
Article:
Horner, K, Hopkins, M orcid.org/0000-0002-7655-0215, Finlayson, G orcid.org/0000-0002-5620-2256 et al. (2 more authors) (2020) Biomarkers of appetite: is there a potential role for metabolomics? Nutrition Research Reviews, 33 (2). pp. 271-286. ISSN 0954-4224

https://doi.org/10.1017/s0954422420000062

Biomarkers of appetite: is there a potential role for metabolomics?

Katy Horner[1], Mark Hopkins[2], Graham Finlayson[3], Catherine Gibbons[3], Lorraine Brennan[4,5]

[1] UCD School of Public Health, Physiotherapy and Sport Science, Institute of Food and Health and Institute of Sport and Health, UCD, Belfield, Dublin 4, Republic of Ireland

[2] School of Food Science and Nutrition, Faculty of Environment, University of Leeds, Leeds, United Kingdom

[3] School of Psychology, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom

[4] UCD School of Agriculture and Food Science, Institute of Food and Health, UCD, Belfield, Dublin 4, Republic of Ireland

[5] UCD Conway Institute of Biomolecular and Biomedical Research, UCD, Belfield, Dublin 4, Republic of Ireland

Corresponding Author:
Dr Katy Horner
School of Public Health, Physiotherapy and Sport Science,
Woodview House,
University College Dublin,
Belfield,
Dublin 4,
Ireland.
Email: katy.horner@ucd.ie
Telephone: + 353 (1) 7163439

Shortened Title: Metabolomics and Biomarkers of Appetite

Keywords: Amino Acids, Appetite, Glucose, Lipids, Metabolites, Satiety

Abstract

Knowing the biological signals associated with appetite control is crucial for understanding the regulation of food intake. Biomarkers of appetite have been defined as physiological measures that relate to subjective appetite ratings, measured food intake, or both. Several metabolites including amino acids, lipids and glucose were proposed as key molecules associated with appetite control over 60 years ago, and along with bile acids are all among possible appetite biomarker candidates. Additional metabolites that have been associated with appetite include endocannabinoids, lactate, cortisol and β-hydroxybutyrate. However, although appetite is a complex integrative process, studies often investigated a limited number of markers in isolation. Metabolomics involves the study of small molecules or metabolites present in biological samples such as urine or blood, and may present a powerful approach to further the understanding of appetite control. Using multiple analytical techniques allows the characterisation of molecules, such as carbohydrates, lipids, amino acids, bile acids and fatty acids. Metabolomics has proven successful in identifying markers of consumption of certain foods and biomarkers implicated in several diseases. However, it has been under-exploited in appetite control or obesity. The aim of this narrative review is to (1) provide an overview of existing metabolites that have been identified in human biofluids and associated with appetite control; and (2) discuss the potential of metabolomics to deepen understanding of appetite control in humans.

Introduction

Both over- and under-nutrition are associated with increased risk of chronic disease[1,2] and represent a major global public health issue. Appetite is the internal driving force for the ingestion of food[3], and results from complex interactions between internal and external factors including biology, psychology and the environment. Along with environmental and psychological factors, knowledge of the biological processes involved in the control of food intake and appetite is therefore essential for better understanding and treatment of a range of conditions associated with poor appetite control, and hence over- and under-nutrition. For example, poor appetite control associated with sedentary lifestyles, obesity or ageing.

Although several early theories attempted to explain appetite based on a single factor such as glucose, it has become increasingly evident that appetite is part of a larger integrative process and single target approaches to understand or modify appetite control are largely ineffective. Appetite control has been conceptualised to consist of three levels of events and processes[4] which interact to form part of a 'psychobiological system' controlling appetite[5]. These include (i) psychological events and behaviour, (ii) peripheral physiology and metabolic events, and (iii) neurotransmitter and metabolic interactions in the brain[4].

Biomarkers of appetite are defined as physiologic measures that relate to subjective appetite ratings, measured food intake, or both and can be considered indicators of appetite or causal factors influencing appetite[3]. Interest in identifying appetite biomarkers has continued to increase due to a number of potential applications. These include contributing to more objective and reliable measurement of appetite, increasing understanding of alterations in appetite across the lifespan and in health and disease, and identifying targets for improving appetite control. However, it should be noted that biomarkers are unable to fully characterise the range of processes involved in appetite control and should only be used to make claims about appetite in combination with behavioural measures[6].

Several comprehensive reviews have previously discussed biomarkers of satiation and satiety in the brain and periphery[3,7] and the role of the GI tract and related peptide signals involved in the control of food intake[8]. Satiation can be defined as the process leading to termination of eating, and satiety as the process leading to inhibition of further eating, a reduction in hunger and increase in fullness after a meal[6]. Examples of known biomarkers and potential biomarkers include gut hormones such as ghrelin and cholecystokinin (CCK), longer term signals arising from adipose tissue such as leptin, along with gastric distension, cytokines such as IL-6 and the thermogenic effect of protein[3,7,8]. Consensus statements note

biomarkers should be valid (clearly linked to appetite), reproducible, specific, sensitive and feasible - measured in accessible or easily obtained material using ethical and minimally invasive methods[9]. This highlights the value that markers in blood or other easily obtained human samples such as saliva might have.

In addition to identification of gut hormones in blood, several circulating metabolites have long been implicated to have a key role in appetite control, with glucose being one of the first to be identified in Mayer's glucostatic theory of appetite in 1953[10]. Since then, many metabolites or small molecules present in biological samples have been proposed to be indirectly or causally associated with appetite, and consequently many studies have included metabolites as outcome measures (e.g.[11,12,13,14,15]). However, most studies have targeted only one or a few specific metabolites. With the advancement of metabolomics in recent years our ability to measure a broad range of metabolites with diverse chemical characteristics has increased.

Given the complexity of appetite control, metabolomics offers great potential to increase understanding of the integrative processes of appetite control and to identify potential biomarkers of appetite. The aim of this review is to (1) provide a collective overview of metabolites that have been identified in biological samples and associated with appetite control in humans and (2) discuss the potential for modern metabolomics techniques to identify appetite biomarkers and deepen understanding of appetite control.

The control of appetite and identified biomarkers

It is first important to acknowledge some of the key physiological processes involved in appetite control. For a seminal review see[3]. The hypothalamus plays a key role; in particular the arcuate nucleus receives and processes signals both from other areas of the brain and the periphery. Briefly, the arcuate nucleus houses 2 sets of neuronal circuits that are functionally antagonistic. A group of neurons co expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) are part of an appetite stimulating (orexigenic) circuit. In contrast, pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) neurons are part of an appetite inhibiting (anorexigenic) circuit[16] and signal to inhibit energy intake by action at specific melanocortin receptors [17]. There are also connections between the two neuronal sub-groups, for example, AgRP neurons can exert an inhibitory effect on POMC neurons [18][19].

Signals from the periphery are often categorised as short term or long term but the connotation episodic and tonic is also appropriate[20]. Tonic signals such as leptin are constantly released, mainly by adipose tissue in proportion to the amount of lipid stores, therefore signalling chronic nutritional state[17]. Insulin, released by pancreatic β cells is also a tonic signal and shares many properties with leptin, with both stimulating POMC and inhibiting NPY to signal satiety. Leptin and insulin bind to their respective receptors on the surface of POMC neurons. This promotes processing of POMC to the mature hormone α-melanocyte-stimulating hormone (α-MSH), which binds to melanocortin-4 receptor and signals to decrease energy intake [21].

However, observations that leptin levels are elevated in many individuals with obesity have led to the hypothesis that most are resistant to the actions of leptin[22], and similarly insulin resistance in individuals who are overweight and obese may mean a blunted effect of insulin on appetite[23]. It should also be noted that while tonic and episodic signals generally appear to have different roles in the control of appetite[24] they can also interact with each other. For example, sensitivity to short-term signals can be influenced by leptin[25], and may provide a mechanism through which long-term energy needs are translated into day-to-day food intake.

Episodic signals including orexigenic (ghrelin) and anorexigenic peptides (e.g. cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide YY (PYY)), arise largely from the GI tract and oscillate periodically in relation to eating[24]. Ghrelin circulates in acylated and deacylated forms, however it is commonly measured as total ghrelin. Ghrelin stimulates appetite and rises before meals suggesting a role in meal initiation[26], whereas anorexigenic peptides are released in response to food ingestion. While there is some inconsistency in findings between studies[27,28], relationships between gut peptides and appetite and energy intake at normal physiological levels have been demonstrated[29,30,31,32]. For example, Gibbons et al.[30] found plasma ghrelin (total and acylated) were positively correlated with changes in hunger and in turn food intake, and GLP-1 was negatively associated with hunger in the late satiety phase and subsequent food intake, following consumption of both high fat and high carbohydrate meals. These data suggest ghrelin (total and acylated) and GLP-1 are significant biomarkers of the phases of satiety. In contrast, others[28] have compared a high protein (25% energy) versus normal protein (10% energy) lunch, and found PYY, GLP-1 or acylated ghrelin did not explain the increased satiety response observed following the high protein meal. Although not measured, other factors such as amino acids or other metabolites were proposed as factors which may explain the

153 increased satiety response to the high protein meal. These studies highlight the complexity of
154 biological signals involved in meal to meal appetite control.
155 The large inter-individual variability in appetite and gut peptide responses to meal
156 ingestion [33] should also be acknowledged (see Figure 1). One peptide biomarker is unlikely
157 to fully explain appetite responses. It is more likely a combination of signals that influences
158 appetite control, which could vary based on a range of factors such as the characteristics of the
159 test meal or of the individual[33]. This highlights the importance of studying individual
160 responses to a treatment or manipulation, as it may help to identify individuals or specific
161 groups who might benefit, even though there may be no mean group changes[33].

162

163

164 [Figure 1]

165

166

167 **Potential Role of Metabolomics**

168 'Omics' methods including genomics, transcriptomics, proteomics and metabolomics have
169 been applied in the search for biomarkers of a range of conditions. Metabolomics, including
170 lipidomics specifically involves the study of small molecules or metabolites present in
171 biological samples[34]. Using multiple analytical techniques such as mass spectrometry
172 coupled with liquid chromatography or gas chromatography, and nuclear magnetic resonance
173 spectroscopy allows the characterisation of molecules such as carbohydrates, lipids, amino
174 acids, bile acids and fatty acids. Each analytical technique has its own advantage and
175 disadvantage and the optimal coverage of metabolites is obtained by use of multiple
176 approaches. Metabolomics can be applied to a range of biological samples such as blood,
177 urine, saliva, faecal water and cerebrospinal fluid.

