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ORIGINAL ARTICLE

Nutrimetabolomics: integrating metabolomics in nutrition to disentangle intake of animal-based foods

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Abstract
Food intake and metabolization of foods is a complex and multi-facetted process that encompasses the introduction of new metabolite compounds in our body, initiation or alterations in endogenous metabolic processes and biochemical pathways, and likely also involving the activity of the gut microbial community that we host. The explorative nature of metabolomics makes it a superior tool for examining the whole response to food intake in a more thorough way and has led to the introduction of the term nutrimetabolomics. Protein derived from animal sources constitutes an important part of our diet, and there is therefore an interest in understanding how these animal-derived dietary sources influence us metabolically. This review aims to illuminate how the introduction of nutrimetabolomics has contributed to gain novel insight into metabolic and nutritional aspects related to intake of animal-based foods.

Keywords Animal protein · Endogenous metabolism · Meat consumption · Milk protein · Foodomics · Dietary biomarkers · Food biomarkers

1 Introduction

Protein is an essential and vital part of our diet, and adequate protein intake is pivotal for health and development (Elmadfa and Meyer 2017). Since ancient time, protein derived from animal sources has constituted an important part of our diet. There are several reasons why it is of fundamental interest to understand how intake of animal-based foods impacts human health. Firstly, world-wide increases in animal protein intake are forecasted (Boland et al. 2013). In fact, globally there is an increased need for dietary protein and it thus of utmost importance to understand how different dietary proteins exert effect on human health. Conventionally, the nutritional value of dietary proteins is evaluated based on their amino acid composition, which determines their amino acid index that is used to assess and compare the nutritional quality of different protein sources. However, still increasing evidence is emerging that the nutritional quality of proteins is not adequately articulated in a simple amino acid index or an even slight more advanced digestibility score. Apparently the nutritional value of a specific dietary protein source is influenced by multiple factors that among others include intrinsic molecular structure, processing and biophysical properties of the food matrix. These factors interact in such a complex manner that the prediction of the nutritional attributes is not trivial. In this review, we attempt to illustrate how metabolomics has emerged as a prominent tool for obtaining novel insight into nutritional aspects of animal-based foods that are hidden and not displayed in more conventional assessments of nutritional protein quality.

2 The nutrimetabolomics concept

What basically differentiates nutrimetabolomics from traditional approaches in nutrition research is the fact that the metabolomics approach attempts to be explorative and untargeted. In dietary intervention studies, nutrition researchers classically operate with a single parameter as their primary outcome and additional parameters may be analyzed as well to enhance the chances of identifying an effect of the dietary component under investigation. Typically, the entire study is founded on a hypothesis, and the purpose of the study is to investigate if the hypothesis can be accepted or rejected, e.g., a classical hypothesis could be that component x lowers

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LDL cholesterol and the primary outcome of the study would be achieved through LDL cholesterol measurements. This basic principle founding the experimental design and defining the outcome of this classical study design is providing a solid and agreed consensus. The approach also allows calculations to determine the statistical power and thereby number of subjects to be enrolled in the study. However, a universal weakness with this established hypothesis-driven approach lies in the fact that it is dependent on a priori knowledge. Any hypothesis requires a priori knowledge to be established; it can only be hypothesized that component x lowers LDL cholesterol if some a priori knowledge exists about component x. What if component x exerts another effect that is not related to LDL cholesterol? Would that ever be discovered if we rely exclusively on hypothesis testing? This problem is to some extent what justifies the introduction of omics technologies in nutrition research where the explorative and untargeted approach is expected to enhance our chances of detecting unforeseen effects and thereby grasp a more complete picture.

Noteworthy, metabolomics is one of several omics technologies; other omics technologies include genomics, transcriptomics, proteomics and epigenomics. No doubt, dependent on the research question that is to be elucidated, each omics technology has its own inherent attributes that are decisive in the choice of omics technology to apply. However, a fact that in many cases makes metabolomics an attractive choice is that metabolomics is in the downstream end of the post-genomic technologies and thus will be reflecting the end-products of complex, hard-to-decipher genetic, epigenetic and environmental stimuli and interactions (Fiehn 2002; Dunn et al. 2011).

