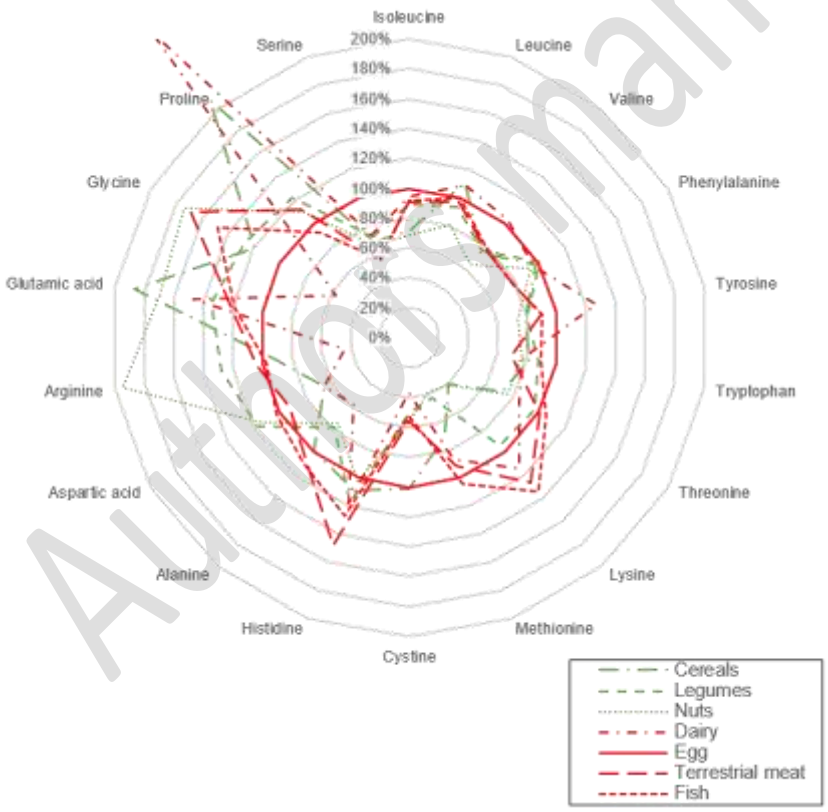

Figure 2: Description of the methodology applied for study selection and data analysis to identify metabolites associated with diets with contrasted AP and PP intake.

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AP: Animal proteins, DASH: Dietary Approach to Stop Hypentension, HEI: Healthy Eating Index, PP: Plant proteins

