

FOCUS: OBESITY

## A Temperature Hypothesis of Hypothalamus-Driven Obesity

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Obesity is a metabolic state in which excess fat is accumulated in peripheral tissues, including the white adipose tissue, muscle, and liver. Sustained obesity has profound consequences on one's life, which can span from superficial psychological symptoms to serious co-morbidities that may dramatically diminish both the quality and length of life. Obesity and related metabolic disorders account for the largest financial burden on the health care system. Together, these issues make it imperative that obesity be cured or prevented. Despite the increasing wealth of knowledge on the etiology of obesity (see below), there is no successful medical strategy that is available for the vast majority of patients. We suggest that brain temperature control may be a crucial component in obesity development and that shortcutting the brain metabolic centers by hypothalamic temperature alterations in a non-invasive remote manner will provide a revolutionary approach to the treatment of obesity.

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†Abbreviations: VMH, ventromedial hypothalamus; PVH, paraventricular hypothalamus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; MC4R, melanocortin receptor 4; POMC, pro-opiomelanocortin; AgRP, arcuate nucleus agouti-related protein; NPY, neuropeptide Y;  $\alpha$ -MSH,  $\alpha$ -melanocyte stimulating hormone; LHRH, luteinizing hormone releasing hormone; BMCP1, brain mitochondrial carrier protein 1; RCR, respiratory control ratio; Tsk, skin temperature; Tc, core temperature; Hcrt, hypocretin/orexin; Glut, glutamate.

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## INTRODUCTION

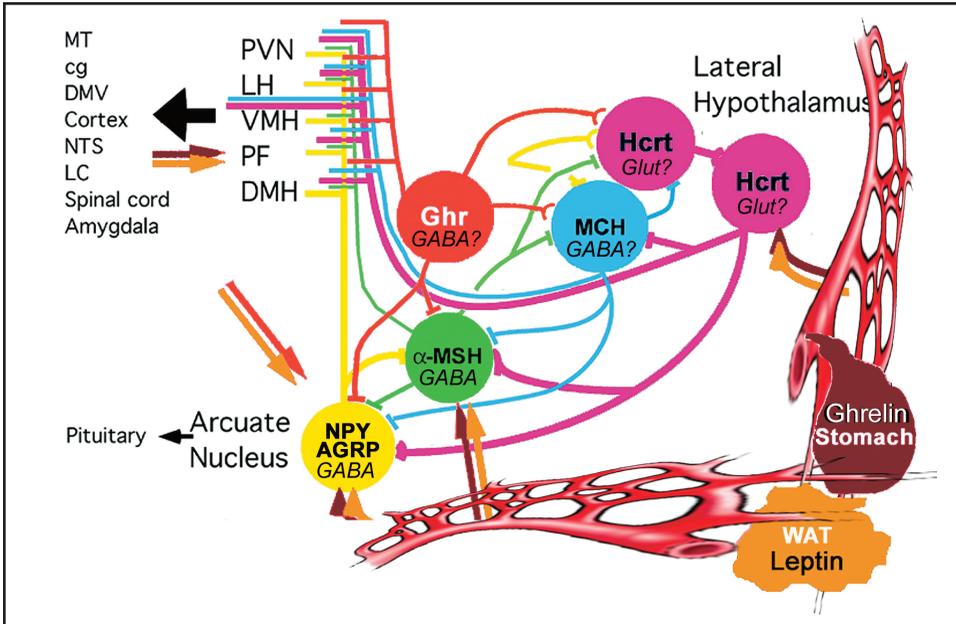
Compelling evidence has been gathered during the last 100 years to suggest that the underlying cause for fat accumulation in peripheral tissues arises, at least in part, from the central nervous system [1-9]. From the dawn of modern neurobiology at the end of the 19th century, both intellectual as well as experimental attempts have been made to identify and isolate the brain's role in energy metabolism regulation. For example, based on the emerging experimental observations on brain stem regulation of respiration at the time, Sherrington suggested that a similar blood link between the periphery and brain should exist for the regulation of food intake [10]. Conclusions on signals from the periphery to the brain regarding feeding regulation were also drawn by others implicating gut-born humoral signals for appropriate brain responses to changing metabolic needs [11,12]. It is still remarkable to realize that these minds, and undoubtedly many more in or before the late 19th and early 20th centuries without the luxury of modern technology and widespread availability of knowledge, had the intellectual capability to predict basic principles that, 100 years later, proved to be correct. These past 100 years, however, have not elapsed without other advancements in the understanding of the brain-obesity relationship.

