Digging deep for nutrients and metabolites derived from high dietary protein intake and their potential functions in metabolic health

Sarah Gilsenan[1,2], Dara Leong[1,2], Paul D. Cotter[1,2,3], Lorraine Brennan[2,4] and Kanishka N. Nilaweera[1,2]

[1]Food Biosciences Department, Teagasc Food Research Centre, Moorepark, Fermoy, County Cork, Ireland; [2]VistaMilk Research Centre, Teagasc, Moorepark, Fermoy, County Cork, Ireland; [3]APC Microbiome Ireland, University College Cork, Cork, Ireland and [4]UCD School of Agriculture and Food Science, UCD Institute of Food and Health, Belfield, UCD, Dublin 4, Ireland

Abstract

Intake of high quantities of dietary proteins sourced from dairy, meat or plants can affect body weight and metabolic health in humans. To improve our understanding of how this may be achieved, we reviewed the data related to the availability of nutrients and metabolites in the faeces, circulation and urine. All protein sources ($\geq$20% by energy) increased faecal levels of branched-chain fatty acids and ammonia and decreased the levels of butyrate. Some metabolites responded to dairy and meat proteins (branched-chain amino acids) as well as dairy and plant proteins (*p*-cresol), which were increased in faecal matter. Specific to dairy protein intake, the faecal levels of acetate, indole and phenol were increased, whereas plant protein intake specifically increased the levels of kynurenine and tyramine. Meat protein intake increased the faecal levels of methionine, cysteine and alanine and decreased the levels of propionate and acetate. The metabolite profile in the faecal matter following dairy protein intake mirrored availability in circulation or urine. These findings provide an understanding of the contrasting gut versus systemic effects of different dietary proteins, which we know to show different physiological effects. In this regard, we provide directions to determining the mechanisms for the effects of different dietary proteins.

Introduction

All living organisms require a constant supply of nutrients that can be metabolised in tissues, acting as fuels for growth and development, as well as regulators of nutrient (energy) homeostasis. This process is controlled, in part, by the small intestine by allowing digestion to take place, breaking complex nutrients into forms that can easily be absorbed into the circulation and/or by producing signalling molecules that communicate the availability of nutrients in the gastrointestinal tract to other tissues [1–3]. By contrast, the colon receives much less nutrient load compared with the small intestine because of absorption through the latter tissue. Yet, a diverse range of metabolites are produced in the colon from metabolism of dietary nutrients by the gut microbiota inhabiting this tissue, resulting in a range of metabolic health outcomes (Fig. 1) [4–9]. In this article, we focused on the nutrient and metabolite profiles created by high dietary protein (HDP) intake, which differ in source, to improve our understanding of how the different dietary proteins influence body weight and metabolic health.

## Effects on physiology and metabolic health

A renewed focus to understand the relationship between diet and metabolic health has arisen in part due to the increased prevalence of obesity and associated comorbidities over the past 100 years, mostly due to high calorie intake, particularly an increased intake of dietary fat, which affects metabolic health [10–15]. A particular interest in protein intake has emerged with many weight loss or weight maintenance recommendations promoting increased protein intake, generally above 20% of total energy intake within the 10–30% acceptable macronutrient range for proteins [16]. Data show that HPD intake reduced body weight gain or cause weight loss up to 10%, with a reduction in fat mass and an increased lean mass in overweight and obese individuals of both sexes (Table 1) [14,30–32]. The effects extended to include reduction in plasma insulin levels, triacylglycerol, high-density lipoproteins and blood pressure (Table 1). Notably, whilst these effects have been shown relative to baseline measurements or in comparison with carbohydrate intake (Table 1), there is evidence that the quality of the protein also impacts metabolic health (Table 1). For instance, whey protein (WP) intake reduced waist circumference and circulating ghrelin and insulin-like growth factor (IGF)1 levels in obese
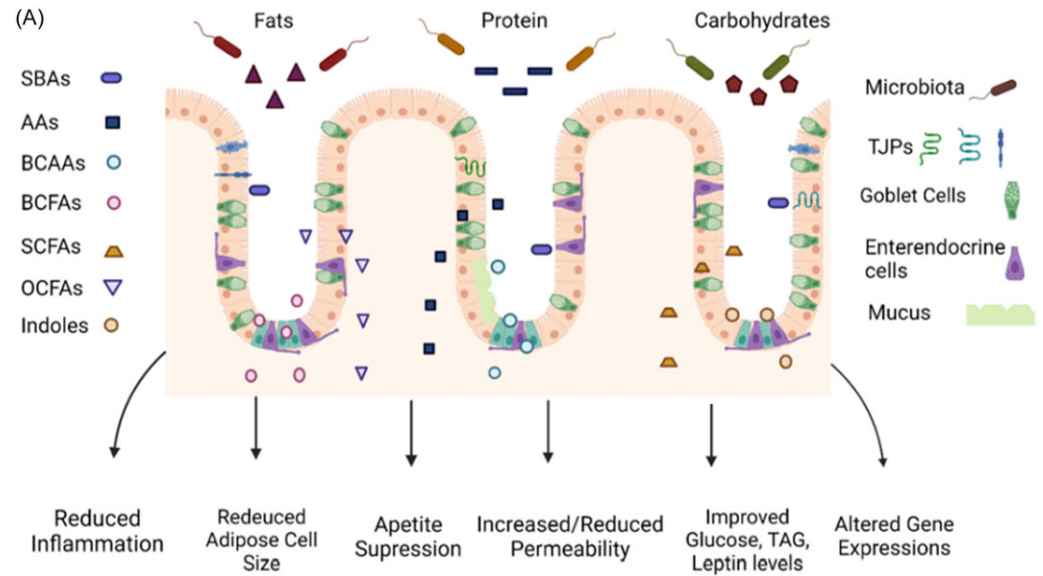
Figure 1. The impact of nutrients on the colonic epithelium. (A) Digested macronutrients either pass through the epithelium or they are metabolised by the gut microbiota, resulting in different metabolites been produced, with diverse roles. (B) A colon intestinal crypt, and associated cells and receptors that respond to nutrients and metabolites involved in many signalling mechanisms. AA, amino acids; BCAA, branched-chain amino acids; BCFA, branched-chain fatty acids; EC, enteroendocrine cells; FXR, Farnesoid X receptor; GPCR, G-protein-coupled receptors; OCFA; odd-chain fatty acids; SBA, secondary bile acids; SCFA, short-chain fatty acids; TAG, triacylglycerol; TLR, Toll-like receptors; LPS, lipopolysaccharides; TJB, tight-junction-associated proteins.
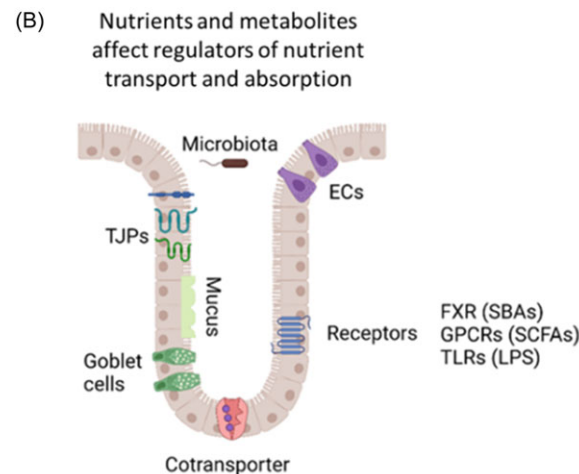
humans in comparison with soy intake (Table 1). Relative to collagen, WP caused a reduction in visceral fat, with similar effects shown for milk proteins, containing both WP and casein, compared with controls fed milk proteins and soy (Table 1). These effects reported for *ad libitum* intake have been extended to include calorie restriction, with WP showing a greater improvement of metabolic health than other protein sources (Table 1), but there are few exceptions (Table 1) [26,29]. It is also important to highlight that there are data showing unhealthy outcomes of HPDs. For instance, red meat intake has been associated with increased risk of colorectal cancers and kidney disease [33,34]. The different effects of proteins on metabolic health can be related to the quantity and composition of the amino acids, how the proteins are digested and absorbed through the gut and the impact on the gut microbiota and their functional capacity to produce metabolites, which ultimately affects host health [35–38]. For this review, we focused attention on dairy, meat or plant protein intake and their impact on the abundance of metabolites produced in the gut (and, hence, detected in faeces) as well as that emerge in circulation/urine to better understand how different proteins affect host metabolic health. Our focus was on data related to human

studies, but in a few cases we have mentioned rodent studies to draw conclusions. The search includes effects of HPD, where the protein content was equal or greater than 20% of total energy intake.

