Fatty acid–induced astrocyte ketone production and the control of food intake

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I- Abstract

Obesity and type 2 diabetes are major worldwide public health issues where there appears to be a relationship between total fat intake and obesity. In addition, the mechanisms of long-term and excessive high-fat diet (HFD) intake in the development of obesity still need to be elucidated. The ventromedial hypothalamus (VMH) is a major site involved in the regulation of glucose and energy homeostasis where “metabolic sensing neurons” integrate metabolic signals from the periphery. Among these signals, fatty acids (FA) modulate the activity of VMH neurons utilizing the FA translocator/CD36 which plays a critical role in the regulation of energy and glucose homeostasis. During low fat diet (LFD) intake, FA are oxidized by VMH astrocytes to fuel their on-going metabolic needs. However, HFD intake causes VMH astrocytes to utilize FA to generate ketones bodies. We postulate that these astrocyte-derived ketone bodies are exported to neurons where they produce excess ATP and ROS which override CD36-mediated FA sensing and act as a signal to decrease short-term food intake. On HFD, VMH astrocyte-produced ketones reduce elevated caloric intake to LFD levels after 3 d in rats genetically-predisposed to resist (DR) diet-induced obesity (DIO), but not leptin resistant DIO rats. This suggests that, while VMH ketone production on HFD can contribute to protection from obesity, the inherent leptin resistance overrides this inhibitory action of ketone bodies on food intake. Thus, astrocytes and neurons form a tight metabolic unit that is able to monitor circulating nutrients in order to alter food intake and energy homeostasis.
2- Introduction

Obesity and type 2 diabetes mellitus have increased drastically in prevalence around the world. Of particular concern is the associated “metabolic syndrome” (hypertension, dyslipidemia, diabetes) which increases the morbidity and mortality of obese individuals. Obesity and diabetes have a major impact upon, and are affected by the overall regulation of energy and glucose homeostasis. The origins of the obesity epidemic are complex but commonly cited factors include the consumption of large quantities of highly palatable, energy-dense food –especially those rich in fat (13, 27). Indeed, a correlation between total fat intake and the development of obesity has been demonstrated (27, 30, 77). However, the effects and underlying mechanisms by which chronic and excessive high fat diet (HFD) intake contributes to the development of obesity are still poorly understood.

Hunger and satiety are two important mechanisms involved in body weight regulation. The brain’s actions to regulate these conditions are influenced by nutrients, hormones, peptides and other metabolically related signaling molecules which cross the blood-brain barrier to alter the activity of specific “metabolic sensing” neurons scattered across diverse anatomical locations in the brain. While the mature human brain weighs only 2-3% of the total body weight (34), neurons themselves store very little fuel and thus depends upon the continuous exogenous supply of glucose as its primary metabolic substrate for the majority of the brain’s energy requirements (90, 100).
While much attention has been given to metabolic sensing neurons as regulators of energy and glucose homeostasis, astrocytes, which provide metabolic support for neurons (23, 109), have received much less attention. Astrocyte foot processes directly abut brain microvessels and thus are the first cells encountered by nutrients entering the brain (1, 105). This makes them a major site for nutrient uptake, storage and processing. Aside from their critical roles in maintaining neuronal transmission (5, 66, 85, 88, 89), astrocytes have several important metabolic functions. These include the storage of glycogen and the production of lactate as important substrates for neuronal metabolism, especially during enhanced neuronal activity (86, 87). Especially in the ventromedial hypothalamus (VMH = ventromedial nucleus (VMN) + arcuate (ARC)), astrocytes also produce ketone bodies from free fatty acids (FA) (50, 51). Unlike lactate production, which occurs as a continuous process (9, 86), astrocyte ketone production occurs predominantly when blood FA levels rise as a result of dietary intake (50, 51). While both neuronal glucose and fatty acid sensing have been reviewed extensively in the past, this review will focus specifically on the newly recognized role of local production of ketones by VMH astrocytes as regulators of food intake during intake of HFD. We also provide novel hypotheses regarding the ways in which astrocytes can regulate FA turnover in the VMH and mechanisms by which astrocyte-produced ketones override normal neuronal FA sensing to regulate feeding.

