The hypothalamic arcuate nucleus and the control of peripheral substrates

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The arcuate nucleus (ARC) of the hypothalamus is particularly regarded as a critical platform that integrates circulating signals of hunger and satiety reflecting energy stores and nutrient availability. Among ARC neurons, pro-opiomelanocortin (POMC) and agouti-related protein and neuropeptide Y (NPY/AgRP neurons) are considered as two opposing branches of the melanocortin signaling pathway. Integration of circulating signals of hunger and satiety results in the release of the melanocortin receptor ligand α-melanocyte-stimulating hormone (α-MSH) by the POMC neurons system and decreases feeding and increases energy expenditure. The orexigenic/anabolic action of NPY/AgRP neurons is believed to rely essentially on their inhibitory input onto POMC neurons and second-orders targets. Recent updates in the field have cast a new light on the role of the ARC neurons in the coordinated regulation of peripheral organs involved in the control of nutrient storage, transformation and substrate utilization independent of food intake.

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Introduction

Obesity and correlated diseases such as hypertension, atherosclerosis, dyslipidemia, coronary diseases and diabetes mellitus are now clearly identified as a worldwide pandemic in both developing and developed countries [1]. Obesity per se, as well as the constellation of associated pathophysiology defined as the metabolic syndrome is inflicting an escalating public health burden that, according to the World Health Organization (WHO) (http://www.who.int/mediacentre/factsheets/fs311/en/) originates mostly from a drastic change in lifestyle involving an increased consumption of energy-rich diet and reduced energy expenditure.

Whereas some genetic loci were clearly identified, and extensively studied as monogenic causes for obesity, it is widely accepted that the metabolic syndrome is in essence a multifactorial diseases that encloses a complex network of molecular, cellular and physiologic alterations [2,3]. Although some debate exists on the exact number, a study from the American Center for Disease Control and Prevention recently made a case for obesity as the next top American killer before tobacco [4]. The urge to understanding the fundamental determinants of obesity and obesity-related disease has therefore generated a frantic race in the scientific community to unravel the mechanism involved and build a potential therapeutic strategy. Appropriate energy homeostasis results from the exquisite balance between energy intake and energy expenditure. In that regard, several determinants of feeding behavior are extensively studied encompassing the homeostatic regulation of nutrient intake, generally attributed to a hypothalamic-brainstem circuitry, but also the hedonic and motivational aspect of feeding relying, at least in part, on dopamine release in the mesocortico-limbic system. Different aspects of energy expenditure that include adaptive thermogenesis, physical activity and basal metabolic rate have also been dissected out as an essential part of obesity etiology. However, a fundamental component of the energy balance involves the ability of the brain to coordinate the activity of peripheral tissue to insure the fate of a nutrient once ingested. It is becoming evident that, aside of excessive energy consumption, obesity-related metabolic complications involve the inappropriate conversion, storage and utilization of nutrients: an integrated process referred as to “nutrient partitioning”.

Feeding inputs, circulating signals reflecting energy stores as well as cognitive and circadian control are among the many parameters that will be integrated at the level of the central nervous (CNS) system which will in turn orchestrate peripheral organ activity through the modulation of the autonomic nervous system (ANS). Although genetic and pharmacological interventions have allowed deciphering key molecular pathways underlying the regulation of energy expenditure and food intake, these studies also provided evidence that obesity-related substrate utilization could be manipulated independently of food intake and body weight.

Bariatric surgery offers a vivid example in which type 2 diabetes can be corrected in a few days after surgery showing a time-lapse that does not correlate with body weight loss. Although the mechanisms are still an active matter of research, it illustrates how obesity and its most common corollary disease can be separated [5–8]. It is tempting to speculate that a rapid reshaping of the reciprocal nervous dialogue between the brain and the periphery could be instrumental in the restoration of proper nutrient partitioning. In that view, the links between obesity and obesity-related diseases such as diabetes and dyslipidemia, could originate from a primary dysfunction in the ability of the brain to orchestrate the activity of peripheral tissues [9,10].