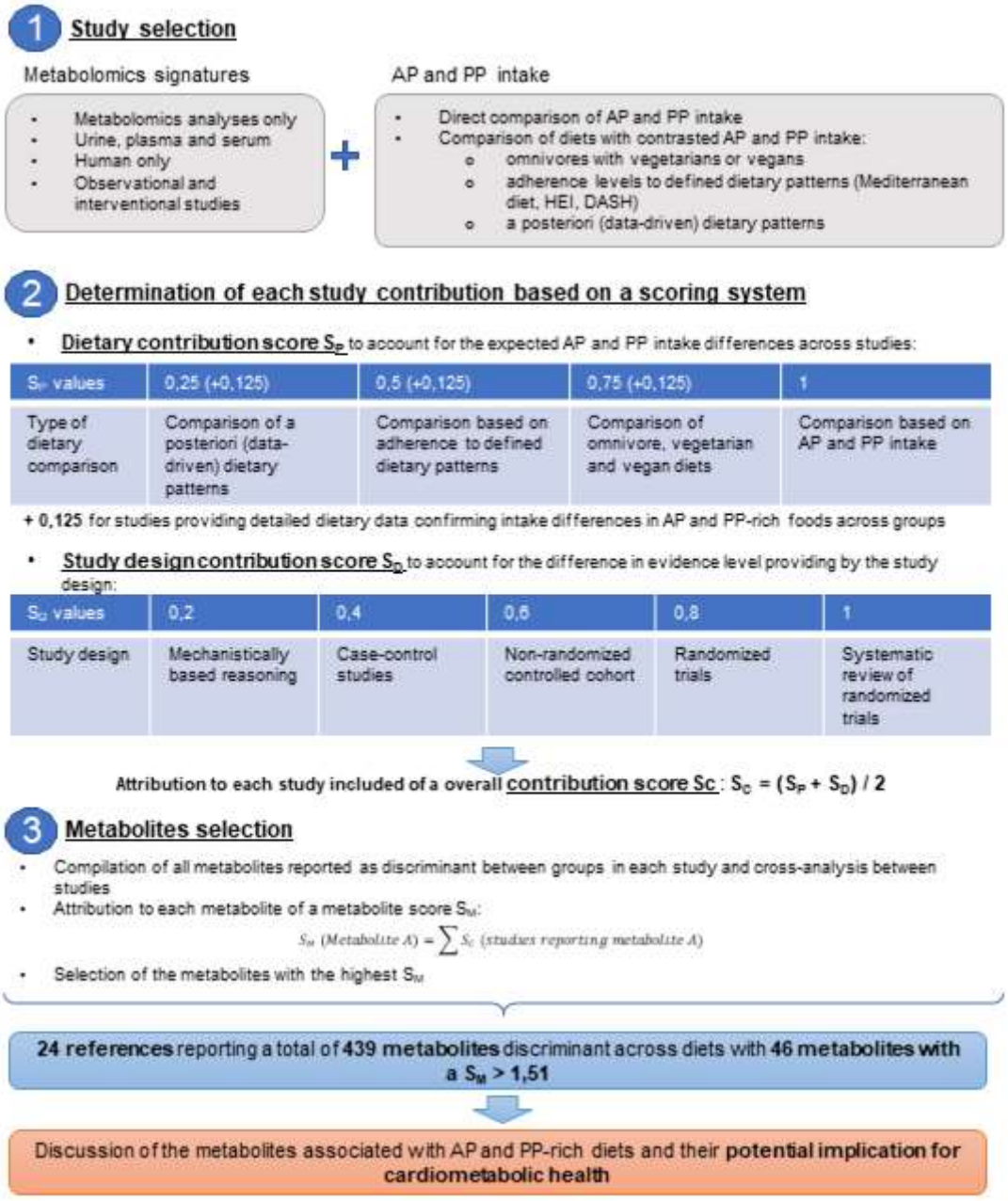
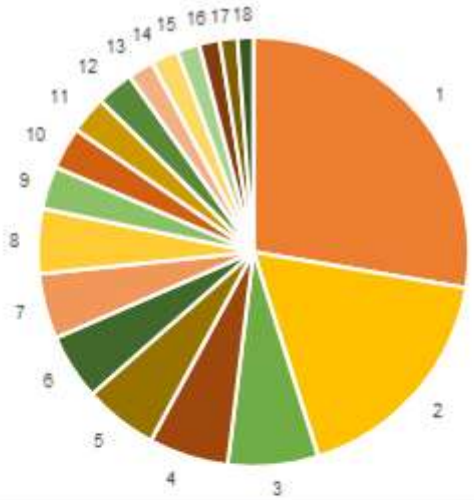


Figure 3: Contribution of each metabolite class to the overall metabolomics signature associated to AP or PP-rich diets, weighted by overall metabolite score.

Sum S_M is the relative weigh attributed to a metabolite class and calculated as the sum of S_M values of each metabolite reported within this class; n is the number of metabolites reported per class; mean S_M is the average S_M value within one metabolite class; % MS represent the

percentage of the Sum S_M value obtained by MS-based studies as opposed to NMR-based studies.

AP: animal protein, PP: plant portein, S_M: metabolite score,



	Metabolite class	Sum S_M	%MS	n	mean S_M
1	protein and amino acid metabolism metabolites	108,11	62	78	1,39
2	phosphatylcholines	68,14	100	92	0,74
3	Energy and carbohydrate metabolism metabolites	26,48	66	28	0,95
4	acylcarnitines	23,35	95	25	0,93
5	plant metabolites	21,73	94	24	0,91
6	mono, di and tryacylglycerides	19,40	100	33	0,59
7	other	19,25	97	27	0,71
8	gut derived metabolites	19,09	62	15	1,27
9	sphingomyelines	12,60	100	15	0,84
10	fatty acids	12,34	90	19	0,65
11	lysophospatidylcholines	11,04	100	12	0,92
12	phosphatidylethanolamines	10,89	100	13	0,84
13	other lipids	7,64	91	12	0,64
14	nucleotides and purine related metabolites	7,96	92	12	0,66
15	bile acids	6,61	100	11	0,60
16	vitamines and associated metabolites	6,04	86	9	0,67
17	lysophospatidylethanolamines	5,19	100	7	0,74
18	cholesterol esters	4,61	100	7	0,66

Figure 4: Main metabolites discriminant between animal protein (AP) and plant protein (PP) rich diets and their potential implication in cardiometabolic risk.