3- **Astrocytes: the major source of brain FA oxidation and ketone body production**
During energy deficits, blood glucose levels decline due to rapid depletion of glycogen stores in the liver and muscle (20, 75, 97). Once glycogen stores are depleted, lipolysis is stimulated in adipose tissue with the release of non-esterified FA. These FA are then converted by the liver into ketone bodies (\(\beta\)-hydroxybutyrate (\(\beta\)OHB) and acetoacetate) through mitochondrial \(\beta\)-oxidation and ketogenesis (95). Recent studies (7, 43) demonstrated that the intestines also produce significant quantities of ketones which can stimulate local visceral afferents regulating feeding. Both liver- and gut-derived ketones, as well as free FA, can be also transported into the brain to serve as an alternate energy source when glycogen stores are severely depleted and blood glucose levels decline during fasting (95). However, when energy intake is sufficient to provide surplus amounts of FA, hypothalamic astrocytes also produce ketone bodies from FA (10, 28, 33, 50). Other areas of the brain can also produce ketone bodies but in a lesser abundance (35).

Astrocytes are the major site of FA oxidation and the only source of ketone body production in the brain (10, 24, 25). When glucose is limiting, astrocytes utilize FA as their major source of ATP production (25). These FA directly enter the mitochondria via CPT1 where they undergo \(\beta\)-oxidation (25, 91). In the presence of excess FA and reduced dietary glucose, hypothalamic astrocytes produce ketone bodies (10, 24, 25, 50). The ketogenic pathways used by hepatocytes and astrocytes are almost identical. As shown in vitro, both cell types prefer FA to glucose as their primary metabolic fuel and produce ketone bodies (25, 33, 69). When excess FA are present, ketone bodies are produced using the rate limiting enzymes 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase.
and HMG-CoA lyase (36, 37). During intake of a low fat diet (LFD), after a 24 hour fast, we have shown (50) that VMH ketone body levels are ~20 μM, whereas the FFA levels are ~37 μM in the presence of ~350 μM and ~300 μM serum ketone and FFA levels, respectively. However, when fed a 60% fat diet (HFD) for several days on a 3 hour/day restricted intake schedule, VMH FA levels average only ~ 22.5 μM, which is actually lower than levels seen during low fat dietary intake (~35 μM) (50). On the other hand, VMH ketone levels spike to ~100 μM for 1 hour during the first 1 ½-2 ½ hour after onset of HFD feeding (50). We postulate (and our preliminary data suggest) that the lower VMH FA and the spike of ketone levels during HFD intake are due to a more avid uptake of FA into astrocytes which have been primed to produce ketone bodies by the chronic exposure to excess dietary FA. Another possibility is that differences in extracellular FA are caused by altered transport of FA across the blood-brain barrier due to differential regulation of FA transporters on cerebral microvessels during intake of LFD vs. HFD. Once FA enter astrocytes from the extracellular space, they produce ketone bodies which are transported out of the astrocytes via monocarboxylate transporter 1 (MCT1) and are then taken up into neurons by MCT2 (6, 23, 25, 99). In neurons, ketone bodies are then metabolized in the mitochondria to produce ATP and ROS though the tricarboxylic acid cycle utilizing 3-hydroxybutyrate dehydrogenase, oxoacid CoA-transferase and acetyl-coenzyme A acetyltransferase (14, 70).

4- Metabolic sensing neurons
The regulation of food intake and energy homeostasis is under the control of specialized “metabolic sensing” neurons which utilize various metabolic substrates, hormones, peptides and neurotransmitters to alter their activity (4, 11, 16, 39, 40, 44, 61, 67, 73, 82, 106). These metabolic sensing neurons are located in the VMH, as well as multiple other brain sites which, collectively, form a distributed network that monitors and regulates the metabolic status of the body (54, 63). The concept of metabolic sensing neurons arose from Jean Mayer’s first proposal in the 1950s that there were specialized glucosensing neurons in the VMH (68). He postulated that such neurons sensed changes in glucose oxidation as a mean of regulating their activity and the control of feeding. But it was not until 1964 that Oomura et al. (81) and Anand et al. (4) actually identified and physiologically characterized such glucosensing neurons. Whereas glucosensing neurons, like all neurons, utilize glucose as their primary fuel, they also have a variety of specialized mechanisms by which they translate alterations in ambient extracellular glucose levels into changes in membrane potential, neuronal activity and neurotransmitter and peptide release (21, 22, 41, 42, 53, 59-61, 94, 102).