The involvement of various hypothalamic regions in the regulation of energy homeostasis stems from degeneration studies in the 1940s and 1950s: destruction of the hypothalamic ventromedial (VMH $\dagger$ ), paraventricular (PVH), and dorsomedial (DMH) nuclei induced hyperphagia [1,13-16]. In contrast, discrete lesions placed in the lateral hypothalamus (LH) [15] reduced food intake. While these approaches may be considered crude by today's standards of research, they were pivotal in pinpointing the hypothalamus as a major regulator of energy homeostasis. In fact, in retrospect to our current knowledge of specific neurotransmitter- and neuromodulator-containing hypothalamic neuronal populations (see below), these lesion studies were strikingly precise in distinguishing between subregions of the hy-

pothalamus that house circuits that either promote or suppress feeding. The degeneration studies in conjunction with physiological observations on strains of animals that were obese [17,18] confirmed the proposition that humoral signals arising from the periphery may exist to inform brain sites about overall energy needs.

A fundamental breakthrough came in 1994 and 1995 when the gene encoding the adipose signal leptin was discovered and its product tested on leptin deficient obese ob/ob mice [19-22]. Subsequently, receptors for leptin were cloned and localized to structures of the hypothalamus [23], some of which had been implicated in metabolism regulation by lesion studies. However, leptin binding and its receptors accumulated predominantly in a ventromedial hypothalamic structure, the arcuate nucleus [24], an area that was not specifically suggested by the lesion studies. The appreciation of this small hypothalamic nucleus gained momentum regarding energy homeostasis when, due partially to serendipity, the melanocortin system was discovered as a player in obesity. In a quest to better understand the role of various melanocortin receptors in the determination of skin color [25,26], the striking revelation was made that when melanocortin receptor 4 (MC4R) was eliminated, animals became morbidly obese [27]. Since the melanocortin system had long been known to exist in neurons of the arcuate nucleus [28], it was reasonable to test whether leptin exerts its effect on metabolism by the mediation of the arcuate nucleus melanocortin system. And this was found to be the case [29]. Further analysis of components of the central melanocortin system [30] delivered an attractive model, which today is considered to be the *primun movens* of metabolism regulation.

The realization of the critical role that the arcuate nucleus melanocortin system plays in mediating leptin's effect on metabolism and the subsequent recognition of a distinct local counterpart of the pro-opiomelanocortin (POMC) cells, the arcuate nucleus agouti-related protein (AgRP)-neuropeptide Y (NPY)-producing cells [31], provided an exceptionally attractive and simple model of



**Figure 1.** Schematic drawing showing known interactions and projection targets of various hypothalamic peptidergic systems, including lateral hypothalamic neurons producing hypocretin/orexin (Hcrt) and melanin concentrating hormone (MCH) and components of the melanocortin system, the NPY/AgRP- and MSH-producing neurons. These neuronal populations are targeted from the circulation by white adipose tissue-derived leptin and stomach-derived ghrelin. They are synaptically interconnected and project, to some degree, in an overlapping manner to intra- and extra-hypothalamic sites. It is a recurring mistake to consider peptide circuits as independent entities. Almost without exception, peptidergic neurons contain various other neuromodulators, including the classical neurotransmitters GABA or glutamate (Glut). With varying artistic characteristics, most of the schemes proposed to date are similar to Figure 1 in that they depict connectivity as a still picture implying hard wiring. While such mapping of connectivity will continue to provide important building blocks for evolving concepts, they lack both synaptic resolution as well as indication of soft wiring or plasticity.

the “heart” of the central feeding center. In this model, activation of the POMC neurons by leptin [32,33] triggers release of  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) from POMC axon terminals, which in turn, appropriately activates MC4Rs, leading to suppressed food intake and increased energy expenditure. Simultaneously, leptin suppresses the activity of arcuate nucleus NPY/AgRP neurons [32,33], which otherwise, through the release of AgRP, antagonizes the effect of  $\alpha$ -MSH on MC4R [30]. The NPY/AgRP system not only antagonizes anorexigenic melanocortin cells at their target sites where MC4Rs are located, but it also very robustly and directly inhibits POMC perikarya using both NPY as well as the small inhibitory amino acid neurotransmitter, GABA [33], an event that occurs through bas-

ket-like synaptic innervations of POMC cells by NPY/AgRP terminals [34]. This unidirectional [34] interaction between the NPY/AgRP and POMC perikarya is of critical significance because it provides a tonic inhibition of the melanocortin cells whenever the NPY/AgRP neurons are active. Because there is no constitutive direct feedback mechanism from the POMC cells to disengage NPY/AgRP neurons, this simple anatomical constellation (Figure 1) may provide the easiest explanation why the baseline blueprint of feeding circuits is more likely to promote feeding than satiety. While this bias toward positive energy balance is a necessity from an evolutionary perspective, it is also a likely contributor to the etiology of metabolic disorders, such as obesity.