The arcuate nucleus (ARC) of the hypothalamus contains at least two crucial populations of neurons that continuously monitor signals reflecting energy status and promote the appropriate behavioral and metabolic responses to changes in energy demand. Neurons making pro-opiomelanocortin (POMC) decrease food intake and increase energy expenditure through activation of G protein-coupled melanocortin receptors (MCR) via the release of α-melanocyte-stimulating hormone (αMSH). Until recently, the prevailing idea was that the neighboring neurons expressing the orexigenic neuropeptides, agouti-related protein (AgRP) and neuropeptide Y (NPY) (NPY/AgRP neurons) increased feeding and decrease energy expenditure primarily by opposing the anorexigenic/catabolic actions of the POMC through both the competitive inhibition of melanocortin tone at the postsynaptic level and via
Fig. 1. The arcuate nucleus and the control of nutrient partitioning Sagittal section (upper panel) of a mouse brain showing interconnected nuclei engaged in the control of energy balance. Arcuate neurons project to second order targets including the paraventricular nucleus (PVN), the lateral hypothalamus (HyLat), the parabrachial nucleus (PBN), the dopaminergic neurons in the ventral tegmental area (VTA) of the midbrain and the nucleus tractus solitarii (NTS) in the brainstem. Hypothalamic nuclei also receive input from the dorsal vagal complex (DMX). The PVN integrates inputs from ARC neurons and from the suprachiasmatic nucleus (SCh). The arcuate nucleus of the hypothalamus (ARC) contains at least two populations of neurons that control energy balance the orexigenic/anabolic neurons producing neuropeptide Y and Agouti-related protein (NPY/AgRP) and the anorexic/catabolic neurons producing pro-opiomelanocortin (POMC) and the natural ligand for the melanocortin receptor. These first order neurons are located exquisitely close to the median eminence (ME), one of the brain’s circumventricular organs that lies at the bottom of the third ventricle (3rdV). The ME represents a microenvironment composed of fenestrated capillaries (red) and glia cells highly structured by tight junctions (green). Together, this creates a privileged region of the blood–brain barrier (BBB) in which macromolecules and energy-related (leptin, insulin, ghrelin) peptides access target neurons in the ARC through a regulated passage involving highly specialized hypothalamic glial cells: the tanycytes. ARC neurons are of diverse nature and display segregated projection to hypothalamic and extra hypothalamic nuclei. Integration of SCh “Clock” input and ARC-relayed nutrient input results in the ANS-mediated coordination of peripheral organ activity. This process can be independent from the regulation feeding, neuroendocrine release or metabolic rate and results in the concerted orchestration of nutrient transformation, storage and utilization.
directed inhibition of POMC firing rate (Fig. 1). This review is an attempt to cover some of the new functions, mechanisms and neurocircuitry by which ARC neurons can specifically control nutrient partitioning trough feeding independent manners.

The two ages of the MONA LISA hypothesis

In the CNS, the hypothalamus has rapidly been recognized as a primary integrator of circulating signal of hunger and satiety and extensively studied for its intimate implication in the control of ANS output, behavior and endocrine release. Several essential physiological functions such as salt & water intake, reproduction, wake & sleep, body temperature, circadian rhythmicity and metabolic rate rely on the coordinated output of a highly differentiated network of hypothalamic nuclei.

In the early 1940s, experiments using electrical stimulation and lesioning allowed the identification of functional different nuclei in the mediobasal hypothalamus (MBH) that had specific actions on energy homeostasis [11,12]. The ventromedial hypothalamus (VMH) was first considered as a satiety area because destruction of the VMH resulted in hyperphagia and obesity, whereas electric stimulation of VMH led to decreased food intake and body weight. Conversely, the destruction of the lateral hypothalamus (LH) led to anorexia, while stimulation of LH caused voracious feeding and obesity. The overall conceptual framework that emerged from these observations, i.e., one in which a “satiety center” kept the “feeding center” in check, was largely abandoned because of the realization that the LH lesions disrupted catecholaminergic nerve tracts passing through the hypothalamus that were essential for normal feeding and movement, and that the VMH lesions had a major impact on the autonomic output [13]. Nevertheless, this experimental paradigm already provided an illustration of the fact that obesity could not be solely attributable to hyperphagia, but rather also involved increased insulinemia [14]. In the same line, mono-sodium-glutamate (MSG) treatment at an early stage after birth results in an extensive damage of the ARC and leads to late onset obesity despite normal feeding [15–17]. A common feature displayed by these obese models was a global decrease in sympathetic tone associated with endocrine alterations, amongst others in corticosterone. These observations prompted the statement by Bray that Most Obesities kNow nAre Low In Sympathetic Activity: the “MONA LISA” hypothesis that already pointed defective nutrient partitioning as a preponderant mechanism driving obesity [18].