Effect on the gut microbiota

The gastrointestinal tract is inhabited by the microbiota, with the colon containing a much higher density of microbiota (10^{10}–10^{11} cells per millilitre of contents), as well as a much more diverse microbial composition compared with other parts of the intestine [39,40]. This complexity in microbial communities is further supported by the colonic structure and functions. Notably, the colon is made up of a number of colonic epithelial cells (Fig. 1), with many of these cells, particularly goblet cells, capable of producing enzymes contributing to the metabolism of nutrients in the intestinal mucosa before they reach the circulation [41]. The colonic microbiome also acts as a vital part of the digestive process by breaking down complex carbohydrates, proteins and fats, which are not broken down enzymatically in the preceding parts of the digestive system [42]. The transit rate in the colon is much slower

Table 1. Impact of protein quantity and quality on body weight and metabolic health in humans

	Test protein	Control	Dur	BMI	Sex	Diet	Health outcomes	Ref
Impact of protein quantity	WP	CHO	23W	~31·1	M/F	Ad lib:	↓ Body mass, fat mass, weight circumference and plasma insulin	[17]
	Soy	CHO	23W	~31·1	M/F	Ad lib:	↓ Plasma insulin	[17]
	Soy	CHO	3W	25–30	M/F	Ad lib:	↓Systolic blood pressure	[53]
	WP	Baseline	16W	~28	M/F	Ad lib:	↓Body weight, body fat, plasma triacylglycerol and HDL	[18]
	Casein	Baseline	12W	~30·5	F	Ad lib:	↑ Total abdominal body fat and subcutaneous fat	[19]
	MP	Baseline	20W	~29	M/F	Ad lib:	↓Body weight, visceral fat and subcutaneous fat and systolic blood pressure	[20]
	Soy	Baseline	20W	~29	M/F	Ad lib:	↓Diastolic blood pressure	[20]
	Red/white meat	High v Low proteins	64W	~32·8	F	CR+ WMD	↑Weight loss	[21]
Impact of protein quality	WP	Soy	23W	~31·1	M/F	Ad lib:	↓Waist circumference, circulatory ghrelin and IGF1	[17]
	MP	Soy + MP	20W	~29	M/F	Ad lib:	↓Visceral fat	[20]
	WP	Collagen	8W	30·9–31·1	F	Ad lib:	↓Visceral fat	[22]
	WP	Casein	12W	~31·3	M/F	Ad lib:	↓Augmentation index (arterial stiffness)	[23]
	WP	Soy	2W	28–50	M/F	CR	Decline in muscle protein synthesis reduced	[24]
	MP + amino acids	Pea + casein	12W	~33	M/F	CR	↓Visceral fat and ↓ subcutaneous fat	[25]
	Gelatin	Whey	8W	~36	F	CR	↓Waist circumference	[26]
	WP + EAA	Casein	8W	~31·3	M/F	CR	↓Body fat	[27]
	WPH	WP	8W	24–35	F	CR	↓HOM-IR	[28]
	Whey	Soy	32W	27·6–40·4	M/F	CR + WMD	CR induced metabolic improvement were not influenced by protein source	[29]

The direction of change is shown by arrows, as increase (↑), decrease (↓) or no change (↔).Ad lib, *ad libitum*; CHO, carbohydrate; CR, calorie restriction; Dur, duration; EAA, essential amino acids; F, female; HDL, high-density lipoproteins; IGF, insulin-like growth factor; M, male; MP, milk proteins; WMD, weight maintenance diet; WP, whey proteins; WPH, whey protein hydrolysate; W, weeks.

compared with the small intestine, allowing increased microbial action on the food material [43]. Indeed, microbial metabolism of digested dietary proteins results in the production of a range of nutrients and metabolites, which have diverse physiological functions (see below).

The microbiota composition plays an important role in metabolic health [37,44,45]. Notably, the alpha and beta microbial diversity, measured by the richness of diversity and evenness and relative differences in the overall diversity of taxa, respectively, highlight the similarities and differences in the microbiota across the different interventions, and associated metabolic states. A rich and diverse gut microbiota composition generally reflects a microbiota that is more resilient and capable of functioning better, with a loss in species diversity a common finding in several disease states [46]. The importance of the gut microbiota in mediating protein effects was highlighted by recent work showing that WP reduced body weight gain in high-fat-fed mice and that this effect can be transferred via faecal matter onto mice fed casein [6,47,48]. In contrast to animal studies [49], only few studies show an impact of dietary proteins on the gut microbiota in humans in the overweight and obese categories (Table 2). Of note, subjects ingesting varied quantities of dietary fats, whilst co-ingesting proteins at 25% energy from various sources (red and white meat and plants), show no effect on the alpha or beta diversities [50].

However, in the latter study, when the main effect of dietary fat on the gut microbiota was removed, an effect of dietary proteins can be seen on these micro-organisms, which were largely due to any source of protein rather than the quality of the protein consumed (Table 2). Similar data have been generated to show an impact of protein quantity on the composition of the gut microbiota (e.g. with or without fish intake or high and low gluten intake; Table 2). Where the impact of the source of proteins was investigated (pork versus chicken intake), the only changes in the gut microbiota was seen relative to baseline intake for each protein type [54] (Table 2). Imposing a calorie restriction for 8 weeks also did not affect the gut microbiota regardless of the hydrolysed state of the WP proteins [28,53] (Table 2). In contrast to the above studies, which used 16S rRNA sequencing to uncover microbial changes, a study by Bel Lassen *et al.* [25] used Metagenomics sequencing to explore the impact of an extended calorie restriction (12 weeks) on subjects consuming milk proteins supplemented with amino acids. The latter intervention was found to increase the microbial potential to produce amino acids compared with pea and casein intake (Table 2). This suggests that the interaction between the quality of the protein and the gut microbiota may be more subtle (at a functional level), requiring a greater depth of sequencing to uncover, but with the potential to influence the luminal pool of amino acids and their derivatives that are accessible by the host.

Table 2. Impact of protein quantity and quality on the composition and functional potential of the gut microbiota in humans

Test protein	Comparison	Dur	BMI	Sex	Intervention:	Impact on gut microbiota	Ref
Red meat, white meat, and plant proteins	High versus low fat	18W	18–26	M/F	Ad lib:	-Dietary fat main driver of changes in the gut microbiota.	[50]
						-Within each level of fat, dietary proteins, regardless of the source, affected *Akkermansia, Bacteroides, Sutterella, Cantenibacterium, Faecalibacterium, Megasphaera, Oscillospira* and *Methanobrevibacter*	
Salmon	No fish	8W	~31	M/F	Ad lib:	-[↓] *Bacteroidetes* Phylum, *Clostridiales* order of *Firmicutes*	[51]
						-[↑] *Selenomonadales* order of *Firmicutes*.	
Low Gluten	High Gluten	22W	~28	?	Ad lib:	-No changes in diversity.	[52]
						-[↓] *B. longum, B. angulatum, B. pseudocatenulatum, B. adolescentis, D. longicatena, B. wexlerae, Lachnospiraceae* 1, *A. hadrus, E. hallii*.	
						-[↑] Clostridiales, Lachnospiraceae 2	
						-[↓] Functional potential for carbohydrate degradation	
Casein	Soy	3W	25–30	M/F	Ad lib:	-No changes in diversity.	[53]
Pork	Chicken	3W	~30	M/F	Ad lib:	-No changes in diversity.	[54]
						-[↑] *Ruminiclostridium 5* pre versus post pork group and [↓] pre versus post chicken group	
MP + amino acid	Pea + casein	12W	~32	M/F	CR	[↑] Microbial function potential to synthesis amino acids	[25]
WPH	WP	8W	24–35	F	CR	No effect on the gut microbiota	[28]

The direction of change is shown by arrows, as increase (↑), decrease (↓) or no change (↔).Ad lib, *ad libitum*; CR, calorie restriction; Dur, duration; F, female; M, male; MP, milk proteins; WP, whey proteins; WPH, whey protein hydrolysate W, weeks.

Effects on nutrients and metabolites

Most digested proteins are absorbed in the small intestine as amino acids, but some undigested proteins, especially following HPD intake, reach the colon where they are further broken down by proteolytic bacteria for the synthesis of other amino acids and/or into amino acid derivatives that have been associated with numerous health outcomes, including regulating digestion and absorption (Table 3). Of note, lysine, arginine, glycine and the branched-chain amino acids (BCAA), namely leucine, iso-leucine and valine, are the most preferred amino acid (AA) substrates of colonic microbiota [83].

BCAA and their derivatives

BCAA are building blocks of lean tissue and are capable of modulating gene expression and signalling pathways, including regulating dietary nutrient absorption, partake in lipolysis, lipogenesis, glucose metabolism and intestinal barrier function [84–86]. However, BCAA have also been shown to have negative effects on metabolism, with increased consumption of BCAA correlated with a more unhealthy metabolic state [87], although these effects may be mediated somewhat by changing the levels of individual BCAAs [88]. Increased BCAA intake has been shown to result in increased insulin resistance [89–91]. Negative effects of BCAA intake may also include an increased risk of cancer [92]. Conversely, a reduction in BCAA intake has been shown to have positive effects on metabolic health [93].

Intake of HPD increased faecal levels of BCAA, specifically following dietary casein and red and white meat intake (Table 4). By contrast, circulating levels of BCAA increased regardless of the type of protein consumed in both fasted and non-fasted states after prolonged intake (3–4 weeks) as well as after acutely challenges, where the post-prandial plasma increase was higher after milk protein consumption compared with plant protein intake (within 5 h), WP intake compared with casein intake (3 h) and following red meat intake compared with baseline measurements (within 4 h) (Table 5). It is interesting that HPD and BCAA have both positive and negative outcomes on metabolic health. Whilst this suggests a potential functional relationship in the way dietary proteins affect metabolic health, it is important to highlight the role of the gut microbiota as a modulator of the effects. This is because these micro-organisms can convert BCAA into short-chain fatty acids (SCFA) and branched-chain fatty acids (BCFA) (Table 3), which have diverse metabolic health effects (discussed below). Indeed, in agreement with the BCAA availability in the faeces and circulation, HPD intake also increases BCFA in faeces with some reaching the urine (Tables 4 and 5). By contrast, the availability of SCFA in faeces and urine was either unaffected or decreased, with the exception of acetate, which was increased in faeces and urine following casein intake (Tables 4 and 5 and further detailed below). The data suggest a potential microbial preference for conversion of amino acids into BCFA over SCFA in a background of HPD intake, which generally accompanies a low carbohydrate intake [53,106,107].

