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Critical Determinants of Hypothalamic Appetitive Neuropeptide Development and Expression: Species Considerations

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Abstract

Over the last decade there has been a striking increase in the early onset of metabolic disease, including obesity and diabetes. The regulation of energy homeostasis is complex and involves the intricate integration of peripheral and central systems, including the hypothalamus. This review provides an overview of the development of brain circuitry involved in the regulation of energy homeostasis as well as recent findings related to the impact of both prenatal and postnatal maternal environment on the development of these circuits. There is surprising evidence that both overnutrition and undernutrition impact the development of these circuits in a similar manner as well as having similar consequences of increased obesity and diabetes later in life. There is also a special focus on relevant species differences in the development of hypothalamic circuits. A deeper understanding of the mechanisms involved in the development of brain circuitry is needed to fully understand how the nutritional and/or maternal environments impacts the functional circuitry as well as the behavior and physiological outcomes.

1. Introduction

In recent decades, obesity during infancy and childhood has become a devastating consequence of the “obesity epidemic” in our country and around the world. According to the recent National Health Examination Survey (NHANES), 33.6% of children within the U.S. are considered overweight, as are 12% of infants [1]. Similar to that in adults, the increased prevalence of childhood obesity has led to an increased risk of hypertension, type II diabetes, dyslipidemia, left ventricular hypertrophy, nonalcoholic With childhood obesity, the resulting metabolic disturbance may permanently disrupt normal development and increase susceptibility to these diseases independently of genetic factors and nutrition in adulthood.

Genetic shifts are not the at the root of the surge in adult obesity

In the United States, it is estimated that nearly two-thirds of adults are either overweight or obese [2;3;4;5]. According to the Center for Disease Control and Prevention (CDC), for adults aged 20-74 years the prevalence of obesity increased from 15.0% in the 1980's to 32.9% in

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the 2006. However, since 2003 the prevalence of obesity has not increased, but the degree of obesity has. Worldwide, excess body-weight affects 1.1 billion adults [6]. In adults, obesity is diagnosed as a body mass index (BMI) of 30 kg/m² or higher (calculated by dividing body-weight by height squared). Obesity is part of a larger disease process termed “metabolic syndrome” which generally includes insulin resistance, impaired glucose tolerance, hypertension, elevated triglycerides and/or cholesterol, and elevated urinary albumin excretion rate, an indicator of renal failure [7;8;9]. If sustained for long periods of time, these conditions expand to to diabetes, cardiovascular disease and neuropathies.

Considering the sheer numbers of individuals dealing with issues of body-weight regulation, only about 5% of obesity is linked to known single-locus gene mutations. Taking into account the dramatic rise in the incidence of obesity in recent decades, Mendelian genetic shifts must be ruled out as the direct causative factor of this incidence. However, a more likely explanation is that the majority of humans are genetically susceptible to obesity when consuming a calorically dense diet. The growing population of obese individuals indicates that the majority of cases of obesity are likely a combination of genetics, epigenetics and environment [10;11].

Escalating prevalence of obesity affects child health

In children, metabolic disease has increased concomitantly with increases in adult populations. Childhood overweight/obesity is defined using BMI in the same calculation as previously described for adults. However, for children the BMI is then plotted on a growth chart - with greater than the 95th percentile considered overweight or obese. A BMI between the 85th percentile and 95th percentile is considered at risk for becoming overweight. Among U.S. children the overall prevalence of risk for overweight is estimated to be 33.6% for the years 2003-2004 [1]. Between studies analyzed in 1999-2000 and 2001-2002, the prevalence of overweight/obese children aged 2-5 years increased from 5.0% to 13.9%; for those aged 6-11 years, prevalence increased from 6.5% to 18.8%; and for those aged 12-19 years, prevalence increased from 5.0% to 17.4%. The serious issue in our modern times is that nearly 15% of the US child population is in the 95% percentile. Many risk factors have been identified for childhood obesity. These include parental obesity, higher birth weight, higher weight gain during the first year and short sleep duration at age 3, neonatal adiposity rebound due to low weight or weight gain at birth, more than 8 hours of television per week at age three, and presence of catch-up growth phases [12;13;14]. Maternal undernutrition during pregnancy, maternal obesity during pregnancy, low birth weight and high birth weight all appear to predispose individuals to obesity and type 2 diabetes later in life [15]. In addition, milk formula feeding as opposed to breastfeeding has a higher association with long-term obesity [16;17]. Smoking during pregnancy is also linked with obesity in offspring [18]. Importantly, population-based human studies reveal correlations between fetal high-fat diet exposure and adult weight regulation [19;20;21]. With so many factors appearing to have a significant impact on long-term body-weight maintenance, it is difficult to estimate the relative contribution of each to the childhood obesity epidemic.

Obesity during childhood negatively impacts many facets of a child's well-being encompassing cardiovascular health, musculoskeletal development, reproduction (including puberty) and psychological health. Though these problems affect the peripheral organs and functions, they are all controlled functions of the central nervous system. In the Bogalusa Heart Study, a study of the long-term cardiovascular health of over 16,000 children and young adults, overweight children were reported to be 4.5 and 2.4 times more likely to have elevated systolic and diastolic blood pressures, respectively [22]. Hypertension was found to be 22% more likely in obese than nonobese children [23]. Overweight children report a greater prevalence of fractures and musculoskeletal discomfort as well as greater impairment of mobility [23]. Various parameters of childhood metabolic syndrome such as elevated cholesterol, glucose and triglyceride levels

are associated with pre-pubertal sleep disordered breathing [24;25]. Children with high body-weight, either at birth or later in childhood, are at increased risk for future asthma [26].

The most immediate yet underemphasized consequences of obesity during childhood are psychosocial, resulting in discrimination and negative self-image during adolescence [27;28]. Obese adolescent females are at especially high risk for major depressive and anxiety disorders [29]. Together, these findings indicate that childhood obesity has damaging implications in the immediate as well as long-term.

Increased prevalence of metabolic syndrome is supported by the fetal origins hypothesis

The *fetal origins of disease hypothesis* or Barker Hypothesis purports that adverse intrauterine conditions can cause permanent changes in the embryo/fetus, predisposing it to chronic disease later in adult life [30;31;32]. Cohort studies have shown that disturbed intrauterine growth has a negative influence on the development of the cardiovascular system and favors the occurrence of hypertension, insulin resistance, hypercholesterolemia, and hyperuricemia in adult life [33;34;35;36]. The most well documented examples are from the Dutch Famine Studies. Evidence from these cohorts revealed that the early nutritional deprivation in pregnant mothers, as a result of famine, produced small-for-gestational-age (SGA) babies. The consequences were irreversible health issues in the offspring. Through longitudinal studies of this population, it came to light that exposure to famine during any period of gestation can lead to glucose intolerance during mid-life (50-60 years) [37]. The worst consequences exist for individuals who experienced famine during early gestation.

These early studies linking low-birth weight and late-onset obesity and diabetes laid the groundwork for the *thrifty phenotype hypothesis*, which proposes that poor nutritional conditions during gestation and early infancy can modify metabolic systems in the offspring to adapt to expectations of chronic undernutrition. With a subsequent calorically dense environment, these offspring are poorly equipped to cope with energy-dense diets and are possibly wired to store as much energy reserves as possible, leading to late-onset metabolic syndrome [30]. Insults such as poor nutrition and decreased maternal calorie or protein consumption are known to cause intrauterine growth retardation in various mammals [38;39;40;41;42]. However, in today's world of overweight and obese adults, the more likely insult during pregnancy is overnutrition and diabetes. Yet overall poor nutrition, such as poor protein sources, definitely contribute to the situation.

Importance of the Prenatal Period

In support of the Barker Hypothesis, it is well established that abnormal fetal growth affects long-term metabolic health [43;44]. High maternal BMI, even in the absence of maternal gestational diabetes, is a risk factor for large-for-gestational-age (LGA) infants who are highly susceptible to lifelong disease [45;46]. Fetal exposure to maternal obesity increases the risk of childhood metabolic syndrome by approximately two-fold [15;45]. In addition, obesity during pregnancy has implications for morbidity and mortality for the mother and the baby. Maternal obesity more than doubles the risk of stillbirth and neonatal death [47;48]. Overweight mothers also have a greater risk of hypertensive complications and preeclampsia [49]. Finally, women with poor glycemic control and/or gestational diabetes have a high risk of giving birth to a macrosomic baby that is more likely to develop obesity and insulin resistance later in life [50;51;52]. Though much work has been done to document the trends and associations between maternal nutrition and health during gestation and long-term metabolic health of offspring, it is still not known which mechanisms are responsible for the increased risk for obesity in adulthood.

Critical periods exist in child development during which nutrition and health status may predispose the individual to life-long body-weight regulation difficulties. One key period is the *in utero*/early infancy period [43]. Hypothalamic appetite regulatory systems develop and mature during the *in utero*/early infancy period. The primary focus of this review is to describe the normal development of hypothalamic appetitive systems as well as their development in the context of genetic, nutritional and environmental manipulations to further understand their impact on body-weight regulation.