The identification of the obese gene encoding a 16 kDa protein called leptin (Lep\textsuperscript{ob}; [19]) has promoted a radical change in the general conceptual framework describing the central regulation of energy balance. The feeding & satiety center hypothesis has been replaced by a notion of intermingled neuronal networks in which highly specialized neurons [20] are able to encode primary signals reflecting blood-borne information about energy stores into synaptic transmission. Leptin levels rise and fall in direct proportion to adipose tissue mass and are relatively insensitive to daily changes in food intake. Food deprivation causes leptin levels to drop as energy stores are utilized, and this decline promotes endocrine and behavioral alterations that result in increased appetite and decreased energy expenditure. Leptin’s targets are found not only in the CNS, but also in peripheral tissues [21,22]. Mice lacking leptin (ob/ob) become morbidly obese as a consequence of metabolic disturbances and hyperphagia; they are also cold intolerant, diabetic and infertile [13,23]. The discovery of leptin and later on the leptin receptor, a single-pass transmembrane protein of the gp130 cytokine receptor family [24], which when mutated is responsible for the db/db phenotype Lep\textsuperscript{db} [25], fueled an active research field in which a leptin-based treatment was hoped to fulfill the promises of a pharmaceutical approach to surfeit the obesity epidemic. Although, leptin deficiency provided a fantastic model to study the metabolic syndrome it accounted as for other monogenic obesity for only rare cases of obesity [26]. However, the identification of the leptin signaling pathway opened up a new era in the understanding of the central control of energy homeostasis and allowed the dissection of several fundamental neurocircuits and molecular mechanisms underpinning the hypothalamic control of feeding, energy expenditure and neuroendocrine control [27]. In the new leptin era, the MONA LISA hypothesis still stands [20]. Leptin, as well as other factors are impinging directly onto ARC neurons to modulate their activity, in turn these “first order” neurons project to several structures that can independently modulate ANS output and separately affect different peripheral organs [9,28,29]. This mechanism provides a circuit-based blueprint to control peripheral substrate utilization and prompted the provocative hypothesis that sees several features of the metabolic syndrome including dyslipidemia,
insulin resistance, high blood pressure, abdominal fat, as a primary defect of brain ANS control [9]. Instead of a global decrease in catecholamine release, the MONA LISA hypothesis could be replaced for a more selective one, i.e., obesity is the reflection of tissue-specific changes in ANS output which will in turn induce a global change in energy fluxes.

The arcuate nucleus of the hypothalamus: exquisite location for exquisite regulation

ARC of the hypothalamus is located at the bottom of the third ventricle (3rdV) in close vicinity to one of the circumventricular organs (CVO): the median eminence (ME), a structure of the blood–brain barrier (BBB) that has evolved to allow selective exchange between blood-borne peptide and cerebrospinal fluid and ARC neurons (Fig. 1). Proper regulation of body weight relies on the ability of peripheral signals to reach and modulate the activity of effector neurons. These neurons will primarily integrate circulating signals and encode energy-related signals into synaptic transmission that will in turn impinge onto secondary target neurons or “second-order neurons”.

The unique specification of the ME was vividly illustrated by the identification of the role of tanyocytes as gatekeepers of the BBB [30]. Tanyocytes are highly specialized hypothalamic glial cells that extend from the ependymal surface of the 3rdV to a plexus of permeable fenestrated capillaries [31] that can accommodate a rapid transport of energy-relevant peptides such as ghrelin [32]. Tanyocytes are layered around tight junctions and represent the first rampart between the blood and the CSF [33]. Changes in nutrient availability were shown to directly affect ME permeability through a glucose sensing-vascular endothelial growth factor A (VEGF-A) releasing mechanism from the tanyocytes to endothelial cells [31]. Tanyocytes were also shown to be the first-and critical-step into leptin’s entry into the hypothalamus [34] (Fig. 1). This work comes in addition to previous observations [35] and emphasizes the role of the ME as an integral component of energy balance regulation. Highly specialized neuronal subsets that define the two branches of the melanocortin system are located in close vicinity to the ME. This anatomical feature allows the ARC neurons to rapidly engage in electrophysiological changes in response to the entry of circulating hunger and satiety hormones, hence their appellation of “first order” neurons (Fig. 1).

