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Control of food intake by metabolism of fuels: a comparison across species

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Research with laboratory species suggests that meals can be terminated by peripheral signals carried to brain feeding centres via hepatic vagal afferents, and that these signals are affected by oxidation of fuels. Pre-gastric fermentation in ruminants greatly alters fuels, allowing mechanisms conserved across species to be studied with different types and temporal absorption of fuels. These fuels include SCFA, glucose, lactate, amino acids and long-chain fatty acid (FA) isomers, all of which are absorbed and metabolised by different tissues at different rates. Propionate is produced by rumen microbes, absorbed within the timeframe of meals, and quickly cleared by the liver. Its hypophagic effects are variable, likely due to its fate; propionate is utilised for gluconeogenesis or oxidised and also stimulates oxidation of acetyl-CoA by anapleurosis. In contrast, acetate has little effect on food intake, likely because its uptake by the ruminant liver is negligible. Glucose is hypophagic in non-ruminants but not ruminants and unlike non-ruminant species, uptake of glucose by ruminant liver is negligible, consistent with the differences in hypophagic effects between them. Inhibition of FA oxidation increases food intake, whereas promotion of FA oxidation suppresses food intake. Hypophagic effects of fuel oxidation also vary with changes in metabolic state. The objective of this paper is to compare the type and utilisation of fuels and their effects on feeding across species. We believe that the hepatic oxidation theory allows insight into mechanisms controlling feeding behaviour that can be used to formulate diets to optimise energy balance in multiple species.

Comparative metabolism: Food intake: Hepatic oxidation

Comparative metabolism can provide a valuable tool to improve understanding of physiological control mechanisms by investigating the similarities and differences across species. This paper addresses comparative aspects of the control of feeding behaviour by peripheral signals generated by oxidation of fuels. Research with rodents and other laboratory species indicates that inhibition of fuel oxidation stimulates feeding, whereas stimulation of oxidation inhibits feeding, and that the signal to brain feeding centres is via hepatic vagal afferents[1]. Feeding behaviour of rats has been related to energy charge in the liver and synergistic effects of metabolic inhibitors suggest an integrated mechanism with a common signal related to hepatic energy status from oxidation of various fuels[2]. However, the liver is only sparsely innervated with afferent fibres and the common hepatic vagus also innervates other tissues, including the duodenum[3]. The enterocyte was recently proposed as the primary sensor for fuel oxidation, casting doubt on a signal from the liver[4]. Evaluation of the ruminant model and comparison with non-ruminant models provides important insight into this issue because pre-gastric fermentation in ruminants greatly alters the type and temporal pattern of absorption of fuels. Rumen microbes ferment organic matter to SCFA that are utilised by different tissues at different rates. Dairy cows have been studied extensively for decades because of their economic

Abbreviations: 2,5-AM, 2,5-anhydromannitol; 2-DG, 2-deoxyglucose; FA, fatty acid; LCFA, long-chain fatty acid; MA, mercaptoacetate; MP, methyl palmoxirate.

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importance and are an ideal model for investigating control of feeding behaviour because of their extraordinary energy requirement and sensitivity to treatments within the physiological range[5]. Excessive energy intake and weight gain in late lactation dairy cows subsequently increases the risk of suppressed appetite in the peri-parturient period, resulting in excessive mobilisation of body energy reserves and a greater incidence of ketosis and hepatic lipidosis. Conversely, energy intake is often a primary constraint to milk yield in peak lactation, and increasing voluntary feed intake can increase productivity of dairy cattle. A better understanding of control of feeding behaviour by hepatic oxidation of fuels will allow diets to be formulated to control energy balance in both domestic animals and human subjects. The objective of this paper is to discuss differences in fuel type and temporal oxidation between ruminant and non-ruminant species and how they relate to the control of feeding behaviour.