2. Central circuits involved in the regulation of energy homeostasis

The neuronal circuitry that controls food intake and energy homeostasis has been extensively studied over the last two decades. Numerous excellent reviews have been published covering this literature [53;54;55;56;⁵⁷;58;⁵⁹;60;61;62;63;64]. Briefly, appetite regulation is a complex system with both homeostatic and hedonic aspects as well as central and peripheral components integrated at the level of the hypothalamus and brainstem [65] (Figure 1). At the crossroads of the central nervous system and peripheral components is the arcuate nucleus of the hypothalamus (ARH). A critical aspect of ARH neurocircuitry is the interplay between the anorexigenic peptide-containing neurons such as α melanocyte-stimulating hormone (α MSH) (a cleavage product of the pro-opiomelanocortin, or POMC, gene) and cocaine and amphetamine-related transcript (CART), and orexigenic peptide-containing neurons, namely neuropeptide Y (NPY) and agouti-related peptide (AgRP) (Figure 1). These neurons are sensitive to blood-borne indicators of energy status (leptin, insulin, glucose, fatty acids, amino acids and gut hormones) and project to other feeding centers in the hypothalamus such as the dorsomedial nucleus of the hypothalamus (DMH) and lateral hypothalamic area (LHA) where they recruit or modulate orexin and melanin concentrating hormone (MCH) neurons (Figure 1). Projections from these nuclei re-converge on the paraventricular nucleus (PVH), which is a site of integration of metabolic signals from many parts of the brain (Figure 1). The efferent outputs of the PVH involved in the regulation of food intake are poorly understood, but is thought to involve inputs onto corticotrophin releasing hormone (CRH) and/or thyrotropin releasing hormone (TRH). The efferent outputs for the regulation of energy expenditure are better understood and involve CRH and oxytocin neurons in the preautonomic area of the parvocellular region of the PVH.

In addition to the critical role of the hypothalamus, various nuclei in the hindbrain contribute to the control of food intake particularly through motor and gustatory mechanisms (Figure 1). Brainstem control of food intake is largely mediated through the dorsal vagal complex (DVC) which includes the dorsal motor nucleus of the vagus (DMV), nucleus of the solitary tract (NTS) and area postrema (AP) (see reviews [66;67]). For example, mastication, swallowing, and salivation are controlled by the caudal brainstem. The DVC acts as a relay site for short-acting gastrointestinal signals, mediated predominantly through the vagus nerve. In addition, gut peptides communicate with other hindbrain nuclei at the AP, a circumventricular organ with decreased blood brain barrier. These gut-hindbrain connections are sufficient to produce satiety on their own, but can be overridden by more cognitive inputs of body-weight regulation.

Superimposed on the homeostatic neurocircuitry of body-weight regulation are the hedonic aspects of food intake (*see review* [65]). This behavioral aspect, based on the palatability and reward of food, can override the normal requirements of daily energy needs to meet normal body weight homeostasis. These hedonic neurocircuits span brain areas such as the nucleus accumbens (NAc), ventral pallidus (VP) ventral tegmental area (VTA) and higher cortical areas such as the prefrontal cortex and converge on the LHA. These pathways utilize biological substrates such as glutamate, opioids, endocannabinoids and dopamine. While these pathways can over-ride homeostatic regulation they are still responsive to peripheral metabolic signals,

most notably, leptin [68;69;70;71;72;73] and ghrelin [74;75]. The well-known effects of these compounds on mood likely contribute significantly to the powerful and overwhelming impact of the hedonic pathways on food intake.

3. Development of homeostatic circuits

Though many of the neuropeptide systems which control food intake are “born” *in utero* in most species, the timing for the development of their projections is dependent on the neuronal phenotype as well as there being a large species difference. The most well studied nucleus in this regards is the ARH. In the rat and mouse ARH neurons have a birth date around E11.5-12.5 [76;77;78] (Dr. L Zeltzer, personal communication). However, ARH neurons do not start to develop their efferent projections until near the end of the first postnatal week, and they are not completed until the end of the 3rd postnatal week [79;80]. The physiological significance of this delayed postnatal development is the lack of homeostatic regulation of energy balance through the ARH. An example of this is the inability of leptin to stimulate energy expenditure and inhibit food intake until after the 3rd postnatal week [81;82;83;84]. This lack of homeostatic feedback on body weight and food intake is not due to a lack of function receptors within the ARH since leptin can stimulate pSTAT3 immunoreactivity and regulate ARH gene expression soon after birth [80;82]. Furthermore, fasting (or rather maternal deprivation) increases ARH-NPY expression within the first postnatal week [85;86]. However, these infant pups lack the ability to generate a rebound hyperphagia to normalize body weight [87]. Models that result in the abnormal development of the ARH circuits lead to obesity. This includes 1) the neonatal MSG-treated animal that leads to an early lesioning of the ARH, resulting in stunted growth and increased adiposity [88]; 2) the obese leptin deficient *ob/ob* mouse which lacks normal development of the ARH circuits [79]; and 3) the Levin high fat diet obese (DIO) Sprague-Dawley rat [89]. In this last model, Bouret *et al.* demonstrated the DIO sensitive rat, which has a polygenic mode of inheritance for diet induced obesity, has a significantly reduced development of ARH circuits compared to the diet resistant animals. The development of specific neuropeptide circuits and the key signals that may be involved in the development of ARH circuits will be discussed below. Overall, this highlights the importance of the ARH circuits in the regulation of energy homeostasis. So while it is recognized that there are many brain regions involved in the regulation of body weight and food intake, disruption of the normal development of the ARH leads to obesity ... at least in rodents.

There is an overwhelming deficiency in our understanding of the development of brain circuits that are involved in the regulation of energy homeostasis in higher species. In humans and nonhuman primates (NHP), the neurons in the ARH can be morphologically distinguished during the 2nd trimester [90;91], surprisingly, not that different from the rodent; however, the development of their projections occurs during the 3rd trimester of pregnancy [91]. This shift in timing between species has many potential implications for the impact of maternal health and nutritional status versus milk quality and availability on the developing offspring. This has been most extensively studied in the rodent. The subsequent portions of this review will focus on studies in rodent models but will include additional discussion on studies performed in higher species, such as in primates and sheep, when available. This review will cover 1) the natural development of the specific neuropeptide circuits involved in the regulation of energy homeostasis, 2) the evidence for the role of peripheral metabolic signals in this development and 3) the effects of maternal and early postnatal nutrition and metabolic health on the development of these circuits.

Hypothalamic neurocircuitry

Neuropeptide Y—In considering the nutrient demands to meet the rapid growth of a neonate, the early appearance of NPY within development is not surprising. In the rodent, NPY mRNA is present in the brainstem and forebrain as early as embryonic day 14 (E14) [92;93]. There is

a relatively abundant level of NPY expression in the ARH throughout life in the rodent, starting at birth. However, NPY mRNA expression does increase from birth, to peak around P15-16, and subsequently slowly declines to adult levels by P30 [94]. While in the normal adult mouse, the expression of the orexigenic neuropeptide within the hypothalamus is restricted to the ARH, during early postnatal development NPY mRNA is also transiently expressed in the DMH, PVH, LHA, perifornical region (PFR), anteroventral periventricular (AVPV) and suprachiasmatic (SCN) nuclei as well as the medial preoptic area (MPO) [85;94;95;96]. The significance of this largely developmental-specific expression of NPY in multiple hypothalamic nuclei has not been elucidated. Yet it is difficult to overlook the fact that many of these regions are key sites for the regulation of energy homeostasis.

While NPY is expressed in neurons in the ARH prior to birth in the rodent, the efferent projection of these neurons develop during the postnatal period [85;94]. In spite of this delayed development of ARH-NPY projections, NPY immunoreactive fibers are abundant throughout the hypothalamus, suggesting that these other transient populations of NPY neurons are significant contributors to the NPY tone early in development. However, the efferent projections for most of these other transient populations of NPY neurons are unknown. The brainstem also contains populations of NPY neurons that project to the hypothalamus. Our group has demonstrated that brainstem NPY projections to the PVH are present as early as P2, but likely develop prior to that [87;95]. It might be assumed that since there is an overwhelming abundance of neurons expressing this orexigenic peptide during early development, that NPY may be a critical factor for maintaining sufficient energy intake during this rapid growth phase. It might be expected that a mutation resulting in the lack of NPY during critical developmental stages would result in stunted growth and development. However, this is not the case; rather mice with a genetic knockout of the NPY gene appear to grow normally and maintain a normal body weight phenotype, yet have an increased susceptibility to seizures [97;98]. Furthermore, loss of NPY receptors also does not cause stunted growth and development, even though some of the receptors are highly expressed in the hypothalamus from birth [87;95;96]. This lack of effect has been attributed to many things, including developmental compensation. Indeed, ARH-NPY neurons also coexpress another potent orexigenic neuropeptide, AgRP; it is possible that AgRP is sufficient to sustain normal food intake necessary for normal growth and development. However, these projections do not develop until later in the postnatal period, and the NPY/AgRP double knockout mouse shows near normal growth and development [99]. So where does this leave us? Does this mean that NPY neurons are not critical for normal growth and development? This depends on how one define “critical”. It is clear that mice can develop to a normal stature without NPY and/or AgRP; however, NPY and NPY/AgRP knockout mice are not completely normal, as they lack the ability to adapt to metabolic challenges, such as fasting and diet induced obesity [100;101]. But the evidence of developmental compensation was highlighted in a study by Richard Palmiter’s group in which they demonstrated that ablation of ARH-NPY/AgRP neurons, which would include the GABA signal from these neurons, in adulthood results in starvation and wasting [102], a phenotype that reinforces the unique importance of these neurons in the regulation of energy homeostasis. Interestingly, ablation of these neurons soon after birth results in a near normal growth and development. What signals allow for this developmental compensation, or exactly what systems are responsible for the compensation remain a mystery. But this adaptation may not be surprising since there are numerous systems that have orexigenic effects.