Among the most well-characterized “first order neurons” are the neurons that make the neuropeptide Y (NPY) and the Agouti-related protein (AgRP) and the neurons that produce the pro-opiomelanocortin (POMC) and cocaine-and amphetamine-related transcript (CART). NPY, initially discovered by Tatemoto et al. [36], was later on found to be a powerful stimulator of feeding [37]. AgRP was discovered as an inverse agonist for the melanocortin receptors [38–40]. Co-localization of the two peptide was described soon after [41], and NPY and AgRP were found to be present in the same processes arising from hunger-associated neurons. In addition, these neurons release gamma aminobutyric acid (GABA) establishing further the inhibitory action of NPY/AgRP neurons [42].

The neighboring POMC neurons are intermingled with NPY/AgRP neurons. POMC neurons are of mixed excitatory and inhibitory nature and release the neurotransmitters glutamate and GABA [43] as well as posttranslational products of the POMC peptide, including α, β, γ-melanocyte-stimulating hormone (MSH) and the adrenocorticotropic hormone (ACTH). POMC and NPY/AgRP neurons have reciprocal antagonistic actions; GABAergic outputs from the NPY/AgRP neurons synaps onto POMC neurons [44–48], moreover the release of NPY by NPY/AgRP neurons leads to activation of the G-coupled NPY-Y1 receptor located onto POMC. Both neuronal population share common “second-order” targets in the CNS that are found not only in the paraventricular (PVN), ventromedial (VMH), dorsomedial (DMH) and lateral (LH) nuclei of the hypothalamus but also in extra hypothalamic targets such as the nucleus of the tractus solitarii (NTS), the parabrachial nucleus (PBN) [16] or the intermediolateral cell column (IML) [49]. In these structures, the release of α-MSH by POMC neurons initiates the anorectic/catabolic melanocortin signaling cascade through the binding of α-MSH to the Gs-coupled melanocortin receptor (MCR). The MCRs (MCR1 to MCR5) are G protein-coupled receptors distributed throughout the body, with MC3R and MC4R having their expression restricted to the CNS [50,51]. Conversely, during energy deprivation electrophysiological NPY/AgRP neurons will increase their firing leading to enhanced release of AgRP, which will oppose α-MSH binding in postsynaptic targets [52] (Fig. 1).
The PVN represents a very important second order structure downstream of ARC neurons outputs. The PVN contains several key neuroendocrine neurons including oxytocin (OT), vasopressin, corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone-synthesizing neurons (TRH). In addition to this endocrine function of many PVN neurons, several pre-autonomic neurons that project to the dorsal motor nucleus of the vagus nerves (DMX) and as well as to the IML allow the PVN to directly contribute to both parasympathetic and sympathetic output as well. In turn the PVN receives input from the NTS and the trigeminal pars caudalis. The PVN receives also inputs from the PBN, DMN and LH. Finally, intra-PVN neuronal encoding is under the control of circadian clock signals emanating from the suprachiasmatic nucleus (SCN) [53].

MC4R is expressed onto CRH, TRH and OT containing neurons [50]. Intra-PVN competition between AgRP and α-MSH tone will directly impact onto energy conservation. During times of plenty α-MSH binding to MCR will positively regulate both the hypothalamic–pituitary–thyroid (HPT) and hypothalamic–pituitary–adrenal (HPA) axis, while the fasting-mediated increase of AgRP release was shown to be instrumental in the adaptive response of the HPT- and HPA-axis during negative energy balance [54,55]. NPY/AgRP and POMC neurons are hence considered as the two opposed branches of the “melanocortin system”, defining a paradigmatic antagonistic regulation of nutrient intake and energy expenditure. Mutations in the melanocortin signaling pathway including MC3R or MC4R null mutants [56] or any enzyme involved in the processing of melanocortin peptide, together with ectopic expression of the MCR antagonist agouti Agouti yellow (Ay) [57], result in increased feeding and decreased energy expenditure and invariably morbid obesity in both humans and experimental animals [58,59].

