Central regulation of glucose metabolism
Rijnsburger, M.

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– part 2 –

The effect of diet interventions on hypothalamic nutrient sensing pathways in rodents

Merel Rijnsburger
Evita Belegri
Leslie Eggels
Unga A. Unmehopa
Anita Boelen
Mireille J. Serlie
Susanne E. la Fleur

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Chapter 1

ABSTRACT

The hypothalamus plays a fundamental role in regulating homeostatic processes including regulation of food intake. Food intake is driven in part by energy balance, which is sensed by specific brain structures through signaling molecules such as nutrients and hormones. Both circulating glucose and fatty acids decrease food intake via a central mechanism involving the hypothalamus and brain stem. Besides playing a role in signaling energy status, glucose and fatty acids serve as fuel for neurons. This review focuses on the effects of glucose and fatty acids on hypothalamic pathways involved in regulation of energy metabolism as well as on the role of the family of peroxisome proliferator activated receptors (PPARs) which are implicated in regulation of central energy homeostasis. We further discuss the effects of different hypercaloric diets on these pathways.
**INTRODUCTION**

Glucose serves as an important fuel for the brain and since glycogen storages are limited, it depends on a continuous supply from circulating glucose. To ensure stable glucose levels, specialized glucose sensing neurons tightly regulate glucose homeostasis [1, 2], by responding to increases and decreases of plasma glucose concentrations. Glucose sensing neurons are either inhibited (glucose-inhibited (GI) neurons) or excited (glucose-excited (GE) neurons) by glucose and these populations of neurons are particularly dense in the hypothalamus and brainstem (reviewed in [3]). Within the hypothalamus, individual nuclei — such as the paraventricular nucleus (PVN), the dorsomedial nucleus (DMH), the ventromedial nucleus (VMN), the arcuate nucleus (Arc) and the lateral hypothalamus (LH) — contribute to glucose homeostasis [2, 4-6].

Although glucose was long considered the only energy source for the brain and the brain favors glucose utilization as a primary source of ATP [7], fatty acids (FA’s) can be metabolized by brain cells and serve as an alternative energy substrate. FA’s cross the blood-brain barrier either by passive diffusion (bound to albumin) or via uptake of lipoprotein particles mediated by lipoprotein receptors on the luminal surface of the cerebrovascular endothelium [8-12]. Furthermore, accumulating evidence suggests that FA’s act as cellular signaling molecules in the brain to control feeding behavior and glucose metabolism [13-16]. Next to the glucose and FA sensing capability of specific populations of neurons, the astrocyte, a type of glia cell is recently being recognized as an important regulator of both glucose and FA sensing mechanisms [17-20].

Our Western style diet contains high amounts of fat and sugar and contributes to the increasing prevalence of obesity. It has been shown that nutrients in excess directly affect the brain and induce hyperphagia by overriding hypothalamic control of feeding behavior but exact mechanisms remain poorly understood. This review focuses on how glucose and FA’s regulate energy metabolism through hypothalamic pathways, and discusses the family of the peroxisome proliferator activated receptors (PPARs) which are implicated in central regulation of energy homeostasis and are nutrient responsive. We further address the effects of different hypercaloric diets on these pathways.
Hypothalamic control of energy metabolism: role of glucose and fatty acids

Many of the cellular mechanisms involved in glucose sensing and cellular handling of glucose and FA’s have been studied extensively in the pancreas and liver, adipose tissue and skeletal muscle respectively. More recently, it has been shown that similar pathways are present in the brain and are involved in regulation of whole body energy metabolism. We provide a summary of findings of central manipulation of these pathways and their effects on energy metabolism in figure 1 and table 1.

Both glucose and fatty acids decrease food intake through common intracellular pathways in the hypothalamus (figure 1, [21]), and in addition to their direct effects on the liver, they decrease glucose production via a central mechanism [15, 22]. Increases in blood glucose, as a result of a meal or after injection (intraperitoneally (i.p) or intracerebroventricularly (ICV, [23, 24]) reduces adenosine monophosphate-activated protein kinase (AMPK) phosphorylation within hypothalamic neurons [13, 23, 24], resulting in dephosphorylation and activation of acetyl-coenzyme A carboxylase (ACC). In turn, ACC increases malonyl-CoA which inhibits carnitine palmitoyltransferase A (CPT1a), a rate limiting enzyme transferring the acyl group from long chain fatty acid-acyl coenzyme A (LCFA-CoA) to carnitine, allowing LCFA to shuttle through a translocase into