Aromatic amino acids (AAA) and derived metabolites

Tryptophan: Evidence is emerging that the dietary supply of tryptophan affects host metabolic health directly or indirectly, the latter following microbial fermentation into numerous metabolites [71,72,108]. Indole, a tryptophan metabolite, acts as a signalling molecule capable of modulating the secretion of the satiety

Table 3. Metabolic effects of dietary protein or microbial-derived amino acids and their metabolites

Dietary protein-derived nutrient	Metabolites	Host metabolic effects of metabolites	References
AA	Microbial-derived AA	Protein synthesis; Immunity; regulation of metabolic pathways; energy homeostasis.	[35,55–63]
Aromatic AA: tryptophan	Tryptamine	Neurotransmitter (immunity and intestinal motility).	[59,60,64–72]
	Indole	Improves intestinal barrier and satiety.	
	Kynurenine/ quinolinic	Immunity.	
	Serotonin	Neurotransmitter (mood, appetite and immunity).	
Aromatic AA: tyrosine	Tyramine	Neurotransmitter linked to hypertension.	[59]
	Phenol	Reduces integrity of gut epithelium.	
	p-Cresol-derivatives	Reduces integrity of gut epithelium.	
Aromatic AA: phenylalanine	Phenylethylamine	Neurotransmitter. Releases catecholamine/serotonin.	[59]
Glutamate, arginine	GABA	Neurotransmitter (stress modulation).	[59,60]
Basic AA: arginine	Agmatine	Anti-inflammatory and inhibits intestinal cell proliferation.	[59]
	Putrescine	Promotes intestinal cell proliferation.	
	Spermidine/ spermine	Reduces oxidative stress.	
Basin AA: histidine	Histamine	Neurotransmitter (wakefulness and learning).	[59,60]
Sulphur AA: cysteine, methionine	H_2S methanethiol	Impedes cellular respiration and cause apoptosis.	[60,73]
Basic AA: lysine, histidine, aromatic AA: tyrosine, tryptophan	Ammonia	Reduces intestinal activity.	[60,73]
BCAA: leucine /isoleucine	BCFA: isovalerate/ isobutyrate	Lipid and glucose metabolism	[74,75]
Glutamine, glutamate, alanine, histidine, serine, threonine cysteine, methionine, lysine	Butyrate (SCFA)	Reduces intestinal permeability and affects cell proliferation; energy source for gut cells; mucin production; immunity; epigenetic effects.	[76–78]
Glutamine/glutamate, alanine, glycine, histidine, serine, threonine, cysteine, proline, lysine	Acetate (SCFA)	Colonic calcium absorption; appetite suppression; liver lipogenesis and cholesterol metabolism	[79,80]
Aspartate, alanine, threonine methionine	Propionate (SCFA)	Production of satiety hormones and appetite suppression; intestinal permeability; impedes liver gluconeogenesis; cholesterol metabolism [60].	[81,82]

AA, amino acids; BCAA, branched-chain amino acids; BCFA, branched-chain fatty acid; GABA, gamma-aminobutyric acid; SCFA, short-chain fatty acid.

hormone, glucagon-like peptide (GLP)-1 from colonic enteroendocrine L cells [67]. Indole improves the intestinal epithelial barrier, upregulating genes responsible for tight-junction organisation, actin cytoskeleton, mucin production and adherens junction, suggesting the strengthening and maintenance of the epithelial barrier, which directly affects intestinal permeability [64]. The tryptophan breakdown also produces indole-3-propionic acid (IPA), indole acetic acid (IAA) and kynurenine, which are also associated with several positive health outcomes (Table 3). Of note, like indole, IPA improves epithelial barrier function and reduces inflammation and body weight [69]. This molecule also improves insulin sensitivity, as does IAA [69]. These effects are in part due to the indole moiety, which acts as a ligand for the aryl hydrocarbon receptor [109], whereby receptor activation can suppress inflammatory response and affect energy metabolism [110]. Serotonin can be synthesised by the gut microbiota from tryptamine, a metabolite of tryptophan, and the latter amino acid and its derivative regulate the serotonin levels in the colon and blood

[71,111]. Tryptamine is also capable of inducing the release of serotonin from enteroendocrine cells as well as potentiating the inhibitory response of cells to serotonin [112,113]. While there are many health benefits of breakdown of tryptophan, in host cells and by microbial activity, the co-production of ammonia is a concern because of the damage caused to the mucosal layer in the colon, which impairs the absorptive capacity of the tissue [73].

In relation to protein source, intake of high quantities of proteins increased the faecal levels of indole derivatives (milk proteins) and ammonia (all protein sources; Table 4). This raises the possibility that these dietary proteins increase the microbial activity related to metabolism of tryptophan in the gut. In support of this suggestion, the intake of milk proteins supplemented with amino acids was found to increase the gut microbial potential to produce amino acids (Table 2). Beyond the gut, indole derivatives have been found to increase in urine following dairy protein (casein) intake (Table 5), whilst other tryptophan metabolites, namely kynurenine and quinolinic acid, show no consistency in

Table 4. Impact of dietary proteins on the metabolite profiles in the faeces in humans

Nutrient or metabolite		High-protein diets			Reference
		Dairy	Meat	Plant	
BCAA		↑CAS (3W)	↑RED/WHT (4W)	?	[53,94]
EAA: methionine		?	↑ RED/WHT (4W)	?	[94]
Non-EAA: cysteine, alanine		?	↑ RED/WHT (4W)	?	[94]
Non-EAA: tyrosine		?	↔ RED/WHT (4W)	↔GLTN (22W)	[52,94]
BCFA		↑ MP (1W)	↑ RED/WHT (4W)	↑ SOY (3W)	[52,53,95–97]
		↑CAS (3W)		↔ GLTN (22W)	
SCFA: butyrate		↓ CAS (3W)	↓ RED/WHT (4W)	↓ SOY (3W)	[52,53,96–98]
		↔ DAIRY/MEAT (1·5W)		↔GLTN (22W)	
		↔ DAIRY/ANIMAL/PLANT (48W)			
SCFA: propionate		↔ CAS (3W)	↓RED/WHT (4W)	↔ SOY (3W)	[52,53,96–98]
		↔ DAIRY/MEAT (1·5W)		↔GLTN (22W)	
		↔ DAIRY/ANIMAL/PLANT (48W)			
SCFA: acetate		↑ CAS (3W)	↓ RED/WHT (4W)	↔ SOY (3W)	[52,53,96–98]
		↔ DAIRY/MEAT (1·5W)		↔GLTN (22W)	
		↔ DAIRY/ANIMAL/PLANT (48W)			
Tryptophan derivatives	Indole derivatives	↑ MP (1W)	↔ RED/WHT (4W)	↔ GLTN (22W)	[52,95–97]
	Kynurenine	?	?	↑ GLTN (22W)	[52]
	Quinolinic	?	?	↔ GLTN (22W)	[52]
Tyrosine derivatives	Phenol	↑ MP (1W)		?	[95,98]
		↔ DAIRY/MEAT (1·5W)			
		↔ DAIRY/ANIMAL/PLANT (48W)			
	p-Cresol-derivatives	↑ MP (1W)	↔ RED/WHT (4W)	↑ SOY (3W)	[53,95,96]
	Tyramine	↔ CAS (3W)	?	↑ SOY (3W)	[52,53]
				↔GLTN (22W)	
Tryptophan, lysine, histidine, tyrosine derivative	Ammonia	↑ MP (1W)	↑RED (3W)	↔ SOY (3W)	[53,95,97–99]
		↔ CAS (3W)	↔ RED/WHT (4W)		
		↑ DAIRY/MEAT (48W)			
		↑ DAIRY/ANIMAL/PLANT (48W)			
Cysteine, methionine derived	Methanethiol	↑ MP (1W)	?	?	[95]

The direction of change is shown by arrows, as increase (↑), decrease (↓) or no change (↔) of metabolites. The length of the dietary challenge is shown in subscript in weeks (W). BCAA, branched-chain amino acids; BCFA, branched-chain fatty acids; CAS, casein; EAA, essential amino acids; MP, milk proteins; RED, red meat; SCFA, short-chain fatty acids; GLTN, gluten; WHT, white meat.

terms of availability in faeces and circulation/urine based on the source of the protein consumed (Tables 4 and 5). The presence of indole in faeces and circulation/urine following chronic intake of dairy proteins (>1 week; Tables 4 and 5), is striking, and this contrasts with the intake of non-dairy proteins, which only seem to increase indole levels only in urine (by soy or meat/plant protein intake; Table 5). The difference may be related to the differential impact of dairy and plant proteins on the functional potential of the gut microbiota (mostly affected by dairy proteins; Table 2) combined with the host tissue accessibility and metabolism of tryptophan that we know to be higher in quantity in milk proteins compared with plant proteins [38].

Tyrosine: Microbial metabolism of tyrosine can lead to the production of phenols, *p*-cresol derivatives and tyramine (Table 3) [59]. Tyramine is a neurotransmitter facilitating norepinephrine release, which is known to affect respiration and glucose levels in blood (Table 3). Both phenol and *p*-cresol are known to decrease the integrity of the gut epithelium [59]. Similar to tryptophan, the faecal availability of this AAA was not influenced by protein source, but the related metabolites, phenol, *p*-cresol derivatives and tyramine were increased in faecal matter by dairy (phenol and *p*-cresol) and plant (*p*-cresol and tyramine) intake (Table 4). Data are limiting on the availability of tyrosine-derived metabolites in circulation, except for the increased urinary levels of *p*-cresol detected following dairy (casein) protein intake (Table 5). The data suggest that the quality of protein associated with HPD, which can deliver high quantities of tryptophan and tyrosine, can provide beneficial effects (by producing indoles) as well potential harmful effects (by producing ammonia, phenol and *p*-cresol).