ARC neurons also project to PVN-pre-autonomic neurons and a direct projection was described for a subset of POMC neurons onto MC4R positive neurons in the IML (Reference). Altogether these observations support the implication of the MCR signaling pathway in the autonomic regulation and predict that both direct ARC-IML and ARC-PVN-IML network might account for autonomic and metabolic effects of melanocortin signaling molecules.

Recently, a reciprocal control from PVN neurons onto ARC NPY/AgRP neurons was described as a novel regulatory loop involved in feeding behavior neurons [60]. This complex interplay of MCR-bearing neuronal subpopulations defines the so-called melanocortin signaling pathway and is a key neurocircuit for the regulation of the energy balance. Hitherto the ARC neurons have been considered as a primary integrative structure for circulating signals entering the ME. NPY/AgRP neurons are classically envisioned as a natural opponent of POMC neurons activity, mostly through their antagonistic action onto the melanocortin signaling pathway [61,62]. It is only recently that the integrated role has expended beyond the strict regulation of food intake, to melanocortin dependent and independent regulation of peripheral nutrient partitioning.

Arcuate control of peripheral nutrient partitioning

ANS-modulation of peripheral organ activity via efferent nerves is a crucial component of an integrated adaptive response that is initiated at the level of the brain as a result of the integration of hormonal and nervous afferent inputs. Appropriate regulation of ANS output onto metabolically active tissues is required to finely orchestrate inter-organ communication during post-absorptive states. Virtually every tissue including pancreas, liver, brown adipose tissue, white depot, as well as striated, cardiac and smooth muscle, intestinal tract and bone tissue receives ANS inputs [9,63]. The increased sympathetic tone during fasting represents a key mechanism for increased adipose tissue lipolysis and liver glucose production, as well as decreased beta-cell insulin release. At the opposite increased parasympathetic activity will promote energy storage and conversion [9,64]. Viral-based tracing studies have shown that pre-autonomic hypothalamic neurons have a distinct organization according to their efferent organs [64]. This organization supports the concept that a discrete subset of neurons could participate in and control the selective ANS outflow to one specific tissue. Both POMC and NPY/AgRP neurons provide dense synaptic inputs to pre-autonomic structures such as the PVN and a direct action onto peripheral tissue activity, independent from acute regulation of feeding are now largely documented. Central manipulation of the melanocortin signaling pathway was shown to affect peripheral cholesterol and lipid metabolism independent from food intake [65–67]. In these experiments
central blockade of MCR signaling, in otherwise pair-fed conditions, led to increased lipid synthesis and storage in the white adipose (WAT) and increased insulin-stimulated glucose uptake in the WAT, but resulted in decreased glucose metabolism in muscle and BAT [65]. This effect was mediated by the sympathetic nervous system and associated with increased triglyceride synthesis in and export from the liver [65]. Conversely central stimulation of MCRs was shown to trigger lipid mobilization in WAT [65,66,68]. In the same line, central manipulation of MCRs selectively increased high-density-circulating lipoprotein cholesterol (HDL) through reduced liver uptake [67]. These results were observed independently form food intake and provide a vivid example of selective substrate changes induced peripherally (lipid vs carbohydrate) through central manipulation. Fasting represents a physiological situation in which NPY/AgRP neurons have the highest firing rate and exert a profound inhibitory tone onto POMC neuron activity, antagonize MCR tone through AgRP release and promote postsynaptic inhibition through NPY release. A recent study, perfectly in line with the results cited above showed that during fasting, AgRP release is required to promote the fasting-induced decrease in hepatic SNS activity and increased liver TG synthesis while central knock-down of AgRP in wild type animal prevented high fat induced liver steatosis [69]. Central injection of NPY was shown to promote increased liver very-low-density lipoprotein secretion [70], reduced insulin inhibitory action onto VLDL secretion [71] and to induce hepatic insulin resistance via sympathetic innervations [71,72]. More recently, the release of NPY by NPY/AgRP neurons was shown to control tyrosine hydroxylase (TH) expression in the PVN and other brainstem regions via NPY-Y1 receptor activation. The overall consequence was to increase BAT thermogenesis [73].