178 Application of metabolomics in nutrition research has increased rapidly in recent
179 years. In particular, it has played a key role in the following areas: (1) identification of
180 biomarkers related to nutrient and food intake (food intake biomarkers), (2) understanding the
181 impact of nutrition interventions to define potential mechanisms of action, (3) understanding
182 diet/disease relationships in nutritional epidemiology and (4) development of personalised
183 nutrition (Figure 2). Putative biomarkers exist for a range of foods including fish, red meat,
184 citrus fruit, apples, and cruciferous vegetables[35,36,37,38,39,40]. The goal of food intake

biomarkers is to aid in the assessment of food intake as self-reported methods have well-accepted limitations[41,42]. Work using proline betaine as a biomarker of citrus intake demonstrated that using these biomarkers one can obtain quantitative information on food intake[35]. In this study participants consumed standardized breakfasts as part of a controlled dietary intervention for three consecutive days over three weeks where citrus intake was changed over the weeks. The urinary proline betaine concentrations were used to develop calibration curves relating citrus intake with biomarker levels. These curves were then used to determine citrus intake in an independent cross-sectional study of 560 individuals and the results demonstrated that the biomarker approach performed extremely well at determining intake. In a similar study setting, Garcia-Perez[43] and colleagues examined the ability of tartaric acid to determine grape intake. While development of these and other biomarkers are important it is also worth noting that they will not completely replace self-reported data and the true value will be in combining both approaches.

[Figure 2]

Metabolites associated with appetite control

Although several metabolites including lipids and amino acids have been shown to be associated with subjective appetite ratings or measured food intake in human studies (Table 1), most studies that have investigated potential biomarkers of appetite have been hypothesis driven and focused on measuring a single metabolite or a limited number of specific metabolites.

[Table 1]

Lipids, lipid-like molecules and fatty acids

Circulating concentrations of total levels of free fatty acids (FFAs) or non-esterified fatty acids (NEFAs) have long been associated with appetite control. In 1960, van Itallie and Hashim[44] showed that patterns of plasma NEFAs in individuals on self-selected diets were similar to patterns of hunger and satiety. More recently, plasma NEFA concentrations have been shown to predict differences in the duration of satiety following ingestion of a pharmacological agent known to inhibit NEFA β-oxidation compared to an agent known to stimulate NEFA β-oxidation[45]. In contrast others have shown plasma NEFA levels to change without changes in appetite in response to overfeeding in humans[46], illustrating that NEFA levels may only be associated with appetite under certain conditions. Regarding individual FFAs, there is substantial mechanistic evidence for a role in appetite control from animal studies. For example, central administration of the long chain fatty acid oleic acid has been shown to reduce food intake in rats[47]. However, excessive intake of long-chain saturated fatty acids can promote hypothalamic insulin and leptin resistance [48] which could in turn blunt effects on appetite. In humans, ingestion of specific fatty acids has been shown to impact appetite[49]. However, whether circulating concentrations of individual FFAs in humans are associated with appetite or food intake remains to be clearly established.

Interestingly, fatty acid ethanolamides, a class of lipid signalling molecules derived from fatty acid precursors, such as oleoylethanolamide (OEA) the ethanolamide of oleic acid[11,50], anandamide (AEA) of arachidonic acid[51] and palmitoylethanolamide (PEA) of palmitic acid[51] have been shown to be associated with appetite in humans (Table 1). OEA is an N-acylethanolcholamine, with most of its reported effects being attributed to activation of peroxisome proliferator-activated receptor-alpha (PPAR- α) [52] in the small intestine [53]. Although the mechanisms linking PPAR- α activation to satiety are incompletely understood, the subsequent activation of apolipoprotein A-IV[54], stimulation of CCK release and inhibition of gastric motility [55] may have a role.

In a human study, Menella et al.[11] examined the effects of fatty acid composition of a meal (white bread with three different test oils), particularly its oleic acid content, on plasma OEA along with four other related lipid molecules, on appetite and energy intake. Meals eliciting the highest plasma OEA response resulted in reduced intake at a lunch meal served three hours later. Others[50] have since shown salivary OEA concentrations to be positively associated with satiety and fullness. Collectively although human studies are limited, and others have not reported such associations[51], these studies combined with mechanistic evidence from animal studies highlight OEA in blood and saliva as a potential biomarker of appetite following consumption of certain foods in humans.

AEA, an endogenous agonist of the cannabinoid CB1 and CB2 receptors, and the AEA metabolically related lipid PEA and agonist of PPAR- α have also been shown to positively correlate with postprandial hunger ratings[51], although not in all studies[50]. The postprandial AUCs of plasma AEA and PEA were shown to positively correlate with postprandial hunger AUC in 10 men with obesity in an already satiated state[51]. Plasma AEA was also shown to positively correlate with ratings of fullness in the fasted state in healthy post-menopausal women[56]. Overall, while there is substantial evidence for the role of these endocannabinoids and related lipid molecules in the control of appetite and food intake in animal studies, studies in humans examining the role of circulating levels of these metabolites are currently limited.

Short chain fatty acids, including acetate, butyrate and propionate are produced when non-digestible carbohydrate is fermented in the colon and appear to have a beneficial role in appetite control and energy homeostasis (for a detailed review see[57]). Various factors must be considered when interpreting their circulating short chain fatty acid concentrations including that they can be produced from both endogenous and exogenous sources. However, in general colonic fermentation is considered the main source of acetate in the blood[58]. A direct role of acetate in central appetite control has been shown in mice, with findings that peripheral administration of acetate increased POMC and reduced AgRP expression in the hypothalamus[59].

Few studies have directly investigated relationships between concentrations in human biofluids of individual short chain fatty acids and appetite or energy intake. In one study, plasma butyrate has been shown to correlate directly with late satiety and fullness 4-6 hours after a range of test breakfasts[60]. Others have shown targeted delivery of propionate to the colon through ingestion of an inulin-propionate ester increased plasma propionate, GLP-1 and PYY levels, and reduced subsequent energy intake[61] but direct correlations between plasma propionate and energy intake were not reported. Elsewhere[62], the non-digestible carbohydrate L-rhamnose was found to significantly increase plasma propionate and PYY, but had no effect on appetite. The latter finding may be due to the small sample size (n=10). As the composition of the gut microbiota can shape the fecal metabolome [63], the fecal metabolome also presents a useful approach for further understanding of the role of fatty acids as biomarkers in appetite control. There is evidence of higher fecal concentrations of proprionate, butyrate and branched chain fatty acids (isobutyrate and isovalerate) in individuals with obesity post gastric bypass[63] suggesting a potential mechanism by which gastric bypass surgery may impact satiety. However, appetite was not assessed.

Dehydroepiandrosterone sulfate (DHEA-S), produced from cholesterol belonging to the class of compounds known as sulphated steroids (sterol lipids with a sulfate group), has been shown to be inversely correlated with satiety ratings during energy deficit over 48 hours in soldiers[15]. DHEA-S modulates the activity of neurotransmitters within regions in the central nervous system involved in appetite-control[64], however, as it does not readily cross the blood brain barrier, changes in peripheral concentrations may not alter concentrations in the brain[15]. Therefore, while it may potentially serve as an indirect marker of satiety under certain conditions, the role of circulating levels in appetite control in humans is currently unclear and needs further investigation.

Taken together, there is emerging evidence for a role of some lipid molecules including fatty acid ethanolamides as potential biomarkers of appetite in humans. Moreover, while there appears to be a clear role for individual fatty acids such as oleic acid and short chain fatty acids in appetite control, with substantial mechanistic evidence in animals, evidence of direct associations between concentrations of individual fatty acids in biofluids such as blood or faeces and appetite/energy intake in humans is currently lacking.

Amino Acids

Circulating concentrations of amino acids have long been implicated to have a role in appetite control. In 1956, Mellinkoff[65] demonstrated that when serum amino acid nitrogen increased, appetite diminished and when amino acid concentration decreased, appetite increased following a breakfast of eggs, milk and toast. Various mechanisms have since been proposed linking amino acids to appetite control, including the original amino-static hypothesis[65] - that amino acid levels in general are monitored by some kind of amino-stat, and that when a certain level is reached this limits the intake of further amino acids (hence food)[66]. Millward's[67] 'protein-stat' theory further suggests that skeletal muscle mass specifically is tightly regulated and food intake is directed to meet the needs for lean tissue growth and maintenance. However, the evidence for such regulation is currently limited, mainly due to a lack of research[68].

Although findings are inconsistent, some studies have since reported associations between single circulating amino acids or multiple amino acids with subjective appetite ratings and energy intake in humans (Table 1). For example, Hall et al.[13] reported that a greater rise in total amino acids, CCK and GLP-1 occurred following whey compared to casein consumption and energy intake at an ad libitum meal 90 minutes later was

317 concomitantly reduced. When separately examined, valine, isoleucine, leucine and threonine
318 were the amino acids that significantly differed. The authors suggested that the influence of
319 the amino acid and gut peptide response on satiety was likely complementary. In contrast, in
320 men with overweight and obesity during energy restriction, no strong association was evident
321 between circulating plasma amino acid concentrations and appetite[69,70]. This indicates no
322 direct action of circulating amino acids on central mechanisms of satiety under these
323 conditions[69,70] leading to the suggestion that complex and redundant pathways may be
324 involved in protein and amino acid induced satiety[70].