Oxidation of fuels and feeding in non-ruminants

Fuel oxidation and feeding

The effect of oxidation of fuels on food intake in laboratory species has been addressed by previous reviews in more detail[1,2] and will be briefly described here. Various metabolic inhibitors have been reported to stimulate feeding by inhibiting glucose or fatty acid (FA) oxidation. Glycolysis is inhibited by 2-deoxy-D-glucose (2-DG), which increased food intake by rabbits when injected into the hepatic portal circulation[6] and inhibition of glucose oxidation by α-cyano-4-hydroxycinnamic acid, which decreases pyruvate transport across the mitochondrial membrane, stimulated feeding in rats[7]. Long-chain FA (LCFA) require carnitine palmitoyltransferase for transport across the mitochondrial membrane and subsequent β-oxidation, and inhibition of carnitine palmitoyltransferase-1 by methyl palmoxirate (MP)[8] and etomoxir[9] stimulated feeding in rats fed diets rich in long-chain TAG. However, MP failed to stimulate feeding when rats were fed a diet rich in medium-chain TAG[10], likely because medium-chain FA do not require carnitine palmitoyltransferase-1 for transport into the mitochondria[11]. Mercaptoacetate (MA) depresses β-oxidation by inhibiting long-chain acyl-CoA dehydrogenase activity and stimulated feeding in rats fed a high-fat diet[12,13]. However, feeding response to MA has been inconsistent[4], likely because of anorectic effects of MA through a β-adrenergic mechanism[14]. Stimulation of fuel oxidation by PPARα agonists decreased food intake in rats[15–17] and knockdown of 11β-hydroxysteroid dehydrogenase type 1 increased hepatic oxidation and decreased food intake in mice[18]. In summary, there is a large body of evidence consistent with involvement of fuel oxidation in the control of food intake.

Signal via hepatic vagal afferents

Hepatic vagal afferents are involved in the transmission of signals from fuel oxidation to brain feeding centres. The discharge rate of hepatic vagal afferents was increased by portal infusion of the metabolic inhibitors 2,5-anhydromannitol (2,5-AM)[19] and MA[20] and hepatic vagotomy blocked the stimulation of feeding by 2-DG in rabbits[6], as well as 2,5-AM[21] and MA[12] in rats. Hepatic vagotomy also blocked the stimulation of satiety by a variety of fuels in rats[22,23], glucose in chickens[24], and propionate in sheep[25]. This research indicates that fuel oxidation in peripheral tissues is involved in the control of feeding and that the transmission of signals to brain feeding centres is via hepatic vagal afferents.

Feeding related to hepatic energy status

A series of experiments conducted by Friedman and colleagues showed that feeding behaviour was related to hepatic energy status. An inverse temporal relationship was reported between hepatic ATP concentration and feeding in rats[26,27] and preventing ATP production by 2,5-AM stimulated feeding in rats[28,29]. This fructose analogue is rapidly phosphorylated in the liver but not metabolised beyond the 1,6-bisphosphate stage, thereby sequestering inorganic phosphate and preventing ATP production[29]. Phosphate loading restored ATP concentrations and eliminated the stimulatory effects of 2,5-AM on feeding[30]. The effect was likely in the liver because the latency for the eating response was less for portal compared with jugular infusion, hepatic vagotomy blocked the response, and radiolabelled 2,5-AM concentrations increased in liver but not the brain[21]. Inhibition of FA oxidation by MP[31] and etomoxir[32] also reduced hepatic ATP concentration and stimulated feeding. Hepatic energy status might be a common link by which oxidation of various fuels affect feeding.