Taken together these studies converge toward the notion that MCR blockage and increased NPY/AgRP activity will promote a concerted change in liver lipid synthesis and export, via the ANS, associated with increased WAT lipid storage and BAT lipid catabolism. These observations also illustrate how central ANS manipulation can simultaneously enhance substrate synthesis in one tissue (liver) to promote its catabolism to another tissue (BAT) via the exquisite coordination of effector organs. From a strict thermodynamic stand point, these changes can be independent from actual nutrient intake and allow the re-direction of energy fluxes from one tissue to another (Fig. 1).

**Neuronal and physiologic timing for ARC neuron activity**

When considering the ARC based neurocircuitry, one has to conciliate the proper timing during which ARC neuron will fire, the differential delay and action of fast-acting neurotransmitters vs slow-acting neuropeptides together with the differential activity of ARC neurons during different physiological conditions. In that view, the critical role for GABA release from NPY/AgRP neurons was provided by several independent studies. The strict necessity for AgRP neurons in the maintenance of feeding came from several studies using selective ablation of NPY/AgRP neurons. Acute depletion of NPY/AgRP neurons leads to profound anorexia [74–76], which was not the consequence of an increased melanocortin tone but rather involved the sudden loss of GABAergic inputs from NPY/AgRP neurons onto the PBN [77]. GABA-A receptor agonist delivery specifically into the PBN could prevent anorexia following NPY/AgRP neurons ablation. This study demonstrated that GABA made by NPY/AgRP neurons was critically required to maintain feeding in a melanocortin independent manner [78,79]. The same group recently identified calcitonin gene-related peptide-expressing neurons in the outer external lateral subdivision of the PBN and their projection to the central nucleus of the amygdala as the neuronal substrate for suppressing appetite [80]. In vivo manipulation of NPY/AgRP neurons through forced expression of designer receptors exclusively activated by designer drugs (DREADD) or photo-activatable channel rhodopsin (ChR2) [81,82] demonstrated the necessity and sufficiency of NPY/AgRP neurons to promote the full feeding sequence [83], but also confirmed that acute feeding evoked by NPY/AgRP neurons activation relied on the combination of NPY and GABA mediated inhibition of PVN oxytocin (OT) neurons independently from MCR inhibition [84]. Interestingly, activation of ARC fibers synapsing in the PVN led to a prolonged asynchroneous GABA release at the NPY/AgRP synapse that sustained inhibition for hundreds of milliseconds after action potential-mediated Inhibitory Postsynaptic Currents (IPSCs) occurred. This timescale is usually associated with neuromodulation, but was here fully recapitulated by biphasic GABA release. Hence, NPY/AgRP neurons that synapse onto the PVN can modulate postsynaptic activity two orders of magnitude longer after action potential occurred [84]. One can therefore envision a delay in fast- and
slow-acting neurotransmitters, initiated by changes in AgRP neuronal activity, but occurring at different timescales and in different postsynaptic structures. A recent study from Krashes et al. definitively established that AgRP release by NPY/AgRP neurons is sufficient to induce feeding during a prolonged period of time, while GABA and NPY co-release is critical to induce rapid feeding events [85].