AP and PP-rich diets are associated with distinct urine, plasma and serum metabolites in humans. BCAA (leucine, isoleucine, valine), tyrosine, phenylalanine, glutamate, short-chain acylcarnitines and gut-derived TMAO were associated with AP-rich diets while glycine and glutamine were associated with PP-rich diets. Regarding their potential implication in

cardiometabolic risk, metabolites associated with AP-rich diets have been shown to be associated with higher risk while metabolites associated with PP-rich diets have been associated with lower risk.

BCAA: branch-chained amino acids, TMAO: trimethylamine-N-oxide

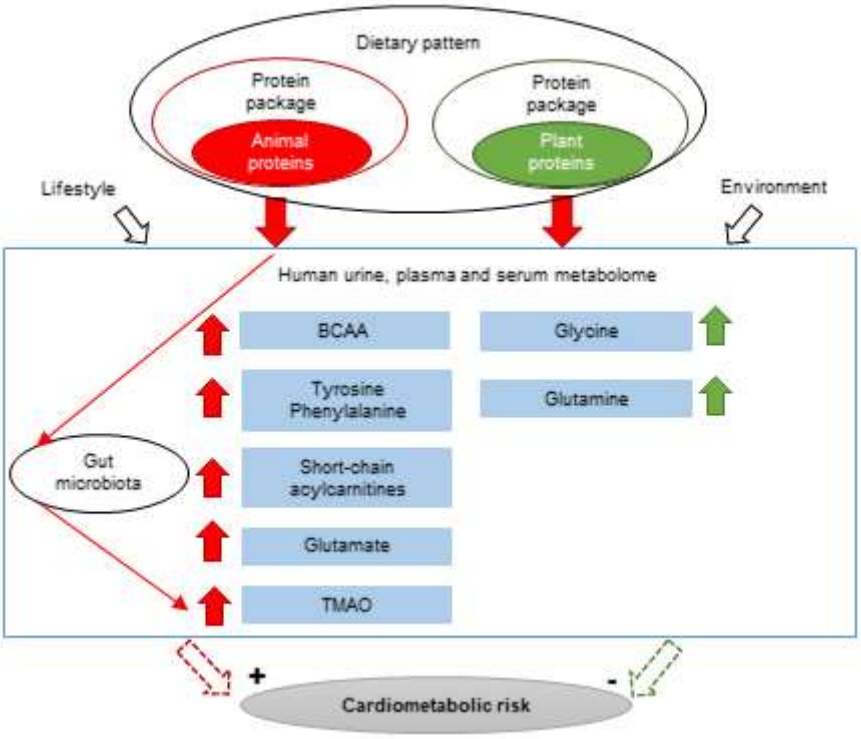


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Table 1: Description of the observational studies comparing the metabolites associated to diets with contrasted AP and PP intakes through a metabolomics approach[1]

Ref	Type of diet compared *(Food intake assessment method)*	Study design and cohort *(Country)*	Population studied	n	Metabolomics analysis	Biofluid	S_C
Comparison based on AP and PP intake							
(42)	Stratification of subjects by AP and PP intake *(FFQ)*	Cross-sectional analysis of the baseline of PREDIMED (Prevención con Dieta Mediterránea) study according to protein type intake *(Spain)*	Men and women with high CVD risk (T2D or at least 3 cardiovascular risk factors)	1833	Untargeted LC-MS	Plasma	0.70
Comparison of omnivores, vegetarians and vegans							
(50)	Habitual omnivores Habitual lactovegetarians Diet followed for at least 5 year *(None)*	Cross-sectional analysis according to vegetarian status *(China)*	Healthy men and women.	180	Untargeted NMR	Urine	0.58
(51)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet *(FFQ)*	Cross-sectional analysis of the EPIC (European Prospective Investigation into Cancer and Nutrition)-Oxford cohort according to vegetarian status *(UK)*	Healthy men only, between 30 and 49 years old	379	Targeted 145 metabolites MS/MS BIOCRATES kit	Plasma	0.58
(52)	Omnivores Lacto-ovo-vegetarians Vegans Diet followed for a at least 6 months *(3-day dietary records)*	Cross-sectional analysis according to vegetarian status *(China)*	Healthy men and women	36	Targeted 132 metabolites GC-MS	Serum	0.58