Integrated mechanism

Synergistic effects of metabolic inhibitors on feeding by rats have been demonstrated. Inhibition of glycolysis by 2-DG and FA oxidation by MP[8] and inhibition of glycolysis by 2-DG and lipolysis by nicotinic acid[33] synergistically increased feeding in rats. In addition, feeding response to specific fuels is dependent on diet. Lactate and pyruvate caused hypophagia when rats were fed chow but not a high-fat diet[34], which the authors attributed to decreased activity of pyruvate dehydrogenase (required for oxidation of pyruvate via conversion to acetyl-CoA) by the high-fat diet[35]. Diet also affected feeding response to metabolic inhibitors. When rats were fed a low-fat, high-carbohydrate diet, 2,5-AM stimulated feeding but MP did not; conversely, when they were fed a high-fat, low-carbohydrate diet, MP stimulated feeding but 2,5-AM did not[36]. These experiments, along with the relationship between hepatic energy status and feeding, suggest that a common integrated mechanism involving the hepatic oxidation of a variety of fuels is involved in the control of food intake as suggested by Friedman and Tordoff[8].

Control of food intake by hepatic oxidation: the case for ruminants

Pre-gastric fermentation: different fuels

Diets consumed by dairy cows and most other ruminants contain a higher concentration of fibre (>25% insoluble

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fibre) and lower concentration of FA (<5%) than diets consumed by human subjects and laboratory species. Pre-gastric fermentation greatly alters both type and temporal supply of fuels. SCFA are produced by fermentation of organic matter by rumen microbes and include acetic, propionic and butyric acids. Acetic acid (mostly acetate at rumen pH) is produced primarily from fermentation of fibre and is the most abundant SCFA. Propionate is produced primarily from fermentation of starch and its rate of production and absorption is much greater than acetate because starch ferments faster than fibre and propionate is absorbed more quickly than acetate. Starch escaping fermentation in the rumen can be digested and absorbed in the small intestine providing glucose and lactic acid (from intestinal metabolism of glucose) as absorbed fuels. The starch concentration of ruminant diets ranges widely from a trace for ruminants grazing mature pastures to more than 50% for feedlot cattle, and site of digestion is easily manipulated by type, concentration and processing of cereal grains in the diet. Unsaturated FA are altered by biohydrogenation in the rumen increasing degree of saturation of FA absorbed compared with FA consumed and producing various FA isomers with physiological effects on energy partitioning[37]. The different fuels produced from pre-gastric fermentation and the ability to manipulate site of digestion combined with differences between species in hepatic metabolism of fuels (below) makes ruminants an important model to investigate mechanisms controlling feeding.

Hepatic metabolism of fuels

Fuels metabolised in bovine liver include NEFA, glycerol, amino acids, lactate and certain SCFA (primarily propionate). Plasma NEFA are important fuels oxidised in the liver of both ruminant and non-ruminant species. They are extracted from the blood in proportion to their concentration[38]. The bovine liver has limited capacity to export TAG as VLDL[39] so most NEFA taken up by the liver are oxidised either immediately or stored as TAG to be oxidised later. Glycerol (from lipolysis of TAG), lactate (from intestinal metabolism of glucose and the Cori cycle) and amino acids are common fuels metabolised by both ruminant and non-ruminant species. The primary difference for hepatic metabolism between ruminants and non-ruminants is metabolism of glucose and propionate. Unlike most laboratory species, hepatic uptake of glucose from the blood is negligible in mature ruminants[40] because glucokinase activity, necessary for activation and subsequent metabolism, is very low[41]. It is notable that plasma glucose concentration is similar for pre-ruminant calves and many non-ruminants species and decreased utilisation of plasma glucose by the liver coincides with rumen development, when absorbed glucose supply is diminished by pre-gastric fermentation. Propionate is rapidly produced by rumen microbes (primarily by fermentation of starch), readily cleared by ruminant liver from the portal vein, and readily metabolised[42], in part because activity of propionyl-CoA synthetase is very high[43]. Propionate as an oxidative fuel in the liver is much less important for non-ruminants; its supply to the liver is low because most starch is digested and absorbed before becoming available for fermentation in the large intestine. Other SCFA are less important than propionate as oxidative fuels in ruminant liver. Uptake and utilisation of plasma acetate by ruminant liver is negligible[42], because acetyl-CoA synthetase activity is very low[43]. Butyrate is cleared and metabolised by ruminant liver, but its supply is much lower than propionate because of a lower rate of production[44] and extensive metabolism by ruminal epithelia[45]. Similar to propionate, butyrate can be oxidised via conversion to acetyl-CoA, but unlike propionate, butyrate cannot stimulate oxidation in the TCA cycle by anaplerosis.