3 Analytical tools

As within other applications of metabolomics in life sciences, different analytical platforms for conducting metabolomics analyses exist. The dominating analytical platforms used for metabolomics in nutrition research include nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based techniques. NMR holds many important and attractive attributes as analytical platform in nutrimetabolomics. Basically, proton (^{1}H) NMR spectroscopy will detect any proton-containing low-molecular weight metabolite present in a sample. Accordingly, ^{1}H NMR spectroscopy is not selective for any specific metabolite classes; it will detect both polar and non-polar metabolites, and thus, probably no other analytical technique has the same explorative and untargeted nature as ^{1}H NMR spectroscopy possess. ^{1}H NMR spectroscopy is also highly reproducible, even across different magnetic field strength, as we have demonstrated on urine samples from a dietary intervention study with milk and protein diets (Bertram et al. 2007). Here we found that same spectral features could discriminate pre- and post-intervention samples when analyses were conducted on 250, 400, 500 and 800 MHz spectrometers, respectively (Bertram et al. 2007). In relation to sample preparation, NMR spectroscopy is also competitive to other analytical techniques, and in principle NMR analyses can be done directly on urine and blood samples without any kind of extraction. The major drawback associated with NMR spectroscopy as an analytical tool in nutrimetabolomics is attached to the fact that its dynamic range together with spectral overlap sets limits for its sensitivity in terms of number of metabolites detected. Thus, metabolites present in highest concentration will dominate and obscure the detection of low-concentration metabolites. As a rough rule of thumb, about ten times more metabolites are detected with MS-based techniques than with ^{1}H NMR spectroscopy of biofluids (Psychogios et al. 2011). Consequently, while MS-based techniques commonly involve more sample preparation, are less reproducible and results obtained to a much higher extent are dependent on analysis/acquisition conditions, MS-based techniques are superior to ^{1}H NMR spectroscopy in terms of sensitivity and number of metabolites that can be detected. It is becoming increasingly common to combine different analytical platforms to enhance metabolite coverage in metabolomics studies. A comprehensive description and review of the different analytical techniques for metabolomics analyses and recent analytical advances can be found elsewhere (Dunn et al. 2011; Zhang et al. 2012; van Duynhoven and Jacobs 2016; Marshall and Powers 2017; Khamis et al. 2017).

4 Milk proteins: appealing in our obesity-prone lifestyle?

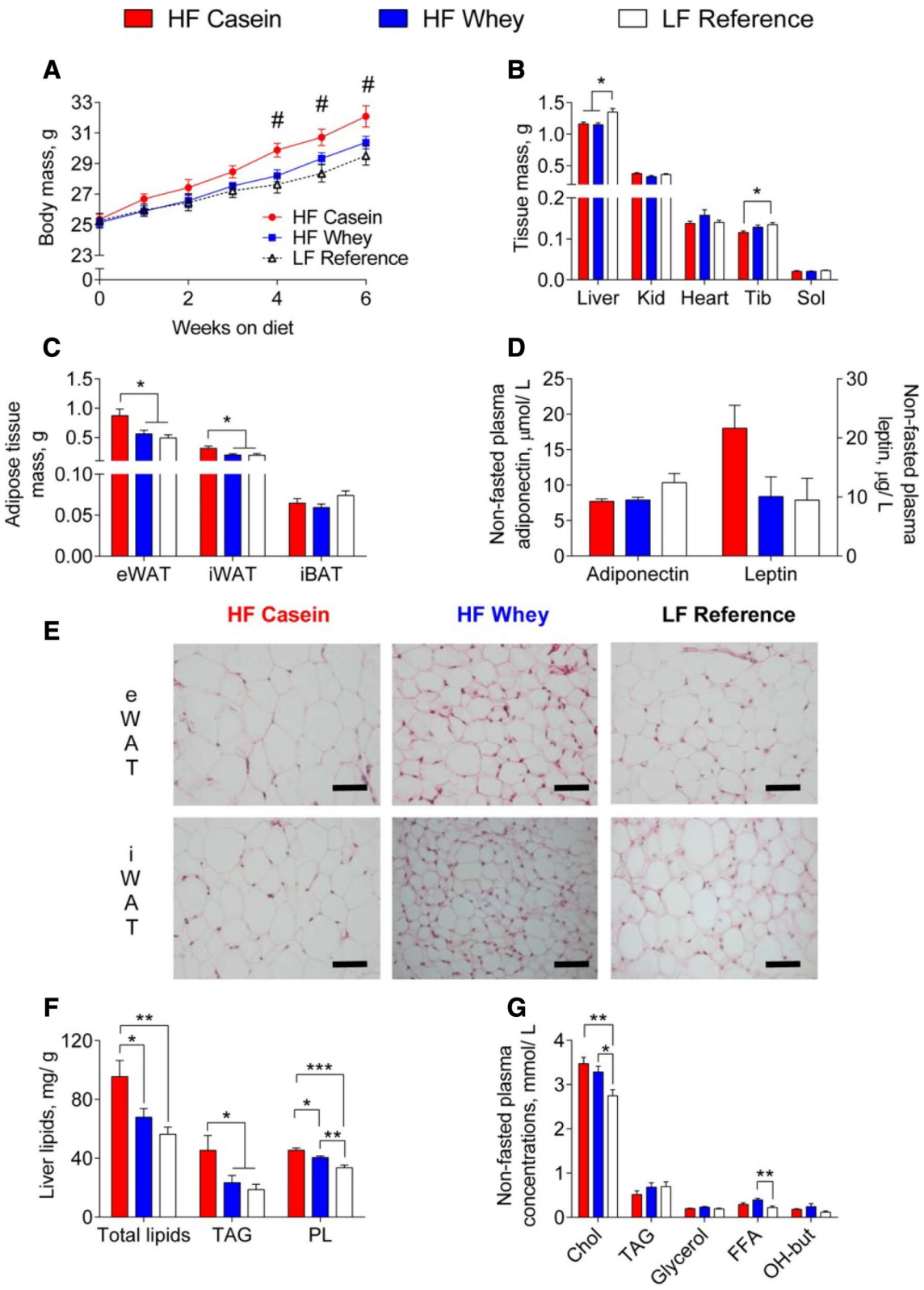
As before mentioned, increasing evidence is emerging that the nutritional attributes of a dietary protein when ingested goes beyond its amino acid index score and depends on multiple factors (Dallas et al. 2017). Milk proteins appear to represent such a class of proteins that exhibits unique properties from a nutritional perspective. Intriguingly, cohort studies have demonstrated associations between intake of dairy products and obesity risk; consumption of dairy products including milk and fermented products is associated with a lower BMI (Mirmiran et al. 2005; Wang et al. 2016; Lee and Cho 2017; Feeney et al. 2017), and results from controlled intervention studies suggest that the milk proteins are fundamental for this association. Consequently, in a randomized trial with obese individuals (BMI 30–42 kg/m^2), a milk protein-based supplement lead to a higher fat loss, overall weight loss as well as higher preservation of lean body mass during a 12-week period