[1] aHEI: alternate Healthy Eating index, aMD: alternate Mediterranean Diet, AP: animal protein, CVD: Cardiovascular diseases, DASH: Dietary Approach to Stop Hypertension, FFQ: Food Frequency Questionnaire, HDI: Healthy Diet indicator, HEI: Healthy Eating Index, MD: Mediterranean Diet, PP: plant protein, S_C: score of study contribution (see text for additional explanations), T2D: Type 2 Diabetes

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(53)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet *(4-day weighted dietary records)*	Cross-sectional analysis according to vegetarian status *(Sweden)*	Healthy men and women	120	Untargeted NMR	Serum	0.58
(54)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet *(4-day weighted dietary records)*	Cross-sectional analysis according to vegetarian status *(Sweden)*	Healthy men and women	118	Untargeted NMR	Urine	0.58
(64)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet *(FFQ)*	Cross-sectional analysis of the EPIC (European Prospective Investigation into Cancer and Nutrition)-Oxford cohort according to vegetarian status *(UK)*	Healthy men only, between 30 and 49 years old	286	Targeted NMR	Serum	0.58
Comparison based on adherence to defined dietary patterns							
(55)	HEI-2010 aMD HDI Baltic Sea Diet Score *(FFQ)*	Cross-sectional analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study according to dietary pattern *(Finland)*	Healthy men aged between 50-69 years and smokers	1336	Untargeted LC-MS	Serum	0.45
(56)	aMD aHEI HEI-2015 DASH *(FFQ)*	Cross-sectional analysis of the Cancer Prevention Study-II Nutrition Cohort according to dietary pattern *(USA)*	Healthy postmenopausal women	1367	Untargeted LC-MS	Serum	0.51
(57)	MD *(FFQ)*	Cross-sectional analysis of the Fenland Study according to dietary pattern and cardiovascular disease risk factors *(UK)*	Healthy men and women	10806	Targeted 188 metabolites BIOCRATES kit	Plasma	0.45

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(58)	MD *(FFQ)*	Cross-sectional analysis according to dietary pattern and longitudinal analysis according to CVD risk *Test cohort:* PREDIMED study, median follow-up=4.8 years (Spain) *Validation cohorts:* Nurse Health Study I, median follow-up=22.3 years Nurse Health Study II, median follow-up=15.3 years Healthy women Health Professionals study, median follow-up=17.6 years *(USA)*	Test cohort: Men and women with high CVD risk (T2D or at least 3 cardiovascular risk factors) Validation cohort: healthy men and women	Test cohort n=1856 Validation cohort n=6868	Untargeted LC-MS	Plasma	0.45
(59)	aHEI DASH MD *(FFQ)*	Cross-sectional analysis of the Framingham Off Springs Study according to dietary pattern *(USA)*	Healthy men and women	2284	Targeted LC-MS	Serum	0.45
(60)	HEI-2015 aHEI-2010 DASH aMD *(FFQ)*	Cross-sectional analysis of the Atherosclerosis Risk in Communities Study according to dietary pattern *(USA)*	Healthy men and women	3862	Untargeted LC-MS and GC-MS	Serum	0.45
Comparison of a posteriori (data-driven) dietary patterns							
(61)	Comparison of 2 dietary patterns: - A pattern with more meat products, less vegetables - A pattern with more vegetables and less meat products. (3-day food records)	Cross-sectional analysis (Ireland)	Healthy men and women	160	Untargeted NMR	Urine	0.36
(62)	Comparison of 2 dietary patterns: - “western / nonvegetarian”: poultry + fish + red meat + coffee + pizza + alcohol	Cross-sectional analysis of the MASALA (Metabolic Syndrome and Atherosclerosis in South Asians Living in America) pilot study (USA)	Healthy men and women	145	Targeted number of metabolites not specified (above 60)	Serum	0.33

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	- "vegetarian": rice + legumes, sweet beverages + snacks (FFQ)				MS		
(63)	Comparison of 2 dietary patterns: - High meat, low vegetable - Low meat, high vegetable (FFQ)	Cross-sectional analysis (Swiss)	Healthy men and women	89	Targeted 87 metabolites in plasma and 103 in urine GC-MS + LC-MS/MS	Urine and plasma	0.36