In contrast to rodents, in both the human and NHP, NPY neurons are born during the middle of the second trimester [90;91]. Like the rodents during early development, NPY expressing neurons are evident throughout the hypothalamus; such as in the SON, PVH, and DMH. However, this expression is not limited to early development in the NHP, but rather these neurons continue to express NPY in the normal adult [103]. In the NHP, ARH-NPY projections begin to develop in the late second trimester, and they continue to develop throughout the third

trimester and into the early postnatal period [91]. While the effects of maternal nutrition on the fetal NPY system have been studied in the sheep, the development of their projections has not been characterized [104;105;106]; however, it would be assumed that it would be similar to the NHP and human. The *in utero* development of the NPY system in higher species again raises the question as to the physiological relevance of the development of an orexigenic system during the prenatal period, prior to the need for independent ingestion. The only evidence of a significant role comes from the sheep model in which icv NPY injections were shown to stimulate swallowing of amniotic fluid in the fetal sheep [107], a function that is thought to be important for fluid balance and for the development of the gastrointestinal tract.

Melanocortins—In adult animals (including most species), AgRP is exclusively colocalized in ARH-NPY neurons; this is also the case throughout development. Surprisingly though, AgRP mRNA levels appear to be first detectable during the first few postnatal days whereas NPY mRNA levels are detected much earlier in gestation [95;108]. This suggests that NPY and AgRP may be differentially regulated within the same cell, possibly by different transcriptional regulators and/or different afferent inputs. Indeed, in the adult rodent, NPY and AgRP are not always regulated in parallel [109;110;111]. However, as expected, the development of AgRP projections follows the pattern of innervation by NPY [95].

In a like manner to NPY, POMC neurons are present within the hypothalamus in the developing rat at E12-13, several days before their appearance in the pituitary [112;113]. At birth, and unlike AgRP, α MSH fibers are evident throughout the hypothalamus, including the PVH [87;112]. At first glance this may suggest that ARH-POMC (α MSH) projections develop sooner than ARH-NPY/AgRP projections. However, we know from studies by Bouret and Simerly [79;80] that this is not likely that case, since they used a nondiscriminant anterograde tracer to demonstrate that all ARH projections are not present at birth. It is more likely that ARH-POMC neurons from the solitary tract nucleus in the brainstem [114;115;116;117] are contributing to the PVH α MSH immunoreactivity at this early age [87]. Though the projections of POMC neurons become evident towards the end of gestation they do not reach their full maturity until weaning.

It may seem counterproductive to have a satiety signal intact at such an early developmental stage, but early work has shown that endogenous α MSH functions to stimulate fetal growth [118;119;120]. When antibodies to α MSH are administered in late gestation, fetal growth retardation results [118]. These data reveal substantial differences in α MSH actions during early development and adulthood, the former being growth promoting and the latter resulting in satiation. These divergent actions have not been further pursued. The growth promoting effect does conflict with evidence that POMC or MC4 receptor knockout mice are larger in both lean and fat mass [121;122]. However, this may just be highlighting the critical role that melanocortin agonists play in the regulation of energy homeostasis later in life.

In the NHP, POMC neurons are readily detectable at gestational day 100 (early 2nd trimester) [91]. Furthermore, the development of ARH-POMC projections, like the NPY/AgRP projections, occurs during the 3rd trimester; however, there is a delay in their development. While ARH-NPY/AgRP projects are readily detectable by gestational day 130 (early 3rd trimester), α MSH immunoreactive fibers are sparse until later in the 3rd trimester when α MSH projections become abundant. This is not simply due to preferential conversion to other POMC derived peptides since ACTH and β -endorphin immunoreactivity are also sparse in the early 3rd trimester (Grayson and Grove, unpublished observations).

Highlighting the importance of the melanocortin system, and especially the MC4 receptor, humans with mutations in this system develop obesity early in childhood. Like the mouse models, these individuals also have increased fat and lean mass and are generally taller [10;

11;123;124;125;126;127;128]. However, while MC4 and POMC mutant mice do develop obesity this does not occur until after or near weaning, paralleling the development of this circuitry.

Cocaine and Amphetamine Related Transcript (CART)—CART is another neuropeptide implicated in diverse physiological functions, which include stress response, feeding, reward and autonomic function. CART is abundantly expressed in the brain, including hypothalamic regions such as the VMH, PVH, ARH, but also the nucleus accumbens, VTA, thalamic nuclei, dorsal raphe, amygdala, olfactory bulbs, spinal cord and NTS [129;130]. In the rodent, CART has been found to co-localize with other prominent neuropeptides and neurotransmitters such as POMC, tyrosine hydroxylase and MCH in the hypothalamus, GABA in the nucleus accumbens and acetylcholine in the gut myenteric system [131;132;133;134]. While CART likely has many physiological roles it has primarily been studied as a feeding-related peptide. The primary limiting factor in studying the CART systems is that its receptor remains unidentified.

CART neurons appear in the hypothalamus as early as E11 in the rodent [134]. During the postnatal period CART immunoreactive neurons are detectable within the LHA as early as P5; however, it is not until between P11 and P15 that neurons in ARH become detectable by this method (Figure 2). This relatively late onset of expression in the ARH correlates with the development of these projections. Because there are so many populations of CART neurons it is difficult to know when each population develops its projections; however within the ARH it seems likely that it coordinates with ARH-POMC projections and within the LHA with MCH projections (see below). Very little is known about the potential role of CART during development, except for a potential role in the neuronal migration of dopamine (DA) neurons [134]. This was evidenced by the presence of DA (tyrosine hydroxylase-positive) neurons migrating laterally from the neuroepithelium through the area containing the CART-immunoreactive neurons. These DA cells exhibited close dendritic appositions with CART perikarya. Mice with a deletion of the CART gene; however, display no abnormal developmental phenotype, although this has not been extensively studied. They do develop obesity later in life, but this is likely secondary to dysfunction in the pancreatic beta cell, which also expresses CART [135].

In the adult rodent, CART is known to colocalize with α MSH in the ARH and MCH in the LHA [136]; however, this does not appear to be phylogenetically conserved. In the human, CART is colocalized with NPY and AgRP and does not colocalize with α MSH [137]. In the NHP, however, CART forms an independent population of neurons within the ARH that does not express either α MSH or AgRP at any stage of development [91] (Figure 3). This significant species difference accents the yet undescribed role of CART in the primate regarding food intake. Because, like the rodent, the NHP contains many populations of CART neurons it is difficult to characterize the development of their projections using histochemistry techniques.

Orexin and Melanin Concentrating Hormone—Orexin (hypocretin) and MCH neurons within the LHA also play key roles in the regulation of energy homeostasis. These two independent populations of neurons are exclusively localized in LHA area, but have a broad distribution throughout this region including cells scattered as medially as the DMH. Furthermore, both populations of neurons have an impressive distribution of efferent projection that extend to most regions of the brain. Orexin neurons are evident in the LHA of the rodent as early as E19 [138]. They have robust synaptic activity around parturition [139], suggesting active afferent inputs, but the projections do not fully develop until the 3rd postnatal week [138]. In contrast, MCH mRNA expression is detectable as early as E13 [140] with immunoreactive fibers being detectable as early as E18; however, MCH fiber projections are not readily detectable throughout the brain until P5 [141].

Both orexin and MCH immunoreactive neurons are readily evident in the LHA in the NHP as early as G100 (late 2nd trimester; Figure 4). However, it seems likely that at least the orexin neurons must exist even earlier since their projections to the ARH (Figure 5), as well as to other brain regions, are apparent as early as G100, even though they are relatively sparse at this age. In contrast, the MCH projections are delayed in their development and don't become prominent until just prior to parturition (Figure 5). It has always been intriguing as to the physiological relevance of such an early development of the orexin system. Why does the 2nd trimester fetal primate brain need orexin projections to such far reaches as the cortex? This implicates the orexin system as an important developmental signal in the primate brain. In the adult NHP, CART and orexin immunoreactive fibers have the highest density projections of all neuropeptide systems we have studied.

Midbrain and Brainstem Circuitry

In direct contrast to hypothalamic circuit maturation in the rodent, the brainstem neurocircuitry matures prenatally and with further refining and direct input from hypothalamic nuclei postnatally. Therefore, in the rodent, these circuits are conceivably far more sensitive to the maternal environment in comparison to the hypothalamic development which appears to be influenced more by the postnatal nutrient environment. This section will only discuss the development of the serotonin, dopamine and norepinephrine systems because of their significant roles in the regulation of energy and glucose homeostasis, [71;142;143;144;145;146;147;148;149;150;151;152;153;154]. However, it should be recognized that these systems are extensive, with many different populations of neurons, making it difficult to distinguish the development of one population versus the next. Also, very little is known about the development of these systems, with regards to energy homeostasis, in higher species.

In the rodent, serotonin-*ir* appears in the raphe at E12 [155] and subsequent expression of the serotonin transporter (5HTT) ensues shortly thereafter [156;157]. By E18-20, serotonin immunoreactivity can be visualized throughout the brain, including the hypothalamus [156]. Because of the ubiquitous expression of serotonin in the brain, nearly every neuropeptide and neurotransmitter system is impacted by serotonin terminal differentiation [158]. However, the exact timing of the development of serotonin projections to hypothalamic circuits involved in the regulation of energy homeostasis has not been directly studied. The melanocortin neurons are a primary efferent target by which serotonin regulates energy homeostasis [142;143;144;145;146;159;160]. Since the serotonin system develops relatively early it could be a key signal for the normal development of the melanocortin system.

Similar to serotonin, noradrenergic neurons appear on E12 in the rat [161]. In general immunoreactivity for the different components of the noradrenergic system (i.e., the density of immunoreactive projections) is surprisingly more robust earlier in development in comparison to the serotonin system even though both types of neurons have similar birth dates [162]. In the rat, a wave of proliferation in fiber density and intensity occurs during the first postnatal week, with adult levels being reached by the third postnatal week [162]. Noradrenergic projections to the hypothalamus, and especially the PVH, have been strongly implicated in glucoprivic feeding - hyperphagia induced by hypoglycemia. Glucoprivic feeding is induced by administration of 2-deoxy-D-glucose, a nonmetabolizable form of glucose. These brainstem catecholaminergic projections, which also contain NPY, develop during the perinatal period in the rodent [95;163;164]. However, glucoprivic feeding responses do not appear to develop in rodents until weaning at approximately 21 days postnatal [165;166]. In general, noradrenergic innervation appears to be crucial to the proliferation of brain astrocytes and glia and therefore neuronal migration is affected throughout the brain when noradrenergic development is affected [161].