325 Others have shown that postprandial plasma concentrations of all 20 amino acids,
326 except cysteine, were positively associated with suppression of subsequent energy intake in
327 healthy lean males following intra-duodenal infusion of whey protein[71], which may argue
328 against independent roles for individual amino acids in appetite control. However, elsewhere,
329 specific plasma amino acid concentrations were demonstrated to correlate with prospective
330 food consumption (glutamate) and EI (histidine, valine, leucine, isoleucine and the amino
331 acid derivative α-aminobutyric acid) following differing liquid preloads with varied
332 macronutrient composition in normal weight and overweight adults[72]. Others have shown
333 taurine was the only amino acid to directly correlate with increased satiety and reduced
334 hunger following consumption of soy protein in humans[73]. Such an effect could also
335 potentially explain the satiating effects of fish that have been previously observed, as seafood
336 is rich in taurine[73,74]. These studies highlight a potential role for different amino acids in
337 appetite control following consumption of different foods.

338 Amino acids and amino acid derivatives have also been recently implicated in the
339 increased satiety response following RYGB surgery[75]. In this cross-sectional study,
340 participants were divided into those with high and low postprandial satiety scores and high
341 and low postprandial reductions in hunger. Those with high satiety scores had a significantly
342 greater total amino acid response and greater response of 10 out of 24 individual amino acids,
343 along with greater GLP-1 and PYY responses. Five of these amino acids were particularly
344 pronounced in those with high GLP-1 and PYY responses, suggesting this may have been the
345 mechanism of action for those amino acids. Whereas, it was proposed the other five amino
346 acids that differed may have a direct action on satiety. Interestingly, when divided into
347 groups based on high and low reductions in hunger, only one metabolite differed - the amino
348 acid derivative α-aminobutyric acid, highlighting that amino acids may have varying
349 associations with different appetite sensations (i.e. hunger versus satiety). However, as

responses were assessed at one time-point only post-operatively, causal relationships between changes in circulating amino acid concentrations and appetite could not be established.

Finally, some studies have investigated the role of specific amino acids in appetite control through intraduodenal infusions of certain amino acids and subsequent measurement of plasma concentrations. Steinert and colleagues[76,77] reported circulating levels of plasma leucine and tryptophan to be positively associated with subsequent test meal EI following intraduodenal infusions of leucine[76] and tryptophan[77] respectively compared to saline. Elsewhere it has been shown that this may be population specific with associations between plasma tryptophan and test meal EI following intra-duodenal tryptophan infusion being only apparent in lean individuals but not in those with obesity[78].

Collectively, these human studies highlight that circulating amino acids could have a role in appetite control and as a potential biomarker of appetite in certain contexts, and that this may occur either indirectly through effects of gut peptides or directly, or a combination of both.

Glucose

The glucostatic hypothesis, proposing a role for blood glucose levels and glucose utilisation in the regulation of appetite was also described in the 1950's[10,79], and has since been the focus of substantial research. An extensive review of the role of glucose in appetite control is beyond the scope of the current review (see[80,81] for reviews). Various studies in humans have demonstrated that postprandial blood glucose concentrations or patterns of blood glucose are associated with appetite ratings, EI at a test meal and/or meal initiation ([12,15,23,29,82,83,84,85,86,87], see Table 1). However, it is essential to note that many studies have also shown no association between blood glucose concentrations and appetite ratings[23,83,88,89,90].

The inconsistent findings may be due to several reasons. Like many postprandial markers, distinguishing between the contributions of individual signals is difficult. For example, during controlled weight loss in women with obesity, when examined in multivariate regression analyses, insulin was the greatest and only independent predictor of satiety[12], supporting an insulinotropic[91] rather than glucostatic hypothesis. Further evidence to suggest the role of glucose in appetite control may occur indirectly through effects on other

mechanisms comes from studies that have shown intraduodenal glucose infusion to suppress appetite and reduce EI[92] but intravenous glucose infusion to have no effect[88,92]. These findings together with evidence from meta-analysis[23] suggest the impact of intestinal glucose is unlikely to be mediated by effects on blood glucose, but instead via effects on insulin and/or incretins[23,88].

A further explanation is that blood glucose concentrations may only impact appetite and energy intake at extreme levels such as during hypoglycaemia or in hyperglycemia, but not when blood glucose levels are within the normal physiological range. Indeed several studies reporting associations between appetite, energy intake and glucose showed such associations existed in individuals with type 1 diabetes[86], during a hypoglycaemic clamp[84], or during acute hyperglycemia[93]. For example, some hypoglycemic clamp studies have shown that hunger ratings are increased at blood glucose levels of 3.0 mmol/L[84,94].

In summary, it is clear that blood glucose may be associated with appetite under certain conditions, such as at extreme levels during hypoglycaemia or hyperglycaemia. However, at normal physiological levels, findings are generally inconsistent and interpreting the association of glucose with appetite is often confounded by covariation with other key postprandial markers involved in appetite control.

Other Organic Acids, derivatives and other metabolites

In addition to fatty acids, amino acids and glucose, several other metabolites such as ketone bodies, lactate and cortisol have been associated with appetite. Alterations in appetite with ketosis have been thought to have a major role in contributing to weight loss. During ketosis, there is an increase in circulating concentrations of ketones (β-hydroxybutyrate, acetoacetate and acetone) that are synthesised as a consequence of a sustained increase in ß-oxidation of free fatty acids in the liver, and provide an alternative fuel source when glucose supply is limited e.g. during prolonged periods of fasting or very low carbohydrate diet[95]. In a meta-analysis examining changes in appetite during ketosis, hunger and desire to eat were shown to be suppressed and fullness/satiety increased, with ketosis appearing to provide a plausible explanation for this[95]. However, whether there is a threshold level of circulating ketone concentrations at which appetite is suppressed could not be determined as the level of β-hydroxybutyrate (~0.5mM) was similar among all studies included[95]. Nevertheless, there is mechanistic evidence from animal studies that intracerebroventricular infusion[96] and

414 subcutaneous injection[97] of β-hydroxybutyrate reduce food intake, along with support from
415 in vitro studies showing that β-hydroxybutyrate under physiological conditions influences
416 AgRP expression and reduces orexigenic signalling via the AMPK pathway[98].

417 Promising data in humans for a direct effect of circulating ketones on appetite comes
418 from evidence of reductions in appetite following consumption of a taste-matched ketone
419 ester drink compared to a dextrose drink[99]. In response to consumption of the ketone ester
420 drink, β-hydroxybutyrate levels increased from 0.2 to 3.3 mM after one hour, the onset of
421 hunger was delayed, desire to eat reduced and there was a delayed rise in plasma total ghrelin
422 levels. Moreover, β-hydroxybutyrate circulating concentrations were strongly inversely
423 correlated with change from baseline in hunger and desire to eat, and positively correlated
424 with fullness. Interestingly, the appetite suppression effect in this study could not be
425 attributed to plasma glucose, insulin, GLP-1 or PYY levels. Overall, although human studies
426 examining direct effects are currently limited, the evidence to date indicates blood β-
427 hydroxybutyrate as a promising potential biomarker for appetite; that may act either directly
428 or indirectly through effects on gut hormones such as ghrelin.

429 Lactate, produced from pyruvate, has a role in many biological processes including as
430 an energy substrate, and is another metabolite that appears to have a role in appetite control.
431 Plasma lactate concentrations have been shown to increase after meals in a similar pattern to
432 insulin, with a greater response following consumption of high compared to low carbohydrate
433 meals[100]. Although appetite ratings were not assessed in that study, others have shown direct
434 correlations between circulating lactate concentrations and hunger ratings following
435 consumption of resistant starch[90]. In a subsequent analysis by the same authors[101] using
436 data from three studies combined, delta mean satiety was most strongly correlated with delta
437 AUC plasma lactate, and was also correlated with glucose, insulin, noradrenaline, gastric
438 inhibitory peptide and carbohydrate oxidation. In a more recent study, blood lactate was
439 implicated to have a role in 'exercise-induced anorexia' – the transient suppression of
440 appetite following high intensity exercise, with an increase in blood lactate inversely
441 correlated with both subjective appetite ratings and acylated ghrelin AUC[14].

442 As lactate binds to the G protein-coupled receptor on gastric cells that produce
443 ghrelin, and inhibits ghrelin secretion[102], this may be one mechanism by which lactate
444 impacts appetite control. In addition, findings from animal studies indicate that lactate may
445 act centrally in the regulation of food intake[103]. The influence of lactate on appetite however
446 may also be dependent on circulating levels of other metabolites such as glucose, with

447 evidence of reductions in EI when lactate was infused during a euglycemic clamp but no
448 effects during a hypoglycemic clamp in humans[104]. Overall, the current evidence in humans,
449 supported by mechanistic studies in both humans and animals, indicates that lactate may have
450 a role in appetite control under certain conditions.

451 Glucocorticoids such as cortisol, a steroid hormone and the major glucocorticoid
452 secreted by the adrenal gland, has been shown to directly correlate with appetite
453 ratings[15,105,106] and energy intake[107] in some, but not all[108] studies. Serum cortisol was
454 inversely correlated with satiety during a period of 48 hours of energy balance in humans,
455 however, there was no relationship during an equivalent period of energy deficit[15]. In
456 addition, salivary and plasma cortisol were shown to be associated with increased hunger[106]
457 and energy intake[107] in women under conditions of stress, but not on a rest day (without
458 stress)[107]. Cortisol responses to food intake have also been correlated with acylated ghrelin
459 in women with obesity suggesting a common neuro-humoral pathway through which stress
460 and anxiety may influence appetite[109]. However, it may be likely that cortisol reflects or
461 modulates other factors that respond to stress, such as leptin, neuropeptide Y, or cytokines,
462 that have a more direct effect on appetite, than directly influencing appetite itself[107].
463
464 *Bile Acids*
465 Bile consists of a range of molecules including bile acids, cholesterol, phospholipids and
466 bilirubin. In addition to key roles in lipid metabolism and cholesterol homeostasis, bile acids
467 can act as signalling molecules and an effect of bile acids on appetite was described in
468 1968[110]. More recent studies have shown bile acids in plasma to correlate with key appetite-
469 related gut hormones. For example, following a standardised test meal in 12 normal weight
470 adults GLP-1 and PYY responses were found to correlate with chenodeoxycholic acid
471 metabolites while total ghrelin was inversely correlated with deoxycholic acid
472 metabolites[111]. However, appetite was not assessed. Therefore, while there is evidence for a
473 role of bile acids in appetite control, and this may occur through direct effects or indirectly
474 through effects on gut hormones (see[112] for a detailed discussion), current findings showing
475 direct associations between bile acid concentrations in biological samples and appetite/energy
476 intake in humans are limited.
477
478 *Untargeted metabolomics approaches and appetite*

In contrast to studies targeting specific metabolites, current metabolomics techniques allow for hundreds of metabolites to be quantified and also allow an untargeted approach to be employed where one has the potential to identify new/novel biomarkers. There are limited studies that have employed metabolomics based approaches to the study of appetite. Using NMR for metabolomics analysis, Malagelada et al.[113] found increased plasma valine and glucose levels to correlate with satiation immediately after ingestion of a test meal to satiation. In addition, circulating metabolites were proposed that could serve as objective biomarkers of hedonic responses to food ingestion[113]. Elsewhere, in a comprehensive study examining a range of mechanisms potentially involved in the appetite response to mycoprotein compared to chicken, Bottin et al.[114] constructed orthogonal projection latent structure (OPLS) models to identify variation in metabolites in urine associated with fullness following each meal. Urinary creatinine (a breakdown product of creatine phosphate) was inversely associated with fullness following both meals. The metabolite α-keto-β-methyl-N-valerate (a deamination product of isoleucine) was also inversely associated with fullness, and β-hydroxybutyrate was positively associated with fullness following the mycoprotein but not chicken meal[114]. The latter finding regarding β-hydroxybutyrate is supported by evidence from ketone ester drinks[99] highlighting one potential mechanism by which mycoprotein may suppress appetite.