with 500 kcal energy restriction than a control diet with identical macronutrient composition (Frestedt et al. 2008). In order to disentangle the metabolic effects associated with intake of milk intake and elucidate how milk proteins may exert effects on body weight management, metabolomics was introduced in a study where obese-prone mice of the C57BL/6J strain were fed high-fat diets with either casein or whey as protein sources (Lillefosse et al. 2014). Intriguingly, after 6 weeks feeding with these diets, mice fed whey protein had a significant lower weight gain than mice fed casein protein, which is consistent with other studies showing that whey protein diets attenuate weight gain in obese-prone C57BL/6J mice fed high-fat diets (Tranberg et al. 2013; McAllan et al. 2013). Even a dose-dependent effect of dietary whey protein on body weight gain has been demonstrated in C57BL/6J mice (Shi et al. 2011). Adipose tissue mass was also lower in the mice fed whey protein whereas no difference was observed in lean mass between whey and casein fed mice (Fig. 1). The reduced weight gain and the lower adipose tissue mass in whey-fed mice were despite a similar energy intake and apparent nitrogen and fat digestibility (Lillefosse et al. 2014). Both NMR-based and LC–MS-based metabolomics analyses were conducted on 48 h urine collections from the mice. OPLS-DA analyses of the NMR metabolomes showed that urine from mice fed whey contained higher levels of formate, allantoin, taurine and the tricarboxylic acid cycle (TCA) intermediates citrate and succinate. Pathway analysis of the data showed that especially the TCA cycle metabolism were differently regulated in whey- and casein-fed mice, respectively, and an examination of the individual TCA intermediates showed that ion counts for all TCA cycle metabolites detected in the urine by LC–MS metabolomics (pyruvic acid, citric acid, *cis*-aconitic acid, isocitric acid and succinic acid) revealed a significantly higher urinary excretion of these metabolites in whey-fed mice compared with casein-fed mice (Lillefosse et al. 2014). Consequently, by employing metabolomics, it has been possible to identify metabolic pathways that are regulated by whey protein intake and thereby obtain novel and essential knowledge that aid to understand the underlying metabolic mechanisms by which whey protein likely exerts effects on body weight regulation under obese-prone conditions. Whey protein is characterized by a high content of branched chain amino acids (BCAA), and it has commonly been assumed that this fact explains the beneficial effects of whey protein on obesity as BCAA can stimulate muscle protein synthesis (McGrogor and Poppitt 2013). While such a muscle synthesis-promoting effect is likely to contribute to the impact of whey protein body weight management, through metabolomics it has thus been discovered that a direct effect on endogenous metabolism through availability of TCA intermediates as substrates for anabolic processes such as lipid synthesis also appears to be a fundamental mechanism by which whey protein exerts effect on body weight regulation.