Table 5. Impact of dietary proteins on the metabolite profiles in circulation or urine in humans

Nutrient or metabolite		High-protein diets			References
		Dairy	Meat	Plant	
EAA: BCAA		↑CAS (3W:PLASMA: FASTED)	↑RED (4H:PLASMA; POSTPRANDIAL)	↑SOY (3W:PLASMA:FASTED)	[94,53,100–103]
		↑ WP (4W:PLASMA; FASTED)	↑RED/WHT (4W:SERUM: NOT FASTED)	↑ MIX PLANT (5H;PLASMA: POSTPRANDIAL)	
		↑ WP (3H:PLASMA; POSTPRANDIAL)			
		↑MP (5H;PLASMA: POSTPRANDIAL)			
EAA: threonine		↑ MP (5H;PLASMA: POSTPRANDIAL)	↑RED (4H:PLASMA; POSTPRANDIAL)	↑ MIX PLANT (5H;PLASMA: POSTPRANDIAL)	[94,102,103]
			↔ RED/WHT (4W:SERUM: NOT FASTED)		
EAA: tryptophan, methionine, lysine		↑ MP (5H;PLASMA: POSTPRANDIAL)	↑ RED (4H:PLASMA; POSTPRANDIAL)	↑ MIX PLANT (5H;PLASMA: POSTPRANDIAL)	[94,102,103]
			↔ RED/WHT (4W:SERUM: NOT FASTED)		
Non-EAA: tyrosine		↑ MP (5H;PLASMA: POSTPRANDIAL)	↑ RED (4H:PLASMA; POSTPRANDIAL)	↑ SOY (3W:PLASMA:FASTED)	[94,53,102,103]
		↑ CAS (3W:PLASMA: FASTED)	↔ RED/WHT (4W:SERUM: NOT FASTED)	↑ MIX PLANT (5H;PLASMA: POSTPRANDIAL)	
Non-EAA: glycine		↑ MP (5H;PLASMA: POSTPRANDIAL)	↑RED (4H:PLASMA; POSTPRANDIAL)	↑ MIX PLANT (5H;PLASMA: POSTPRANDIAL)	[94,102,103]
			↔ RED/WHT (4W:SERUM: NOT FASTED)		
BCFA		↑ CAS (3W:URINE: FASTED)	↑ RED/WHT (4W:SERUM:NOT FASTED)	↑ SOY (3W:URINE:FASTED)	[52,53,94]
				↔ GLTN (22W:URINE;FASTED)	
SCFA: butyrate		↓ CAS (3W:URINE: FASTED)	?	↓ SOY (3W:URINE:FASTED)	[52,53]
				↔ GLTN (22W:URINE;FASTED)	
SCFA: propionate		↔ CAS (3W:URINE: FASTED)	?	↔ SOY (3W:URINE:FASTED)	[52,53]
				↔ GLTN (22W:URINE;FASTED)	
SCFA: acetate		↑CAS (3W:URINE: FASTED)	?	↔ SOY (3W:URINE:FASTED)	[52,53]
				↔ GLTN (22W:URINE;FASTED)	
Tryptophan derivatives	Indole derivatives	↑ CAS (3W:URINE: FASTED)	↔ RED/WHT (4W:SERUM: NOT FASTED)	↑ SOY (3W:URINE:FASTED)	[52,53,94,104,105]
				↔ GLTN (22W:URINE;FASTED)	
			↑ MEAT/PLANT (2W:URINE/SERUM;FASTED)		
	Kynurenine	?	↔ RED/WHT (4W:SERUM: NOT FASTED)	↓ GLTN (22W:URINE;FASTED)	[52,94]
	Quinolinic	?	?	↔ GLTN (22W:URINE;FASTED)	[52]
Tyrosine derivatives	*p*-Cresol derivatives	↑CAS (3W:URINE: FASTED)	↔RED/WHT (4W:SERUM:NOT FASTED)	↔SOY (3W:URINE:FASTED)	[53,94,104]
			↔MEAT/PLANT (2W:URINE/SERUM;FASTED)		
Tryptophan, tyrosine, histidine-Derivatives	Ammonia	↔CAS (3W:URINE:FASTED)	?	↔SOY (3W:URINE:FASTED)	[53]

The direction of change is shown by arrows, as increase (↑), decrease (↓) or no change (↔) of metabolites. The length of the dietary challenge is shown in subscript in weeks (W) or hours (H) along with the medium in which the metabolite was detected and whether the subjects were fasted or non-fasted. BCAA, branched-chain amino acids; BCFA, branched-chain fatty acids; CAS, casein; EAA, essential amino acids; MP, milk proteins; RED, red meat; SCFA, short-chain fatty acids; GLTN, gluten; SCFA, short-chain fatty acids; WHT, white meat.

Non-essential amino acids: Dietary AAs are absorbed through the gut or act as substrates for microbial production of AAs, for their own utilisation and/or for supply to the host (Table 3). For instance, glutamine supplementation is found to impact the overall AA composition and content in the gastro-intestine, including raising the concentration of Asp, Glu and Ala in the blood [114–116]. Similarly, in the host, serine can be used to produce glycine or this process can be reversed [117]. Glycine has many biological effects, including being used for protein synthesis and bile acid metabolism and, hence, contributing to the digestion and absorption of dietary lipids and vitamins, as well as reducing body weight and fat and leading to an associated improvement in insulin sensitivity [117]. Given the wide range of routes of amino acid synthesis (host tissue metabolism and the gut microbiota), it is no surprise that the intake of dietary proteins should cause an increase in the levels of tyrosine and glycine in circulation following chronic and acute challenges (all protein sources; Table 5). Interestingly, whilst intake of red and white meat did not cause any changes in circulatory levels of these amino acids, it should be noted that the related data were generated from non-fasted state following 4 weeks of intervention [94] (Table 5), contrasting with other studies showing a post-prandial increase in the AAs (4–5h) following an acute dietary protein challenge (Table 5), presumably reflecting a greater absorption in the small intestine.

Short-chain fatty acids: There is a large body of evidence relating to the beneficial health impacts of SCFA, namely acetate, butyrate and propionate, in particular in regulating energy metabolism, specifically in reducing hepatic glucose production and adiposity and stimulating the release of satiety related hormones such as peptide YY [79,81,118,119]. The SCFA also partake in the maintenance of the gut, including improving the integrity of intestinal epithelial cells, promoting the expression of tight-junction-associated proteins, cell proliferation and increasing mucin production [120,121]. These effects are dependent upon the type of SCFA produced and how and where they act. Of note, SCFA are absorbed into the colonocytes or those that escape metabolism in cells are transported into the liver via the portal system. It should be mentioned that only a minor fraction of SCFA produced in the colon reach the circulatory system. Despite this, some contrasting responses of SCFAs need to be highlighted. Of note, acetate can be utilised for cholesterol synthesis, while propionate decreases the activity of the related pathway in the liver [60]. The higher levels of SCFA also decrease the production of hydrogen sulphide, which is well established to be detrimental to colonic health (Table 2), including as a contributing factor to ulcerative colitis [122,123] and as a potential trigger of colorectal cancer [124]. The effects of SCFA are mediated by G-protein-coupled receptors, namely GPR41, GPR43 and GPR109a, which are expressed in different tissues within the body [125]. It should also be noted that some SCFA have negative effects on health. Notably, propionate has been shown to increase liver lipogenesis [126]. In addition, acetate, propionate and butyrate have been shown to reduce gut dysbiosis-driven lung inflammation, as well as cause a pro-inflammatory response in human primary lung fibroblasts [126].

The SCFA synthesised in the colon are produced mainly by the microbial fermentation of indigestible carbohydrates such as fibre, with increasing fibre intake increasing SCFA-producing bacteria and colonic SCFA [127–130]. However, AA can also function as synthetic precursors of SCFA in the colon [60], with the type and quantity of SCFA produced depending on the AA substrate available (Table 1) as well as the microbiota present [131–133]. Likely, as a result of the availability of AA in the colon, HPD with low carbohydrates have been shown to influence the production of SCFA [53,106,107]. Of note, proteins from dairy (casein), meat (red and white meat) and plant (soy) all decreased butyrate-producing microbiota, and further decreased butyrate levels in faeces (Table 4). By contrast, dairy (casein) proteins increased acetate levels in the faecal matter (Table 4) and also in urine (Table 5). Available evidence suggests that the source of protein influences the type of SCFA produced in the gut, with some (acetate) reaching the urine, presumably via the circulation.

Branched-chain fatty acids (BCFAs): Further microbial fermentation of BCAA results in the formation of branched SCFA (BSCFA) or BCFA, including isovalerate and isobutyrate. The latter can also be produced from bacterial fermentation of some amino acids such as glycine (which can produce acetate), threonine (which can produce butyrate) or alanine (which can produce propionate) [60]. The levels of BCFA in the colon highlight proteolytic fermentation, as BCFA are elevated when saccharolytic fermentation is minimal and protein fermentation is significantly enhanced in the colon [73,134]. Similarly to SCFA, BCFA are shown to have positive impacts on metabolic health (Table 3), being associated with weight loss and maintenance [75] as well as showing an inverse correlation with lipotoxicity and improved insulin sensitivity [74,75]. Likely due to the availability of BCAA in the colon (Table 4), HPD increased the levels of BCFA in the faecal matter and circulation, including urine (Tables 4 and 5). This effect was seen for proteins sourced from dairy, meat and plants, with the exception of gluten (Table 5). The largely similar effects of different proteins on the availability of BCFA both in faecal matter and in circulation suggest an important role for these metabolites in mediating the metabolic health effects of HPDs.