**Figure 1.** Effects of glucose and fatty acids on hypothalamic pathways involved in food intake. Glucose inhibits AMPK which in turn dephosphorylates and activates ACC. ACC increases malonyl-CoA favouring lipid synthesis and reducing mitochondrial β-oxidation, leading to a reduction in food intake. Fatty acids are activated to LCFA-CoA by ACS and also inhibit food intake via inhibition of NPY/AGRP. PPARs stimulate β-oxidation and increase the orexigenic peptides NPY and AGRP, stimulating food intake. CPT1a increases food intake via stimulation of NPY/AGRP and inhibition of POMC/CART neurons.
the mitochondrion to undergo β-oxidation. The increase in malonyl-CoA results in lipid storage through activation of fatty acid synthase (FAS). Thus, glucose reduces β-oxidation of LCFA-CoA and enhances lipid storage. Long chain fatty acids that enter the cell are converted to LCFA-CoA, a process that is catalyzed by long chain acyl CoA synthetase (ACS) and, depending on the AMP/ADP ratio and AMPK phosphorylation, are either oxidized or stored. Thus, during a positive energy balance after a meal, both glucose and FA’s increase the LCFA-CoA pool in the hypothalamus through different routes: by activation of FAS, by inhibition of β-oxidation and/or by direct conversion of fatty acids into LCFA-CoA. The net result is a decrease in food intake and glucose production [21, 25, 26]. On the other hand, a negative energy balance, i.e. during fasting, which increases the amount of free fatty acids in the plasma, has been shown to increase hypothalamic AMPK phosphorylation resulting in phosphorylation and hence inactivation of ACC and enhanced β-oxidation. This is associated with stimulation of food intake [13]. More recently a novel brain specific CPT1 homologue (CPT1c) was identified. Although CPT1c seems not to be involved in fatty acid oxidation, it binds malonyl-CoA but does not catalyze fatty acid acyl transfer to carnitine like other homologues. CPT1c knock-out mice have lower body weight and food intake. Paradoxically they are more susceptible to diet-induced obesity (DIO) [27]. Thus, although mechanisms are unclear, these data in CPT1c k.o. mice do indicate that this brain CPT1 homologue is necessary for the regulation of energy metabolism.

**Peroxisome proliferator activated receptors**

Peroxisome proliferator-activated receptors (PPARs) are ligand activated transcription factors that regulate expression of genes involved in cell differentiation and various metabolic processes, especially lipid and glucose homeostasis. The PPAR family comprises three isoforms: PPARγ, PPARα and PPARβ/δ and, it has been shown that unsaturated fatty acids serve as their natural ligands [28].

PPARγ regulates genes involved in fatty acid metabolism such as ACC, FAS and CPT1 and is widely expressed throughout the body [29] including the brain where it is expressed within the VMN and Arc of the hypothalamus [30]. It has recently been established that PPARγ is an important CNS lipid sensor involved in the regulation of energy metabolism [31]. Selective overexpression of central PPARγ increases food intake and abdominal fat [31] and activity of the neuropeptides neuropeptide Y (NPY) and pro-opiomelanocortin (POMC) expressed in the Arc are controlled by PPARγ [32].

Less is known about central PPARα and PPARβ/δ and their involvement in energy metabolism. Activation of PPARα results in increased FA oxidation and it is therefore not surprising that, in the periphery, it is mainly expressed in tissues with a high capacity for FA oxidation, e.g. the liver, heart, and skeletal muscle. It also plays a role in glucose homeostasis and insulin resistance development [28]. In addition, a centrally administered PPARα agonists, WY14643, either infused acutely or chronically by ICV infusions reduced whole body glucose turnover [33] without effects on body weight and food intake [33, 34], pointing to a role for central PPARα in glucose regulation. It should be noted however that WY14643 also agonizes PPARγ
and therefore the effects on glucose turnover might not be solely attributable to PPARα [35].