Both neuronal systems are directly targeted by leptin and can also be affected by other peripheral metabolic signals, such as ghrelin, glucose, insulin, and peptide YY [35-38]. Their localization in ventromedial aspects of the arcuate nucleus in close vicinity to the neurohemal (regions without blood-brain barrier) median eminence warrants that these neurons may be reached by circulating metabolic signals in the earliest and most effective manner. In fact, it is not unusual to find either NPY/AgRP or POMC perikarya or dendrites in direct contact with capillaries (Figure 1), furthering the likelihood that these neurons are in excellent position to respond to changes in characteristics of the blood, such as its temperature fluctuation.

The simplicity of the putative hypothalamic feeding center is remarkable and suggests that manipulation of either component of the melanocortin system should shift energy balance. More importantly, if this system is the *primum movens* of energy homeostasis, then it should be possible to correct metabolic disorders such as obesity by altering either the AgRP/NPY or POMC circuit. Indeed, genetic manipulations of either component can lead to altered metabolic phenotype [39]. To date, no successful medical strategies have emerged that target this system for the treatment of either positive (obesity) or negative (cachexia) energy balance, in part because of the redundant interaction of the melanocortin system with other hypothalamic and extrahypothalamic neuronal systems. Developments of the past decade also suggest that the flexibility of the hypothalamic center of metabolism is not only due to redundant interconnectivity but also the consequence of its recently uncovered soft wiring [9,40].

#### **AN APPARENT PARADOX: THE INCREASING BODY OF KNOWLEDGE ON PERCEIVED FEEDING CENTERS AND THE LACK OF SUCCESSFUL THERAPIES FOR OBESITY**

The discovery of hypophyseotropic hormones in the 1960s rapidly helped reveal the regulation of various peripheral tissues, in-

cluding the ovaries and testes, by the brain and how the feedback from these tissues influenced the brain. For example, the characterization and synthesis of luteinizing hormone releasing hormone (LHRH) and its influence on the pituitary had immediate academic as well as clinical implications for issues associated with ovarian cycles and infertility [41]. This rapid translation of newfound principles for clinical medicine occurred at a time when genetics and molecular biology, in general, were not common practice and, by and large, were not utilized. Thus, the anticipation since the discovery of leptin in 1994/1995 that medical approaches for metabolic disorders would soon follow seemed to be well founded. These medical breakthroughs have not yet occurred, however, and it is unlikely that the status quo will change in the immediate future. Nevertheless, the paradox remains, considering the amount of intellectual talent and monetary resources being directed at this issue.

The argument can be made that, from an evolutionary perspective, the regulation of energy balance is at least as important as reproduction. Thus, if it turns out to be overly redundant, a method should be found to successfully shortcut the central nervous system component of metabolism regulation similar to what was done regarding reproduction. It is worthwhile to note that although the principal hypothalamic neurons in the regulation of ovarian and testicular function, the LHRH cells, have been found and extensively studied [42], the blueprint of gonadal feedback on the hypothalamus remains largely ill-defined. This fascinating and biologically critical subject, however, is becoming more academic as successful approaches bypassing the brain to regulate reproduction have emerged [43]. Whether or not it is reasonable to anticipate similar breakthroughs in metabolism regulation is yet to be seen. Controlling hypothalamic temperature may offer that very possibility.

#### **THE TEMPERATURE HYPOTHESIS**

We suggest that hypothalamic temperature is a determinant of the output of the arcuate nucleus metabolic center, which, in

turn, underlies feeding behavior and energy expenditure. It is intriguing to note that a similar hypothesis was proposed more than 50 years ago by Brobeck [44], albeit his impetus was more intuition than experimentally based. It is important to clarify that our temperature hypothesis (and that of Brobeck) is not to be mistaken (although it most likely relates) with the regulation and role of body temperature in metabolism regulation.

### ***Core Body Temperature and Metabolism***

The primary mode of energy expenditure in mammals is by dissipation of energy in the form of heat. Thus, body temperature and its regulation is a major determinant of a metabolic phenotype. Humans rely primarily on behavioral thermoregulation to protect themselves against the cold. They wear clothing, use various heat generating devices, and seek out a warm environment. When these behaviors are unavailable or inadequate to defend body temperature homeostasis during cold exposure, lower peripheral tissue temperatures stimulate peripheral thermoreceptors, and the resultant afferent flow elicits autonomic homeostatic responses (thermogenesis and vasoconstriction) to restore body temperature [45-49]. Both core and skin temperatures modulate temperature regulation responses [50-52], which are finely tuned by thermoregulation centers located in the preoptic/anterior hypothalamus. The hypothalamus contributes to all core temperature signals, both hypothalamic and extrahypothalamic, such as spinal cord or blood temperature. The hypothalamus also regulates metabolism (see above), so hypothalamic temperature and metabolic responses may be tightly coupled. Intriguingly, this possibility has never been explored by prospective studies, and, consequently, our current understanding of the effect of hypothalamic temperature on thermoregulatory and metabolic responses is limited.