Table 2: Description of the interventional studies comparing the metabolites associated to diets with contrasted AP and PP intakes through a metabolomics approach[1]

Ref	Study design	Type of diet compared	Population studied *(Country)*	n	Metabolomics analysis	Biofluid	S_C
(65)	Cross-over 15 days of controlled diet	-High meat -Low meat -Vegetarian diet	healthy men *(UK)*	12	Untargeted NMR	Urine	0.78
(66)	Parallel 6-month intervention A large proportion of food provided through a dedicated shop + dietary counselling	-NND: high content of vegetables, fruits, whole grains, nuts, fish and seafood products -Control: Average Danish Diet: close to actual Danish food consumption	Men and women with high waist circumference *(Denmark)*	145	Untargeted LC-MS	Urine	0.71
(67)	Parallel 6-month intervention A large proportion of food provided through a dedicated shop + dietary counselling	-NND: high content of vegetables, fruits, whole grains, nuts, fish and seafood products -Control: Average Danish Diet: close to actual Danish food consumption	Men and women with high waist circumference *(Denmark)*	145	Untargeted GC-MS	Plasma	0.71
(68)	Parallel 6-month intervention	-NND: high content of vegetables, fruits, whole grains, nuts, fish and seafood products	Men and women with high waist circumference *(Denmark)*	145	Untargeted LC-MS	Plasma	0.71

[1]AP: animal protein, MD: Mediterranean diet, NND: new Nordic Diet, PP: plant protein, PP-to-AP ratio: plant protein to animal protein ratio, S_C: score of study contribution (see text for additional explanations), TMAO: trimethylamine-N-oxide

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	A large proportion of food provided through a dedicated shop + dietary counselling	-Control: Average Danish Diet: close to actual Danish food consumption					
(69)	Parallel 1 to 3-year intervention Dietary counselling with nuts and olive oil provided	-Low fat diet: instruction to reduce all type of fats and recommendations according to American heart association -MD and olive oil supplementation (1L/week) -MD and nuts supplementation (30g/day)	PREDIMED study Men and women, non-diabetics but with high cardiovascular risk *(Spain)*	98	Untargeted NMR	Urine	0.65
(70)	Cross-over Postprandial follow up after the consumption of different test meal (3 hours after meal consumption)	-Omnivore meal -Vegetarian meal -Vegan meal	Healthy men and women *(Sweden)*	32	Untargeted NMR	Serum	0.84
(71)	Parallel 8-week intervention Combination of dietary counselling and food supply	-MD: induced an increase in PP-to-AP ratio by 2.5 -Control diet: habitual food consumption Isocaloric diets adjusted for each participant	Obese and overweighed men and women (mean BMI = 30) *(Italy)*	62	Untargeted combined with targeted approach for TMAO, carnitine, choline, creatinine and betaine LC-MS	Urine and Serum	0.71
(72)	Cross-over 4 weeks of controlled diet	-Meat-based diet -Vegetarian diet	Mostly men (94%) with ischemic heart disease *(Sweden)*	27	Untargeted LC-MS/MS	Plasma	0.84

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Table 3: Short-list of metabolites associated with AP and PP-rich diets with a sufficient level of evidence, as defined by a S_M value above 1.51[1]