Peripheral PPARβ/δ is involved in FA oxidation and reducing adiposity, which could contribute to prevention of obesity development. Indeed, in animals, PPARβ/δ activation in adipocytes leads to resistance to diet-induced obesity (DIO) via increased FA oxidation and utilization [36, 37]. One brain specific knock-down model for PPARβ/δ using Nest-Cre-loxP technology has been described. Knock-down mice gained more weight on a high-fat diet (HFD) compared to the wild types and showed decreased leptin sensitivity without changes in food intake. Furthermore, they showed increased expression of genes involved in lipid uptake, lipid synthesis and FA oxidation in the hypothalamus [38].

Taken together, the maintenance of glucose homeostasis and food intake are under control of central signaling of glucose and FA’s sharing common pathways and the PPARs are important regulators of these pathways [37, 39-43].

**Nutrients serve as signaling molecules to alter hypothalamic actions**

Glucose sensing neurons have been identified in many hypothalamic nuclei, including the Arc and VMN. Glucose, transported into the neuron via glucose transporters (GLUT) subtypes 2, 3 or 4, is metabolized to generate ATP. The increase in ATP/ADP ratio leads to depolarization of the cell membrane and this causes influx of Ca²⁺ and thereby release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA)[2, 5, 6, 44]. Although some components of glucose sensing in GI neurons are similar to those found in GE neurons, the signal transduction pathway by which GI neurons decrease their activity to changes in glucose levels is less well understood [2, 44]. Alternatively to the classical ATP dependent sensing in GE neurons, a non-metabolic glucose sensing mechanism is proposed with the sodium–glucose cotransporters (SGLTs). These transmembrane proteins operate as sodium-dependent transporters of glucose and certain other sugars, coupling the uptake of a sugar molecule to the influx of Na⁺ ions [45, 46]. The SGLT subtype 1 in the VMH has been shown to play an important role in the counter regulatory response to hypoglycemia [47-49]. FA’s are shown to alter neuronal activity and gene expression, via FA translocase/CD36, which in the VMN acts as receptor and at least 50% of VMN and Arc neurons are FA responsive [50, 51]. FA’s excite or inhibit activity of Arc neurons, depending on extracellular glucose levels as measured by patch clamping of Arc neurons [25, 52, 53].

Within the Arc two main cell populations are directly excited or inhibited by glucose. One cell population expresses the orexigenic peptides NPY and agouti-related peptide (AGRP) whereas another cell population expresses the anorexigenic POMC and cocaine-and amphetamine-regulated transcript (CART) [54]. It has been shown that i.p. or ICV infusion of glucose increases mRNA expression of POMC [23, 24] and CART [23] and decreases mRNA expression of NPY and AGRP [23, 24, 49]. ICV infusion of the LCFA oleic acid has also been shown to decrease NPY and AGRP mRNA expression [15, 55] which together might explain the reduction in food intake observed after glucose and fatty acids infusions. Although these effects of single FA’s within the brain on neuropeptide expression and metabolism point towards a role for dietary FA’s in centrally mediated effects on
energy metabolism, ICV injections of only one or several FA’s does not necessarily reflect the actual composition of a Western style diet. Since different FA’s do not have similar effects on peripheral outcomes when injected into the brain, such as glucose production [56], infusing a combination of FA’s would be preferable. Furthermore, infusing glucose and FA’s via ICV cannula is a sub-optimal method because it is not the route by which nutrients physiologically reach the brain and it is a challenge to use concentrations that resemble actual brain and even neuronal levels and thus in most studies concentrations provided to the brain are far higher than physiological levels. On the other hand, it can add to our knowledge in understanding central nutrient sensing, without the influence of changes in circulating glucose and FA levels.

The nutrient-induced changes observed in Arc neuropeptide expression could result from changes in gene expression and/or activity of enzymes involved in fuel sensing as described earlier, and changes in NPY/AGRP or POMC/CART themselves could also affect expression of these Arc genes.

When increasing lipid biosynthesis by ICV infusion of citrate, which increases the ATP/AMP ratio, phosphorylation of AMPK/ACC is reduced, POMC mRNA expression is increased, and NPY mRNA expression is decreased [57]. In addition, hypothalamic inhibition of CPT1a in the Arc using a plasmid to decrease expression, or specific inhibitors, results in decreased NPY/AGRP mRNA expression [22] and fasting, known to increase FFA, increases both NPY and CPT1a mRNA expression [58]. Moreover, the same authors showed that in vitro incubation of hypothalamic cells with NPY increased CPT1a mRNA expression. In addition, FAS inhibition with C75, which also activates CPT1a, increased NPY/AGRP and decreased POMC/CART mRNA in DIO mice [59]. Intraperitoneal administration of the PPARγ agonist rosiglitazone increased AGRP and NPY mRNA in a similar way as food deprivation, and administration of a PPARγ antagonist attenuated the increase in AGRP mRNA after food deprivation [32]. In line with the increased weight gain, NPY mRNA expression is increased in PPARβ/δ knock out mice [38].