The dopamine system within the VTA is intricately involved in the rewarding and/or addictive properties of food [167;168;169;170;171]. The dopamine neurons within the VTA have been shown to be directly regulated by important peripheral metabolic signals such as leptin and ghrelin [71;154;167]. Like the above noradrenergic and serotonin monoamine transmitter systems, the VTA dopamine projections appear to develop early in the postnatal period in the rodent, as indicated by the presence of dopamine terminals within the nucleus accumbens, a major target site for VTA-dopamine neurons [172]. However, synaptic maturation within the nucleus accumbens does not fully develop until the 3rd postnatal week [172], indicating that both maternal and neonatal nutrition and environment may impact their development. While little is known about the signals that are important for the normal development of this system, the implications for abnormal development are very broad, including changes in food preferences and increased drive to seek palatable or more “rewarding” or “comforting” foods, as is observed with drug and alcohol addictions [171]. Maternal obesity in rodents, induced by high fat diet (HFD) feeding, has recently been shown to have long term effects on the VTA-dopamine system [173]. In these studies HFD offspring, that were weaned and maintained on a standard chow diet, had increased tyrosine hydroxylase expression in the VTA and increased DA content within the nucleus accumbens. Furthermore, the HFD offspring were relatively and significantly insensitive to amphetamine induced activity, compared to control offspring. What components of the HFD exposure or of the maternal phenotype (obesity, hyperinsulinemia, hyperleptinemia, etc) responsible for these effects on the DA system were not determined; however the changes the HFD offspring point to potential life-long changes in behavior.

Leptin and insulin

Though leptin is largely considered an anorectic hormone, there are dynamic changes in leptin expression and levels during the pre- and postnatal period in rodents that suggest a more critical role in early developmental processes. As mentioned above, although neurons within the ARH can respond to leptin early in the postnatal period, as indicated by pSTAT3 activation, these signals are not transmitted to downstream sites [81]. Therefore, leptin can not modulate food intake or energy expenditure through actions in the ARH until after the 3rd postnatal week [82]. However, there is a sizable (more than 5 fold) surge in leptin production and secretion during the 2nd postnatal week that is independent of the amount of fat present [174], suggesting that leptin must be playing some development role. The most striking demonstration of a functional role of leptin as a neurotrophic factor is its importance for the normal development of ARH projections. The obese leptin deficient *Lep^{ob}/Lep^{ob}* mouse has a significant impairment in the development of the both ARH-NPY/AgRP and ARH-POMC projections [79]. While it has not been systematically investigated, this deficiency is not apparent in some other hypothalamic projections (such as the DMH). Furthermore, treatment of *Lep^{ob}/Lep^{ob}* mice with exogenous leptin specifically during the early postnatal period, but not in adulthood, normalizes the development of these projections, indicating that there is a critical window for leptin actions [79]. Further demonstration that the postnatal leptin surge is important for “programming” metabolic systems, recent work using a leptin antagonist specifically during the time of the postnatal leptin surge results in adult-onset leptin resistance as well as increased overall adiposity and hyperleptinemia, in addition to greater propensity to obesity and diet-induced weight gain [175]. This study did not investigate the effect on the development of the hypothalamic circuitry. Unfortunately, the story is not as simple as the leptin surge being important for the normal development of metabolic systems. The studies in the *Lep^{ob}/Lep^{ob}* mouse indicate that no leptin is definitely “bad” for the development of ARH-melanocortin circuits; however it is now apparent that too much or too little and the timing of the leptin surge can all affect the development of these circuits. For example, our group and others have demonstrated that early postnatal overfeeding, using a “small litter” manipulation, can result in an exaggerated leptin surge that results in early leptin resistance in ARH neurons

(unpublished observations)[176;177] (This model is discussed in greater detail below). Importantly, this leptin resistance persists past the point that endogenous leptin levels normalize. Bouret and Simerly have recently demonstrated that this rather subtle overfeeding manipulation early in life also results in reduced development of ARH projections, similar to that observed in the Lep^{ob}/Lep^{ob} mouse (Bouret and Simerly, personal communication). Furthermore, Yura et al. demonstrated that offspring from dams with food restriction during pregnancy have a premature leptin surge during the early postnatal period [178]. These offspring develop a hypersensitivity to a HFD displaying increased weight gain and adiposity. Surprisingly, mimicking the premature leptin surge with exogenous leptin administration resulted in a similar hypersensitivity to the HFD. Both groups of offspring showed abnormalities as adults in neuropeptide terminal densities in the PVH; thus, simply shifting the timing of the leptin surge is sufficient to cause developmental abnormalities in the hypothalamic circuitry.

To date, there is no evidence supporting the importance of leptin for the development of ARH circuitry in higher species. In the NHP, leptin levels are very low to undetectable until the middle of the 3rd trimester, which correlates with the late development of adipose tissue. This is the case even in fetal offspring of pregnant animals that are obese and hyperleptinemic [179]. Thus, significant leptin in the developing fetus is not evident until after the initiation of the ARH-NPY/AgRP projections [91]. However, ARH-POMC projections are more delayed in their development; thus leptin could have more of an impact on this specific projection. Furthermore, leptin still may play a role in the refinement or arborization of the projections [180;181;182], which occurs closer to parturition.

In addition to its other roles, insulin is also an important neurotrophic factor during development along with insulin-like growth factor (IGF) and brain derived nerve factor (BDNF) [183]. The local production of insulin within the hypothalamus and high level of receptor expression confirms the importance of insulin signaling for neuronal maturation in addition to its neuromodulatory functions. Insulin immunoreactivity and insulin receptors are present very early during fetal life [184]. However, the function of insulin within the hypothalamus has not been emphasized in the literature predominately because of the difficulties in separating central actions versus the robust peripheral expression and activity of insulin.

4. Neurocircuitry put to the test: Dietary manipulations exacerbate the system

Animal models

Many animal models have been developed to investigate the effects of dietary manipulations on fetal and postnatal development and growth. Models resulting in an adult obese phenotype have relied on maternal overnutrition and undernutrition during gestation. These models change the hormonal and nutrient status of the mother that are then reflected in fetal growth and development. Conversely, models of placental insufficiency result in suppressed blood flow and hence nutrients to the growing fetus in the absence of maternal health status being altered. This also leads to maturity-onset obesity due to a series of catch-up growth periods. Alternatively, obesity can also result from neonatal overnutrition or undernutrition. This can be accomplished by creating either very small litters of several pups where an abundance of milk is available, or very large litters whereby milk availability is spread thinly among litter mates, thus limiting developmental growth but again spurring catch-up growth phases. Other groups have formula fed pups with milk of varying composition, thus controlling the nutritional quality of the milk. These are less natural models but do mimic the altered nutrition that might be received in formula-fed infants. In the following sections, each of these types of manipulations will be described in light of their impact on the origin of developmental obesity; however, we will focus primarily on maternal and neonatal overnutrition because of their

relevance to the current epidemic of childhood obesity. Furthermore, because of the extensive number of such studies in some of these areas is not possible to discuss each one in detail, thus we will focus on recent literature in the area.

Maternal overnutrition

Models in which overall content and quality of the maternal diet is altered prior to and during gestation demonstrate long-lasting effects on energy balance in the offspring. While the results from many different groups all support an effect by maternal HFD, there is often varying effects on brain neurochemistry, hormone levels and the extent of obesity as well as sex differences. This is likely attributed to the different types of diets (either different types or percent of fat in the diet), different exposure periods (i.e., chronic vs acute) as well as different rodent models (mouse vs rat). A recent study by Patel's group using rats has shown that fetuses exposed to maternal HFD have elevated serum leptin and insulin levels as well as increased expression of NPY, AgRP, POMC and MC4R in the hypothalamus [185]. The reason for elevations in both orexigenic and anorexigenic neuropeptides is unknown, but suggests that this nutritional manipulation is driving some common excitatory signal within the hypothalamus, possibly increased cytokines and/or corticosterone, that are known to be elevated in response to obesity. It should be recognized that while we understand much of how different hormonal (i.e., leptin, insulin, glucocorticoids and cytokines) and nutrient (i.e., glucose and fatty acids) signals modulate the activity of the ARH circuit in the adult animal, very little is known about how these signals impact neurons during fetal and neonatal life. It is entirely possible that receptor signaling within these different neurons changes throughout development. While the long-term implications on the development of the hypothalamic signal was not investigated the Patel study [185] it is reasonable to speculate that this chronic abnormal drive on these neurons during critical periods of development will impact the manner in which this circuit is programmed. Indeed, others have shown that offspring in this type of model have higher insulin/glucose ratios, higher body fat percentage, and greater triglyceride levels at birth [186], and go on to develop obesity [173;187]. Others have also used a "junk food diet" which is high not only in fat but preferentially higher in sugars and salt. This diet results in enhanced adiposity specifically in female offspring as well as a host of changes in metabolic parameters [188]. The most striking results and strongest evidence of maternal HFD causing long-term reprogramming within the hypothalamus comes from studies performed by Leibowitz and colleagues in which they demonstrated that maternal HFD consumption stimulated neuroepithelium to proliferate and preferentially become orexigenic peptide-producing neurons of the LHA in the offspring [189]. Furthermore, there was increased expression and increased cell numbers of several neuropeptide systems including galanin, dynorphin and enkephalin in the PVH and MCH and orexin in LHA [189]. From these studies it is suggested that the increased neurogenesis in the developing fetus, stimulated by the maternal HFD, would be predicted to cause a broad range of behavioral and physiological complications. Supporting this conclusion, Walker and colleagues [173], as highlighted earlier, have demonstrated that maternal HFD consumption has long-term effects on the VTA-dopamine system, causing desensitization to amphetamines.