In another untargeted metabolomics study of plasma pre and post rye bread consumption (at least 20% of daily energy intake) over 8 weeks, 540 metabolites were profiled and specific metabolites identified were suggested to have a role in the satiety response to rye bread[115]. Among other metabolites, ribonic acid increased with rye bread consumption and was positively correlated with tryptophan - a precursor for the biosynthesis of serotonin which in turn impacts appetite[115]. However, appetite was not measured in this study, therefore an association of these metabolites with appetite could only be speculated upon.

In a study examining appetite in female hemodialysis patients a range of endocannabinoids and fatty acids were measured by gas chromatography and liquid chromatography mass spectrometry. A significant association between specific endocannabinoids and appetite measured through a Simplified Nutritional appetite questionnaire was reported[116]. In particular, docosatetraenoyl ethanolamide was positively correlated with appetite and a ratio of two specific endocannabinoids was inversely associated with appetite. Although the cross-sectional design prevents any causal inference, these findings nevertheless highlight a link between circulating endocannabinoids and

appetite in hemodialysis patients and provide several avenues for further research in this patient population.

Overall, although limited in number, these studies highlight the potential for metabolomic approaches to deepen understanding of the complex effects of different nutrients and foods on appetite and in understanding alterations in appetite in different conditions in humans.

Methodological Considerations and Future Directions

A role for additional metabolites

This review has focused on metabolites that have been identified in human biofluids, and have shown direct associations with appetite and/or energy intake in humans. Several additional metabolites have been measured in different biofluids in humans and implicated to have a role in appetite control, however often appetite was not assessed or direct associations between the metabolite concentration and appetite or energy intake were not reported. For example, in addition to bile acids, plasma acylcarnitines and phospholipid levels have been shown to change in response to bariatric surgery but associations with appetite were not determined[117]. Plasma acylcarnitines may likely be a consequence of alterations in insulin resistance and metabolic flexibility[118,119], rather than a marker of appetite. Additional relevant metabolites may include enterostatins which have been shown to reduce food intake, particularly from high fat foods in rats[120]. In women with obesity, a blunted enterostatin response has been shown after a meal[121], although appetite was not measured in that study. It should also be acknowledged that some metabolites may indirectly impact appetite or provide insight into the role of other metabolites in appetite control. For example, tissue and circulating ceramides may indirectly disrupt the hypothalamic control of food intake by increasing insulin resistance[122]. In addition, as the present review is a narrative review it is possible that some relevant articles were missed. Future systematic reviews on specific metabolites or groups of metabolites may yield further information.

A role for a range of biofluids

Blood is the predominant biofluid used in studies to date, although some have studied metabolites in saliva[50,107] and urine[114]. The latter are attractive samples to identify potential

appetite biomarkers due to the non-invasive nature of collection and warrant further study. Although invasive to collect, the characterisation of metabolites in other biofluids such as cerebrospinal fluid may also offer further insights into biological processes of appetite control. For example, neurotransmitter metabolite levels in cerebrospinal fluid have been shown to differ in individuals with bulimia, with lower serotonin and dopamine metabolite concentrations compared to healthy controls[123]. Given that the metabolite concentrations were inversely correlated with frequency of binge eating, higher concentrations of these metabolites could potentially contribute to a blunted satiety response in individuals with bulimia[123]. The fecal metabolome largely reflects gut microbial composition[124] and therefore presents a key method of gaining further insight into the role of the gut microbiome in appetite control. Gut microbes are able to produce various metabolites which may exert their effects on appetite either directly by interacting with receptors on L-cells in the intestine or by translocating from the intestine into the peripheral circulation (for a detailed review, see[125]). For example, GABA, an inhibitory neurotransmitter and naturally occurring amino acid, has been identified in human feces (as well as saliva, urine, blood and cerebrospinal fluid), and can be produced in the intestine by strains of lactobacillus and bifidobacterium [126].

What influences peripheral metabolites implicated in appetite control?

The long-term molecular link between energy needs and daily intake has been commonly thought to be driven by feedback signals arising from adipose tissue such as leptin; and gut and adipose tissue derived peripheral signals have been the focus of the majority of studies investigating appetite and obesity[127]. However, while these signals clearly have an important role in different aspects of appetite control, other signals such as a molecular signalling pathway arising from lean tissue such as skeletal muscle may also feature [128,129,130], but to date has received little attention. Recent evidence has linked fat free-mass, resting metabolic rate[129,131,132] and activity energy expenditure[133] to the tonic drive component of appetite control that is thought to reflect biological energy requirements. However, the molecular signalling pathways that link the energy demands arising from metabolically active tissues to energy intake remain unclear. This highlights that in addition to signals arising from the gut and adipose tissue, other tissues and factors should also be considered when identifying biological signals and potential metabolites involved in appetite control.

Providing insight into individual variability in appetite and appetite responses

In addition to increasing understanding of appetite responses to ingestion of different foods, metabolomics could also have a role in increasing understanding of different appetite and behavioural phenotypes. For example, the 'low satiety phenotype' i.e. those with lower satiety responsiveness to a test meal is often assessed using the satiety quotient which relates the suppression of hunger, fullness and desire to eat to the amount of energy consumed[134], and has been characterised by a tendency towards a higher anxiety level and blunted cortisol response[135], along with a higher level of disinhibition and greater wanting of high fat food[136], among other characteristics. Similarly metabolomics may help to understand different binge-eating subtypes and a range of other phenotypes such as those susceptible or resistant to weight gain[137].

Metabolomics could provide further insight into the wide individual variability that occurs in appetite and energy intake responses to different interventions. Some studies have classified individuals as compensators or non-compensators based on whether they ate more or less in response to exercise[138,139]. Although metabolomics analysis was not conducted in these studies, baseline postprandial profiles of appetite-related peptides have been shown to identify those susceptible or resistant to exercise-induced weight loss[140]. Elsewhere in response to dietary intervention[141], metabolomics analysis was carried out on 70 healthy individuals during a mixed meal tolerance test over 8 hours, and ~300 metabolites in plasma were profiled. Two distinctive 'metabotype' clusters were found, with only participants from one 'metabotype' showing positive changes in the glycaemic response after 12 weeks. Although, appetite was not assessed in this study, this highlights the potential for metabolomics to aid in understanding individual variability in responses to different interventions.

A role as part of a larger panel of appetite-related assessments

As noted for many metabolites discussed, there is covariation with other postprandial metabolites, and with the release of appetite-related gut peptides, which often mirror postprandial appetite ratings. However, the relative contribution of the different markers to appetite control is unknown. There is no composite peptide or metabolite measure that is similar to a rating of subjective appetite, raising the question of whether peptide biomarkers provide stronger evidence than subjective ratings[33]. However, while clearly biomarkers

should not replace subjective ratings of appetite or actual measures of food intake, they may be extremely useful used in conjunction with a larger assessment involving a range of psychological, physiological and behavioural measures to improve mechanistic understanding behind changes in appetite and energy intake, along with other components of appetite not addressed in this review such as food reward. An example study workflow is shown in Figure 3.

[Figure 3]

A role in conjunction with other 'omics' methods

While this review has focused on metabolomics and relationships between metabolites and appetite ratings or energy intake, a combination of different 'omics' methods, may also yield further information. For example, in combination with metabolomics, metagenomics and epigenomics may provide important insights into links between stress, appetite and obesity (see[142] for a comprehensive review). Elsewhere, proteomics analysis of plasma in the fasting state revealed apoliprotein A-IV as a putative satiety factor that rises following gastric bypass surgery and could potentially contribute to weight loss[143]. Moreover, the salivary proteome may be used to identify proteins and peptides which can be used as biomarkers of satiety[144]. Although cost and other logistical aspects is a limiting factor for widespread feasibility, to gain greater insight into underlying mechanisms and initially discover potential biomarkers, metabolomics profiles should ideally be considered alongside a range of other biological signals.

Conclusion

Appetite is a complex integrative process. In addition to environmental, psychological and behavioural factors, appetite is influenced by a range of short and long term biological signals. While the list of biological signals and potential biomarkers of appetite in humans is continuing to increase, many metabolites including glucose and amino acids were proposed as key signalling molecules in appetite over 60 years ago. Some metabolites appear to be associated

with appetite under specific conditions, such as in energy deficit, or be dependent on concentrations of other metabolites such as glucose. However, while several studies to date show associations between circulating metabolites and appetite, in many cases causal relationships in humans remain to be established highlighting the need for longitudinal studies. Furthermore, many studies have targeted single metabolites or a limited number of metabolites and/or gut peptides. Modern metabolomics facilitates the measurement of hundreds of metabolites using a targeted and/or untargeted approach, and has significant potential to identify potential biomarkers and deepen understanding of the complex biological signals involved in appetite control. This could in turn aid in improving strategies targeting appetite control and in tailoring strategies more effectively to individuals.