To conclude, circulating glucose and FA’s serve as signaling molecules in the hypothalamus to control energy metabolism. Increased substrate availability in general increases POMC and decreases AGRP and NPY mRNA expression resulting in a reduction in food intake. Besides, intracellular pathways involved in metabolic handling of glucose and FA’s have independent effects on expression of these neuropeptides. The main concern with the studies described above is that it remains uncertain which cell types are exactly involved in the signaling of glucose and FA’s. In recent years, a significant role for the astrocyte is recognized in glucose and FA sensing and the hypothesized mechanism will be discussed below.

The role of astrocytes in regulation of glucose and FA metabolism

Recently a role for glia-to-neuron signaling in the hypothalamus has gained lot of attention. An ‘astrocyte-neuron lactate shuttle’ has been postulated [60] which implies that glucose that is taken up by astrocytes is partly oxidized and partly metabolized to lactate. Lactate is then released into the extracellular space via monocarboxylate
Table 1. Effects of manipulating genes involved in central lipid metabolism in rodents

<table>
<thead>
<tr>
<th>Gene</th>
<th>Rodent type/species</th>
<th>Gene intervention</th>
<th>Brain area</th>
<th>Diet</th>
<th>Fat%&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Sucrose%&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Effects on FI</th>
<th>BW/fat mass</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Wistar rats</td>
<td>Overexpression</td>
<td>ICV</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>SD rats/zucker rats</td>
<td>Antagonist</td>
<td>ICV</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>=</td>
<td>=</td>
<td>[57, 75, 86, 87]</td>
</tr>
<tr>
<td>FAS</td>
<td>BALB/c, C57BL/6, DIO or ob/ob mice</td>
<td>Antagonist (C75)</td>
<td>i.p. or ICV</td>
<td>Chow/HFD</td>
<td>varied</td>
<td>varied</td>
<td>+</td>
<td>+</td>
<td>[59, 80, 88-93]</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 and 129</td>
<td>Knock out</td>
<td>hypothalamus</td>
<td>Chow/HFD</td>
<td>13/42</td>
<td>3.8/34</td>
<td>-/-</td>
<td>-/-</td>
<td>[94]</td>
</tr>
<tr>
<td>CPT1a</td>
<td>SD rats</td>
<td>Antagonist</td>
<td>ICV</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>n.m</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>SD rats</td>
<td>Overexpression</td>
<td>VMN</td>
<td>Chow</td>
<td>13</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>[76, 77]</td>
</tr>
<tr>
<td>CPT1c</td>
<td>C57BL/6 mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow/HFD</td>
<td>10/60</td>
<td>7/7</td>
<td>-/-</td>
<td>+/-</td>
<td>[95]</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 mice</td>
<td>Antagonist</td>
<td>ICV</td>
<td>Chow</td>
<td>3.8</td>
<td>14*</td>
<td>-</td>
<td>-</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow/HFD</td>
<td>10/60</td>
<td>34/7</td>
<td>-/+</td>
<td>-/+</td>
<td>[27, 96]</td>
</tr>
<tr>
<td></td>
<td>BALB/c mice</td>
<td>Overexpression</td>
<td>v. HT</td>
<td>HFD</td>
<td>45</td>
<td>17</td>
<td>=</td>
<td>-</td>
<td>[97]</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>=</td>
<td>n.m</td>
<td>[78]</td>
</tr>
<tr>
<td>PPARγ</td>
<td>LE rats</td>
<td>Agonist</td>
<td>ICV</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>LE rats</td>
<td>Overexpression</td>
<td>m. HT</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>n.m</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>LE rats</td>
<td>Antagonist</td>
<td>ICV</td>
<td>Chow/HFD</td>
<td>?/40</td>
<td>?/8</td>
<td>-/-</td>
<td>+/-</td>
<td>[31]</td>
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<tr>
<td></td>
<td>Sib hamsters</td>
<td>Agonist</td>
<td>ICV</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>n.m</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Mice</td>
<td>Knock out</td>
<td>POMC neurons</td>
<td>HFD</td>
<td>45</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>[98]</td>
</tr>
<tr>
<td>C57BL/6 mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow/HFD</td>
<td>60</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>[99]</td>
<td></td>
</tr>
<tr>
<td>C57BL/6 mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>=</td>
<td>=</td>
<td>[30]</td>
<td></td>
</tr>
<tr>
<td>PPARα</td>
<td>C57BL/6 mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>n.m</td>
<td>+</td>
<td>[33]</td>
</tr>
<tr>
<td>PPARβδ</td>
<td>C57BL/6 mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>LFD/HFD</td>
<td>45</td>
<td>17</td>
<td>=</td>
<td>+</td>
<td>[38]</td>
</tr>
<tr>
<td>PGC1α</td>
<td>Mice</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>HFD</td>
<td>60</td>
<td>7</td>
<td>+</td>
<td>-</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 and 129</td>
<td>Knock out</td>
<td>Whole brain</td>
<td>Chow/HFD</td>
<td>42</td>
<td>34</td>
<td>+/-</td>
<td>+/-</td>
<td>[100]</td>
</tr>
<tr>
<td>BDNF</td>
<td>Wistar rats</td>
<td>Chronic infusion</td>
<td>ICV &amp; PVN</td>
<td>Chow</td>
<td>12.6</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>SD rats</td>
<td>Infusion</td>
<td>PVN</td>
<td>Chow/HFD</td>
<td>10/45</td>
<td>34/17</td>
<td>+/-</td>
<td>+/-</td>
<td>[37, 43]</td>
</tr>
<tr>
<td></td>
<td>SD rats</td>
<td>Infusion</td>
<td>VMN</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>[101]</td>
</tr>
<tr>
<td>C57BL/6 and 129</td>
<td>Knock out</td>
<td>VTA</td>
<td>Chow/HFD</td>
<td>5/45</td>
<td>5/17</td>
<td>+/-</td>
<td>+/-</td>
<td>[100]</td>
<td></td>
</tr>
<tr>
<td>C57BL/6 and 129</td>
<td>Deletion</td>
<td>VMN &amp; DMN</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td>C57BL/6 and 129</td>
<td>Knock out</td>
<td>Whole body</td>
<td>Chow</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>[102]</td>
<td></td>
</tr>
</tbody>
</table>
transporter 1 and 4 (MCT), which in turn is transported into neurons via MCT2. Lactate in this manner provides a supplemental energy source for neurons and in this way can affect neuronal activity [61].