Hypophagic effects of fuels in ruminants

Hypophagic effects of fuels have been investigated by infusion studies with ruminants and these results are consistent with effects of diet on feeding behaviour. The most hypophagic fuels include propionate, medium-chain FA and unsaturated LCFA and the least hypophagic include glucose and acetate. Propionate decreased feed intake compared with acetate when isosmotic solutions were infused into the rumen in many studies reported in the literature[46]. While a reduction of feed intake by propionate could occur simply because propionate has greater energy concentration than acetate, propionate also linearly decreased total energy intake when the energy of infusates was considered[47]. Glucose has been shown to be hypophagic in a variety of non-ruminant species[48] but did not reduce feed intake when infused abomasally[49,50] or intravenously[51] in cows. Unsaturated C_{18} FA were more hypophagic than 18 : 0 when infused abomasally in lactating cows[52] and coconut oil (primarily medium-chain TAG) was more hypophagic than LCFA when fed to lactating dairy cows[53]. Variation in the hypophagic effects of fuels in ruminants allows insight into mechanisms controlling feeding across species.

Hypophagia from hepatic oxidation?

It is notable that the most hypophagic fuels for ruminants are those most readily oxidised in the liver. Glucose and acetate have little effect on feeding relative to other fuels and do not stimulate oxidation in the liver, while propionate is hypophagic and can be oxidised as well as stimulate oxidation. Unsaturated C_{18} FA are more hypophagic than stearate and more readily oxidised in ruminant liver[54]. Medium-chain TAG are more hypophagic than LCFA and are quickly and completely hydrolysed, delivered quickly to the liver via the portal vein compared to the lymphatic system for LCFA, and unlike LCFA, can bypass carnitine palmitoyltransferase for transport into the mitochondria[1]. Hypophagic effects of specific fuels in ruminants are consistent with their effects on hepatic oxidation.

The temporal supply to the liver varies greatly among fuels even when cows are fed *ad libitum*. Propionate is most variable and is most likely to be the primary fuel to stimulate satiety in ruminants fed diets containing starch[55]. Propionate is the primary end-product of rumen starch digestion and rumen production rates vary greatly

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among diets because of variation in starch concentration and fermentability[46]. It is rapidly absorbed within the timeframe of meals[56] and rapidly extracted by the liver[45]. Much of the glucose from intestinal starch digestion is oxidised to lactate or CO_2 by enterocytes, and that which is absorbed is not oxidised in the liver. Lactate is metabolised by ruminant liver, but it is unlikely to be the primary fuel oxidised causing satiety; relatively little reaches the liver during meals because of latency for transit of starch to the small intestine, and extraction of lactate by the liver is lower than propionate[40]. Similarly, the time required for passage from the rumen to the small intestine decreases the likelihood that amino acids or LCFA in feed consumed is oxidised in the liver within the timeframe of meals. However, these fuels can contribute to satiety and delay hunger by supplying acetyl-CoA for oxidation, and lactate, glycerol, and some amino acids can also stimulate oxidation in the TCA cycle by anapleurosis, thereby extending oxidation between meals. In addition, absorbed glucose, as well as glucose spared by metabolism of acetate, β-hydroxybutyrate, and LCFA by extra-hepatic tissues, can elevate plasma insulin concentration and affect hepatic oxidation indirectly by decreasing gluconeogenesis.