Glucosensing neurons are also sensitive to many other nutrients such as FA, lactate and ketone bodies (21, 41, 42, 48, 52, 102), as well as peripheral hormones such as leptin (38, 39, 103), insulin (96, 104, 107, 108) and ghrelin (17); thus, the term “metabolic sensing” neurons. Glucose-excited neurons increase, whereas glucose-inhibited neurons decrease their firing rate as ambient glucose levels rise (93). During the diurnal cycle, brain glucose levels vary from ~10-20% of blood levels. After an overnight fast, VMH levels are ~0.5-0.7 mM and after a full meal, they rise to 2.0-2.5 mM (22, 71, 98, 101).
Glucose-excited neurons are activated at 2.5 mM and inhibited at 0.5 mM VMH glucose levels, while glucose-inhibited neurons are excited and inhibited at 0.5 mM and 2.5 mM glucose, respectively (41, 42, 93).

Metabolic sensing neurons also possess specialized pathways by which they utilize FA as signaling molecules to regulate their activity (52). Nanomolar concentrations of oleic acid (OA) either excite or inhibit 70% of dissociated VMN neurons (52). A subset of these FA sensing neurons responds to both glucose and FA, often with opposing effects; i.e., when glucose excites, FA often inhibit these neurons (52). Since fasting is associated with low glucose and high blood FA levels, while feeding is associated with raised glucose and FA levels, these specialized FA- and glucosensing neurons should be able to differentiate between the fasted and fed states. While 20 to 40% of VMH neuronal FA sensing is due to the metabolism of FA through β-oxidation and the formation of ROS and ATP (52), we have shown that 60-80% of VMH neuronal FA sensing neurons utilize the FA translocator/receptor FAT/CD36 (CD36) to regulate their FA sensing (48, 52, 76). CD36 is a member of class B scavenger receptor proteins that displays preferential binding to long-chain fatty acids (LCFA) (8, 26). Depleting CD36 in VMH neurons in vivo increases food intake, body weight and fat mass and leads to insulin resistance in lean and obese rats. It also shifts the deposition of fat from visceral to subcutaneous depots. This demonstrates that VMH CD36-mediated FA sensing plays an important role in the regulation of energy and glucose homeostasis and fat deposition in rats (47, 48).

4. Regulation of food intake by astrocyte-derived ketone bodies
Manipulating brain FA oxidation can alter food intake (2, 18, 19, 74, 76, 78, 91). Indeed, hypothalamic levels of LCFA-CoAs can be increased by enhancing esterification of circulating or central lipids (45, 79) and/or by the local inhibition of lipid oxidation (78). These interventions also result in marked inhibition of eating and liver glucose fluxes (3, 45, 78, 79, 91). These observations suggest that inhibiting FA synthase and stimulating the entry of FA into the mitochondria through CPT1 can reduce food intake and body weight in rodents. However, the majority of such studies have failed to address the issue of which cells were affected by these manipulations of FA oxidation.

Given their prominent role in both FA oxidation and ketone production in the brain (25), our studies (50, 51) suggest that it is likely that such manipulations affect mainly astrocytes rather than neurons. These studies focused on the VMH because of its high concentration of metabolic sensing neurons (80, 83, 101), and its well-established role in the control of ingestive behaviour (15, 64, 67, 72). To understand the role of VMH astrocyte-derived ketone bodies in the regulation of food intake, we developed novel methods to simultaneously monitor food intake, VMH and blood FA and ketone body levels. In an initial set of studies, lean rats were trained to eat all of their daily calories on a 13.5% LFD or 60% HFD for 3 hours/day. On testing day, they were assessed for continuous food intake, serum and VMH ketone or FFA levels over a 6 hour period after dark onset (50). While rats fed LFD and HFD ate the same amount over the first 3 hours, those on HFD ate only 50% as much and consumed fewer meals during the second 3 hour period after dark onset. Unexpectedly, extracellular VMH FA levels were lower in HFD
than LFD rats during the entire 6 hour period (50). Given these somewhat paradoxical
findings, we postulated that astrocytes from rats fed HFD had increased their FA uptake
at a rate which was greater than the influx of FA from blood, i.e. there was a greater flux
into astrocytes leading to lowered extracellular FA levels (Fig. 1).