Most studies in this field focus on one type of manipulation (i.e., maternal HFD); however, in real situations individuals are faced with multiple types of stressors. Recent studies by Tamashiro's group have investigated the interaction of different types of insults during pregnancy. In these studies, pregnant animals were either exposed to a HFD, repeated novel variable stress or both [190]. Compared to controls, offspring from all three experimental groups appeared normal if they were raised (after weaning) on standard chow diet; however, offspring from all three groups displayed a hypersensitivity to a HFD, in that they exhibited exacerbated weight gain and insulin resistance compared to control offspring. Although predicted, it was surprising from these studies that there was not a significant interaction

between the maternal variable stress and the HFD; however, it is possible that the levels of insult used in each model (60% HFD or 3 weeks of repeated variable stress) elicited maximal effects individually. It is more likely that an interaction would have been observed if a diet lower in fat or a more modest stress regime had been used (K. Tamashiro, personal communication).

The effect of maternal overnutrition has also been studied in higher species. In the sheep, infusions of glucose directly into the fetus for 10 consecutive days, mimicking maternal hyperglycemia, or overfeeding the pregnant sheep results in increased insulin levels, adiposity and increased POMC gene expression in the fetal offspring [105;106;191]. Interestingly, the effect of both fetal glucose infusion and maternal overnutrition appeared to have a relatively specific effect on the POMC system, since NPY, AgRP and CART were unaffected by these treatments. The long-term implications of these fetal changes have not been reported.

Our group has used a NHP model of maternal overnutrition, using a chronic maintenance on a diet high in fat and calories [179]. Like humans, some adult animals became obese and insulin resistant on the HFD, while others maintained a normal body weight, adiposity and insulin sensitivity, even after 4-6 years on the HFD. The primary findings from these initial studies were the observation that 100% of the fetal and neonatal offspring developed signs of fatty liver disease, as evidenced by increased liver triglycerides, oxidative damage and activation of the gluconeogenic pathway. Furthermore, we demonstrated that the fetal offspring also have elevations in a broad range of circulating cytokines. The offspring from the HFD animals also displayed increased adiposity and liver triglycerides through the early postnatal period. What was concerning and surprising from these studies was that the fetal lipotoxicity was evident whether the mother was obese or lean, suggesting that the high fat/high caloric diet alone was significantly contributing to this effect. It remains to be determined to what extent fetal lipotoxicity leads to abnormalities in the development of hypothalamic circuits; although unpublished data from our group indicates that there are abnormalities in the hypothalamic melanocortin system as well as the serotonin system in the dorsal raphe (Grayson and Grove, unpublished observations). To date, no long-term studies have been done in any higher species; however, we can speculate with some confidence that maternal overnutrition or HFD consumption, as has been observed in the rodent models, can and will have long-term effects on the function and efficiency of metabolic systems in the offspring.

Neonatal overnutrition

There are numerous studies investigating the effects of nutritional manipulations during the early postnatal period in rodents, primarily by groups led by Andres Plagemann [192;193;194; 195] and by Mulchand Patel [196;197;198;199;200]. Plagemann's group has performed extensive studies on the metabolic characteristics and hypothalamic programming of pups reared in small vs normal litters. The small litter model (i.e. 3 pups per litter) results in reduced competition for nursing time and, thus, increased milk consumption and increased weight gain during the early postnatal period [201;202]. These animals are hyperphagic and hyperleptinemic and hyperinsulinemic as early as P10 [203]. These offspring maintain their increased body weight throughout life even when weaned on to a standard chow diet; however, the adult phenotype is rather mild, with the animals being only about 10% heavier and having normal leptin levels [204]. In spite of this mild phenotype young adult offspring from small litters have been shown by Plagemann's group to have a shift in the hypothalamic signaling to insulin and leptin as well as numerous neuropeptide and neurotransmitter systems [176;177; 205;206;²⁰⁷;208;209;210;211]. Furthermore, our group has demonstrated that these postnatally overfed animals have reduced adaptive thermogenesis that is likely secondary to abnormalities in regulation of sympathetic outflow to brown adipose tissue [212]. As mentioned earlier, recent studies from our group have shown that the postnatally overfed

animals develop leptin resistance in the ARH, but not the DMH, during the postnatal period (unpublished observations). This may not be surprising since these animals are hyperleptinemic during this period; however, the leptin resistance in the ARH persists into adulthood, even though leptin levels normalize. While these animals have a relatively mild metabolic phenotype as adults, they do display an exacerbated phenotype when given a HFD. The increased sensitivity to the HFD appears to be partially due a prolonged initial hyperphagic response when placed on the HFD. Most animals normalize their caloric intake within two days of being placed on a HFD; however, the postnatally overfed animals take 7-9 days. Furthermore, they fail to increase their activity when placed on the HFD and have a slightly lower BAT temperature, indicating that early life overnutrition has functionally altered the tone of the neurons within the ARH to overall sensitivity to leptin and insulin and perhaps other nutrients such as fatty acids and glucose. Their altered ability to respond to incoming signals is consistent with abnormalities in the development of the ARH circuits, as mentioned earlier (Bouret and Simerly, personal communication).

Patel's groups, using the "pup in a cup" model, have investigated the effects of milk diets either high in lipids (mimicking natural milk) or high in carbohydrates [196;197;198;199;200]. While this artificially raised animal model has its limitations, the importance of these studies is that the investigators are simply altering fat and carbohydrate levels, while the caloric intake and other nutrients are kept constant. In this model, pups that are reared on a high carbohydrate milk throughout the postnatal period are relatively hyperphagic and develop obesity even when weaned onto a standard chow diet [213]. The hyperphagia and obesity have been attributed to the increased expression of several orexigenic neuropeptides and decreased expression of anorexigenic systems; these changes are observed as early as P12, and persist into adulthood. It is worth noting that this group has compared pups raised artificially on a milk diet high in fat with pups naturally raised and reported no significant differences in the hypothalamic gene expression or the metabolic phenotype. These studies indicate that simply changing nutrient content, without changing caloric intake, can cause significant reprogramming within the hypothalamus, increasing the susceptibility to obesity and diabetes. However, it still remains to be determined to what extent the hypothalamic circuits or wiring are altered. This group has also shown that high carbohydrate milk reared female offspring, when raised to adulthood and bred, pass on their metabolic abnormalities to their offspring [214]. However, this may not be surprising since the adult pregnant females display characteristics consistent with gestational diabetes, which itself is well known to cause complications in the developing fetus.

Maternal undernutrition

In studies in which maternal nutrient restriction is imposed during early and mid-gestation, obesity and energy balance dysregulation also results. This in many ways is paradoxical considering that undernutrition also results in obesity. However, though the result is similar, the mechanism may indeed be different. Offspring of dams placed on a 50% food restriction during gestation have a dampened leptin surge during the first two postnatal weeks in comparison to control animals and have suppressed POMC as well as a reduction in endorphin-*ir* at weaning [215;216]. These animals show decreased body-weight during early postnatal life. Since leptin has been shown to be crucial to the maturation of ARH circuits, the dampened leptin surge likely results in disruptions in the development of the hypothalamic circuits. Pregnant dams exposed to low protein diets during pregnancy give rise to offspring who have a preference for fatty foods as opposed to high protein and carbohydrate [217]. By reducing caloric intake by the pregnant dam, the sparing that occurs to support fetal growth appears to result in wiring that favors fuel storage, or increased metabolic efficiency. This is consistent with the *thrifty phenotype* hypothesis.

Neonatal undernutrition

One of the most widely used models of neonatal undernutrition is to alter pup milk availability by placing an extraordinary demand on maternal milk production through maintenance of very large litter (14-24 pups) [218;219;220]. This model describes early stunted growth and overall lower body-weight early in life with catch-up growth and late-onset insulin resistance and glucose intolerance [221;222]. These animals are thus exposed to inadequate nutrition during the time of hypothalamic development and have dampened levels of leptin during this critical time period.

Placental Insufficiency

Intrauterine growth retardation (IUGR) is a common gestational condition that results in increased risk of infant morbidity and mortality. In animal models IUGR is accomplished through bilateral uterine artery ligation resulting in reduced blood flow to the placenta and placental insufficiency. This model has been characterized in the rodent as well as the sheep. IUGR rodents become glucose intolerant and insulin resistant at an early age which eventually results in diabetes [223]. In the sheep, IUGR results in variety of metabolic alterations in the offspring which include accelerated “catch-up” growth after birth, reduction in lean body mass and adult obesity [224;225;226].

5. Conclusion

From the studies discussed in this review it is clear that early life events (prenatal and postnatal) can significantly impact the development of metabolic systems, including hypothalamic circuitry, causing long-term consequences on body weight and energy homeostasis in adult life. It seems logical that the maternal environment should be a key signal for the developing fetus to provide programming for the adaptations to shifting nutritional environments. However, when developing fetus is impacted by a strong acute signal this can lead to increased health risks later in life; this is the basis of the “Barker Hypothesis”. This part of the equation is clear, but there are many questions that remain to be answered. For example, what are the mechanisms and signals for the development of the hypothalamic circuitry that control food intake and energy homeostasis? Is it simply changes in leptin levels signaling the development of the ARH circuits? The leptin signal, at least for the rodent, appears to be somewhat selective for the ARH circuits. Are there yet unidentified metabolic signals that are critical for the development of other hypothalamic or brainstem circuits involved in energy homeostasis? Undoubtedly. Much focus has been applied to the function and development of the ARH circuits; more effort needs to be spent on other areas of the brain as well as how the complete integrated circuit develops, including peripheral metabolic systems.