		Associated to AP-rich diets		**Associated to PP-rich diets**	
Metabolite	S_M	Obs	Int	Obs	Int
Leucine*	4.83	(51,52,62)[a,#] (53,64)[a,§]	(71)[a,#] (70)[a,§]		(69)[b,§]
Creatinine*	4.24	(51,57)[a,#] (53,64)[a,§] (54)[b,§]	(70)[a,§]		(69)[b,§]
Valine*	4.16	(42,51,52,62)[a,#] (53,64)[a,§]	(70)[a,§]		
Creatine*	4.11	(42,51)[a,#] (53)[a,§]	(70)[a,§] (65)[b,§]		(69)[b,§]
Carnitine	4.05	(42,51,57)[a,#]	(72)[a,#] (71)[b,#] (65)[b,§]		
Glycine*	4.03			(51)[a,#] (53)[a,§] (50,54,61)[b,§]	(67)[a,#] (69)[b,§]
TMAO*	3.79	(50,61)[b,§]	(65,69)[b,§]		(68)[a,#] (66)[b,#]
Proline betaine	3.69		(68)[a,#] (66)[b,#] (69)[b,§]	(55,60)[a,#]	(71)[a,#]
Alanine	3.60	(52,62)[a,#]	(67)[a,#]	(51)[a,#] (64)[a,§]	(70)[a,§]
Threonine	3.41	(57)[a,#]	(67,68)[a,#] (70)[a,§]	(42)[a,#]	
Citrate*	3.09		(67)[a,#]	(64)[a,§] (50,54)[b,§]	(69)[b,§]
Isoleucine*	2.76	(52,57,62)[a,#] (53)[a,§]	(70)[a,§]		
Lysine	2.56	(51,52)[a,#] (53)[a,§]	(70)[a,§]		
Lysophosphatidyl-choline a C16:1	2.56	(51,57)[a,#]	(72)[a,#]	(42)[a,#]	
Glutamine*	2.50		(65)[b,§]	(51)[a,#] (53,64)[a,§]	
Proline	2.45	(57,58,62)[a,#] (53)[a,§]			(69)[b,§]
Methionine	2.44	(51,62)[a,#]	(70)[a,§]	(42)[a,#]	
Acylcarnitine C4*	2.44	(42,51,58)[a,#]	(68)[a,#]		
Phenylacetyl-glutamine*	2.43	(42)[a,#]	(71)[b,#]	(61)[b,§]	(69)[b,§]

[1] AP: animal protein, CMPF: 3-Carboxy-4-methyl-5-propyl-2-furanpropanoate, DHA: docosahexaenoic acid, GABA: gamma-aminobutyrate: Interventional studies, Obs: observational studies, PP: plant protein, S_M: metabolite score reflecting the degree of concordance of its identification as a discriminant metabolite between studies of good relevance (see text for additional explanations), TMAO: trimethylamine-N-oxide

[*] Metabolite discussed in the present review

[a] Analysis conducted in plasma or serum

[#] MS-based analysis

[§] NMR-based analysis

[b] Analysis conducted in urine

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Glutamate*	2.41	(52,57,58,63)[a,#]		(51)[a,#]	
Hippurate	2.39		(69)[b,§]	(59)[a,#] (50)[b,§]	(66)[b,#]
Phenylalanine*	2.31	(62)[a,#] (50)[b,§]	(67)[a,#]	(42)[a,#]	
Tryptophan*	2.19	(51)[a,#]	(71)[a,#]	(57,59)[a,#]	
Acylcarnitine C2*	2.18	(62)[a,#] (61)[b,§]	(71)[b,#] (65)[b,§]		
Lysophosphatidyl-choline a C16:0	2.13	(51)[a,#]	(72)[a,#]		(68)[a,#]
GABA	2.09	(42,52)[a,#] (63)[b,#]		(58)[a,#]	
p-cresol*	2.08		(71)[a,b,#]		(69)[b,§]
3-methylhistidine	2.06	(50)[b,#]	(72)[a,#] (69)[b,§]		
Tyrosine*	2.05	(51,52,62)[a,#] (64)[a,§]			
Betaine	2.00		(70)[a,§]	(59)[a,#]	(71)[a,#]
Choline	1.99	(59)[a,#]	(70)[a,§]	(42)[a,#]	
Succinate*	1.99	(52)[a,#]		(50)[b,§]	(70)[a,§]
Taurine	1.93	(53)[a,§] (50)[b,§]	(65)[b,§]		
1-methylhistidine	1.93	(42)[a,#] (50)[b,§]	(71)[a,#]		
Tryptophan betaine	1.88			(55)[a,#]	(71)[a,b,#]
Citrulline*	1.74	(57)[a,#]		(51)[a,#]	(71)[a,#]
Kynurenine	1.73	(51,58)[a,#]		(42)[a,#]	
Acylcarnitine C18:2	1.73			(42,51,57)[a,#]	
CMPF	1.68			(55,56)[a,#]	(71)[a,#]
Threonic acid	1.61			(55,56,60)[a,#]	(67)[a,#]
Acylcarnitine C18	1.60	(42,57,58)[a,#]			
Lactate*	1.55		(67)[a,#]		(70)[a,§]
Lysophosphatidyl-ethanolamine C22:0	1.54		(72)[a,#]	(42)[a,#]	
DHA	1.54	(64)[a,§]		(55,56)[a,#]	
Sphingomyelin (d18:2/18:1)	1.54	(51,56,59)[a,#]			
Isocitrate*	1.51	(59)[a,#] (63)[b,#]		(42)[a,#]	

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