Exploring the potential mechanisms

All protein sources increased BCFA in faecal content, probably from the increased gut availability of BCAA (Fig. 2A), suggesting a greater bacterial conversion of BCAA to BCFA with the intake of different proteins, but we cannot exclude the contribution of other amino acids for this process. Dairy protein intake specifically increased faecal levels of indole and acetate (Fig. 2A). Alongside these health-promoting metabolites, several other metabolites emerge in faecal matter with known unhealthy outcomes. These were phenol (dairy), *p*-cresol derivatives (dairy and plant), ammonia (all protein sources) and tyramine (plant). In circulation, and regardless of the source of proteins, amino acids, including BCAA, increased (Fig. 2B). In addition, for dairy proteins, the impact on the faecal availability of acetate, indole, *p*-cresol, BCFA and butyrate mirrored availability in the circulatory system or urine (Fig. 2B), suggesting both gastro-intestinal and systemic effects of these metabolites. This contrasts with metabolites produced following plant and meat protein intake, which show fewer common responses in faecal matter and circulation (and urine) (Fig. 2). The contrasting levels of metabolites in the gut and circulation/urine following dairy, meat or plant protein intake could be related to the differences in the amino acid composition and the three-dimensional structure of the proteins accessible for enzymatic digestion and how the resulting digested peptides and amino acids are utilised by the dietary protein-sensitive gut microbiota to produce metabolites, which ultimately reach the gut and/or enter the circulatory system [35–38].
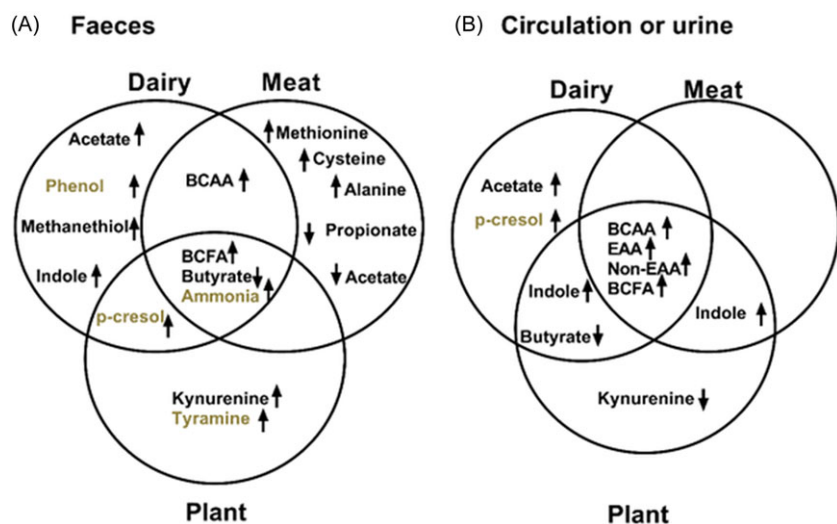
Figure 2. Impact of protein quality on the metabolites in (A) faeces and (B) circulation or urine. The direction of change is shown by arrows, as increased (↑) or decreased (↓). Metabolites highlighted in red colour are known to cause unhealthy outcomes in humans. BCAA; branched-chain amino acids; BCFA, branched-chain fatty acids; EAA; essential amino acids.

In exploring the mechanisms for the physiological outcomes of HPD intake, the post-prandial increase in circulatory levels of AA including BCAA is notable because their increase has been associated with increased satiety in human subjects, in particular following WP consumption compared with casein intake. The effect can be related to increased circulatory levels of satiety related hormones, namely cholecystokinin, (GLP)-1 and glucose-dependent insulinotropic polypeptide [101]. Whilst these data have emerged from acute challenges, the long-term intake of HPD does not appear to cause changes in energy intake in humans, suggesting that there are other mechanisms at play [135]. In this regard, the increased availability of indole in the gut lumen by dairy protein intake is interesting because this metabolite and its derivatives are known to increase the release of GLP-1 from enteroendocrine cells [69], and the activity of GLP-1 has been linked to roles beyond the reduction in food intake to include effects on adiposity [136]. In addition, and separate from effects on GLP-1, indoles and their derivatives have a direct impact on adiposity, reducing adipogenesis and increasing thermogenesis [69], and their detection in urine following dairy (and plant) protein, presumably by the crossover from circulation, supports circulatory effects. Given that BCFA have been associated with a reduction in body weight [75], it is not surprising that these should also increase in circulation following HPD intake (Fig. 2). The data suggest that indole and BCFA generated by HPD intake may contribute to the reduction in body weight through effects on intake, effects on energy expenditure and/or direct effects on lipid metabolism. These metabolites could account, at least in part, for the greater impact of dairy proteins on metabolic health compared with other sources of proteins. In contrast to body weight and adiposity, the effect of HPD on insulin sensitivity is inconstantly reported [14,21,137,138]. This may be due in part to increased circulatory levels of BCAA and increased faecal and circulatory levels of *p*-cresol and its derivatives, which are known to reduce insulin sensitivity [89,90], counterbalanced by the increased levels of acetate detected mainly following dairy protein intake. Whilst HPD intake is known to reduce or cause no change in butyrate and propionate levels, the increased acetate level in faecal matter is significant because of important roles of these SCFA in energy balance regulation and insulin sensitivity [139–141]. Overall, it is

clear that proteins from different sources produce common and distinct metabolites, and their unique mechanisms of actions in the gut and/or via circulation presumably underlie the differences in physiological and metabolic outcomes of HPD.

Future directions

There are limited data on the effect of high protein intake on the composition and functional potential of the gut microbiota. Further studies are also needed to ascertain how plant proteins other than soy and gluten influence the nutrients and metabolite profiles in the gut, given the increased focus on these proteins as a sustainable production source for human consumption [142]. Extending these lines of investigation, work is also needed to clarify the role of sex, since this parameter influences the protein quantity consumed, the composition and the functional potential of gut microbiota and physiological and metabolic parameters [143–145]. While the focus of our review was on human data, cross-species investigations can provide a greater understanding of the role played by nutrients and metabolites identified here as potential mediators of metabolic health effects of HPD. These studies could involve the transfer of faecal matter from humans to other species such as rodents within each sex and/or supplementation or depletion of the nutrients or metabolites in the diet to ascertaining their biological significance.

Conclusions

The amino acids derived from dietary proteins play important roles in physiological processors and, in turn, in metabolic health and, in some instances, in the pathophysiology of metabolic disorders. This functional relationship extends to include metabolites formed in the gut by the activity of the microbiota. A comparison of HPD, which included the limited number of studies on plant proteins (soy and gluten), revealed similarities and differences in the metabolite profiles in faeces and circulation/urine, highlighting the contrasting gut versus circulatory effects of protein source within HPD. This understanding will help to elucidate the complex mechanisms of action of HPD and, in turn, improve the efficacy of the interventions.

References

[1] Ma ZF & Lee YY (2020) Chapter 7 – Small intestine anatomy and physiology. In Rao SSC, Lee YY & Ghoshal UC (eds), *Clinical and basic neurogastroenterology and motility*. Cambridge, MA: Elsevier, Academic Press. pp. 101–111.

[2] Martinez-Guryn K, Hubert N, Frazier K, Urlass S, Musch MW, Ojeda P, *et al.* (2018) Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. *Cell Host Microbe* **23**, 458–469.e5.

[3] Saps M & Miranda A (2017) Gastrointestinal pharmacology. *Handb Exp Pharmacol* **239**, 147–176.

[4] Clarke SF, Murphy EF, Nilaweera K, Ross PR, Shanahan F, O'Toole PW & Cotter PD (2012) The gut microbiota and its relationship to diet and obesity: new insights. *Gut Microbes* **3**, 186–202.

[5] Covasa M, Stephens RW, Toderean R & Cobuz C (2019) Intestinal sensing by gut microbiota: targeting gut peptides. *Front Endocrinol (Lausanne)* **10**, 82.

[6] Nychyk O, Barton W, Rudolf AM, Boscaini S, Walsh A, Bastiaanssen TFS, *et al.* (2021) Protein quality and quantity influence the effect of dietary fat on weight gain and tissue partitioning via host-microbiota changes. *Cell Rep* **35**, 109093.

[7] Rajilić-Stojanović M & de Vos WM (2014) The first 1000 cultured species of the human gastrointestinal microbiota. *FEMS Microbiol Rev* **38**, 996–1047.

[8] Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R & Gordon JI (2009) The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* **1**, 6ra14.

[9] Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, *et al.* (2019) Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. *Gut* **68**, 1417.

[10] Hu S, Wang L, Yang D, Li L, Togo J, Wu Y, *et al.* (2018) Dietary fat, but not protein or carbohydrate, regulates energy intake and causes adiposity in mice. *Cell Metab* **28**, 415–431 e4.

[11] Le TH, Disegna M & Lloyd T (2020) National food consumption patterns: converging trends and the implications for health. *EuroChoices* n/a.