5 Processed milk and dairy products

A large proportion of dairy milk undergoes further processing into a variety of dairy products, and some common processing technologies applied include enzymatic or non-enzymatic protein hydrolysis, heat treatments, drying and fermentation. During hydrolysis, proteins are broken down to oligopeptides and free amino acids while the gross molecular composition is not altered, and hydrolysis may be applied to milk proteins for infant formulas as it typically reduces the allergenicity (Bu et al. 2013). In addition, hydrolysates have found use in commercial products for sports nutrition where there is an interest in a rapid absorption of amino acids to stimulate muscle synthesis (Manninen 2009), and in products targeted for persons suffering from malnutrition or for persons who for other clinical reasons have a lower protein digestibility and insufficient protein supply (Abd El-Salam and El-Shibiny 2017). Hindmarch et al. (2012) were among the first to investigate the impact of hydrolysis of milk proteins on the metabolic response by a metabolomic approach. The study was compiled as a 7-day intervention study with pigs that were either fed intact or an enzymatic-derived casein hydrolysate, and urine samples collected directly from the bladder were analyzed by using ^{1}H NMR spectroscopy. The study showed that the hydrolyzed casein diet was associated with higher urinary excretion of leucine, valine, taurine and glycine as compared with the intact casein diet (Hindmarch et al. 2012). These unanticipated results suggest that the exogenous protein hydrolysis prior to consumption influenced the subsequent endogenous metabolism and thereby demonstrated how the explorative nature of metabolomics can give rise to new knowledge that would not have been obtained from conventional, targeted analyses.

Metabolomics has also been applied to elucidate the metabolic impact of an extensive hydrolysis of casein using the obese-prone 657BL/6J mouse model. Employing NMR-based metabolomics in a multi-compartmental approach that included blood, urine, fecal, liver, spleen and muscle tissue samples, Yde et al. (2014) investigated the detailed endogenous response to intake of casein and hydrolyzed casein in an 8-week intervention study. Measurement of body weight and fat mass showed that the hydrolyzed casein feeding was associated with a lower weight gain and fat mass than intact casein feeding (Lillefosse et al. 2013), and in the metabolomics data this was reflected in lower tissue and plasma lipid contents as well as higher fecal lipid content (Yde et al. 2014). Consequently, the metabolomics data could unveil



that a lower fat uptake in the gastrointestinal tract likely contributes to the reduced fat mass and weight gain in mice fed hydrolyzed casein. In addition, across different sample types, metabolomics data also evidenced that the hydrolyzed casein feeding was associated with an altered amino acid metabolism; the level of alanine was reduced in feces, liver

◂**Fig. 1** Reduced energy deposition as adipose tissue mass and hepatic lipids in mice fed HF whey diets. From weeks 4–6 of the feeding trial, HF casein had higher body mass than HF whey and LF reference fed mice (**a**). Both HF-fed groups had lower liver tissue mass, whereas only HF-casein-fed mice had reduced tibialis anterior (skeletal muscle) mass compared with LF reference fed mice (**b**). Both HF whey and LF reference fed mice had reduced white adipose masses, relative to HF-casein-fed mice (**c**). No difference was observed in nonfasted plasma concentrations of adiponectin or leptin (**d**). Adipocyte size in eWAT and iWAT seemed reduced in HF whey, relative to HF-casein-fed mice. Scale bar represents 50 µm (**e**). Reduced liver total lipids and TAG concentration in HF whey and LF reference compared with HF-casein-fed mice (**f**). Both HF groups have higher nonfasted plasma total cholesterol, and HF-whey-fed mice also had higher free fatty acid (FFA) concentration, relative to LF reference fed mice (**g**). Abbreviations: Kid, kidneys; Tib, tibialis anterior; Sol, soleus; eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue; iBAT, interscapular brown adipose tissue; TAG, triacylglycerol; PL, phospholipids; FFA, free fatty acids, OHbut, hydroxybutyrate. Values are given as mean ± SEM ($n = 12$–14: **a**, Liver, Tib in **b, c**; $n = 5$–8: Kid, Heart, Sol in **b, d, f, g**). Significant differences marked by # ($P < 0.02$ HFC versus LF and HFW); * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$). Reprinted from Lillefosse et al. (2014). J. Proteome Research, 13, 2560–2570: http://pubs.acs.org/doi/abs/10.1021%2Fpr500039t with permission from ACS. Further permissions related to the material excerpted should be directed to the ACS