Effects of altering site of starch digestion on feed intake and feeding behaviour of dairy cows is consistent with the temporal supply of oxidative fuels to the liver[5]. Shifting the site of starch digestion post-ruminally by substituting less fermentable starch sources increased feed intake in several experiments reported in the literature[46]. Increased feed intake by cows offered a less fermentable starch source is consistent with a slower rate of propionate production and metabolism within the timeframe of meals, resulting in increased meal size. To investigate mechanisms underlying these feed intake responses, feeding behaviour was measured in an experiment comparing a more fermentable to a less fermentable starch source fed to lactating cows[57]. The more rapidly fermentable starch source reduced feed intake 8% by reducing meal size 17%, despite a numerical decrease in the time between meals. The more fermentable treatment increased organic matter fermented in the rumen and likely increased the contribution of propionate, and decreased glucose and lactate as fuels. The effect of treatment on meal size and feed intake is consistent with the temporal supply of oxidative fuels to the liver.

Alternative mechanisms

Differences between ruminants and non-ruminants for hypophagic effects of glucose infusion allow insight into the existence of sensory neurons for glucose[55]. It seems improbable that glucose utilisation in sensory neurons is involved in the hypophagic effects of glucose in non-ruminants because ruminal neural tissue metabolises glucose[58], yet glucose *per se* does not cause hypophagia in ruminants[46]. It is more likely that differences in hypophagic effects of glucose between ruminants and non-ruminants are because of differences in hepatic oxidation of glucose.

Specific receptors have been identified for SCFA[59]; G-protein-coupled receptors (41 and 43) are widely expressed in bovine tissue including small intestine and liver and are activated similarly by acetate and propionate[60]. Greater rumen production and flux through the portal drained viscera for acetate compared with propionate as well as very high extraction of propionate by the liver results in much lower peripheral concentrations of propionate than acetate. This decreases the likelihood that these receptors are involved in the mechanism for hypophagia from propionate relative to acetate.

Propionate is an insulin secretagogue[61] and insulin is a putative satiety hormone[62,63]. However, propionate has depressed dry matter intake without altering plasma insulin[50,64] and insulin's putative effects on feeding are through receptors in the central nervous system[65], yet hepatic vagotomy eliminated hypophagic effects of propionate[25]. Therefore, it is unlikely that the hypophagic effects of propionate are through direct effects of insulin. Rather, insulin is more likely involved indirectly through its effects on gluconeogenesis and removal of fuels from the blood that are potentially oxidised in the liver[55]. While insulin is known to down-regulate transcription of gluconeogenic genes[66], short-term increases in plasma insulin concentration caused by propionate during meals likely have little effect on gluconeogenesis[67]. Rapid response to insulin for nutrient uptake by peripheral tissues and decreased lipolysis results in decreased supply of NEFA and other fuels to the liver[5]. Depression in feed intake by a highly fermentable starch source was related to plasma insulin concentration and insulin response to a glucose challenge in an experiment with lactating dairy cows[67]. The more fermentable starch source depressed feed intake but response varied greatly among cows. Response in feed intake to the more fermentable starch source was negatively related to plasma insulin concentration (r^2 0·28, $P<0·01$) consistent with enhanced propionate oxidation due to down-regulation of gluconeogenesis. In addition, intake response was related positively (quadratically) to insulin response to a glucose challenge (r^2 0·40, $P<0·01$), consistent with decreased availability of fuels for hepatic oxidation. Therefore, insulin is likely involved indirectly in hypophagia from propionate by affecting oxidation of fuels in the liver.

Weaknesses in the model

Much of the support for a signal from the liver to brain feeding centres relies on experiments in which treatment effects on feed intake were eliminated by hepatic vagotomy. However, the common hepatic branch of the vagus innervates other tissues besides the liver including the proximal duodenum, distal stomach, pylorus, portal vein and pancreas; others have suggested that results of experiments with hepatic vagotomy must be interpreted with caution[3,68]. Langhans[4] suggested that the signal linking FA oxidation to feeding centres in the brain via the hepatic vagus originates in enterocytes rather than the liver. This was based on the following observations: (1) vagotomy of the common hepatic vagus nerve typical of rat experiments is not specific to the liver only, (2) liver parenchyma is

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sparsely innervated in rats, (3) infusion of MA into the pancreatico-duodenal artery (targeting the proximal duodenum) increased the discharge rate of hepatic vagal afferents and the response was blocked by infusion of lidocaine into the intestinal lumen and (4) inhibition of β-oxidation by MA failed to stimulate feeding when FA oxidation was elevated by fasting or adrenoreceptor agonists.