While reduced transport of FA transport across the blood-brain barrier in rats fed HFD
could also contribute to these lower extracellular FA levels, the fact that, VMH ketone
levels spiked during the first 1 hour after dark onset in HFD-fed rats suggests that
increased FA uptake by astrocytes was the primary mechanism underlying lowered
extracellular FA levels (51). The mechanism by which this early spike in VMH ketones
might decrease food intake 3-6 hours later has yet to be elucidated but might be explained
by altered gene transcription or translation or the secretion of anorexic gut hormones.
Regardless of the cause, we confirmed that this initial spike in VMH astrocyte ketone
production was responsible for the delayed reduction in food intake since local inhibition
of VMH ketone production during the last 2 hours prior to dark onset fully restored the
intake of HFD to that of LFD during the second 3 hour epoch after feeding onset (50). To
understand how such local VMH ketone production might alter the activity of VMH
neurons responsible for mediating food intake, we assessed the effects of FA and ketones
on dissociated VMH neurons using calcium imaging as a surrogate for changes in
neuronal activity (52). We found that ketone bodies override normal FA sensing in many
of these neurons, primarily by exciting neurons that are either activated or inhibited by
FA. We postulate, but have not proven, that this predominantly excitatory effect of excess
ketone bodies is due to the overproduction of ATP and ROS in neuronal mitochondria that overrides the CD36-mediated FA sensing mechanism (Fig. 1).

However, our original method of assessing the effects of LFD vs. HFD on VMH FA levels, ketone production and food intake were relatively artificial given the utilization of a highly restricted feeding regimen. Thus, we turned to a model of normal spontaneous diurnal feeding in rats selectively bred to develop diet-induced obesity (DIO) or to be diet-resistant (DR) when fed a moderate fat (31%), high energy (HE) diet. DIO rats are larger but not fatter than DR rats when fed a low-fat chow diet but rapidly become hyperphagic, obese, and insulin resistant when fed an HE diet (57, 62, 92). Importantly, the DIO phenotype is inherited as a polygenic trait (55, 58, 92). We previously showed (58) that, when DIO and DR rats are switched from chow to HE diet, both become hyperphagic for 3 days. However, after 3 days on HE diet, DR rats reduce their intake to chow-fed levels while DIO rats remain hyperphagic for an additional 6–8 weeks, despite their early, marked and persistent increase in leptin levels (32). Importantly, DIO rats have defective VMN neuronal FA sensing while on LFD (53) while 3 days of HE diet intake markedly alters their VMN neuronal responses to both FA (49) and ketone bodies (51), but does not affect neurons in DR rats.

In fact, on day 3 of HE diet, DIO and DR rats have comparable VMH ketone levels over the entire 6 hour period after dark onset feeding. However, only DR rats reduce their intake over this 6 hour period, as well as the entire 24 hours of day 3 (51). In keeping with the hypothesis that raised VMH ketone levels are responsible for the decreased day
3 intake of HE diet intake by DR rats, local inhibition of their VMH astrocyte ketone production for 2 hours prior to dark onset feeding completely reversed their reduced 6 hour food intake and substantially increased their overall intake over the entire 24 hours (51). Since DR rats take 3 days to reduce their intake of HE diet, we postulate that it takes this long for the elevated fat content of the HE diet to prime VMH astrocytes to make ketone bodies in both DIO and DR rats. In fact, our unpublished data do suggest that DR VMH ketone levels are not elevated on day 2 of HE diet intake but, when VMH ketone levels are elevated to day 3 levels on day 2, DR rats reduce their intake to chow-fed levels. We further postulate that, since both DIO and DR rats have similarly elevated VMH ketone levels on day 3 but only DR rats respond to these elevated levels by reducing their food intake at that time, DR FA sensing neurons are more sensitive to the overriding effects of ketones on normal FA sensing. Taken together, our data strongly support a role for elevated VMH ketone body levels in the reduction in food intake of both outbred rats fed 60% fat, 6% sucrose diet for 2 weeks and DR rats fed 31.5% fat, 25% sucrose HE diet for 3 days. Such data suggest that defective VMH neuronal FA and ketone sensing might contribute to the persistent hyperphagia of DIO rats on HE diet (Fig. 1). However, the additional resistance to the markedly increased levels of the catabolic hormone leptin that occurs on day 3 of HE diet intake (58) is likely to be a major cause of the persistent hyperphagia and development of obesity in DIO rats on such diets (84). In other words, FA and ketone sensing by VMH neurons are likely to be important mediators of food intake and energy homeostasis in outbred and DR rats, but they play a less important role in regulating these processes in DIO rats which are leptin resistant from an early age (12, 32, 58). Thus, while the short-term inhibitory effects of
HFD on feeding appear to rely on an interplay between neuronal FA and ketone sensing, the long-term hyperphagia of animals and humans ingesting HFD may be due factors such as the development of leptin resistance as obesity progressively develops.