[12] Lee JH, Duster M, Roberts T & Devinsky O (2021) United States dietary trends since 1800: lack of association between saturated fatty acid consumption and non-communicable diseases. *Front Nutr* **8**, 748847.

[13] Morselli E, Criollo A, Rodriguez-Navas C & Clegg DJ (2014) Chronic high fat diet consumption impairs metabolic health of male mice. *Inflamm Cell Signal* **1**, e561.

[14] Santesso N, Akl EA, Bianchi M, Mente A, Mustafa R, Heels-Ansdell D & Schunemann HJ (2012) Effects of higher- versus lower-protein diets on health outcomes: a systematic review and meta-analysis. *Eur J Clin Nutr* **66**, 780–788.

[15] Wang L, Wang H, Zhang B, Popkin BM & Du S (2020) Elevated fat intake increases body weight and the risk of overweight and obesity among Chinese adults: 1991–2015 trends. *Nutrients* **12**, 1–13.

[16] Wolfe RR, Cifelli AM, Kostas G & Kim IY (2017) Optimizing protein intake in adults: interpretation and application of the recommended dietary allowance compared with the acceptable macronutrient distribution range. *Adv Nutr* **8**, 266–275.

[17] Baer DJ, Stote KS, Paul DR, Harris GK, Rumpler WV & Clevidence BA (2011) Whey protein but not soy protein supplementation alters body weight and composition in free-living overweight and obese adults. *J Nutr* **141**, 1489–1494.

[18] Arciero PJ, Baur D, Connelly S & Ormsbee MJ (2014) Timed-daily ingestion of whey protein and exercise training reduces visceral adipose tissue mass and improves insulin resistance: the PRISE study. *J Appl Physiol (1985)* **117**, 1–10.

[19] Sites CK, Cooper BC, Toth MJ, Gastaldelli A, Arabshahi A & Barnes S (2007) Effect of a daily supplement of soy protein on body composition and insulin secretion in postmenopausal women. *Fertil Steril* **88**, 1609–1617.

[20] Takahira M, Noda K, Fukushima M, Zhang B, Mitsutake R, Uehara Y, *et al.* (2011) Randomized, double-blind, controlled, comparative trial of formula food containing soy protein vs. milk protein in visceral fat obesity. -FLAVO study. *Circ J* **75**, 2235–2243.

[21] Clifton PM, Keogh JB & Noakes M (2008) Long-term effects of a high-protein weight-loss diet. *Am J Clin Nutr* **87**, 23–29.

[22] Giglio BM, Schincaglia RM, da Silva AS, Fazani ICS, Monteiro PA, Mota JF, *et al.* (2019) Whey protein supplementation compared to collagen increases blood Nesfatin concentrations and decreases android fat in overweight women: a randomized double-blind study. *Nutrients* **11**, 1–14.

[23] Pal S & Ellis V (2010) The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. *Obesity* **18**, 1354–1359.

[24] Hector AJ, Marcotte GR, Churchward-Venne TA, Murphy CH, Breen L, von Allmen M, Baker SK & Phillips SM (2015) Whey protein supplementation preserves postprandial myofibrillar protein synthesis during short-term energy restriction in overweight and obese adults. *J Nutr* **145**, 246–252.

[25] Bel Lassen P, Attaye I, Adriouch S, Nicolaou M, Aron-Wisnewsky J, Nielsen T, *et al.* (2021) Protein intake, metabolic status and the gut microbiota in different ethnicities: results from two independent cohorts. *Nutrients* **13**, 1–15.

[26] Piccolo BD, Comerford KB, Karakas SE, Knotts TA, Fiehn O & Adams SH (2015) Whey protein supplementation does not alter plasma branched-chained amino acid profiles but results in unique metabolomics patterns in obese women enrolled in an 8-week weight loss trial. *J Nutr* **145**, 691–700.

[27] Coker RH, Miller S, Schutzler S, Deutz N & Wolfe RR (2012) Whey protein and essential amino acids promote the reduction of adipose tissue and increased muscle protein synthesis during caloric restriction-induced weight loss in elderly, obese individuals. *Nutr J* **11**, 105.

[28] Sun Y, Ling C, Liu L, Zhang J, Wang J, Tong X, *et al.* (2022) Effects of whey protein or its hydrolysate supplements combined with an energy-restricted diet on weight loss: a randomized controlled trial in older women. *Nutrients* **14**, 1–13.

[29] Kjolbaek L, Sorensen LB, Sondertoft NB, Rasmussen CK, Lorenzen JK, Serena A, Astrup A & Larsen LH (2017) Protein supplements after weight loss do not improve weight maintenance compared with recommended dietary protein intake despite beneficial effects on appetite sensation and energy expenditure: a randomized, controlled, double-blinded trial. *Am J Clin Nutr* **106**, 684–697.

[30] Moon J & Koh G (2020) Clinical evidence and mechanisms of high-protein diet-induced weight loss. *J Obes Metab Syndr* **29**, 166–173.

[31] Pesta DH & Samuel VT (2014) A high-protein diet for reducing body fat: mechanisms and possible caveats. *Nutr Metab (Lond)* **11**, 53.

[32] Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, *et al.* (2009) Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med* **360**, 859–873.

[33] Aykan NF (2015) Red meat and colorectal cancer. *Oncol Rev* **9**, 288.

[34] Lew QJ, Jafar TH, Koh HW, Jin A, Chow KY, Yuan JM & Koh WP (2017) Red meat intake and risk of ESRD. *J Am Soc Nephrol* **28**, 304–312.

[35] Agus A, Clement K & Sokol H (2021) Gut microbiota-derived metabolites as central regulators in metabolic disorders. *Gut* **70**, 1174–1182.

[36] Bilsborough S & Mann N (2006) A review of issues of dietary protein intake in humans. *Int J Sport Nutr Exerc Metab* **16**, 129–152.

[37] Cunningham AL, Stephens JW & Harris DA (2021) A review on gut microbiota: a central factor in the pathophysiology of obesity. *Lipids Health Dis* **20**, 65.

[38] Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB & van Loon LJC (2018) Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids* **50**, 1685–1695.

[39] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, *et al.* (2005) Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638.

[40] Vuik F, Dicksved J, Lam SY, Fuhler GM, van der Laan L, van de Winkel A, *et al.* (2019) Composition of the mucosa-associated microbiota along the entire gastrointestinal tract of human individuals. *United Eur Gastroenterol J* **7**, 897–907.

[41] Husted AS, Trauelsen M, Rudenko O, Hjorth SA & Schwartz TW (2017) GPCR-mediated signaling of metabolites. *Cell Metab* **25**, 777–796.

[42] Tremaroli V & Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* **489**, 242–249.

[43] Roager HM, Hansen LB, Bahl MI, Frandsen HL, Carvalho V, Gobel RJ, et al. (2016) Colonic transit time is related to bacterial metabolism and mucosal turnover in the gut. *Nat Microbiol* **1**, 16093.

[44] Ley RE (2010) Obesity and the human microbiome. *Curr Opin Gastroenterol* **26**, 5–11.

[45] Ley RE, Peterson DA & Gordon JI (2006) Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* **124**, 837–848.

[46] Manor O, Dai CL, Kornilov SA, Smith B, Price ND, Lovejoy JC, Gibbons SM & Magis AT (2020) Health and disease markers correlate with gut microbiome composition across thousands of people. *Nat Commun* **11**, 5206.

[47] Boscaini S (2020) *Novel insights into the mechanisms underlying the anti-obesity effects of whey protein.* Cork, Ireland: Doctoral University College Cork.

[48] Boscaini S, Cabrera-Rubio R, Golubeva A, Nychyk O, Fulling C, Speakman JR, et al. (2021) Depletion of the gut microbiota differentially affects the impact of whey protein on high-fat diet-induced obesity and intestinal permeability. *Physiol Rep* **9**, e14867.

[49] Wu S, Bhat ZF, Gounder RS, Mohamed Ahmed IA, Al-Juhaimi FY, Ding Y & Bekhit AEA (2022) Effect of dietary protein and processing on gut microbiota – a systematic review. *Nutrients* **14**.

[50] Lang JM, Pan C, Cantor RM, Tang WHW, Garcia-Garcia JC, Kurtz I, et al. (2018) Impact of individual traits, saturated fat, and protein source on the gut microbiome. *mBio* **9**, 1–14.

[51] Bratlie M, Hagen IV, Helland A, Erchinger F, Midttun O, Ueland PM, et al. (2021) Effects of high intake of cod or salmon on gut microbiota profile, faecal output and serum concentrations of lipids and bile acids in overweight adults: a randomised clinical trial. *Eur J Nutr* **60**, 2231–2248.

[52] Hansen LBS, Roager HM, Sondertoft NB, Gobel RJ, Kristensen M, Valles-Colomer M, et al. (2018) A low-gluten diet induces changes in the intestinal microbiome of healthy Danish adults. *Nat Commun* **9**, 4630.

[53] Beaumont M, Portune KJ, Steuer N, Lan A, Cerrudo V, Audebert M, et al. (2017) Quantity and source of dietary protein influence metabolite production by gut microbiota and rectal mucosa gene expression: a randomized, parallel, double-blind trial in overweight humans. *Am J Clin Nutr* **106**, 1005–1019.

[54] Dhakal S, Moazzami Z, Perry C & Dey M (2022) Effects of lean pork on microbiota and microbial-metabolite trimethylamine-*N*-oxide: a randomized controlled non-inferiority feeding trial based on the dietary guidelines for Americans. *Mol Nutr Food Res* **66**, e2101136.