and spleen, and the levels of the BCAA isoleucine, leucine and valine were higher in both urine and feces from mice fed hydrolyzed casein as compared with mice fed intact casein. The metabolomics data also indicated an effect on glucose metabolism; a higher glycogen level and a concomitant lower glucose level in blood and feces was identified in the mice fed hydrolyzed casein as compared with mice fed intact casein (Yde et al. 2014). Collectively, by merging metabolomics data from different organs and biofluids, the multi-compartmental approach enabled to pinpoint changes in the metabolism of the whole organism that probably are linked with the reduced energy efficiency and lower weight gain and fat mass in mice fed hydrolyzed casein.

LC–MS-based metabolomics analyses have also been conducted on urine samples from the exact same study that the multi-compartmental NMR-based metabolomics study was based on. Comparison of results demonstrates how different and complementary information clearly can be extracted from the two analytical platforms. Thus, PCA of the LC–MS data showed a very pronounced effect of intact versus hydrolyzed casein feeding on the urine metabolome (Clausen et al. 2015). Elucidation of the 50 most important m/z features for discrimination of intact and hydrolyzed casein in an orthogonal partial least squares discriminant analysis (O-PLS-DA) showed that the majority could be assigned as molecules conjugated with either glucuronic acid (193 or 175 m/z fragments), sulphate (80 or 97 m/z fragments) or glycine (74 m/z fragment). Tandem mass spectrometry of these discriminatory features showed that hydrolyzed casein feeding induced excretion of glucuronic acid conjugates and sulphate conjugates, and many of these metabolites were completely absent in urine from mice fed intact casein. Glucuronic acid conjugates and sulphate conjugates originate from phase II metabolism that is often associated with xenobiotic metabolism, however, as indicated in this study by Clausen et al. (2015) and also previous studies (Butler and Dauterman 1988; Woodall et al. 1996), dietary protein may affect phase II metabolism as well. A possible explanation may be that with the hydrolyzed casein intake, free amino acids may be absorbed directly in the intestine, and amino acids may be taken up more rapidly and reach a higher concentration in the portal vein and liver.

A high intake of saturated fat has for a long time been regarded as an important risk factor for cardiovascular diseases (CVD). Because of the fact that dairy products generally have a relatively high content of saturated fat, a high consumption of dairy products have been speculated to impact human health negatively, and dietary guidelines recommend avoiding high-fat dairy products as parts of a balanced diet to prevent CVD (Perk et al. 2012; Eckel et al. 2014). However, evidence is accumulating that the food structure and intrinsic nutrients within a food matrix influence the digestion and absorption and thereby the overall nutritional properties of the food (Thorning et al. 2017), which likely explains why dairy foods have an attenuating effect on CVD risk (Givens 2017). Epidemiological data especially points at cheese consumption having a different impact on CVD risk than other sources of animal fat (Artaud-Wild et al. 1993; Alexander et al. 2016; Chen et al. 2017). Furthermore, research has shown that the cheese matrix apparently possess distinctive properties that influence the response to cheese ingestion. Thus, studies that have compared intake of cheese and butter, respectively, in controlled dietary interventions found that cheese intake resulted in a significantly lower LDL cholesterol level than butter even though the diets were adjusted to similar energy content and macronutrient composition (Biong et al. 2004; Tholstrup et al. 2004; Nestel et al. 2005). Zheng et al. (2015) demonstrated how metabolomics enables the elucidation of the general endogenous response to cheese intake in a study where NMR-based metabolomics analyses were conducted on urine and fecal samples collected from healthy young men that had consumed isocaloric diets with a high content of cheese (120 g/day), a high content of milk (670 mL/day) or a dairy-free diet, respectively, in a 2-week controlled dietary intervention. PCA demonstrated a pronounced effect of the dietary interventions on the metabolome; cheese and milk consumption decreased urinary choline and TMAO levels. In addition, fecal excretion of the short-chain fatty acid (SCFA) butyrate was elevated after cheese consumption while levels of acetate and propionate in the feces were enhanced after both milk and cheese consumption. SCFAs have been found to exert beneficial effects on lipid