As mentioned in the previous section, FA’s are sensed by neurons and consequently alter activity using FA translocase/CD36 as a receptor. However, FA oxidation occurs predominantly in astrocytes rather than in neurons and thus astrocytes appear to play an important role in FA sensing [62]. In addition, astrocytes are the only source of ketone body production in the brain, which increases when FA levels rise as is the case with a HFD [63]. Le Foll and colleagues recently established a role for ketone bodies in adjusting caloric intake. They showed that inhibiting ketone production within the VMN and Arc increased intake of a HFD and that ketones can even override the normal glucose and FA sensing in VMN neurons [18, 64].

In summary, it appears that the metabolic products of astrocytes are important additional players in the regulation of the activity of glucose and FA sensing neurons and are part of an interplay between non-neuronal and neuronal populations.

**Effect of different hypercaloric diets on hypothalamic metabolic gene expression**

Central over- or under expression of genes involved in fatty acid and glucose homeostasis can alter food intake in animals that are on a HFD or are obese. The question arises whether hypercaloric diets change the genes involved in central FA and glucose sensing. It has been hypothesized that an overload of central fatty acids or disturbed central fat metabolism contribute to obesity [26] and it has been shown that direct central infusion of fatty acids via intracarotid catheters increases glucose production [65] and decreases food intake [66].

In table 1 we summarize studies describing the effect of specific nutrients on some metabolic gene expressions in the hypothalamus. Surprisingly, little is known on how brain circuits involved in energy sensing respond specifically to diets with increased content of fat plus sugar, which represent a typical Western style diet. Feeding a high carbohydrate/low fat diet as well as i.p. glucose injections decrease hypothalamic pAMPK and pACC [23, 24]. To our knowledge, there are no data describing effects of hypercaloric diets on CPT1a, CPT1c or FAS gene expression.