[55] Alamshah A, Spreckley E, Norton M, Kinsey-Jones JS, Amin A, et al. (2017) l-phenylalanine modulates gut hormone release and glucose tolerance, and suppresses food intake through the calcium-sensing receptor in rodents. *Int J Obes* **41**, 1693–1701.

[56] Chandel NS (2021) Amino acid metabolism. *Cold Spring Harb Perspect Biol* **13**, 1–17.

[57] Cruzat V, Macedo Rogero M, Noel Keane K, Curi R & Newsholme P (2018) Glutamine: metabolism and immune function, supplementation and clinical translation. *Nutrients* **10**, 1–31.

[58] Cuomo P, Capparelli R, Iannelli A & Iannelli D (2022) Role of branched-chain amino acid metabolism in type 2 diabetes, obesity, cardiovascular disease and non-alcoholic fatty liver disease. *Int J Mol Sci* **23**, 1–11.

[59] Oliphant K & Allen-Vercoe E (2019) Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome* **7**, 91.

[60] Portune KJ, Anne-Marie D, Daniel T, François B, Martin B & Yolanda S. (2016) Gut microbiota role in dietary protein metabolism and health-related outcomes: the two sides of the coin. *Trends Food Sci Technol* **57**, 213–232.

[61] Tomé D (2021) Amino acid metabolism and signalling pathways: potential targets in the control of infection and immunity. *Nutr Diabetes* **11**, 20.

[62] Vermeulen MA, de Jong J, Vaessen MJ, van Leeuwen PA & Houdijk AP (2011) Glutamate reduces experimental intestinal hyperpermeability and facilitates glutamine support of gut integrity. *World J Gastroenterol* **17**, 1569–1573.

[63] Ye Z, Wang S, Zhang C & Zhao Y (2020) Coordinated modulation of energy metabolism and inflammation by branched-chain amino acids and fatty acids. *Front Endocrinol* **11**, 1–12.

[64] Bansal T, Alaniz RC, Wood TK & Jayaraman A (2010) The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc Natl Acad Sci U S A* **107**, 228–233.

[65] Bischoff SC, Mailer R, Pabst O, Weier G, Sedlik W, Li Z, et al. (2009) Role of serotonin in intestinal inflammation: knockout of serotonin reuptake transporter exacerbates 2,4,6-trinitrobenzene sulfonic acid colitis in mice. *Am J Physiol-Gastrointest Liver Physiol* **296**, G685–G695.

[66] Cheng Y, Jin UH, Allred CD, Jayaraman A, Chapkin RS & Safe S (2015) Aryl hydrocarbon receptor activity of tryptophan metabolites in young adult mouse colonocytes. *Drug Metab Dispos* **43**, 1536–1543.

[67] Chimerel C, Emery E, Summers DK, Keyser U, Gribble FM & Reimann F (2014) Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. *Cell Rep* **9**, 1202–1208.

[68] Dehhaghi M, Kazemi Shariat Panahi H & Guillemin GJ (2019) Microorganisms, tryptophan metabolism, and kynurenine pathway: a complex interconnected loop influencing human health status. *Int J Tryptophan Res* **12**, 1178646919852996.

[69] Hu W, Yan G, Ding Q, Cai J, Zhang Z, Zhao Z, Lei H & Zhu YZ (2022) Update of indoles: promising molecules for ameliorating metabolic diseases. *Biomed Pharmacother* **150**, 112957.

[70] Jennis M, Cavanaugh CR, Leo GC, Mabus JR, Lenhard J & Hornby PJ (2018) Microbiota-derived tryptophan indoles increase after gastric bypass surgery and reduce intestinal permeability *in vitro* and *in vivo*. *Neurogastroenterol Motil* **30**.

[71] Roth W, Zadeh K, Vekariya R, Ge Y & Mohamadzadeh M (2021) Tryptophan metabolism and gut-brain homeostasis. *Int J Mol Sci* **22**, 1–23.

[72] Su X, Gao Y & Yang R (2022) Gut microbiota-derived tryptophan metabolites maintain gut and systemic homeostasis. *Cells* **11**, 1–20.

[73] Yao CK, Muir JG & Gibson PR (2016) Review article: insights into colonic protein fermentation, its modulation and potential health implications. *Aliment Pharmacol Ther* **43**, 181–196.

[74] Heimann E, Nyman M, Palbrink AK, Lindkvist-Petersson K & Degerman E (2016) Branched short-chain fatty acids modulate glucose and lipid metabolism in primary adipocytes. *Adipocyte* **5**, 359–368.

[75] Taormina VM, Unger AL, Schiksnis MR, Torres-Gonzalez M & Kraft J (2020) Branched-chain fatty acids-an underexplored class of dairy-derived fatty acids. *Nutrients* **12**, 1–16.

[76] Davie JR (2003) Inhibition of histone deacetylase activity by butyrate. *J Nutr* **133**, 2485S–2493S.

[77] Parada Venegas D, de la Fuente MK, Landskron G, Gonzalez MJ, Quera R, Dijkstra G, et al. (2019) Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol* **10**, 277.

[78] Salvi PS & Cowles RA (2021) Butyrate and the intestinal epithelium: modulation of proliferation and inflammation in homeostasis and disease. *Cells* **10**, 1–13.

[79] Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, et al. (2014) The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* **5**, 3611.

[80] Trinidad TP, Wolever TM & Thompson LU (1996) Effect of acetate and propionate on calcium absorption from the rectum and distal colon of humans. *Am J Clin Nutr* **63**, 574–578.

[81] Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, et al. (2015) Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* **64**, 1744.

[82] Yoshida H, Ishii M & Akagawa M (2019) Propionate suppresses hepatic gluconeogenesis via GPR43/AMPK signaling pathway. *Arch Biochem Biophys* **672**, 108057.

[83] Dai ZL, Wu G & Zhu WY (2011) Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. *Front Biosci (Landmark Ed)* **16**, 1768–1786.

[84] Doi M, Yamaoka I, Fukunaga T & Nakayama M (2003) Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem Biophys Res Commun* **312**, 1111–1117.

[85] Nishimura J, Masaki T, Arakawa M, Seike M & Yoshimatsu H (2010) Isoleucine prevents the accumulation of tissue triglycerides and upregulates the expression of PPARalpha and uncoupling protein in diet-induced obese mice. *J Nutr* **140**, 496–500.

[86] Zhang S, Zeng X, Ren M, Mao X & Qiao S (2017) Novel metabolic and physiological functions of branched chain amino acids: a review. *J Anim Sci Biotechnol* **8**, 10.

[87] Orozco-Ruiz X, Anesi A, Mattivi F & Breteler MMB (2022) Branched-chain and aromatic amino acids related to visceral adipose tissue impact metabolic health risk markers. *J Clin Endocrinol Metab* **107**, e2896–e2905.

[88] Yu D, Richardson NE, Green CL, Spicer AB, Murphy ME, Flores V, *et al.* (2021) The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. *Cell Metab* 33, 905–922.e6.

[89] Bishop CA, Machate T, Henning T, Henkel J, Püschel G, Weber D, *et al.* (2022) Detrimental effects of branched-chain amino acids in glucose tolerance can be attributed to valine induced glucotoxicity in skeletal muscle. *Nutr Diabetes* **12**, 20.

[90] De Bandt J-P, Coumoul X & Barouki R (2023) Branched-chain amino acids and insulin resistance, from protein supply to diet-induced obesity. *Nutrients* **15**, 68.

[91] Vanweert F, Schrauwen P & Phielix E (2022) Role of branched-chain amino acid metabolism in the pathogenesis of obesity and type 2 diabetes-related metabolic disturbances BCAA metabolism in type 2 diabetes. *Nutr Diabetes* **12**, 35.

[92] Rossi M, Turati F, Strikoudi P, Ferraroni M, Parpinel M, Serraino D, Negri E & La Vecchia C (2023) Dietary intake of branched-chain amino acids and pancreatic cancer risk in a case-control study from Italy. *Br J Nutr* **129**, 1574–1580.

[93] Cummings NE, Williams EM, Kasza I, Konon EN, Schaid MD, Schmidt BA, *et al.* (2018) Restoration of metabolic health by decreased consumption of branched-chain amino acids. *J Physiol* **596**, 623–645.

[94] Connolly-Schoonen J, Danowski L, Bistricer M, Campo Catalan L, Ailawadi S, Sicinski EM, *et al.* (2023) A pilot controlled feeding trial modifying protein intake in healthy subjects to assess adherence and the metabolome. *Nutr Cancer* **75**, 1499–1510.

[95] Geypens B, Claus D, Evenepoel P, Hiele M, Maes B, Peeters M, Rutgeerts P & Ghoos Y (1997) Influence of dietary protein supplements on the formation of bacterial metabolites in the colon. *Gut* **41**, 70–76.

[96] Gratz SW, Hazim S, Richardson AJ, Scobbie L, Johnstone AM, Fyfe C, *et al.* (2019) Dietary carbohydrate rather than protein intake drives colonic microbial fermentation during weight loss. *Eur J Nutr* **58**, 1147–1158.

[97] Russell WR, Gratz SW, Duncan SH, Holtrop G, Ince J, Scobbie L, *et al.* (2011) High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr* **93**, 1062–1072.

[98] Moller G, Andersen JR, Jalo E, Ritz C, Brand-Miller J, Larsen TM, *et al.* (2020) The association of dietary animal and plant protein with putative risk markers of colorectal cancer in overweight pre-diabetic individuals during a weight-reducing programme: a PREVIEW sub-study. *Eur J Nutr* **59**, 1517–1527.