### ***Hypothalamic Temperature and Metabolism***

Our interest in hypothalamic temperature emerged when the mitochondrial un-

coupler, UCP2, was discovered and localized to the central nervous system [53]. Uncoupling proteins encoded by nuclear DNA are located in the inner membrane of the mitochondria, and their primary function is thought to be to leak hydrogen protons from the intermembrane space to the matrix of the mitochondria. Through this process, they deprive the driving force of ATP synthase from catalyzing ATP synthesis, dissipate energy in the form of heat, and by enhancing the speed of oxidation, they may diminish the production of superoxides and block entry of calcium to the mitochondrial matrix [35,36]. The most well-characterized uncoupling protein, UCP1, is expressed solely in the brown adipose tissue and is responsible for thermogenesis in small rodents. In the last few years, however, several other members of the uncoupling protein family have been discovered and found to promote partial uncoupling of oxidation from phosphorylation *in vitro*. These proteins include UCP2, UCP3, UCP4, and brain mitochondrial carrier protein 1 (BMCP1). The five UCPs differ greatly in tissue distribution and regulation and may have distinct physiological roles. While UCP1 and UCP3 are expressed only in peripheral tissues (UCP1 only in brown adipose tissue, and UCP3 solely in skeletal muscle and the heart in humans), and UCP4 and BMCP1 are predominantly expressed in the CNS, UCP2 is expressed in muscle, adipose tissue, spleen, and the CNS [41,47,48]. In the healthy brain, UCP2 is expressed predominantly in neuronal populations of subcortical regions that are involved in the central regulation of autonomic, endocrine, and metabolic processes. These regions include the arcuate nucleus, a key brain region in determining energy balance.

We hypothesized that if UCP2 in neuronal circuits is a functional uncoupler in a manner similar to what was found regarding UCP1 in the brown fat, the proton leak of mitochondria in UCP2-containing brain regions should be increased. Indeed, we found that the mitochondrial respiratory control ratio (RCR) in rat mitochondrial extracts from regions with abundant UCP2 expression (hypothalamus) was significantly

higher than that measured in regions that lack UCP2 expression (the striatum-lateral thalamus region). The presence of decreased mitochondrial energy coupling efficiency (increased proton leak) in UCP2 containing brain regions supported our hypothesis that a thermogenic mechanism is intrinsic to distinct neuronal pathways. To test this further, brain tissue temperature in the face of steady core body temperature was determined at several dorso-ventral and medio-lateral locations in rats, and it was found that UCP2-containing brain regions have a significantly higher local temperature when compared to other sites or to the core body temperature. These observations raised the possibility that a mitochondrial uncoupling mechanism participates in the central regulation of whole body metabolism and that in this process, temperature is a determinant factor [53]. To address this, we analyzed feeding responses of UCP2 overexpressing and UCP2 knockout animals. In these studies, we found that overexpression of UCP2 in the hypothalamus, where it is co-expressed with the most potent orexigenic neuropeptide, NPY [53], results in elevated levels of hypothalamic NPY levels and food intake. In contrast, UCP2 knockout animals exhibited blunted rebound feeding after a short fast [54,55]. In further support of the idea that a hypothalamic thermogenic mechanism may contribute to the regulation of feeding circuits is our observation during the development of diet-induced obesity of the induction of mRNA levels of a heat-inducible protein, heat-responsive protein 12, in the hypothalamus [56]. This is a time when peroxisome-driven beta oxidation of fats appears to be increased in the hypothalamus [57], and mitochondrial fusion emerges [58-60].

### **PROPOSED APPROACH FOR THE TESTING OF THE CONCEPTUAL AND PRACTICAL BASIS OF THE TEMPERATURE HYPOTHESIS**

If hypothalamic temperature is a major determinant of the outflow of the arcuate nucleus metabolic circuitry, then approaches that selectively up- or down-regulate local

temperature would provide non-invasive and non-pharmacological tools to regulate energy expenditure. If such a methodology succeeds, therapeutic approaches to obesity and related disorders would be dramatically changed and enhanced. Our proposal aims to clarify the conceptual and practical correlates of such approaches.