**Legend Table 1.** ICV, intracerebroventricular; i.p., intraperitoneal; VMH, ventromedial part of hypothalamus; Arc, arcuate nucleus; v. HT, ventral hypothalamus; m. HT, medial hypothalamus; VTA, ventral tegmental area; DMH, dorsomedial part of hypothalamus; PVN, paraventricular nucleus; HFD, high fat diet; LFD, low fat diet. Only data from obesity prone rats are shown. Data are grouped by rodent type/strain (SD= Sprague–Dawley, LE=Long Evans). When only stated ‘mice’, background was not mentioned. N.r.= not reported. FI = food intake. * Disaccharide content. 1 percentage fat and sucrose in diet.
Diet resistant male rats on a HFD (defined as rats not increasing body weight upon a HFD) show decreased hypothalamic mRNA expression of PPARγ after 5 weeks while DIO rats show no alterations suggesting that decreasing hypothalamic PPARγ contributes to a healthy energy balance [67]. The effects of diets on hypothalamic PPARα or PPARβ/δ mRNA expression have not been studied yet. The peroxisome proliferator-activated receptor gamma, coactivator 1 α (PGC1α) is a transcriptional coactivator involved in multiple metabolic pathways including regulation of gluconeogenesis, and targets the whole PPAR family [68] and one study showed that a 16 week HFD diet decreased PGC1α mRNA expression and protein levels in mice hypothalami [69]. Furthermore, treatment of primary astrocytes or hypothalamic neurons with palmitic or stearic acid reduced PGC1α expression [69], both in line with the effects found after chronic HFD exposure and of decreased PPARγ after HFD [41, 70-72]. To our knowledge no data are available that describe the effects of hypercaloric high fat high sugar (HFHS) diets on hypothalamic metabolic gene expression. We therefore decided to take advantage of brain material collected during our ongoing studies on the free-choice hypercaloric (fcHFHS) diet and measured hypothalamic metabolic gene expression after 1 week of diet exposure of which the results are described below.

**EFFECTS OF A FREE-CHOICE HIGH-FAT HIGH-SUGAR DIET ON HYPOTHALAMIC GENE EXPRESSION**

Rats on a free choice high fat high sugar diet (fcHFHS), consisting of a 30% liquid sugar (sucrose) component and a fat component (lard) in addition to their regular chow, have been shown to develop hyperphagia within a week. This was associated with increased NPY mRNA and decreased POMC expression in the Arc [73]. When provided either with the fat or the sugar component separately, rats initially overeat but normalize their caloric intake and show no changes in NPY or POMC expression [73, 74]. We investigated which fuel sensing genes in the hypothalamus are affected by the fcHFHS diet. Since animals are hyperphagic and show increased NPY and decreased POMC expression, we expected the genes stimulating β-oxidation and thus food intake, to be increased. Indeed, hypothalamic expression of many genes involved in fatty acid oxidation were increased in rats after one week on the fcHFHS diet (see figure 2 for gene expression profiles, and table 3 for animal characteristics). These changes might be related to persistent hyperphagia observed in animals on the fcHFHS diet, as increased ACC and PPARβ/δ are associated with increased food intake and/or body weight (table 1, [38, 75]). Interestingly, the increase in PGC1α after 1 week of fcHFHS diet points towards PPAR activation but is contrary to others that found increased food intake and body weight after centrally knocking out PGC1α [38] and lower expression after a HFD of 16 weeks [69]. Higher expression of PGC1α could explain the sustained hyperphagia through an increase in NPY since PGC1α knock-out obese mice show a blunted NPY response to fasting [69]. We observed a trend for an increase in CPT1c expression, which could be driven by the observed changes in PPARβ/δ known
Diet interventions and hypothalamic nutrient sensing

In turn, CPT1c has been shown to increase NPY mRNA which is in line with our earlier observed increases in NPY mRNA when animals are exposed to a fcHFHS diet for a week [76-78]. mRNA expression of PPARγ and PPARα was not altered after one week of fcHFHS. Hypothalamic PPARγ has been shown to decrease but only in diet-resistant (DR) rats and after 5 weeks of HFD [67], suggesting that the 1 week diet might be too short to induce changes in PPARγ. The only report showing central effects of PPARα [33] shows increased fat mass in the knock-out animal but effects on food intake are unknown, and in our hands there are no effects of the short term fcHFHS diet on PPARα mRNA expression [40].