One key and valid criticism of the hepatic oxidation theory is the fact that afferent innervation of the liver parenchyma is relatively sparse, at least in the rat[69]. If we assume that these findings indicate that most hepatocytes do not directly interface with afferent nerve endings, does this mean that hepatic sensing of energy status could not be communicated to the brain? Although little work has been done to investigate potential modes of communication, several possibilities are feasible. Hepatocytes contain gap junctions that allow intercellular movement of ions, including Ca^{2+} [70], which can cause membrane depolarisation. Rawson *et al.*[71] reported that 2,5-AM caused a phospholipase C-mediated release of intracellular Ca^{2+} stores in hepatocytes in a timeframe that coincided with decreases in cellular ATP concentration. However, gap junctions between hepatocytes and afferent receptor nerves have not been found[3], making it unlikely that direct transport of ions between hepatocytes and neurons occurs. It may be more important that increased intracellular Ca^{2+} can stimulate exocytosis of signalling molecules. Hepatocytes are known to utilise ATP as an autocrine–paracrine signalling molecule[72,73] and some afferent nerves are activated by ATP[74]. Depolarisation of a hepatocyte could trigger release of intracellular Ca^{2+} stores[75], leading to exocytosis of ATP, which could then move through the extracellular space to nearby parasympathetic nerves, binding purinergic receptors and leading to the generation of action potentials. Although some findings have been consistent with such a proposed mechanism[76], others have not[77].

Another potential signalling mechanism is glutamate release by hepatocytes. This process is thought to be mediated by the recently characterised organic anion transporter 2[78], and effects of liver failure on systemic glutamate concentrations suggest that the release is quantitatively significant. Although it is presently unclear how (or if) hepatic glutamate release is regulated, there is strong evidence for the presence of glutamate sensors in the hepatic–portal region that signal via the hepatic vagus nerve; hepatic portal vein administration of monosodium glutamate increased vagal afferent tone, and this response was eliminated by vagotomy[79]. Therefore, it is at least possible that alterations in hepatocyte energy status influence glutamate efflux and, in turn, vagal signals to the central nervous system. The relatively large efflux of glutamate from the liver[78] could support adequate concentrations to induce sensory responses even in the absence of synapse-like interfaces between hepatocytes and sensory neurons.

An inability to clearly define mediators for liver communication with the central nervous system certainly should not be interpreted as evidence of a lack of such communication. In a series of studies unrelated to peripheral regulation of feeding behaviour, Imai *et al.*[80] clearly demonstrated that nerve signals originating from the liver reach the brain and subsequently impact other peripheral organs. In this work, adenoviral-mediated overexpression of extracellular-regulated kinase in liver parenchyma resulted in increased insulin secretion. Imai *et al.*[80] first discovered that this response was driven by pancreatic β-cell proliferation, despite the lack of transgene expression in that organ. They further showed that the pancreatic effects could be eliminated by ablation of afferent splanchnic nerve signalling, pancreatic vagotomy or bilateral midbrain transection, clearly demonstrating the involvement of a liver–brain–pancreas axis mediated by the nervous system. Although the splanchnic nerve does not exclusively innervate the liver, the transgene was not detected in any portion of the gastrointestinal tract in this experiment[80]. This demonstrated signalling pathway highlights the possibility that nutrient sensors expressed in hepatocytes, including AMP-dependent protein kinase[81,82] and mammalian target of rapamycin[83], can respond to cellular energy status and communicate this status to the central nervous system. These signals are not necessarily coupled to hepatocyte depolarisation.