metabolism in various tissues through a stimulation of lipogenesis (Schoenfeld and Wojtczak 2016) and SCFAs generation is proposed to be one of the pivotal mechanisms by which the gut microbiome impacts health of the host (Woting and Blaut 2016). Hong et al. (2015) also performed correlation analyses between the metabolomics data and LDL cholesterol, and demonstrated a negative and significant correlation between the subjects' LDL cholesterol level and their fecal excretion of butyrate as well as propionate. Thus, the metabolomics data substantiate the impact of SCFAs in lipid metabolism and point at this as a principal mechanism by which the cheese matrix moderates the general metabolic response to saturated fat intake.

6 Post prandial studies

Some of the mechanisms by which foods exert effects on endogenous metabolism are strongly linked to the post prandial processes, uptake and absorption, and it has been proposed that the key mechanistic events by which food components affect human health are to be found in the post prandial state (Burton-Freeman 2010). Stanstrup et al. (2014a) investigated the post prandial response to intake of casein, whey, gluten and fish-derived protein, respectively, using an LC–MS-based metabolomics approach. The study identified a faster and larger postprandial peak in several aromatic and branched-chain amino acids and their metabolites in the plasma after whey intake that could not exclusively be explained by differences in the amino acids composition of the different protein sources studied (Stanstrup et al. 2014a). Several of these amino acids found to be elevated postprandial after intake of whey protein are recognized to be insulinotropic. For lipophilic compounds, Stanstrup et al. (2014a) found lower postprandial levels of several fatty acids, in particular medium-chain fatty acids, in the plasma after whey intake, which may also be linked to a projected effect on insulin. Insulin is decisive for the endogenous energy storage post prandially and involved in the regulation of metabolic switches in several organs like liver, muscle and adipose tissue, which is considered pivotal for the development of life-style related diseases with metabolic roots. Thus, it is intriguing the metabolomics data reported by Stanstrup et al. (2014a) unravel that whey protein intake appears to exert a central effect on postprandial insulin.

The postprandial response to intake of four different milk-derived protein products has also been investigated by an LC–MS-based metabolomics approach (Stanstrup et al. 2014b). The four different protein sources were whey protein, hydrolyzed whey protein, α-lactalbumin and caseinglycomacro-peptide (CGMP)-enhanced milk protein, and the study depicted how the post prandial kinetics of various amino acids in plasma differed for the different protein products, and also demonstrated how metabolomics may provide novel information about amino acid derivatives generated through the endogenous metabolism. Furthermore, the study unraveled that whey hydrolysate intake gave rise to the presence of cyclic dipeptides that may be involved in hypoglycemic effects (Stanstrup et al. 2014b) and thereby elegantly demonstrated how the explorative nature of metabolomics may generate new knowledge that are not accessible by conventional, targeted analyses.

Ross et al. (2015) employed a GC–MS metabolomics approach to elucidate the postprandial response to intake of fish (two product types) and beef. The study successfully identified differences in the post prandial plasma metabolites between the three diets, and the study could confirm previous work that has established docosahexaenoic acid (DHA) as an exposure marker related to fish intake. In addition, some of the major findings reported included that the beef-based meal was associated with a higher postprandial level of 4-hydroxyproline, β-alanine, and 2-aminoadipic acid in plasma (Ross et al. 2015). The higher plasma level of β-alanine is probably reflecting a higher content of this metabolite in the beef meal than in the fish-based meals, and the higher level 4-hydroxyproline may also be derived directly from the meals or from degradation of collagen present in the beef meals. The authors suggested that 2-aminoadipic acid, which origin is uncertain but possibly related to lysine degradation, may have impact on insulin and thereby involved in an increased diabetes risk proposed to be associated with red meat intake (Aune et al. 2009).

7 Nutrimetabolomics to shed light on the friend or foe question raised with meat ingestion

Meat represents a very nutritional food item that provides us with all essential amino acids, iron, zinc and other minerals with a very high bioavailability as well as important vitamins such as vitamin B12 (Elmadfa and Meyer 2017). However, meat is also a food item, which impact on human health is widely debated. Based on epidemiological data, it has been proposed that intake of red and processed meat is associated with increased mortality and cancer risk (Larsson and Wolk 2006; Rohrmann et al. 2013). At the same time, other meta-analysis studies point at only rather weak associations between intake of red meat and colorectal cancer risk (Alexander et al. 2015). In relation to meat intake and metabolic and cardiovascular health, this also appears to be an unsolved matter. Some studies have proposed associations between consumption of red and processed meat and obesity, cardiovascular disease risk, and diabetes (Wang and Beydoun 2009; Micha et al. 2012; Pan et al. 2013) while other studies found opposing results (O'Connor et al. 2017)