Acknowledgements

No sources of funding were received for this review.

KH and LB contributed to the conception and design of the review. KH and LB drafted the initial manuscript. KH, MH, CG, GF and LB reviewed and edited the manuscript.

There are no conflicts of interest to declare.

References

1. Soenen S, Chapman IM (2014) Body Weight, Anorexia, and Undernutrition in Older People. *Journal of the American Medical Directors Association* **14**, 642-648.
2. Michalakis K, Goulis DG, Vazaiou A *et al.* (2013) Obesity in the ageing man. *Metabolism: Clinical and Experimental* **62**, 1341-1349.
3. de Graaf C, Blom WA, Smeets PA *et al.* (2004) Biomarkers of satiation and satiety. *American Journal of Clinical Nutrition* **79**, 946-961.
4. Blundell J (1991) Pharmacological approaches to appetite suppression. *Trends Pharmacol Sci* **12**, 147-157.
5. Blundell JE, King NA (1996) Overconsumption as a cause of weight gain: Behavioural-physiological interactions in the control of food intake (appetite). *CIBA Foundation Symposia*, 138-158.
6. Blundell J, De Graaf C, Hulshof T *et al.* (2010) Appetite control: methodological aspects of the evaluation of foods. *Obesity reviews* **11**, 251-270.
7. Havel PJ (2001) Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. *Experimental Biology and Medicine* **226**, 963-977.
8. Delzenne N, Blundell J, Brouns F *et al.* (2010) Gastrointestinal targets of appetite regulation in humans. *Obesity reviews* **11**, 234-250.
9. Diplock A, Aggett P, Ashwell M *et al.* (1999) The European Commission concerted action on functional foods science in Europe (FUFOSE). Scientific concepts of functional foods in Europe. Consensus document. *Br J Nutr* **81**, S1-27.
10. Mayer J (1953) Glucostatic mechanism of regulation of food intake. *New England Journal of Medicine* **249**, 13-16.
11. Mennella I, Savarese M, Ferracane R *et al.* (2015) Oleic acid content of a meal promotes oleoylethanolamide response and reduces subsequent energy intake in humans. *Food & function* **6**, 203-209.
12. Heini AF, Kirk KA, Lara-Castro C *et al.* (1998) Relationship between hunger-satiety feelings and various metabolic parameters in women with obesity during controlled weight loss. *Obesity research* **6**, 225-230.
13. Hall W, Millward D, Long S *et al.* (2003) Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *British Journal of Nutrition* **89**, 239-248.
14. Islam H, Townsend LK, McKie GL *et al.* (2017) Potential involvement of lactate and interleukin-6 in the appetite-regulatory hormonal response to an acute exercise bout. *Journal of Applied Physiology* **123**, 614-623.
15. Karl JP, Smith TJ, Wilson MA *et al.* (2016) Altered metabolic homeostasis is associated with appetite regulation during and following 48-h of severe energy deprivation in adults. *Metabolism* **65**, 416-427.
16. Wren AM, Bloom SR (2007) Gut Hormones and Appetite Control. *Gastroenterology* **132**, 2116-2130.
17. Blundell JE (2006) Perspective on the central control of appetite. *Obesity (Silver Spring)* **14**, 160S-163S.
18. Lau J, Herzog H (2014) CART in the regulation of appetite and energy homeostasis. *Frontiers in neuroscience* **8**, 313.
19. Atasoy D, Betley JN, Su HH *et al.* (2012) Deconstruction of a neural circuit for hunger. *Nature* **488**, 172.
20. Halford JC, Blundell JE (2000) Separate systems for serotonin and leptin in appetite control. *Annals of Medicine* **32**, 222-232.

21. Baldini G, Phelan KD (2019) The melanocortin pathway and control of appetite-progress and therapeutic implications. *Journal of Endocrinology* **241**, R1-R33.
22. Caro JF, Sinha MK, Kolaczynski JW *et al.* (1996) Leptin: the tale of an obesity gene. *Diabetes* **45**, 1455-1462.
23. Flint A, Gregersen NT, Gluud LL *et al.* (2007) Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. *British Journal of Nutrition* **98**, 17-25.
24. Blundell JE, Levin F, King NA *et al.* (2008) Overconsumption and obesity: Peptides and susceptibility to weight gain. *Regulatory Peptides* **149**, 32-38.
25. McMinn JE, Sindelar DK, Havel PJ *et al.* (2000) Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology* **141**, 4442-4448.
26. Cummings DE, Purnell JQ, Frayo RS *et al.* (2001) A Preprandial Rise in Plasma Ghrelin Levels Suggests a Role in Meal Initiation in Humans. *Diabetes* **50**, 1714-1719.
27. Diepvens K, Häberer D, Westerterp-Plantenga M (2008) Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *International journal of obesity* **32**, 510.
28. Smeets AJ, Soenen S, Luscombe-Marsh ND *et al.* (2008) Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *The Journal of nutrition* **138**, 698-702.
29. Lemmens SG, Martens EA, Kester AD *et al.* (2011) Changes in gut hormone and glucose concentrations in relation to hunger and fullness1–3. *The American Journal of Clinical Nutrition* **94**, 717-725.
30. Gibbons C, Caudwell P, Finlayson G *et al.* (2013) Comparison of postprandial profiles of ghrelin, active GLP-1, and total PYY to meals varying in fat and carbohydrate and their association with hunger and the phases of satiety. *The Journal of Clinical Endocrinology & Metabolism* **98**, E847-E855.
31. Erdmann J, Hebeisen Y, Lippl F *et al.* (2007) Food intake and plasma ghrelin response during potato-, rice-and pasta-rich test meals. *European journal of nutrition* **46**, 196-203.
32. Adam T, Westerterp-Plantenga M (2005) Nutrient-stimulated GLP-1 release in normal-weight men and women. *Hormone and Metabolic Research* **37**, 111-117.
33. Gibbons C, Hopkins M, Beaulieu K *et al.* (2019) Issues in Measuring and Interpreting Human Appetite (Satiety/Satiation) and Its Contribution to Obesity. *Current obesity reports* **8**, 77-87.
34. Brennan L (2013) Metabolomics in nutrition research: current status and perspectives: Portland Press Limited.
35. Gibbons H, Michielsen CJ, Rundle M *et al.* (2017) Demonstration of the utility of biomarkers for dietary intake assessment; proline betaine as an example. *Molecular nutrition & food research* **61**, 1700037.
36. Cheung W, Keski-Rahkonen P, Assi N *et al.* (2017) A metabolomic study of biomarkers of meat and fish intake. *The American journal of clinical nutrition* **105**, 600-608.
37. Edmands WM, Beckonert OP, Stella C *et al.* (2011) Identification of human urinary biomarkers of cruciferous vegetable consumption by metabonomic profiling. *Journal of proteome research* **10**, 4513-4521.
38. Heinzmann SS, Brown IJ, Chan Q *et al.* (2010) Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption. *The American journal of clinical nutrition* **92**, 436-443.
39. Ulaszewska M, Vázquez-Manjarrez N, Garcia-Aloy M *et al.* (2018) Food intake biomarkers for apple, pear, and stone fruit. *Genes & nutrition* **13**, 29.

40. Andersen M-BS, Kristensen M, Manach C *et al.* (2014) Discovery and validation of urinary exposure markers for different plant foods by untargeted metabolomics. *Analytical and bioanalytical chemistry* **406**, 1829-1844.
41. Bingham SA (2002) Biomarkers in nutritional epidemiology. *Public health nutrition* **5**, 821-827.
42. Kipnis V, Midthune D, Freedman L *et al.* (2002) Bias in dietary-report instruments and its implications for nutritional epidemiology. *Public health nutrition* **5**, 915-923.
43. Garcia-Perez I, Posma JM, Chambers ES *et al.* (2016) An analytical pipeline for quantitative characterization of dietary intake: application to assess grape intake. *Journal of agricultural and food chemistry* **64**, 2423-2431.
44. Van Itallie T, Hashim S (1960) Biochemical concomitants of hunger and satiety in man. *The American Journal of Clinical Nutrition* **8**, 587-594.
45. Gatta B, Zuberbuehler C, Arnold M *et al.* (2008) Acute effects of pharmacological modifications of fatty acid metabolism on human satiety. *British journal of nutrition* **101**, 1867-1877.
46. Deighton K, King AJ, Matu J *et al.* (2019) A single day of mixed-macronutrient overfeeding does not elicit compensatory appetite or energy intake responses but exaggerates postprandial lipaemia during the next day in healthy young men. *British Journal of Nutrition* **121**, 945-954.
47. Obici S, Feng Z, Morgan K *et al.* (2002) Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* **51**, 271-275.
48. Sergi D, Williams LM (2019) Potential relationship between dietary long-chain saturated fatty acids and hypothalamic dysfunction in obesity. *Nutrition Reviews*.
49. Lawton CL, Delargy HJ, Brockman J *et al.* (2000) The degree of saturation of fatty acids influences post-ingestive satiety. *British Journal of Nutrition* **83**, 473-482.
50. Kong X, Ferracane R, De Luca L *et al.* (2016) Salivary concentration of N-acylethanolamines upon food mastication and after meal consumption: Influence of food dietary fiber. *Food Research International* **89**, 186-193.
51. Rigamonti AE, Piscitelli F, Aveta T *et al.* (2015) Anticipatory and consummatory effects of (hedonic) chocolate intake are associated with increased circulating levels of the orexigenic peptide ghrelin and endocannabinoids in obese adults. *Food & Nutrition Research* **59**, 29678.
52. Fu J, Gaetani S, Oveisi F *et al.* (2003) Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. *Nature* **425**, 90.
53. Fu J, Kim J, Oveisi F *et al.* (2008) Targeted enhancement of oleoylethanolamide production in proximal small intestine induces across-meal satiety in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **295**, R45-R50.
54. Tso P, Liu M (2004) Apolipoprotein A-IV, food intake, and obesity. *Physiology & behavior* **83**, 631-643.
55. Glatzle J, Darcel N, Rechs AJ *et al.* (2004) Apolipoprotein A-IV stimulates duodenal vagal afferent activity to inhibit gastric motility via a CCK1 pathway. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **287**, R354-R359.
56. Stone NL, Millar SA, Herrod PJ *et al.* (2018) An analysis of endocannabinoid concentrations and mood following singing and exercise in healthy volunteers. *Frontiers in behavioral neuroscience* **12**.
57. Byrne CS, Chambers ES, Morrison DJ *et al.* (2015) The role of short chain fatty acids in appetite regulation and energy homeostasis. *International Journal Of Obesity* **39**, 1331.
58. Wolever TM, Josse RG, Leiter LA *et al.* (1997) Time of day and glucose tolerance status affect serum short-chain fatty concentrations in humans. *Metabolism* **46**, 805-811.