Why does overnutrition and undernutrition during the prenatal and postnatal periods result in similar adult phenotypes - overweight/obese and diabetic? Are similar mechanisms involved? While the thrifty phenotype hypothesis can explain undernutrition early programming adaptations, it is difficult to apply this to the situations of overnutrition. This paradox has been shown for many species including rodents, sheep, NHPs and humans. Our studies in the NHP have indicated that maternal HFD results in increased circulating cytokines in the developing fetus (termed fetal lipotoxicity) which can cause broad oxidative damage. Furthermore, we have demonstrated that this model has reduced placental blood flow (unpublished observations), which is similar to the placental insufficiency models in sheep and rodents. Does both undernutrition and overnutrition cause similar abnormalities in placental function? This is a definite possibility that warrants further investigation.

A final consideration; while we have and will continue to gain important insight using rodent species, it is important to consider the significant species differences in the development of

metabolic systems. It still remains to be determined if we can completely model the complex circuitry and function of the human using rodents. This may especially be true for complex behaviors. Ultimately the goal is to understand the underlying cause and consequences of the dramatic increase in childhood obesity.

REFERENCES

- [1]. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006;295:1549-55. [PubMed: 16595758]
- [2]. Baskin ML, Ard J, Franklin F, Allison DB. Prevalence of obesity in the United States. *Obes Rev* 2005;6:5-7. [PubMed: 15655032]
- [3]. Flegal KM. Epidemiologic aspects of overweight and obesity in the United States. *Physiol Behav* 2005;86:599-602. [PubMed: 16242735]
- [4]. Flegal KM. Estimating the impact of obesity. *Soz Praventivmed* 2005;50:73-4. [PubMed: 15900958]
- [5]. Flegal KM. Commentary: the epidemic of obesity--what's in a name? *Int J Epidemiol* 2006;72-4. discussion 81-2. [PubMed: 16339593]
- [6]. Haslam DW, James WP. Obesity. *Lancet* 2005;366:1197-209. [PubMed: 16198769]
- [7]. De Ferranti SD, Osganian SK. Epidemiology of paediatric metabolic syndrome and type 2 diabetes mellitus. *Diab Vasc Dis Res* 2007;4:285-96. [PubMed: 18158698]
- [8]. Pinto NM, Marino BS, Wernovsky G, de Ferranti SD, Walsh AZ, Laronde M, Hyland K, Dunn SO Jr, Cohen MS. Obesity is a common comorbidity in children with congenital and acquired heart disease. *Pediatrics* 2007;120:e1157-64. [PubMed: 17974711]
- [9]. de Ferranti SD, Rifai N. C-reactive protein: a nontraditional serum marker of cardiovascular risk. *Cardiovasc Pathol* 2007;16:14-21. [PubMed: 17218210]
- [10]. Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *N Engl J Med* 2003;348:1085-95. [PubMed: 12646665]
- [11]. Farooqi IS, Yeo GS, O'Rahilly S. Binge eating as a phenotype of melanocortin 4 receptor gene mutations. *N Engl J Med* 2003;349:606-9. author reply 606-9. [PubMed: 12908459]
- [12]. Reilly JJ. Physical activity and obesity in childhood and adolescence. *Lancet* 2005;366:268-9. [PubMed: 16039314]
- [13]. Reilly JJ. Descriptive epidemiology and health consequences of childhood obesity. *Best Pract Res Clin Endocrinol Metab* 2005;19:327-41. [PubMed: 16150378]
- [14]. Reilly JJ, Armstrong J, Dorosty AR, Emmett PM, Ness A, Rogers I, Steer C, Sherriff A. Early life risk factors for obesity in childhood: cohort study. *BMJ* 2005;330:1357. [PubMed: 15908441]
- [15]. Wei JN, Sung FC, Li CY, Chang CH, Lin RS, Lin CC, Chiang CC, Chuang LM. Low birth weight and high birth weight infants are both at an increased risk to have type 2 diabetes among schoolchildren in taiwan. *Diabetes Care* 2003;26:343-8. [PubMed: 12547860]
- [16]. Moreno LA, Rodriguez G. Dietary risk factors for development of childhood obesity. *Curr Opin Clin Nutr Metab Care* 2007;10:336-41. [PubMed: 17414504]
- [17]. Mayer-Davis EJ, Rifas-Shiman SL, Zhou L, Hu FB, Colditz GA, Gillman MW. Breast-feeding and risk for childhood obesity: does maternal diabetes or obesity status matter? *Diabetes Care* 2006;29:2231-7. [PubMed: 17003298]
- [18]. Salsberry PJ, Reagan PB. Taking the long view: the prenatal environment and early adolescent overweight. *Res Nurs Health* 2007;30:297-307. [PubMed: 17514704]
- [19]. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *JAMA* 2004;291:2847-50. [PubMed: 15199035]
- [20]. Ong KK, Ahmed ML, Dunger DB. Lessons from large population studies on timing and tempo of puberty (secular trends and relation to body size): the European trend. *Mol Cell Endocrinol* 2006;254-255:8-12. [PubMed: 16757103]

- [21]. Schaefer-Graf UM, Pawliczak J, Passow D, Hartmann R, Rossi R, Buhner C, Harder T, Plagemann A, Vetter K, Kordonouri O. Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes. *Diabetes Care* 2005;28:1745–50. [PubMed: 15983329]
- [22]. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics* 1999;103:1175–82. [PubMed: 10353925]
- [23]. Sorof J, Daniels S. Obesity hypertension in children: a problem of epidemic proportions. *Hypertension* 2002;40:441–7. [PubMed: 12364344]
- [24]. Verhulst SL, Desager K, Van Gaal L, De Backer W. Sleep duration and insulin resistance in obese children: when variables are sleeping too close together. *J Pediatr* 2007;151:e14. author reply e14–5. [PubMed: 17889055]
- [25]. Verhulst SL, Schrauwen N, Haentjens D, Rooman RP, Van Gaal L, De Backer WA, Desager KN. Sleep-disordered breathing and the metabolic syndrome in overweight and obese children and adolescents. *J Pediatr* 2007;150:608–12. [PubMed: 17517244]
- [26]. Flaherman V, Rutherford GW. A meta-analysis of the effect of high weight on asthma. *Arch Dis Child* 2006;91:334–9. [PubMed: 16428358]
- [27]. Eisenberg ME, Neumark-Sztainer D, Story M. Associations of weight-based teasing and emotional well-being among adolescents. *Arch Pediatr Adolesc Med* 2003;157:733–8. [PubMed: 12912777]
- [28]. Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics* 1998;101:518–25. [PubMed: 12224658]
- [29]. Anderson SE, Cohen P, Naumova EN, Jacques PF, Must A. Adolescent obesity and risk for subsequent major depressive disorder and anxiety disorder: prospective evidence. *Psychosom Med* 2007;69:740–7. [PubMed: 17942847]
- [30]. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001;60:5–20. [PubMed: 11809615]
- [31]. Barker DJ. Fetal programming of coronary heart disease. *Trends Endocrinol Metab* 2002;13:364–8. [PubMed: 12367816]
- [32]. Barker DJ, Eriksson JG, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol* 2002;31:1235–9. [PubMed: 12540728]
- [33]. Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME. Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. *BMJ* 1989;298:564–7. [PubMed: 2495113]
- [34]. Barker DJ, Osmond C, Law CM. The intrauterine and early postnatal origins of cardiovascular disease and chronic bronchitis. *J Epidemiol Community Health* 1989;43:237–40. [PubMed: 2607302]
- [35]. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;2:577–80. [PubMed: 2570282]
- [36]. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 1991;303:1019–22. [PubMed: 1954451]
- [37]. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* 1998;351:173–7. [PubMed: 9449872]
- [38]. Morrison JL, Botting KJ, Dyer JL, Williams SJ, Thornburg KL, McMillen IC. Restriction of placental function alters heart development in the sheep fetus. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R306–13. [PubMed: 17428893]
- [39]. Loeliger M, Duncan J, Louey S, Cock M, Harding R, Rees S. Fetal growth restriction induced by chronic placental insufficiency has long-term effects on the retina but not the optic nerve. *Invest Ophthalmol Vis Sci* 2005;46:3300–8. [PubMed: 16123433]
- [40]. Schlubritz-Loutsevitch NE, Dudley CJ, Gomez JJ, Nevill CH, Smith BK, Jenkins SL, McDonald TJ, Bartlett TQ, Nathanielsz PW, Nijland MJ. Metabolic adjustments to moderate maternal nutrient restriction. *Br J Nutr* 2007;98:276–84. [PubMed: 17391566]
- [41]. Desai M, Gayle D, Babu J, Ross MG. The timing of nutrient restriction during rat pregnancy/lactation alters metabolic syndrome phenotype. *Am J Obstet Gynecol* 2007;196:555 e1–7. [PubMed: 17547893]