Another key criticism of the hepatic oxidation theory is based on recent studies in which MA failed to promote food intake even when it apparently suppressed hepatic FA oxidation[84]. The logic underlying these experiments was that if oxidation of FA is blocked, then the resulting decreased energy status should promote greater food intake, according to the hepatic oxidation theory. Indeed, much of the evidence for this theory has been built on the use of metabolic inhibitors, so such findings must be taken seriously. However, in our opinion drug treatments (as opposed to palatable dietary interventions) provide little insight when they suppress intake or have no effect, simply because of the possibility that they had off-target effects and/or generated an aversive response[85]. In fact, several such problems have been reported with the use of MA[86,87]. This problem is not exclusive to results obtained for MA; we likewise put little emphasis on suppression of food intake by activators of PPARα or other drugs that result in increased hepatic oxidation. In fact, it is only an increase in food intake in response to a metabolic modifier that is unusual and, as such, these findings are far more valuable for discerning the biology of the sensory systems than negative results are.

One frustrating aspect of research on mechanisms regulating feeding behaviour is the vast degree of redundancy and overlap in the system, which can make even cleverly designed experiments difficult to interpret. Investigating the effects of a compound such as MA in the small intestine is an example. Langhans *et al.*[84] reported that intestinal infusion of MA altered vagal discharge rate, and that intestinal lidocaine administration eliminated this response. However, these responses and intake modulation by MA could be mediated by alterations in gut peptide signalling rather than through signals derived from oxidation in enterocytes. MA is known to act as a β-adrenergic agonist[86], and the β-adrenergic agonist isoproterenol was shown to increase cholecystokinin secretion[88]. As cholecystokinin release was decreased by anaesthetics[89], effects of MA on vagal discharge rate could have been

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exclusively through gut peptides. Although intrajejunal infusion of MA stimulated feeding, the site of administration might have minimised response from vagal afferents and cholecystokinin release in the duodenum and the feeding response might have been through effects of absorbed MA on hepatic oxidation. This might explain the quandary for a signal from enterocytes discussed by Langhans[84] that MA and cholecystokinin have similar effects on vagal discharge rate of afferents from the duodenum but opposite effects on feeding. Langhans *et al.*[84] did report that the increase in food intake stimulated by intestinal MA infusion was eliminated by sub-diaphragmatic deafferentation; however, no evidence was presented that disconnecting afferents in this region of the vagus leaves the hepatic branch proper of the vagus nerve intact. Therefore, responses to intestinal MA infusions may well be explained by a combination of effects on gut peptide release and signalling from the liver.

Signal from liver, enterocytes, or both?

Although the link between oxidation of fuels and the firing of hepatic vagal afferents is not known, evidence for a common mechanism by oxidation of various fuels is compelling. While a signal from oxidation of fuels in enterocytes via the hepatic vagus is plausible and demands further investigation, there are some inconsistencies for both ruminant and non-ruminants models and it is unlikely that enterocytes are the primary signal by which peripheral oxidation of fuels affect feeding. Evidence across species provides strong support for a signal from the liver linked to oxidation of a variety of fuels. We base this on the following observations:

(1) Although it is nearly impossible to selectively transect only those vagal fibres of the hepatic branch proper in rats[3], it appears to be feasible in ruminants. Vagotomy of the hepatic branch of the vagus in sheep decreased hypophagic effects of propionate[25].

(2) Inhibition of a satiety signal from oxidation of glucose by 2-DG in rabbits was more likely from the liver than enterocytes because eating was increased to a greater extent and with shorter latency when 2-DG was injected into the hepatic–portal system compared with both the jugular vein in normal rabbits and the hepatic–portal system of vagotomised rabbits[6].

(3) Glucose is oxidised by enterocytes and is partially oxidised in the portal drained viscera in ruminants when infused abomasally[90]. However, abomasal infusion of glucose did not decrease feed intake in cows[49,50]. It is likely that the difference in hypophagic effects of infused glucose between ruminants and non-ruminants is because of differences in hepatic oxidation as previously discussed.