59. Frost G, Sleeth ML, Sahuri-Arisoylu M *et al.* (2014) The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature communications* **5**, 3611.
60. Hartvigsen M, Lærke H, Overgaard A *et al.* (2014) Postprandial effects of test meals including concentrated arabinoxylan and whole grain rye in subjects with the metabolic syndrome: a randomised study. *European journal of clinical nutrition* **68**, 567.
61. Chambers ES, Viardot A, Psichas A *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**, 1744-1754.
62. Byrne CS, Preston T, Brignardello J *et al.* (2018) The effect of L-rhamnose on intestinal transit time, short chain fatty acids and appetite regulation: a pilot human study using combined 13CO2/H2 breath tests. *Journal of breath research* **12**, 046006.
63. Ilhan ZE, DiBaise JK, Isern NG *et al.* (2017) Distinctive microbiomes and metabolites linked with weight loss after gastric bypass, but not gastric banding. *The ISME journal* **11**, 2047.
64. Maninger N, Wolkowitz OM, Reus VI *et al.* (2009) Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Frontiers in Neuroendocrinology* **30**, 65-91.
65. Mellinkoff SM, Frankland M, Boyle D *et al.* (1956) Relationship between serum amino acid concentration and fluctuations in appetite. *Journal of applied physiology* **8**, 535-538.
66. Hill A, Blundell J (1988) Role of amino acids in appetite control in man. In *Amino acid availability and brain function in health and disease*, pp. 239-248: Springer.
67. Millward DJ (1995) A protein-stat mechanism for regulation of growth and maintenance of the lean body mass. *Nutrition research reviews* **8**, 93-120.
68. Hopkins M, Blundell JE (2017) Energy Metabolism and Appetite Control. In *Appetite and Food Intake: Central Control 2nd edition*: CRC Press/Taylor & Francis.
69. Lobley GE, Holtrop G, Horgan GW *et al.* (2015) Responses in gut hormones and hunger to diets with either high protein or a mixture of protein plus free amino acids supplied under weight-loss conditions. *British Journal of Nutrition* **113**, 1254-1270.
70. Neacsu M, Fyfe C, Horgan G *et al.* (2014) Appetite control and biomarkers of satiety with vegetarian (soy) and meat-based high-protein diets for weight loss in obese men: a randomized crossover trial. *The American journal of clinical nutrition* **100**, 548-558.
71. Luscombe-Marsh N, Hutchison A, Soenen S *et al.* (2016) Plasma free amino acid responses to intraduodenal whey protein, and relationships with insulin, glucagon-like peptide-1 and energy intake in lean healthy men. *Nutrients* **8**, 4.
72. Korompokis K, Östman E, Dougkas A (2016) The impact of liquid preloads varying in macronutrient content on postprandial kinetics of amino acids relative to appetite in healthy adults. *Appetite* **107**, 511-520.
73. Veldhorst MAB, Nieuwenhuizen AG, Hochstenbach-Waelen A *et al.* (2009) Dose-dependent satiating effect of whey relative to casein or soy. *Physiology & Behavior* **96**, 675-682.
74. Uhe AM, Collier GR, O'Dea K (1992) A comparison of the effects of beef, chicken and fish protein on satiety and amino acid profiles in lean male subjects. *The Journal of nutrition* **122**, 467-472.
75. van den Broek M, de Heide LJ, Emous M *et al.* (2018) Satiety and gastrointestinal hormones during a Mixed Meal Tolerance Test after gastric bypass surgery: association with plasma amino acid concentrations. *Surgery for Obesity and Related Diseases* **14**, 1106-1117.
76. Steinert RE, Landrock MF, Ullrich SS *et al.* (2015) Effects of intraduodenal infusion of the branched-chain amino acid leucine on ad libitum eating, gut motor and hormone functions, and glycemia in healthy men. *The American journal of clinical nutrition* **102**, 820-827.

77. Steinert RE, Luscombe-Marsh ND, Little TJ *et al.* (2014) Effects of intraduodenal infusion of L-tryptophan on ad libitum eating, antropyloroduodenal motility, glycemia, insulinemia, and gut peptide secretion in healthy men. *The Journal of Clinical Endocrinology & Metabolism* **99**, 3275-3284.
78. Ullrich S, Fitzgerald P, Giesbertz P *et al.* (2018) Effects of intragastric administration of tryptophan on the blood glucose response to a nutrient drink and energy intake, in lean and obese men. *Nutrients* **10**, 463.
79. Mayer J (1955) Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. *Annals of the New York Academy of sciences* **63**, 15-43.
80. Campfield LA, Smith FJ (2003) Blood glucose dynamics and control of meal initiation: a pattern detection and recognition theory. *Physiological Reviews* **83**, 25-58.
81. Chaput J, Tremblay A (2009) The glucostatic theory of appetite control and the risk of obesity and diabetes. *International journal of obesity* **33**, 46.
82. Anderson GH, Catherine NL, Woodend DM *et al.* (2002) Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. *The American Journal of Clinical Nutrition* **76**, 1023-1030.
83. Flint A, Møller BK, Raben A *et al.* (2006) Glycemic and insulinemic responses as determinants of appetite in humans. *The American Journal of Clinical Nutrition* **84**, 1365-1373.
84. Schultes B, Oltmanns KM, Kern W *et al.* (2003) Modulation of hunger by plasma glucose and metformin. *The Journal of Clinical Endocrinology & Metabolism* **88**, 1133-1141.
85. Campfield LA, Smith FJ, Rosenbaum M *et al.* (1996) Human eating: evidence for a physiological basis using a modified paradigm. *Neuroscience & Biobehavioral Reviews* **20**, 133-137.
86. Jones K, Horowitz M, Berry M *et al.* (1997) Blood glucose concentration influences postprandial fullness in IDDM. *Diabetes Care* **20**, 1141-1146.
87. Melanson KJ, Westerterp-Plantenga MS, Saris WH *et al.* (1999) Blood glucose patterns and appetite in time-blinded humans: carbohydrate versus fat. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **277**, R337-R345.
88. Schultes B, Panknin A-K, Hallschmid M *et al.* (2016) Glycemic increase induced by intravenous glucose infusion fails to affect hunger, appetite, or satiety following breakfast in healthy men. *Appetite* **105**, 562-566.
89. Sepple C, Read N (1989) Gastrointestinal correlates of the development of hunger in man. *Appetite* **13**, 183-191.
90. Raben A, Tagliabue A, Christensen NJ *et al.* (1994) Resistant starch: the effect on postprandial glycemia, hormonal response, and satiety. *The American journal of clinical nutrition* **60**, 544-551.
91. Porte D, Woods S (1981) Regulation of food intake and body weight by insulin. *Diabetologia* **20**, 274-280.
92. Lavin J, Wittert G, Sun W *et al.* (1996) Appetite regulation by carbohydrate: role of blood glucose and gastrointestinal hormones. *American Journal of Physiology-Endocrinology And Metabolism* **271**, E209-E214.
93. Gielkens HAJ, Verkijk M, Lam WF *et al.* (1998) Effects of hyperglycemia and hyperinsulinemia on satiety in humans. *Metabolism* **47**, 321-324.
94. Schultes B, Kern W, Oltmanns K *et al.* (2005) Differential adaptation of neurocognitive brain functions to recurrent hypoglycemia in healthy men. *Psychoneuroendocrinology* **30**, 149-161.
95. Gibson AA, Seimon RV, Lee CM *et al.* (2015) Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obesity Reviews* **16**, 64-76.

96. Arase K, Fisler JS, Shargill NS *et al.* (1988) Intracerebroventricular infusions of 3-OHB and insulin in a rat model of dietary obesity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **255**, R974-R981.
97. Langhans W, Egli G, Scharrer E (1985) Selective hepatic vagotomy eliminates the hypophagic effect of different metabolites. *Journal of the autonomic nervous system* **13**, 255-262.
98. Laeger T, Pöhland R, Metges CC *et al.* (2012) The ketone body β-hydroxybutyric acid influences agouti-related peptide expression via AMP-activated protein kinase in hypothalamic GT1-7 cells. *Journal of Endocrinology* **213**, 193-203.
99. Stubbs BJ, Cox PJ, Evans RD *et al.* (2018) A ketone ester drink lowers human ghrelin and appetite. *Obesity* **26**, 269-273.
100. Havel PJ, Townsend R, Chaump L *et al.* (1999) High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes* **48**, 334-341.
101. Raben A, Holst J, Christensen NJ *et al.* (1996) Determinants of postprandial appetite sensations: macronutrient intake and glucose metabolism. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity* **20**, 161-169.
102. Engelstoft MS, Park W-m, Sakata I *et al.* (2013) Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells. *Molecular metabolism* **2**, 376-392.
103. Lam CK, Chari M, Wang PY *et al.* (2008) Central lactate metabolism regulates food intake. *American Journal of Physiology-Endocrinology and Metabolism* **295**, E491-E496.
104. Schultes B, Schmid SM, Wilms B *et al.* (2012) Lactate infusion during euglycemia but not hypoglycemia reduces subsequent food intake in healthy men. *Appetite* **58**, 818-821.
105. Lawson EA, Holsen LM, Desanti R *et al.* (2013) Increased hypothalamic-pituitary-adrenal drive is associated with decreased appetite and hypoactivation of food-motivation neurocircuitry in anorexia nervosa. *Eur J Endocrinol* **169**, 639-647.
106. Geliebter A, Carnell S, Gluck ME (2013) Cortisol and ghrelin concentrations following a cold pressor stress test in overweight individuals with and without night eating. *International Journal of Obesity* **37**, 1104.
107. Epel E, Lapidus R, McEwen B *et al.* (2001) Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. *Psychoneuroendocrinology* **26**, 37-49.
108. Raspopow K, Abizaid A, Matheson K *et al.* (2014) Anticipation of a psychosocial stressor differentially influences ghrelin, cortisol and food intake among emotional and non-emotional eaters. *Appetite* **74**, 35-43.
109. Sarker MR, Franks S, Caffrey J (2013) Direction of post-prandial ghrelin response associated with cortisol response, perceived stress and anxiety, and self-reported coping and hunger in obese women. *Behavioural Brain Research* **257**, 197-200.
110. Bray G, Gallagher JR T (1968) Suppression of appetite by bile acids. *The Lancet* **291**, 1066-1067.
111. Roberts RE, Glicksman C, Alaghband-Zadeh J *et al.* (2011) The relationship between postprandial bile acid concentration, GLP-1, PYY and ghrelin. *Clinical endocrinology* **74**, 67-72.
112. Kuhre RE, Albrechtsen NJW, Larsen O *et al.* (2018) Bile acids are important direct and indirect regulators of the secretion of appetite-and metabolism-regulating hormones from the gut and pancreas. *Molecular metabolism* **11**, 84-95.
113. Malagelada C, Barba I, Accarino A *et al.* (2016) Cognitive and hedonic responses to meal ingestion correlate with changes in circulating metabolites. *Neurogastroenterology & Motility* **28**, 1806-1814.