### *Conceptual Questions Regarding Hypothalamic Temperature and Metabolic Output*

The hypothesis predicts that if hypothalamic temperature is critical for the output of the central metabolic circuitry, then metabolic malfunctions (such as obesity and type 2 diabetes) may be accompanied by altered hypothalamic temperature. Thus, it will be important to determine hypothalamic temperature and its correlation to core body temperature in lean and obese subjects. Brain temperature monitoring has been very difficult to accomplish. Although small temperature probes are available for deep brain temperature measurements [53], it nevertheless remains an invasive approach that involves destruction of blood vessels and induction of acute inflammatory responses, all of which will have a direct impact on local temperature. Of course, assessing fluctuating hypothalamic temperature in relation with metabolic parameters will provide only temporal relationship between these events. For this, it will be necessary to assess the effect of hypothalamic temperature rise and fall on feeding behavior, energy expenditure, electrophysiology, and synapse organization of the melanocortin system.

### *Practical Aspects of Hypothalamic Temperature and Metabolic Output*

While animal models will allow for the testing of conceptual and some practical issues, they will not answer the critical question, i.e., how hypothalamic temperature manipulations could aid the human metabolic condition. In humans, environmental cold exposure produces peripheral vasoconstriction resulting in decreases in peripheral blood flow, thus reducing convective transfer between the body's core and shell (i.e.,

skin, peripheral fat and skeletal muscle). Responses to cold exposure are moderated by factors that influence heat production and heat loss, including the magnitude of the metabolic response, and individual characteristics such as body composition, age, and sex [49,61-63]. Individuals with a large surface area to mass ratio (lean) experience greater declines in body temperature during environmental cold exposure than those with smaller surface area to mass ratio (obese), and insulation is often correlated with subcutaneous fat thickness. Fatter individuals shiver less and experience smaller declines in body temperature during cold exposure than lean individuals [64]. In normal weight individuals, whole-body cold exposure results in vasoconstriction in the periphery when oral temperature reaches  $\sim 35^{\circ}\text{C}$  and becomes maximal when skin temperature is  $\sim 31^{\circ}\text{C}$  [65]. The metabolic response to mild exposure in both moderate and extremely obese adults [66] is reduced relative to lean subjects [67]. Similar data in our laboratory support these findings in children in whom metabolic rate was much lower than expected during cold exposure [68]. Moreover, tympanic temperature vasoconstriction threshold is delayed in obese subjects ( $35.5^{\circ}\text{C}$  versus  $36.0^{\circ}\text{C}$ , for lean and obese, respectively) during general anesthesia [69].

The attenuated thermogenic response to cold exposure in obese subjects [66-68] is to conserve energy so this may also predispose some individuals to obesity. A number of studies have demonstrated the phenomenon of low or normal body temperature combined with low metabolic rate in individuals prone to obesity, leading to the description of an "obesity prone syndrome" [70]. This syndrome includes the hypothesis that individuals who are not yet obese but are prone to obesity might start with a lower body temperature. As their body weight increases, their temperature might rise and become normal, thus diminishing any relationship between body temperature and obesity. Part of this normalization of body temperature might be related to the insulating properties of body fat or related to defects at the hypo-

thalamic level. Studies in Pima Indians demonstrated that individuals with low metabolic rate and low body temperature were especially prone to become obese [70-71]. Moreover, low sympathetic nervous system activity is associated with body weight gain, and low activity of the adrenal medulla is associated with the development of central adiposity [72]. The data in humans are consistent with those in ob/ob mice, in whom characteristics of this syndrome include poor temperature responses to cold and low metabolic rate, both present before the animals become obese [73]. Although studies in obese humans have measured metabolic responses to acute cold exposure [70,71], these studies have not included metabolic rate, sympathetic nerve stimulation (SNS) responses, and peripheral vasoconstriction measurements in conjunction with skin ( $T_{sk}$ ) and core ( $T_c$ ) temperature measurements. More importantly, there has been no attempt to isolate the effect of hypothalamic cooling on metabolic or sympathetic responses in a population at risk for obesity.

Although the monitoring of hypothalamic temperature of humans is difficult, it is possible to determine the temporal and causal relationship between skin temperature, esophageal temperature, and the metabolic phenotype. Testing heating and cooling devices that can be used on human subjects for the purpose of evoking metabolic alterations.

## CONCLUDING REMARKS

We suggest that brain temperature control is a crucial component in obesity development and that shortcutting the brain metabolic centers by hypothalamic temperature alterations in a non-invasive remote manner could provide a revolutionary approach to the treatment of obesity and related metabolic disorders.

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