- [42]. Desai M, Gayle D, Han G, Ross MG. Programmed hyperphagia due to reduced anorexigenic mechanisms in intrauterine growth-restricted offspring. *Reprod Sci* 2007;14:329–37. [PubMed: 17644805]
- [43]. Dietz WH. Critical periods in childhood for the development of obesity. *Am J Clin Nutr* 1994;59:955–9. [PubMed: 8172099]
- [44]. Dietz WH. Periods of risk in childhood for the development of adult obesity--what do we need to learn? *J Nutr* 1997;127:1884S–1886S. [PubMed: 9278575]
- [45]. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005;115:e290–6. [PubMed: 15741354]
- [46]. Ehrenberg HM, Mercer BM, Catalano PM. The influence of obesity and diabetes on the prevalence of macrosomia. *Am J Obstet Gynecol* 2004;191:964–8. [PubMed: 15467573]
- [47]. Cnattingius S, Bergstrom R, Lipworth L, Kramer MS. Prepregnancy weight and the risk of adverse pregnancy outcomes. *N Engl J Med* 1998;338:147–52. [PubMed: 9428815]
- [48]. Kristensen J, Vestergaard M, Wisborg K, Kesmodel U, Secher NJ. Prepregnancy weight and the risk of stillbirth and neonatal death. *BJOG* 2005;112:403–8. [PubMed: 15777435]
- [49]. Sattar N, Clark P, Holmes A, Lean ME, Walker I, Greer IA. Antenatal waist circumference and hypertension risk. *Obstet Gynecol* 2001;97:268–71. [PubMed: 11165593]
- [50]. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dörner G. Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes. *Int J Obes Relat Metab Disord* 1997;21:451–6. [PubMed: 9192228]
- [51]. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal gestational diabetes, birth weight, and adolescent obesity. *Pediatrics* 2003;111:e221–6. [PubMed: 12612275]
- [52]. Lindsay RS, Hanson RL, Bennett PH, Knowler WC. Secular trends in birth weight, BMI, and diabetes in the offspring of diabetic mothers. *Diabetes Care* 2000;23:1249–54. [PubMed: 10977014]
- [53]. Watts AG, Salter DS, Neuner CM. Neural network interactions and ingestive behavior control during anorexia. *Physiol Behav* 2007;91:389–96. [PubMed: 17531275]
- [54]. Schwartz MW. Central nervous system regulation of food intake. *Obesity (Silver Spring)* 2006;14 (Suppl 1):1S–8S. [PubMed: 16642956]
- [55]. Woods SC, Schwartz MW, Baskin DG, Seeley RJ. Food intake and the regulation of body weight. *Annu Rev Psychol* 2000;51:255–77. [PubMed: 10751972]
- [56]. Woods SC, Seeley RJ. Adiposity signals and the control of energy homeostasis. *Nutrition* 2000;16:894–902. [PubMed: 11054594]
- [57]. Woods SC, Seeley RJ, Porte D Jr, Schwartz MW. Signals that regulate food intake and energy homeostasis. *Science* 1998;280:1378–83. [PubMed: 9603721]
- [58]. Elmquist JK. Hypothalamic pathways underlying the endocrine, autonomic, and behavioral effects of leptin. *Int J Obes Relat Metab Disord* 2001;25(Suppl 5):S78–82. [PubMed: 11840221]
- [59]. Elmquist JK. Anatomic basis of leptin action in the hypothalamus. *Front Horm Res* 2000;26:21–41. [PubMed: 10767939]
- [60]. Elmquist JK. CNS regulation of energy balance and body weight: insights from rodent models. *Lab Anim Sci* 1998;48:630–7. [PubMed: 10090088]
- [61]. Elmquist JK, Coppari R, Balthasar N, Ichinose M, Lowell BB. Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. *J Comp Neurol* 2005;493:63–71. [PubMed: 16254991]
- [62]. Cone RD. The Central Melanocortin System and Energy Homeostasis. *Trends Endocrinol Metab* 1999;10:211–216. [PubMed: 10407394]
- [63]. Enriori PJ, Evans AE, Sinnayah P, Cowley MA. Leptin resistance and obesity. *Obesity (Silver Spring)* 2006;14(Suppl 5):254S–258S. [PubMed: 17021377]
- [64]. Horvath TL. The hardship of obesity: a soft-wired hypothalamus. *Nat Neurosci* 2005;8:561–5. [PubMed: 15856063]
- [65]. Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. *Neuron* 2002;36:199–211. [PubMed: 12383777]

- [66]. Grill HJ. Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. *Obesity* (Silver Spring) 2006;14(Suppl 5):216S–221S. [PubMed: 17021370]
- [67]. Berthoud HR. Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. *Obesity* (Silver Spring) 2006;14(Suppl 5):197S–200S. [PubMed: 17021366]
- [68]. Brunetti L, Michelotto B, Orlando G, Vacca M. Leptin inhibits norepinephrine and dopamine release from rat hypothalamic neuronal endings. *Eur J Pharmacol* 1999;372:237–40. [PubMed: 10395017]
- [69]. Cota D, Barrera JG, Seeley RJ. Leptin in energy balance and reward: two faces of the same coin? *Neuron* 2006;51:678–80. [PubMed: 16982412]
- [70]. Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, Flier JS. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* 2006;51:811–22. [PubMed: 16982425]
- [71]. Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, DiLeone RJ. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 2006;51:801–10. [PubMed: 16982424]
- [72]. Krugel U, Schraft T, Kittner H, Kiess W, Illes P. Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *Eur J Pharmacol* 2003;482:185–7. [PubMed: 14660021]
- [73]. Szczypka MS, Rainey MA, Palmiter RD. Dopamine is required for hyperphagia in *Lep(ob/ob)* mice. *Nat Genet* 2000;25:102–4. [PubMed: 10802666]
- [74]. Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschoop MH, Gao XB, Horvath TL. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* 2006;116:3229–39. [PubMed: 17060947]
- [75]. Brunetti L, Recinella L, Orlando G, Michelotto B, Di Nisio C, Vacca M. Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus. *Eur J Pharmacol* 2002;454:189–92. [PubMed: 12421646]
- [76]. Shimada M, Nakamura T. Time of neuron origin in mouse hypothalamic nuclei. *Exp Neurol* 1973;41:163–73. [PubMed: 4743483]
- [77]. Ifft JD. An autoradiographic study of the time of final division of neurons in rat hypothalamic nuclei. *J Comp Neurol* 1972;144:193–204. [PubMed: 5029133]
- [78]. Khachaturian H, Lewis ME, Alessi NE, Watson SJ. Time of origin of opioid peptide-containing neurons in the rat hypothalamus. *J Comp Neurol* 1985;236:538–46. [PubMed: 2865278]
- [79]. Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 2004;304:108–10. [PubMed: 15064420]
- [80]. Bouret SG, Draper SJ, Simerly RB. Formation of projection pathways from the arcuate nucleus of the hypothalamus to hypothalamic regions implicated in the neural control of feeding behavior in mice. *J Neurosci* 2004;24:2797–805. [PubMed: 15028773]
- [81]. Ahima RS, Hileman SM. Postnatal regulation of hypothalamic neuropeptide expression by leptin: implications for energy balance and body weight regulation. *Regul Pept* 2000;92:1–7. [PubMed: 11024558]
- [82]. Proulx K, Richard D, Walker CD. Leptin regulates appetite-related neuropeptides in the hypothalamus of developing rats without affecting food intake. *Endocrinology* 2002;143:4683–92. [PubMed: 12446596]
- [83]. Schmidt I, Fritz A, Scholch C, Schneider D, Simon E, Plagemann A. The effect of leptin treatment on the development of obesity in overfed suckling Wistar rats. *Int J Obes Relat Metab Disord* 2001;25:1168–74. [PubMed: 11477501]
- [84]. Mistry AM, Swick A, Romsos DR. Leptin alters metabolic rates before acquisition of its anorectic effect in developing neonatal mice. *Am J Physiol* 1999;277:R742–7. [PubMed: 10484491]
- [85]. Grove KL, Brogan RS, Smith MS. Novel expression of neuropeptide Y (NPY) mRNA in hypothalamic regions during development: region-specific effects of maternal deprivation on NPY and Agouti-related protein mRNA. *Endocrinology* 2001;142:4771–6. [PubMed: 11606443]
- [86]. Kowalski TJ, Houtp TA, Jahng J, Okada N, Chua SC Jr, Smith GP. Ontogeny of neuropeptide Y expression in response to deprivation in lean Zucker rat pups. *Am J Physiol* 1998;275:R466–70. [PubMed: 9688681]