955 114. Bottin JH, Swann JR, Cropp E *et al.* (2016) Mycoprotein reduces energy intake and
956 postprandial insulin release without altering glucagon-like peptide-1 and peptide tyrosine-
957 tyrosine concentrations in healthy overweight and obese adults: a randomised-controlled trial.
958 *British Journal of Nutrition* **116**, 360-374.
959 115. Lankinen M, Schwab U, Seppänen-Laakso T *et al.* (2010) Metabolomic Analysis of
960 Plasma Metabolites That May Mediate Effects of Rye Bread on Satiety and Weight
961 Maintenance in Postmenopausal Women. *The Journal of Nutrition* **141**, 31-36.
962 116. Friedman AN, Kim J, Kaiser S *et al.* (2016) Association between plasma
963 endocannabinoids and appetite in hemodialysis patients: A pilot study. *Nutrition Research*
964 **36**, 658-662.
965 117. Fiamoncini J, Fernandes Barbosa C, Arnoni Junior J *et al.* (2018) Roux-en-Y Gastric
966 Bypass Surgery Induces Distinct but Frequently Transient Effects on Acylcarnitine, Bile
967 Acid and Phospholipid Levels. *Metabolites* **8**, 83.
968 118. Ramos-Roman MA, Sweetman L, Valdez MJ *et al.* (2012) Postprandial changes in
969 plasma acylcarnitine concentrations as markers of fatty acid flux in overweight and obesity.
970 *Metabolism* **61**, 202-212.
971 119. Adams SH, Hoppel CL, Lok KH *et al.* (2009) Plasma Acylcarnitine Profiles Suggest
972 Incomplete Long-Chain Fatty Acid β-Oxidation and Altered Tricarboxylic Acid Cycle
973 Activity in Type 2 Diabetic African-American Women. *The Journal of Nutrition* **139**, 1073-
974 1081.
975 120. Erlanson-Albertsson C, Mei J, Okada S *et al.* (1991) Pancreatic procolipase propeptide,
976 enterostatin, specifically inhibits fat intake. *Physiology & behavior* **49**, 1191-1194.
977 121. Prasad C, Imamura M, Debata C *et al.* (1999) Hyperenterostatinemia in Premenopausal
978 Obese Women1. *The Journal of Clinical Endocrinology & Metabolism* **84**, 937-941.
979 122. Summers SA, Goodpaster BH (2016) CrossTalk proposal: Intramyocellular ceramide
980 accumulation does modulate insulin resistance. *The Journal of physiology* **594**, 3167.
981 123. Jimerson DC, Lesem MD, Kaye WH *et al.* (1992) Low serotonin and dopamine
982 metabolite concentrations in cerebrospinal fluid from bulimic patients with frequent binge
983 episodes. *Archives of General Psychiatry* **49**, 132-138.
984 124. Zierer J, Jackson MA, Kastenmüller G *et al.* (2018) The fecal metabolome as a
985 functional readout of the gut microbiome. *Nature genetics* **50**, 790.
986 125. van de Wouw M, Schellekens H, Dinan TG *et al.* (2017) Microbiota-gut-brain axis:
987 modulator of host metabolism and appetite. *The Journal of nutrition* **147**, 727-745.
988 126. Barrett E, Ross R, O'toole P *et al.* (2012) γ-Aminobutyric acid production by culturable
989 bacteria from the human intestine. *Journal of applied microbiology* **113**, 411-417.
990 127. Lean M, Malkova D (2016) Altered gut and adipose tissue hormones in overweight and
991 obese individuals: cause or consequence? *International Journal of Obesity* **40**, 622.
992 128. Hopkins M, Blundell JE (2016) Energy balance, body composition, sedentariness and
993 appetite regulation: pathways to obesity. *Clinical Science* **130**, 1615-1628.
994 129. Blundell JE, Caudwell P, Gibbons C *et al.* (2012) Body composition and appetite: fat-
995 free mass (but not fat mass or BMI) is positively associated with self-determined meal size
996 and daily energy intake in humans. *British Journal of Nutrition* **107**, 445-449.
997 130. Grannell A, De Vito G, Murphy JC *et al.* (2019) The influence of skeletal muscle on
998 appetite regulation. *Expert review of endocrinology & metabolism* **14**, 267-282.
999 131. Hopkins M, Finlayson G, Duarte C *et al.* (2016) Modelling the associations between fat-
1000 free mass, resting metabolic rate and energy intake in the context of total energy balance.
1001 *International Journal of Obesity* **40**, 312-318.
1002 132. Hopkins M, Finlayson G, Duarte C *et al.* (2019) Biological and psychological mediators
1003 of the relationships between fat mass, fat-free mass and energy intake. *International Journal*
1004 *of Obesity* **43**, 233-242.

133. Hopkins M, Duarte C, Beaulieu K *et al.* (2019) Activity energy expenditure is an independent predictor of energy intake in humans. *International Journal of Obesity*, 1.
134. Drapeau V, King N, Hetherington M *et al.* (2007) Appetite sensations and satiety quotient: predictors of energy intake and weight loss. *Appetite* **48**, 159-166.
135. Drapeau V, Blundell J, Gallant A *et al.* (2013) Behavioural and metabolic characterisation of the low satiety phenotype. *Appetite* **70**, 67-72.
136. Dalton M, Hollingworth S, Blundell J *et al.* (2015) Weak satiety responsiveness is a reliable trait associated with hedonic risk factors for overeating among women. *Nutrients* **7**, 7421-7436.
137. Dalton M, Finlayson G, Esdaile E *et al.* (2013) Appetite, satiety, and food reward in obese individuals: a behavioral phenotype approach. *Current Nutrition Reports* **2**, 207-215.
138. Hopkins M, Blundell JE, King NA (2014) Individual variability in compensatory eating following acute exercise in overweight and obese women. *Br J Sports Med* **48**, 1472-1476.
139. King NA, Caudwell PP, Hopkins M *et al.* (2009) Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety. *The American journal of clinical nutrition* **90**, 921-927.
140. Gibbons C, Blundell JE, Caudwell P *et al.* (2017) The role of episodic postprandial peptides in exercise-induced compensatory eating. *The Journal of Clinical Endocrinology & Metabolism* **102**, 4051-4059.
141. Fiamoncini J, Rundle M, Gibbons H *et al.* (2018) Plasma metabolome analysis identifies distinct human metabotypes in the postprandial state with different susceptibility to weight loss–mediated metabolic improvements. *The FASEB Journal* **32**, 5447-5458.
142. Michels N (2019) Biological underpinnings from psychosocial stress towards appetite and obesity during youth: research implications towards metagenomics, epigenomics and metabolomics. *Nutrition research reviews* **32**, 282-293.
143. Culnan DM, Cooney RN, Stanley B *et al.* (2009) Apolipoprotein A-IV, a putative satiety/antiatherogenic factor, rises after gastric bypass. *Obesity* **17**, 46-52.
144. Harthoorn LF, Schipper RG, Loof A *et al.* (2007) Salivary biomarkers associated with perceived satiety and body mass in humans. *PROTEOMICS–Clinical Applications* **1**, 1637-1650.

Table 1. Metabolites that have been identified in human biofluids and associated with subjective appetite ratings and/or energy intake[1]

	Study Details				
Metabolite(s)	**Reference**	**Platform[2]**	**Biofluid**	**Participants**	**Association with Appetite Ratings and/or EI**
Lipids and lipid like molecules					
Oleoylethanolamine (OEA)	Mennella et al. (2015)[11]	LCMS	Blood	n=15 NW	**EI:** Highest postprandial OEA response elicited by test meals (bread + oil) associated with greatest reductions in EI at next meal
	Kong et al. (2016)[50]	LCMS/MS	Saliva	n=18 NW, OW	**Fullness:** Salivary OEA concentration positively correlated with fullness and satiety at 30min post breakfast
Anandamide (AEA)	Rigamonti et al. (2015)[51]	LCMS	Blood	n=10 OB	**Hunger:** AEA positively associated with postprandial AUC hunger VAS score
	Stone et al. (2018)[56]	LCMS/MS	Blood	n=9 weight status not reported	**Fullness:** Plasma AEA positively correlated with fullness across all days and time-points (pre- and 30min post- dancing, reading, singing or cycling in the fasted state)
Palmitoylethanolamide (PEA)	Rigamonti et al. (2015)[51]	LCMS	Blood	n=10 OB	**Hunger:** PEA positively associated with postprandial AUC hunger VAS score
Butyrate	Hartvigsen et al. (2014)[60]	GC	Blood	n=15 OW & OB	**Satiety and Fullness:** Plasma butyrate associated with late satiety & fullness ($AUC_{240\text{-}360min}$) following test meals containing concentrated arabinoxylan, rye kernels or control porridge; plasma total FFAs, acetate or proprionate did not correlate with appetite scores
Dehydroepiandrosterone -sulfate (DHEA-S)	Karl et al. (2016)[15]	Automated Immunoassay	Blood	n=23 NW, OW	**Satiety:** Serum DHEA-S inversely associated with satiety during energy deficit, assessed by satiety labeled intensity magnitude scale
Amino acids and derivatives					