It is unclear why a fcHFHS diet induces ongoing hyperphagia along with increased hypothalamic NPY and decreased POMC expression [73]. Together with the observed changes in metabolic gene expression presented here, we hypothesize that combining fat and sugar leads to increased hypothalamic β-oxidation. It has been shown that diet-induced obesity downregulates the GLUT2 transporter and increases pAMPK in the hypothalamus [79]. The latter would lead to a decrease in malonyl-CoA and thus less inhibition of CPT1a resulting in increased β-oxidation, favoring food intake.

LIMITATIONS AND FUTURE DIRECTIONS

Although effects of high fat diets on hypothalamic fatty acid and glucose homeostatic genes have been studied extensively, comparing results and drawing firm conclusions is difficult due to several factors related to the diets used. First, most diets considered to be a high fat diet are also rich in sucrose and depending on the diet chosen, sucrose
content ranges between 3 and 34%. For example, some studies report on effects of a HFD versus a LFD with the sucrose content being 7% in the HFD while 34% in the LFD [24, 80]. In addition, most diets used are non-choice diets, meaning that the animals automatically ingest fat as they fulfill their other dietary needs, or consume fat at a time of day when it would not otherwise be the preferred macronutrient. It has been shown that having the choice between different diet components is an important contributor in the development of hyperphagia and obesity [81].

Furthermore, most interventions using hypercaloric diets induce peripheral metabolic changes including hyperinsulinemia and hyperleptinemia, which can have independent effects on cerebral nutrient sensing pathways. Some studies account for differences in adiposity in HFD animals by pair feeding the animals to equalize body weight but while this corrects for body weight, it does not necessarily correct for adiposity. Direct infusion of nutrients into the brain using the ICV approach may be a solution for this problem, but this represents a non-physiological route of nutrient sensing. The doses of glucose and FA’s used for ICV infusions vary and it is unknown how they represent the actual circulating levels or the levels within the neuron or astrocytes. Several experiments have been conducted using an intracarotid cannula with the tip pointing towards the brain and via this route substances can be infused directly towards the brain and their independent effects on nutrient sensing pathways can be studied [82]. This method aims to represent a more physiological route to study direct effects of nutrients on the brain although again, doses vary and it is unknown what percentage of the infused dose passes the blood-brain-barrier (BBB). Short term (10 min) infusion of triglycerides using this approach decreases food intake [71] and 24 hour triglyceride infusion decreases hepatic insulin sensitivity [65]. Future studies are needed to dissect independent and integrated effects of hypercaloric diets on nutrient sensing pathways throughout the brain to get insight in

### Table 2. Description of the Primers Used for qPCR

<table>
<thead>
<tr>
<th>gene</th>
<th>sequence</th>
<th>Annealing temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS</td>
<td>Forward 5'-GAT GAT CAA GGC CAG CTT GT – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GCC TTC CCG TAG ACT CAC TG – 3'</td>
<td></td>
</tr>
<tr>
<td>ACC</td>
<td>Forward 5'-GAT GAT CAA GGC CAG CTT GT – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-CAG GCT ACC ATG CCA ATC CGT CTT – 3'</td>
<td></td>
</tr>
<tr>
<td>PPARγ</td>
<td>Forward 5'-CAG GAA AGA CAA CAG ACA AAT CA – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GGG GGT GAT ATG TTT GAA CTT G – 3'</td>
<td></td>
</tr>
<tr>
<td>PPARβ/δ</td>
<td>Forward 5'-CTCCTGCTCAGCATGAGATG – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-TCTCCCTCCTGAGTTCGTCATG – 3'</td>
<td></td>
</tr>
<tr>
<td>PPARα</td>
<td>Forward 5'-TCA CAC AAT GCA ATG TT – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GGC GCT GAG CTT GTG CAT GT – 3'</td>
<td></td>
</tr>
<tr>
<td>PGC1α</td>
<td>Forward 5'-TGCGATGGATAGACCCGAG – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GGTACATTGGTGACCTCTG – 3'</td>
<td></td>
</tr>
<tr>
<td>CPT1α</td>
<td>Forward 5'-ATA GGA CAT TCC AGG AG – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-AAA GAC TGG CGC TGC TCA – 3'</td>
<td></td>
</tr>
<tr>
<td>CPT1c</td>
<td>Forward 5'-CTGCGGT6TCTGAAATGACT – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-CCCAAACAGGGGGAACAG – 3'</td>
<td></td>
</tr>
<tr>
<td>cyclophilin</td>
<td>Forward 5'-ATG TGG TCT TGT GGA AGG TG – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-GAA GGA ATG GTT TGA TGG GT – 3'</td>
<td></td>
</tr>
<tr>
<td>HPRT</td>
<td>Forward 5'-GCA GTA CAG CCC CAA AAT GG – 3'</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Reverse 5'-AAC AAA GTC TGG CCT GTA TCC AA – 3'</td>
<td></td>
</tr>
</tbody>
</table>