In addition to the intrinsic nutrient-entrained activity of NPY/AgRP and POMC neurons, the PVN also integrates circadian-related signals relayed from the SCN [53,64]. SCN input and energy-related input conveyed to ARC neurons could therefore be instrumental in the control of carbohydrate vs lipid-substrate production and utilization [86]. For instance SCN input to the PVN was shown to critically operate SNS-mediated liver glucose production [87] and the simple manipulation of feeding schedule, with no change in calories consumed, was shown to be sufficient in preventing liver steatosis, obesity and diabetes associated with high fat feeding [88]. In the latter experiment, animals subjected to restricted feeding schedules displayed a drastic change in the respiratory quotient (RQ) indicative of the nature of the substrate being used by an organism (RQ = 1 for glucose utilization and RQ = 0.7 for lipid utilization). The drastic RQ changes indicate a rapid change in peripheral substrate selectivity and utilization, while the smaller changes in RQ observed in animals fed ad libitum indicate a limited – essentially gluco-lipidic – metabolic plasticity [88]. Hence, manipulating the timing of nutrient input is sufficient to change the inter-organ dialog and nutrient partitioning. This manipulation illustrates the concept that nutrient excess per se, does not necessarily translates into metabolic disease if appropriate manipulation of nutrient partitioning is applied to optimize nutrient fate and utilization. Finally, the intrinsic timing during which the ARC neurocircuitry is active, i.e., besides external clues of acute energy deprivation or nutrient oversupply, is still a matter of debate. In that regards, NPY/AgRP neurons were shown to exhibit pacemaker activity [89] suggesting that their activity might extend beyond the period of energy deprivation, that is also during the post-prandial period. In that period, the coordinated input from NPY/AgRP neurons to pre-autonomic structures could be instrumental in the determination of peripheral carbohydrate handling independently from nutrient intake, for instance through long-lasting GABAergic inhibitory post-synaptic currents (IPSCs)’s at the synaptic button.

One can therefore envision a role for both POMC and NPY/AgRP neurons in a segregated action onto efferent organ activity, beside their antagonistic regulation of feeding, through the control of ANS output. Both neuronal networks could then operate on different timescales by the combinatorial use of slow and fast-acting neurotransmitters and peptides, in a melanocortin dependent and independent manner to independently affect peripheral nutrient partitioning (carbohydrate vs fat) in a coordinated, but not necessarily opposite manner.

NPY/AgRP neurons: a central switch for peripheral lipid vs carbohydrate utilization

Using an animal model allowing for the selective neonatal ablation of NPY/AgRP neurons [76,90] our group substantiated this hypothesis by showing that mice lacking NPY/AgRP neurons from the perinatal period onwards display a drastic change in ANS output to peripheral tissues characterized by a decreased norepinephrine turn-over rate (an indirect readout of sympathetic outflow) onto the pancreas, liver and white-glycolytic muscle, and an increased norepinephrine turn-over rate in oxidative fat-burning muscle. When fed a regular chow (carbohydrate rich) diet, animal lacking NPY/AgRP neurons displayed increased feeding efficiency together with hyperinsulinemia and late onset obesity. Obesity was not the result of increased caloric intake, but rather involved a shift in substrate utilization due to simultaneous metabolic changes in a number of peripheral tissues. The RQ revealed a change in substrate utilization towards lipid oxidation that correlates with enhanced conversion of carbohydrate in the liver associated with increased TG synthesis and export [91]. These change in RQ clearly followed a daily pattern with a lowest value at the entry of the dark period, a time at which NPY/AgRP neurons should be activated by nutrient deprivation. The shift towards lipid-substrate preference was evidenced by both increased peripheral lipid synthesis and export by the liver, and enhanced lipid-substrate preference at the level of soleus muscle mitochondrial respiration.

Mice lacking NPY/AgRP neurons were then shifted on a high fat diet in order to challenge the hypothesis that these changes in ANS output would indeed change the global adaptation to lipid fuel. NPY/AgRP-ablated mice normalized their feed efficiency and had both a paradoxical improvement of glucose tolerance and a reduction in body weight gain compared to control mice [92,93].
Furthermore, RQ profile and white adipose tissue (WAT) gain in mice lacking NPY/AgRP neurons fed a regular chow diet could be selectively normalized through GABA-A receptor agonist treatment. One can hypothesize that ANS output by NPY/AgRP neurons relies more prominently on the slower, tonic GABAergic inputs that are long-lasting currents compatible with the partitioning of nutrients that occurs after the meal, during post-ingestive processes. Thus, beyond their well described implication in the acute regulation of food intake, NPY/AgRP neurons might also directly regulate the fate of nutrients once ingested through the orchestration of a coordinated dialog between organs including post-prandial insulin release from pancreas, nutrient conversion and storage in the liver and adipose tissue, and glucose vs lipid utilization in muscle [65,66,94].