5. Summary

The chronic overconsumption of a palatable, HFD contributes to the excess caloric intake that leads to the development of obesity in many individuals. However, some animals reduce their intake of HFD and are obesity-resistant depending upon dietary content, intake schedule and genetic background. Specialized hypothalamic metabolic sensing neurons monitor changes in ambient brain levels of substrates such as glucose, FA and ketone bodies by utilizing them as signaling molecules which alter their activity (11, 16, 29, 40, 41, 44, 46, 48, 50). The effect of ketone bodies on VMH neuronal FA sensing to alter short-term food intake differs as a function of both diet and genetic background. Outbred rats fed a LFD exhibit little increase in VMH ketone bodies during ingestion and have a consistent level of food intake which maintains a stable weight. When those same rats are fed a HFD on a restricted schedule, their VMH ketone bodies spike transiently during early intake and this leads to reduced intake after a 3-6 hour delay. Since rats fed 3 hours per day on HFD maintain the same caloric intake and body weight as those on LFD, this suggests that monitoring both VMH FA and ketone levels might be an important factor in preventing them from overeating and becoming obese.
Genetically predisposed, selectively bred DIO rats are inherently leptin resistant before they become obese (12, 31, 56) and have abnormal VMH neuronal FA sensing after exposure to HFD (51, 53). While DR rats respond to elevated VMH ketone levels that arise after 3 days on HE diet by reducing their energy intake back to their LFD levels, DIO rats fail to respond to these same ketone elevations, remain hyperphagic and become obese. This is presumably due to a combination of defective FA and ketone sensing and their inherent leptin resistance (58). Thus, while several issues relating to FA and ketone regulation of both short- and long-term control of food intake remain to be elucidated, the monitoring of VMH FA and the production of ketones by VMH astrocytes appear to be important regulators of food intake and energy homeostasis in leptin-responsive DR rats, but not in leptin-resistant DIO rats in the presence of HFD.

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References


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Figure 1: Hypothetical model for the interaction of CD36-mediated neuronal FA sensing and astrocyte-produced ketone bodies. A. During LFD intake, FA entering the VMH from the blood across the blood-brain barrier (BBB) are taken up and oxidized by astrocytes to produce ATP to fuel their on-going metabolic needs. LCFA bind to CD36 as a FA “receptor” on metabolic sensing neurons to excite or inhibit them by activating store-operated Ca\textsuperscript{2+} channels (SOCC). On a LFD, VMH metabolic sensing neurons are either excited or inhibited by FA, partially dependent upon ambient glucose levels. B. After continued HFD intake, extracellular FA levels are decreased (vs. LFD in A) because flux into VMH astrocytes is increased since they have been “primed” to rapidly take up and utilize extracellular FA to generate ketones bodies (KB). These are then exported via MCT1 (or MCT4 (65)), taken up by neurons via MCT2 and utilized to produce excess ATP and ROS which override CD36-mediated FA sensing by activating or inhibiting the K\textsubscript{ATP} channel. LCFA levels were derived by zero net flux determinations and probe efficiency calculations (50). In the setting of long-term HFD intake and the development of obesity and elevated leptin levels, ketone bodies and FA play a secondary role in the control of food intake due to the development of leptin resistance which promotes continued hyperphagia on HFD.