that might reflect confounding with other lifestyle factors (Rohrmann and Linseisen 2016). Thus, this puzzling picture that appears from epidemiological surveys studying meat intake calls for more mechanistic studies to understand more exactly how the human body responds to meat ingestion, and to unravel differences associated with intake of different meat sources. Jakobsen et al. (2017) introduced NMR-based metabolomics in a study where Sprague-Dawley rats for 2 weeks were fed diets based on either chicken or beef and two levels of fat inclusion. From the urine metabolome it was evident that chicken and beef diets could be differentiated from the urinary excretion of anserine and carnosine, which represent characteristic dipeptides for poultry and red muscle (Peiretti et al. 2011), respectively, and thus can be considered a type of exposure markers directly derived from the diets. A metabolomics study based on a human intervention study with increasing quantities of chicken intake (88, 187, and 290 g/day) for 1 week in three successive weeks also identified anserine as the most specific marker for chicken meat intake (Cheung et al. 2017). Another study that examined the dose–response to intake of chicken using NMR-based metabolomics found guanidoacetate as a candidate for quantitative determination of chicken intake in free-living subjects (Yin et al. 2017). Cheung et al. (2017) also conducted metabolomics analyses on urine after dietary interventions with red meat, processed meat and fish and found that carnosine was increased most pronounced after red meat intake, but as increases in carnosine were also detected after chicken intake, the authors concluded that carnosine is less specific for red meat intake than anserine is for chicken intake (Cheung et al. 2017). In the rat study, the metabolites carnitine, trimethylamine (TMA) and trimethylamine-N-oxide (TMAO) were also excreted at a significantly higher level in urine from rats fed beef as compared with rats fed chicken (Jakobsen et al. 2017). Carnitine, TMA, and TMAO are interconnected; bacteria in the gut will convert carnitine into TMA, which in the liver will be converted into TMAO through an enzymatic process involving flavin-containing monooxygenase (FMO) (Fig. 2). The importance of the gut microflora in the formation of TMAO from dietary carnitine or choline has been shown in germ-free mice (Wang et al. 2011), and TMAO has been proposed to play a key role in the harmful effects that red meat intake may impose on cardiovascular health (Wang et al. 2011; Koeth et al. 2013). Thus, metabolomics may become a crucial tool to illuminate the associations between red meat intake and carnitine, TMA and TMAO metabolites and thereby aid in understanding potential biochemical links between meat consumption and human health. However, current knowledge obtained from metabolomics studies that have addressed connections between diet and TMAO response indicates that the associations between dietary-derived TMAO and cardiovascular health are multifaceted. Thus, beside red meat intake, fish

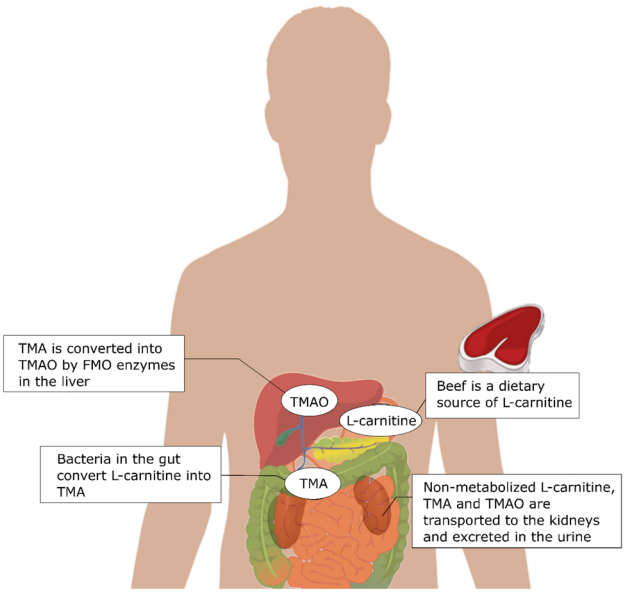


Fig. 2 Illustration of the endogenous metabolization of L-carnitine into TMA (trimethylamine) and TMAO (trimethylamine-N-oxide). FMO, flavin-containing monooxygenase

intake likewise gives rise to pronounced increases in urinary and plasma TMAO (Schmedes et al. 2016; Cho et al. 2017; Cheung et al. 2017). This circumstance must be considered paradoxical as fish consumption is recognized to be beneficial for cardiovascular health (Ussher et al. 2013), and it therefore shows that there are many aspects around TMAO and impact on cholesterol metabolism and cardiovascular health that remain unexplained.