(4) Decreasing rumen digestibility of starch often increases feed intake in dairy cows[46]. However, this increases starch flow to the duodenum and glucose supply to enterocytes, increasing oxidation[90]. The effect on feed intake is the opposite of that expected if oxidation of glucose in enterocytes causes a satiety signal.

(5) Intraruminal infusions of mixtures of acetate and propionate increase concentrations of these SCFA reaching the duodenum and unlike hepatocytes, enterocytes oxidise both acetate and propionate[91–93]. Therefore, oxidation of SCFA by enterocytes is an unlikely mechanism explaining differences in their hypophagic effects in ruminants.

(6) Abomasal infusion of propionate depressed feed intake in cows while glucose infused at twice the rate did not[50]. Although both absorbed fuels are oxidised by enterocytes only propionate is oxidised by ruminant liver[55] consistent with a signal from hepatic oxidation.

(7) Infusion of propionate into the jugular vein of sheep had no effect while infusion into the portal vein at the same rate decreased feed intake by more than 80%[94]. While propionate can be metabolised by enterocytes, it is more likely that the signal was from the liver; propionate supply to enterocytes is much greater when infused in the jugular vein than the portal vein because of the very high extraction (>90%) of propionate by the liver from portal supply[45].

(8) Hypophagia during the transition from pregnancy to lactation is associated with elevated plasma NEFA and β-hydroxybutyrate concentrations in dairy cows[55]. Although oxidation of plasma NEFA by enterocytes is possible[95], hypophagia is more likely from hepatic oxidation of NEFA. This is because hypophagic effects of propionate are enhanced for cows immediately postpartum compared with cows later in lactation that are not in a lipolytic state, likely by stimulating hepatic oxidation of acetyl-CoA by TCA anapleurosis[96]. This is consistent with our recent observation that hypophagic effects of propionate increased linearly with hepatic acetyl-CoA concentrations[97].

(9) While MA stimulates feeding in rats fed a high-fat diet, failure to stimulate feeding in cattle[85] is likely because MA must be activated to MA CoA; activity of acetyl-CoA synthetase in liver is low in ruminants[43] but high in non-ruminants[98]. The hypophagic effect of FA oxidation is more likely from a signal from the liver because acetate is metabolised by enterocytes[92] indicating availability of acetyl-CoA synthetase. The dramatic (>70%) depression of feed intake in dairy cattle over several hours following injection of MA[85] might have been due to a β-adrenergic mechanism as previously discussed[14].

(10) From a purely teleological viewpoint, hepatic sensing of energy status has several advantages over enterocyte sensing. While it is true that enterocytes could offer a more rapid signal of nutrient availability after meals for animals such as rats, this is not the case for animals that rely on either foregut or hindgut fermentation for the majority of their energy supply. Additionally, as a key anabolic organ, the

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liver could offer the unique advantage of sensing not just energy availability, but energy balance relative to nutrient demands. For example, since the bovine liver uses much of its oxidisable substrate to support gluconeogenesis, a high rate of hepatic nutrient metabolism does not necessarily lead to elevated ATP concentrations or suppression of feed intake[47]. Although insulin has central effects on feeding, its ability to integrate long-term energy status and feeding behaviour by hepatic oxidation through regulation of hepatic gluconeogenesis provides a simple control mechanism that is not available by the insulin-independent glucose transporter in enterocytes.

Concluding remarks

If peripheral mechanisms are involved in intake regulation, as many studies suggest, hepatic oxidation is the only proposed mechanism that can accommodate both differences in fuels absorbed and sites of absorption across species while remaining consistent with food intake responses to diets. Although the complexity of feeding behaviour control mechanisms makes it likely that multiple mechanisms are involved, no other proposed peripheral feedback system offers the 'broad explanatory power'[99] of the hepatic oxidation theory. Understanding the mechanisms by which feeding is affected by metabolism of fuels will allow dietary and pharmaceutical approaches to be developed to control energy intake across species.

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