Total AA's (13 assessed), leucine, isolecuine, threonine, valine	Hall et al. (2003)[13]	HPLC	Blood	n=16 NW	**EI:** Total AAs and postprandial leucine, isoleucine, threonine and valine higher and subsequent test meal EI lower after whey compared to casein
Leucine, lysine, tryptophan, isoleucine, and threonine	Veldhorst et al. (2009)[73]	HPLC	Blood	n=25 NW, OW	**Hunger:** Increased postprandial leucine, lysine, tryptophan, isoleucine, and threonine with whey than after a breakfast with casein or soy coincided with greater decrease in postprandial hunger
All 19 out of 20 Amino Acids assessed (except cysteine)	Luscombe-Marsh et al. (2016)[71]	HPLC	Blood	n=16 NW	**EI:** Plasma AUC concentrations of 19/20 AA's (except cysteine) correlated with subsequent test meal EI after intraduodenal infusion of whey protein at different doses versus saline
Total AAs, Alanine, arginine, asparagine, glutamine, glycine, histidine, lysine, phenylalanine, serine, threonine, α-aminobutyric acid	Van den Broek et al. (2018)[75]	HPLC	Blood	n=42 OB 31-76 months post RYGB	**Satiety:** Increased total AA response, plasma alanine, arginine, asparagine, glutamine, glycine, histidine, lysine, phenylalanine, serine and threonine in individuals with a high satiety response compared to low satiety response following mixed meal test. **Hunger: I**ncreased plasma α-aminobutyric acid in individuals with greatest decrease in hunger
Glutamate, histidine, valine, lysine, leucine, isoleucine, α-aminobutyric acid	Korompokis et al. (2016)[72]	GC	Blood	n=36 NW, OW, AAs measured in n=7	**Prospective food consumption:** Plasma glutamate positively associated with prospective food consumption, **EI:** histidine positively associated with test meal EI and valine, leucine, isoleucine and α-aminobutyric acid inversely associated with EI; after liquid preloads varying in macronutrient content
Taurine	Veldhorst et al. (2009)[73]	HPLC	Blood	n=25 NW, OW	**Satiety and hunger:** Higher postprandial taurine after high soy compared to low soy protein breakfast associated with increased satiety AUC and reduced hunger AUC

Leucine	Steinert et al. (2015)[76]	HPLC	Blood	n=12 NW	**EI:** Plasma leucine AUC inversely associated with subsequent test meal EI, following intraduodenal infusions of different loads and saline
Tryptophan	Steinert et al. (2014)[77]	HPLC	Blood	n=10 NW	**Fullness and EI:** Plasma tryptophan AUC positively correlated with AUC fullness, and inversely associated with subsequent test meal EI after intraduodenal infusion of L-tryptophan compared to saline
	Ullrich et al. (2018)[78]	LCMS/MS	Blood	n= 16 NW, and n=16 OB	**EI:** Plasma tryptophan AUC inversely associated with subsequent test meal EI in lean but not obese individuals, following intragastric infusion of tryptophan compared to saline
Glucose					
	Campfield et al. (1996)[85]	Not stated	Blood	n=18 NW & OW	**Hunger and meal initiation: T**ransient declines in blood glucose positively correlated with hunger ratings and meal initiation
	Jones et al. (1997)[86]	Portable glucose analyser	Blood	n = 40 T1D patients NW, OW	**Fullness:** Blood glucose concentration positively associated with pre- and post-prandial fullness following solid + liquid test meal
	Heini et al. (1998)[12]	Hexokinase method	Blood	n=25 OB tested at twice at 3 and 5 weeks during controlled weight loss	**Satiety:** Under postprandial (but not fasting) conditions mean serum glucose across 2 visits positively correlated with satiety following high CHO preload

Gielkens et al. (1998)[93]	Glucose analyser (glucose oxidase method)	Blood	n=6 NW	**Appetite Ratings:** Acute hyperglycemia associated with reduced hunger, wish to eat and prospective feeding following hyperglycemic clamp compared to euglycemic hyperinsulinemia (using euglycemic insulin clamp technique) or control (IV saline) over 4h
Melanson et al. (1999)[87]	Glucose analyser	Blood	n = 10 NW	**Hunger and meal initiation: R**apid declines in blood glucose following fat and carbohydrate preloads associated with hunger ratings and meal initiation
Anderson et al. (2002)[82]	Handheld monitor and reagent strips	Blood	n=18 NW	**Appetite ratings and EI:** 60min postprandial AUC blood glucose following 5 different test CHO drinks inversely associated with mean appetite score (combining 4 appetite ratings) and with EI at lunch meal at 60min
Schultes et al. (2003)[84]	Glucose analyser	Blood	n=15 NW	**Hunger:** Decreased plasma glucose levels associated with increased hunger during hypoglycaemic clamp
Flint et al. (2006)[83]	Automated clinical chemistry analyser	Blood	n=28 NW	**EI:** 3h iAUC postprandial plasma glucose following 14 different breakfasts containing 50g CHO positively correlated with EI at subsequent ad libitum test lunch meal. **Appetite ratings:** No association with plasma glucose
Lemmens et al. (2011)[29]	Hexokinase method	Blood	n=38, NW, OW	**Fullness:** VAS fullness and plasma glucose concentrations changed synchronously, with a mean explained variation of 40% following a 4 course mixed macronutrient lunch meal consumed over 0.5 or 2h
Karl et al. (2016)[15]	Automated clinical chemistry analyser	Blood	n=23 NW, OW	**Satiety:** Serum glucose inversely associated with satiety during energy deficit, assessed by satiety labeled intensity magnitude scale

Other organic acids, derivatives and other metabolites					
β-hydroxybutyrate	Stubbs et al. (2018)[99]	Handheld monitor and reagent strips	Blood	n=28 NW, OW	**Hunger, fullness, desire to eat:** Blood β-hydroxybutyrate levels correlated with change from baseline in hunger, fullness and desire to eat ratings, following ketone ester drink compared to dextrose
Lactate	Raben et al. (1994)[90]	Standard enzymatic methods	Blood	n=10 NW	**Hunger:** Differences in hunger ratings between meals correlated with differences in delta peak lactate concentrations following consumption of resistant starch compared to non-resistant digestible starch
	Islam et al. (2017)[14]	Handheld blood lactate analyser	Blood	n= 8 NW, OW	**Appetite ratings:** Greatest change in blood lactate from pre to post exercise inversely correlated with overall appetite (calculated from mean of 4 different VAS questions)
Cortisol	Epel et al. (2001)[107]	Radioimmunoassay kit	Saliva	n=59 NW, OW & OB	**EI:** Change in salivary cortisol after stress associated with total EI consumed, but not in rest condition
	Lawson et al. (2013)[105]	Chemiluminescent Immunoassay	Blood	n = 36 (w/anorexia, weight recovered and healthy controls)	**Appetite ratings:** Fasting and AUC plasma cortisol significantly inversely associated with hunger and desire to eat
	Geliebter et al. (2013)[106]	Radioimmunoassay kit	Blood	n=28 OW w/ and w/o night eating	**Appetite ratings:** Peak plasma cortisol positively correlated with AUC hunger ratings assessed during and over 60min following cold pressor (stress) test

	Karl et al. (2016)[15]	Automated Immunoassay	Blood	n=23 NW, OW	**Satiety:** Serum cortisol inversely associated with satiety (assessed by satiety labeled intensity magnitude scale) during 48h energy balance, but no association during energy deficit
Metabolites identified using an untargeted approach					
Glucose, valine	Malagelada et al. (2016)[113]	NMR	Blood	n= 18 NW & OW	**Satiation:** Increase in satiation from fasting level was positively correlated with an increase in glucose and valine peaks in the NMR spectra
Creatinine and paracetamol glucuronide	Bottin et al. (2016)[114]	NMR	Urine, Blood	n=14 OW & OB	**Fullness: C**reatinine inversely associated with fullness, paracetamol glucuronide positively associated with fullness following chicken meal
Creatinine, α-keto-β-methyl-N-valerate, β-hydroxybutyrate	Bottin et al. (2016)[114]	NMR	Urine, Blood	n=14 OW & OB	**Fullness:** Creatinine, α-keto-β-methyl-N-valerate inversely associated with fullness, β-hydroxybutyrate positively associated with fullness following mycoprotein meal

[1]Metabolites were identified using targeted searches of the Human Metabolome database, research databases including OVID Medline and Google Scholar and reference lists of key articles. Combinations of the following key terms were included: metabolites; metabolomics; glucose; amino acids; fatty acids; bile acids; carbohydrates; appetite; hunger; fullness; satiation; satiety; energy intake; food intake. Articles that reported direct associations between metabolites and appetite and/or energy intake in humans were included.

[2]*Platform/method of analysis as described in original paper*

AA, amino acid; CHO, carbohydrate; EI, energy intake; FFA, free fatty acid; GC, gas chromatography, HPLC, high performance liquid chromatography, LCMS, liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance; NW, normal weight; OW, overweight; OB, obese; T1D, type 1 diabetes; VAS, visual analogue scale.

Figure Legend

Figure 1. Individual profiles for changes in (a) hunger and (b) total ghrelin in response to a high fat test meal. From Gibbons et al.[33].

Figure 2. Potential applications of metabolomics in nutrition research. Metabolomics has been used to identify biomarkers of food intake; examples now exist for a range of foods including but limited to fish, red meat, citrus fruit, apples, and cruciferous vegetables. Diet-disease relationships can be examined through application of metabolomics. In addition, through the identification of metabolic pathways altered following nutrition interventions mechanistic insights can be obtained.

Figure 3. Illustration of a study workflow investigating biomarkers of appetite using metabolomics. The time-intervals for assessing appetite and energy intake represent one example in this illustration and will vary depending on the objectives and characteristics of a study.