To gain mechanistic insight into how meat intake may impact colon health, Rombouts et al. (2017) employed metabolomics on in vitro colonic digests to study the specific metabolites anticipated to be generated in vivo in the colon upon intake of beef and chicken meat, respectively. The study identified 22 metabolites that were specific and unequivocally for in vitro digestion of beef. Several of these metabolites could be associated with tryptophan metabolism. Tryptophan content is not significantly higher in beef than in chicken. But in complementary assays the authors demonstrated that the formation of the tryptophan-derived metabolites (dityrosine, kynurenic acid, *N*-formylkynurenine, and L-kynurenine) associated with in vitro digestion of beef was stimulated by the addition of myoglobin (Rombouts et al. 2017), suggesting that the presence of heme–iron in beef plays a crucial role in the formation of these tryptophan-derived metabolites. Rombouts et al. (2017) suggested an association between the kynurenine pathway of tryptophan metabolism and cancer progression, however, this is only speculative as a direct link has not be proven, and the role that these colon metabolites play for human health remain unknown.

In summary, metabolomics has enabled to put forward prospective markers of meat intake that ultimately may assist in understanding associations between meat intake and human health. However, the validity of these biomarkers under realistic conditions where interference from unknown dietary factors will be present has only been sparsely studied (Yin et al. 2017) and needs to further proven. In addition, metabolomics has exposed metabolites that are related to meat intake and that may exert endogenous effects, but it remains unclear to what extent meat consumption overall imposes negative effects on human health and also the underlying endogenous mechanisms that may be the main causes for potential associations between meat consumption and human health are not yet cracked.

8 Conclusions and perspectives

This review aimed to display how metabolomics has become a valuable tool for elucidating nutritional aspects of animal-based foods, a scientific discipline that we refer to as nutrimetabolomics: the explorative study of food ingestion. We have attempted to cover the majority of nutrimetabolomics approaches related to intake of animals foods, but additional studies not included here likely exist. From the current state, it is evident that nutrimetabolomics has emerged as a promising discipline that can aid to obtain an amended understanding of the underlying mechanisms by which foods may impact the endogenous metabolic response. The explorative nature of metabolomics represents a genuine strength; it allows the discovery of metabolic effects without any a priori knowledge and thereby eliminates the limitations that an exclusively targeted approach inherently will have. An example where the strength of metabolomics is manifested is the discovery of an association between intake of whey protein and urinary excretion of TCA intermediates (Lillefosse et al. 2014), suggesting that the intake of whey protein exerts a regulating effect on the TCA cycle. These findings were based on a mouse model, and many nutrimetabolomics studies reported here rely on animal models. Complementary human studies validating findings from animals models would strengthen the ability to draw firm conclusions from nutrimetabolomics approaches. In addition, the nutrimetabolomics approaches applied could be further refined to gain even more explicit data, among others through the introduction of analyses that enable to follow fluxes (fluxomics) in metabolic pathways to obtain a direct functional and dynamic readout (Aon and Cortassa 2015; Vaitheesvaran et al. 2015). Another field where nutrimetabolomics in future may renovate current methodologies and practices involve cohort studies where data on food intake currently in most cases rely on self-reporting food questionnaires. Nutrimetabolomics has shown an eminent potential for discovery of markers for intake of specific food items, and obtaining objective and quantitative data on food intake, e.g. based on relatively simple biofluid analyses, will enhance the reliability in cohort studies examining dietary patterns. Current bottlenecks in establishment of food markers is undoubtedly to overcome challenges from interference from the background diet and that many markers are not specific for one food item but may originate from several food items, which makes it demanding to establish quantitative markers (Münger et al. 2017). In conclusion, while it likely will take another decade or two to overcome these challenges, it is anticipated that nutrimetabolomics through the knowledge that the discipline is capable of generating, will be pivotal in the future development of tools for more personalized nutrition.

Compliance with ethical standards

Conflict of interest Author Hanne Christine Bertram has received financial support for research activities from Arla Foods amba, the Danish Dairy Research Foundation, and Arla Food for Health, which is as a consortium between Arla Foods amba, Arla Foods Ingredients Group P/S, Aarhus University and University of Copenhagen. Author Louise M.A. Jakobsen has received financial support for research activities from Arla Foods amba.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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