[99] Bingham SA, Pignatelli B, Pollock JR, Ellul A, Malaveille C, Gross G, *et al.* (1996) Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* **17**, 515–523.

[100] Chiu S, Williams PT, Dawson T, Bergman RN, Stefanovski D, Watkins SM & Krauss RM (2014) Diets high in protein or saturated fat do not affect insulin sensitivity or plasma concentrations of lipids and lipoproteins in overweight and obese adults. *J Nutr* **144**, 1753–1759.

[101] Hall WL, Millward DJ, Long SJ & Morgan LM (2003) Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr* **89**, 239–248.

[102] Pinckaers PJM, Kouw IWK, Gorissen SHM, Houben LHP, Senden JM, Wodzig W, *et al.* (2023) The muscle protein synthetic response to the ingestion of a plant-derived protein blend does not differ from an equivalent amount of milk protein in healthy young males. *J Nutr* **152**, 2734–2743.

[103] Prodhan UK, Pundir S, Chiang VS, Milan AM, Barnett MPG, Smith GC, *et al.* (2020) Comparable postprandial amino acid and gastrointestinal hormone responses to beef steak cooked using different methods: a randomised crossover trial. *Nutrients* **12**, 1–10.

[104] Dahl WJ, Hung WL, Ford AL, Suh JH, Auger J, Nagulesapillai V & Wang Y (2020) In older women, a high-protein diet including animal-sourced foods did not impact serum levels and urinary excretion of trimethyl-amine-*N*-oxide. *Nutr Res* **78**, 72–81.

[105] Mutch DM, Fuhrmann JC, Rein D, Wiemer JC, Bouillot JL, Poitou C & Clement K (2009) Metabolite profiling identifies candidate markers reflecting the clinical adaptations associated with Roux-en-Y gastric bypass surgery. *PLoS One* **4**, e7905.

[106] Andriamihaja M, Davila A-M, Eklou-Lawson M, Petit N, Delpal S, Allek F, *et al.* (2010) Colon luminal content and epithelial cell morphology are markedly modified in rats fed with a high-protein diet. *Am J Physiol-Gastrointest Liver Physiol* **299**, G1030–G1037.

[107] Sattari Najafabadi Z, Skau Nielsen T & Skou Hedemann M (2019) Dietary protein source and butyrylated high-amylose maize starch included in a high-protein diet determines the urinary metabolome of rats. *Int J Food Sci Nutr* **70**, 255–266.

[108] Sridharan GV, Choi K, Klemashevich C, Wu C, Prabakaran D, Pan LB, *et al.* (2014) Prediction and quantification of bioactive microbiota metabolites in the mouse gut. *Nat Commun* **5**, 5492.

[109] Vyhlidalova B, Krasulova K, Pecinkova P, Marcalikova A, Vrzal R, Zemankova L, *et al.* (2020) Gut microbial catabolites of tryptophan are ligands and agonists of the aryl hydrocarbon receptor: a detailed characterization. *Int J Mol Sci* **21**, 1–17.

[110] Girer NG, Tomlinson CR & Elferink CJ (2020) The aryl hydrocarbon receptor in energy balance: the road from dioxin-induced wasting syndrome to combating obesity with ahr ligands. *Int J Mol Sci* **22**, 1–13.

[111] Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC & Siuzdak G (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A* **106**, 3698–3703.

[112] Gao J, Xu K, Liu H, Liu G, Bai M, Peng C, Li T & Yin Y (2018) Impact of the Gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front Cell Infect Microbiol* **8**, 1–22.

[113] Takaki M, Mawe GM, Barasch JM, Gershon MD & Gershon MD (1985) Physiological responses of guinea-pig myenteric neurons secondary to the release of endogenous serotonin by tryptamine. *Neuroscience* **16**, 223–240.

[114] Corpeleijn WE, Riedijk MA, Zhou Y, Schierbeek H, Huang Y, Chen C & van Goudoever JB (2010) Almost all enteral aspartate is taken up in first-pass metabolism in enterally fed preterm infants. *Clin Nutr* **29**, 341–346.

[115] Ma N & Ma X (2019) Dietary amino acids and the gut-microbiome-immune axis: physiological metabolism and therapeutic prospects. *Compr Rev Food Sci Food Saf* **18**, 221–242.

[116] Walker MC & van der Donk WA (2016) The many roles of glutamate in metabolism. *J Ind Microbiol Biotechnol* **43**, 419–430.

[117] Wang W, Wu Z, Dai Z, Yang Y, Wang J & Wu G (2013) Glycine metabolism in animals and humans: implications for nutrition and health. *Amino Acids* **45**, 463–477.

[118] Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK & Bultman SJ (2011) The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* **13**, 517–526.

[119] Hong Y-H, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C, *et al.* (2005) Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* **146**, 5092–5099.

[120] Louis P, Scott KP, Duncan SH & Flint HJ (2007) Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* **102**, 1197–1208.

[121] Wang G, Huang S, Wang Y, Cai S, Yu H, Liu H, *et al.* (2019) Bridging intestinal immunity and gut microbiota by metabolites. *Cell Mol Life Sci* **76**, 3917–3937.

[122] Khalil NA, Walton GE, Gibson GR, Tuohy KM & Andrews SC (2014) *In vitro* batch cultures of gut microbiota from healthy and ulcerative colitis (UC) subjects suggest that sulphate-reducing bacteria levels are raised in UC and by a protein-rich diet. *Int J Food Sci Nutr* **65**, 79–88.

[123] Teigen LM, Geng Z, Sadowsky MJ, Vaughn BP, Hamilton MJ & Khoruts A (2019) Dietary factors in sulfur metabolism and pathogenesis of ulcerative colitis. *Nutrients* **11**, 1–20.

[124] Wolf PG, Cowley ES, Breister A, Matatov S, Lucio L, Polak P, *et al.* (2022) Diversity and distribution of sulfur metabolic genes in the human gut microbiome and their association with colorectal cancer. *Microbiome* **10**, 64.

[125] Layden BT, Angueira AR, Brodsky M, Durai V & Lowe WL Jr (2013) Short chain fatty acids and their receptors: new metabolic targets. *Transl Res* **161**, 131–140.

[126] Gao Y, Yao Q, Meng L, Wang J & Zheng N (2024) Double-side role of short chain fatty acids on host health via the gut-organ axes. *Anim Nutr* **18**, 322–339.

[127] Cani PD (2014) Metabolism in 2013: the gut microbiota manages host metabolism. *Nat Rev Endocrinol* **10**, 74–76.

[128] Den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, Oosterveer MH, Jonker JW, Groen AK, Reijngoud DJ & Bakker BM (2015) Short-chain fatty acids protect against high-fat diet-induced obesity via a PPARgamma-dependent switch from lipogenesis to fat oxidation. *Diabetes* **64**, 2398–2408.

[129] Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ & Lobley GE (2007) Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol* **73**, 1073–1078.

[130] Lu Y, Fan C, Li P, Lu Y, Chang X & Qi K (2016) Short chain fatty acids prevent high-fat-diet-induced obesity in mice by regulating G protein-coupled receptors and gut microbiota. *Sci Rep* **6**, 37589.

[131] Blachier F, Mariotti F, Huneau JF & Tome D (2007) Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. *Amino Acids* **33**, 547–562.

[132] Louis P & Flint HJ (2017) Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* **19**, 29–41.

[133] Topping DL & Clifton PM (2001) Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* **81**, 1031–1064.

[134] Diether NE & Willing BP (2019) Microbial fermentation of dietary protein: an important factor in diet–microbe–host interaction. *Microorganisms* **7**, 1–14.

[135] Nilaweera KN & Cotter PD (2023) Can dietary proteins selectively reduce either the visceral or subcutaneous adipose tissues? *Obes Rev* **24**, e13613.

[136] Nogueiras R, Perez-Tilve D, Veyrat-Durebex C, Morgan DA, Varela L, Haynes WG, *et al.* (2009) Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. *J Neurosci* **29**, 5916–5925.

[137] Drummen M, Tischmann L, Gatta-Cherifi B, Adam T & Westerterp-Plantenga M (2018) Dietary protein and energy balance in relation to obesity and co-morbidities. *Front Endocrinol (Lausanne)* **9**, 443.

[138] Gannon MC & Nuttall FQ (2004) Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. *Diabetes* **53**, 2375–2382.

[139] Hernandez MAG, Canfora EE, Jocken JWE & Blaak EE (2019) The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients* **11**, 1–32.

[140] Morrison DJ & Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7**, 189–200.

[141] Pham NHT, Joglekar MV, Wong WKM, Nassif NT, Simpson AM & Hardikar AA (2024) Short-chain fatty acids and insulin sensitivity: a systematic review and meta-analysis. *Nutr Rev* **82**, 193–209.

[142] Langyan S, Yadava P, Khan FN, Dar ZA, Singh R & Kumar A (2021) Sustaining protein nutrition through plant-based foods. *Front Nutr* **8**, 772573.

[143] Bennett E, Peters SAE & Woodward M (2018) Sex differences in macronutrient intake and adherence to dietary recommendations: findings from the UK Biobank. *BMJ Open* **8**, e020017.

[144] Bredella MA (2017) Sex differences in body composition. *Adv Exp Med Biol* **1043**, 9–27.

[145] Santos-Marcos JA, Haro C, Vega-Rojas A, Alcala-Diaz JF, Molina-Abril H, Leon-Acuna A, *et al.* (2019) Sex differences in the gut microbiota as potential determinants of gender predisposition to disease. *Mol Nutr Food Res* **63**, e1800870.