Genes, primer sequences, and annealing temperatures are shown.
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1

adaptive and mal-adaptive responses in times of nutrient excess. Including the role of astrocytes in studying nutrient sensing would add to this knowledge, especially studying the combination of glucose and FA’s, since so far the literature on glucose and FA sensing in astrocytes describes separate pathways.

It has been shown recently that DIO impairs VMN glucose sensing, despite increased hypothalamic extracellular glucose levels [83]. Thus, next to central insulin and leptin resistance, DIO also causes central glucose resistance. It would be interesting to further study which cell type(s) become resistant to glucose, and whether central resistance to FA’s develop on Western style diets. Furthermore investigating consequences of disturbed nutrient sensing for metabolic health is necessary to elucidate key pathways involved in central (dys)regulation of peripheral metabolism. Finally it would be informative to directly measure glucose and FA concentrations within brain tissue after an acute (HFHS) meal in rodents to get more insight in how much of the circulating glucose and fatty acids reaches the brain and in which parts of the brain. One determinant of this is active transport and passive diffusion through the blood brain barrier, a process highly dependent on feeding conditions since this influences the permeability of the BBB [84].

Conclusions

In summary, circulating glucose and fatty acids act on a common pathway in the hypothalamus to regulate energy balance and further research is necessary to unravel the exact underlying mechanisms within specific hypothalamic nuclei and cell types. Recently it has been shown that the PPAR family is involved in the central regulation of energy balance. We provided an overview of interventions affecting metabolic genes within the hypothalamus and their effects on food intake and body weight. Interestingly not much is known about specific hypercaloric diet interventions, mimicking the Western style diet, on hypothalamic gene expression. We here show that a one week free choice high fat high sugar diet increases expression of genes in the hypothalamus favoring β-oxidation as well as PPARβ/δ and PGC1α. These changes might be related to the observed increase in food intake, i.e. hyperphagia.

Table 3. Animal characteristics per diet group

<table>
<thead>
<tr>
<th>diet</th>
<th>BW (g) ± SE</th>
<th>aWAT (g) ± SE</th>
<th>Kcal/day ± SE</th>
<th>chow (%) ± SE</th>
<th>lard (%) ± SE</th>
<th>sucrose (%) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow (n=12)</td>
<td>333.8 ± 4.7</td>
<td>2.1 ± 0.07</td>
<td>63.7 ± 2.6</td>
<td>63.7 ± 2.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>fCHFHS (n=11)</td>
<td>330.4 ± 4.7</td>
<td>2.8 ± 0.04</td>
<td>89.5 ± 4.2</td>
<td>39.4 ± 2.2</td>
<td>11.0 ± 2.4</td>
<td>39.1 ± 2.0</td>
</tr>
<tr>
<td>p</td>
<td>0.56</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

BW, bodyweight; aWAT, abdominal fat (epididymal, mesenteric and perirenal fat) per 100 g BW; Kcal/day, mean amount of kilo calories ingested per day followed by mean intake per component (chow, lard and sucrose); p, p-values, t-tests were performed to detect differences between diet groups.
Chapter 1

References

Chapter 1


83. de Andrade, I.S., et al., *Diet-induced obesity impairs hypothalamic glucose sensing but not glucose hypothalamic extracellular levels, as measured by microdialysis*. Nutr Diabetes, 2015. 5: p. e162.