Remarkably, despite marked obesity mice lacking NPY/AgRP neurons retained the ability to expand their adipose mass and displayed improved glucose tolerance and insulin sensitivity upon high fat feeding. This counter-intuitive observation can be solved by considering the role of NPY/AgRP neurons, beyond feeding, as a central switch operating the metabolic balance between carbohydrate and lipid utilization. In that view, the lack of NPY/AgRP neurons might have translated into better adaptation to high fat diet and the optimization of carbohydrate vs lipid oxidation [93]. This result is in line with a recent study showing that Sirt1 invalidation selectively in NPY/AgRP neurons results in a shift in the overall metabolic profile, the impairment of metabolic adaptation to fasting and a change in ghrelin-induced excitability [95].

Energy-relevant neurons of the ARC are not limited to the POMC and NPY/AgRP population and more recently other players have been revealed. GABAergic RIP-Cre neurons are a newly described population whose activity directly controls energy expenditure but not feeding [96]. In addition, AgRP, NPY and α-MSH containing fibers are distributed widely in the brain [16,46] and the existence of segregated populations within NPY/AgRP neurons projecting to specific second order targets has been suggested. Using cell-type-specific neuron manipulation and projection-specific anatomical analysis Betley et al. found that NPY/AgRP neurons indeed display segregated axonal projections that target different brain regions and originate from distinct ARC subpopulations among which a subset could control feeding [97]. It is formally possible that the control of nutrient partitioning independent of food intake also originates from a segregated, non-feeding related, neuronal network based in the ARC.

ARC control of nutrient partitioning: the link between obesity and obesity-related disorders?

The limit between a healthy obesity and an obesity associated with corollary diseases could be the result of a defective central switch and aberrant ANS output and nutrient partitioning as stated by the MONA LISA hypothesis [18,20] and Buijs and Kreier [9]. Proper balancing of ANS tone into peripheral tissues could be a primary set-point defined during postnatal development to establish the balance between carbohydrate vs lipid adaptation of an organism for the rest of adulthood. The weaning period is a critical period of development in which a switch between essentially lipid nutrients to carbohydrate-based feeding occurs in a short period of time. One could envision that NPY/AgRP neurons are critical for this behavioral and metabolic switch from lipid to carbohydrate metabolism. Indeed NPY/AgRP and POMC network development coincides with weaning in rodents (i.e., the switch from a lipid-rich milk diet to a carbohydrate-rich solid diet) [98] and the metabolic imprinting of ARC projections have been shown to rely on the trophic action of leptin [99–101]. Mice lacking NPY/AgRP neurons could have remained in a “default” metabolic mode in which lipid substrates are still preferred. In that regards we found that the body weight curves of naïve and NPY/AgRP-ablated mice were similar when fed a HFD since weaning [92]. Similarly, in adulthood an attempt to modify or restore ARC mediated coordination of peripheral substrate utilization could be a promising avenue to treat diabetes, dyslipidemia or cardiovascular diseases independently from actual body weight loss or feeding manipulation.

Summary

The arcuate nucleus of the hypothalamus contains several neuronal populations that are exquisitely positioned, close to a blood–brain barrier entry point, to integrate circulating signals of hunger and satiety. Among ARC neurons, POMC and NPY/AgRP neurons are considered as two opposed branches of activity...
the melanocortin signaling pathway. NPY/AgRP neurons are segregated populations that have different and overlapping projections dedicated to food-related and non-food-related functions [102]. Manipulation of individual neurotransmitters produced by these neurons, as well as in vivo activity have highlighted their implication in ANS-mediated control of peripheral organ activity. Activity of ARC neurons can now be considered to extend beyond the strict regulation of feeding to the control of nutrient storage, utilization and transformation. This fundamental process is a cornerstone to a new conceptual framework that sees obesity and obesity associated disorders as parallel diseases, inherited through the simultaneous alteration of discrete hypothalamic neurocircuitry.

### Practice points

- Obesity and obesity-related disorders can be separated through central manipulation of ARC neurocircuitry.
- The establishment and activity of a hypothalamic network can define the equilibrium between carbohydrate and lipid preference.

### Research agenda

- Pursue the characterization of neural substrate for central control of nutrient partitioning.
- Define the limit of hypothalamic network plasticity in adulthood.
- Explore the dichotomy between obesity per se, and obesity-related disorders.
- Explore pharmacotherapy that would take into account the internal clock and the proper timing for neuronal activation.

### Declaration of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as potential conflict of interest.

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### References