- [87]. Grove KL, Smith MS. Ontogeny of the hypothalamic neuropeptide Y system. *Physiol Behav* 2003;79:47–63. [PubMed: 12818709]
- [88]. Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proc Natl Acad Sci U S A* 1998;95:15043–8. [PubMed: 9844012]
- [89]. Bouret SG, Gorski JN, Patterson CM, Chen S, Levin BE, Simerly RB. Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. *Cell Metab* 2008;7:179–85. [PubMed: 18249177]
- [90]. Koutcherov Y, Mai JK, Paxinos G. Hypothalamus of the human fetus. *J Chem Neuroanat* 2003;26:253–70. [PubMed: 14729128]
- [91]. Grayson BE, Allen SE, Billes SK, Williams SM, Smith MS, Grove KL. Prenatal development of hypothalamic neuropeptide systems in the nonhuman primate. *Neuroscience* 2006;143:975–86. [PubMed: 17029798]
- [92]. Allen JM, McGregor GP, Woodhams PL, Polak JM, Bloom SR. Ontogeny of a novel peptide, neuropeptide Y (NPY) in rat brain. *Brain Res* 1984;303:197–200. [PubMed: 6547364]
- [93]. Woodhams PL, Allen YS, McGovern J, Allen JM, Bloom SR, Balazs R, Polak JM. Immunohistochemical analysis of the early ontogeny of the neuropeptide Y system in rat brain. *Neuroscience* 1985;15:173–202. [PubMed: 3892361]
- [94]. Singer LK, Kuper J, Brogan RS, Smith MS, Grove KL. Novel expression of hypothalamic neuropeptide Y during postnatal development in the rat. *Neuroreport* 2000;11:1075–80. [PubMed: 10790885]
- [95]. Grove KL, Allen S, Grayson BE, Smith MS. Postnatal development of the hypothalamic neuropeptide Y system. *Neuroscience* 2003;116:393–406. [PubMed: 12559095]
- [96]. Grove KL, Grayson BE, Glavas MM, Xiao XQ, Smith MS. Development of metabolic systems. *Physiol Behav* 2005;86:646–60. [PubMed: 16289141]
- [97]. Palmiter RD, Erickson JC, Hollopeter G, Baraban SC, Schwartz MW. Life without neuropeptide Y. *Recent Prog Horm Res* 1998;53:163–99. [PubMed: 9769708]
- [98]. Erickson JC, Clegg KE, Palmiter RD. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 1996;381:415–21. [PubMed: 8632796]
- [99]. Qian S, Chen H, Weingarth D, Trumbauer ME, Novi DE, Guan X, Yu H, Shen Z, Feng Y, Frazier E, Chen A, Camacho RE, Shearman LP, Gopal-Truter S, MacNeil DJ, Van der Ploeg LH, Marsh DJ. Neither agouti-related protein nor neuropeptide Y is critically required for the regulation of energy homeostasis in mice. *Mol Cell Biol* 2002;22:5027–35. [PubMed: 12077332]
- [100]. Patel HR, Qi Y, Hawkins EJ, Hileman SM, Elmquist JK, Imai Y, Ahima RS. Neuropeptide Y deficiency attenuates responses to fasting and high-fat diet in obesity-prone mice. *Diabetes* 2006;55:3091–8. [PubMed: 17065347]
- [101]. Segal-Lieberman G, Trombly DJ, Juthani V, Wang X, Maratos-Flier E. NPY ablation in C57BL/6 mice leads to mild obesity and to an impaired refeeding response to fasting. *Am J Physiol Endocrinol Metab* 2003;284:E1131–9. [PubMed: 12582011]
- [102]. Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 2005;310:683–5. [PubMed: 16254186]
- [103]. Grove KL, Chen P, Koegler FH, Schiffmaker A, Susan Smith M, Cameron JL. Fasting activates neuropeptide Y neurons in the arcuate nucleus and the paraventricular nucleus in the rhesus macaque. *Brain Res Mol Brain Res* 2003;113:133–8. [PubMed: 12750015]
- [104]. Muhlhausler BS, McMillen IC, Rouzaud G, Findlay PA, Marrocco EM, Rhind SM, Adam CL. Appetite regulatory neuropeptides are expressed in the sheep hypothalamus before birth. *J Neuroendocrinol* 2004;16:502–7. [PubMed: 15189324]
- [105]. Muhlhausler BS, Adam CL, Marrocco EM, Findlay PA, Roberts CT, McFarlane JR, Kauter KG, McMillen IC. Impact of glucose infusion on the structural and functional characteristics of adipose tissue and on hypothalamic gene expression for appetite regulatory neuropeptides in the sheep fetus during late gestation. *J Physiol* 2005;565:185–95. [PubMed: 15661821]
- [106]. Muhlhausler BS, Adam CL, Findlay PA, Duffield JA, McMillen IC. Increased maternal nutrition alters development of the appetite-regulating network in the brain. *FASEB J* 2006;20:1257–9. [PubMed: 16684802]

- [107]. Roberts TJ, Caston-Balderrama A, Nijland MJ, Ross MG. Central neuropeptide Y stimulates ingestive behavior and increases urine output in the ovine fetus. *Am J Physiol Endocrinol Metab* 2000;279:E494–500. [PubMed: 10950815]
- [108]. Nilsson I, Johansen JE, Schalling M, Hokfelt T, Fetissov SO. Maturation of the hypothalamic arcuate agouti-related protein system during postnatal development in the mouse. *Brain Res Dev Brain Res* 2005;155:147–54.
- [109]. Ross AW, Johnson CE, Bell LM, Reilly L, Duncan JS, Barrett P, Heideman PD, Morgan PJ. Divergent regulation of hypothalamic neuropeptide Y and agouti-related protein by photoperiod in F344 rats with differential food intake and growth. *J Neuroendocrinol* 2009;21:610–9. [PubMed: 19490367]
- [110]. Kas MJ, Bruijnzeel AW, Haanstra JR, Wiegant VM, Adan RA. Differential regulation of agouti-related protein and neuropeptide Y in hypothalamic neurons following a stressful event. *J Mol Endocrinol* 2005;35:159–64. [PubMed: 16087729]
- [111]. Xu J, Kirigiti MA, Grove KL, Smith MS. Regulation of food intake and gonadotropin-releasing hormone/luteinizing hormone during lactation: role of insulin and leptin. *Endocrinology* 2009;150:4231–40. [PubMed: 19470705]
- [112]. Khachaturian H, Alessi NE, Munfakh N, Watson SJ. Ontogeny of opioid and related peptides in the rat CNS and pituitary: an immunocytochemical study. *Life Sci* 1983;33(Suppl 1):61–4. [PubMed: 6141503]
- [113]. Bugnon C, Fellmann D, Gouget A, Cardot J. Ontogenesis of pro-opiomelanocortin neurons in the rat. *C R Seances Soc Biol Fil* 1982;176:503–10. [PubMed: 6295565]
- [114]. Perello M, Stuart RC, Nillni EA. Differential effects of fasting and leptin on proopiomelanocortin peptides in the arcuate nucleus and in the nucleus of the solitary tract. *Am J Physiol Endocrinol Metab* 2007;292:E1348–57. [PubMed: 17227963]
- [115]. Ellacott KL, Halatchev IG, Cone RD. Characterization of leptin-responsive neurons in the caudal brainstem. *Endocrinology* 2006;147:3190–5. [PubMed: 16601142]
- [116]. Babic T, Townsend RL, Patterson LM, Sutton GM, Zheng H, Berthoud HR. Phenotype of neurons in the nucleus of the solitary tract that express CCK-induced activation of the ERK signaling pathway. *Am J Physiol Regul Integr Comp Physiol* 2009;296:R845–54. [PubMed: 19176891]
- [117]. Appleyard SM, Bailey TW, Doyle MW, Jin YH, Smart JL, Low MJ, Andresen MC. Proopiomelanocortin neurons in nucleus tractus solitarius are activated by visceral afferents: regulation by cholecystokinin and opioids. *J Neurosci* 2005;25:3578–85. [PubMed: 15814788]
- [118]. Swaab DF, Visser M, Tilders FJ. Stimulation of intra-uterine growth in rat by alpha-melanocyte-stimulating hormone. *J Endocrinol* 1976;70:445–55. [PubMed: 185310]
- [119]. Swaab DF, Boer GJ, Boer K, Dogterom J, van Leeuwen FW, Visser M. Fetal neuroendocrine mechanisms in development and parturition. *Prog Brain Res* 1978;48:277–90. [PubMed: 746160]
- [120]. Swaab DF, Boer GJ, Visser M. The fetal brain and intrauterine growth. *Postgrad Med J* 1978;54 (Suppl 1):63–73. [PubMed: 569298]
- [121]. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 1997;88:131–41. [PubMed: 9019399]
- [122]. Smart JL, Tolle V, Low MJ. Glucocorticoids exacerbate obesity and insulin resistance in neuron-specific proopiomelanocortin-deficient mice. *J Clin Invest* 2006;116:495–505. [PubMed: 16440060]
- [123]. Farooqi IS, O'Rahilly S. Recent advances in the genetics of severe childhood obesity. *Arch Dis Child* 2000;83:31–4. [PubMed: 10868996]
- [124]. O'Rahilly S, Farooqi IS, Yeo GS, Challis BG. Minireview: human obesity-lessons from monogenic disorders. *Endocrinology* 2003;144:3757–64. [PubMed: 12933645]
- [125]. Coll AP, Farooqi IS, Challis BG, Yeo GS, O'Rahilly S. Proopiomelanocortin and energy balance: insights from human and murine genetics. *J Clin Endocrinol Metab* 2004;89:2557–62. [PubMed: 15181023]
- [126]. Farooqi IS, O'Rahilly S. Monogenic human obesity syndromes. *Recent Prog Horm Res* 2004;59:409–24. [PubMed: 14749512]

- [127]. Farooqi IS, Drop S, Clements A, Keogh JM, Biernacka J, Lowenbein S, Challis BG, O'Rahilly S. Heterozygosity for a POMC-null mutation and increased obesity risk in humans. *Diabetes* 2006;55:2549–53. [PubMed: 16936203]
- [128]. Farooqi IS, Volders K, Stanhope R, Heuschkel R, White A, Lank E, Keogh J, O'Rahilly S, Creemers JW. Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *J Clin Endocrinol Metab* 2007;92:3369–73. [PubMed: 17595246]
- [129]. Koylu EO, Couceyro PR, Lambert PD, Kuhar MJ. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. *J Comp Neurol* 1998;391:115–32. [PubMed: 9527537]
- [130]. Smith Y, Koylu EO, Couceyro P, Kuhar MJ. Ultrastructural localization of CART (cocaine- and amphetamine-regulated transcript) peptides in the nucleus accumbens of monkeys. *Synapse* 1997;27:90–4. [PubMed: 9268069]
- [131]. Elias CF, Lee CE, Kelly JF, Ahima RS, Kuhar M, Saper CB, Elmquist JK. Characterization of CART neurons in the rat and human hypothalamus. *J Comp Neurol* 2001;432:1–19. [PubMed: 11241374]
- [132]. Couceyro P, Paquet M, Koylu E, Kuhar MJ, Smith Y. Cocaine- and amphetamine-regulated transcript (CART) peptide immunoreactivity in myenteric plexus neurons of the rat ileum and colocalization with choline acetyltransferase. *Synapse* 1998;30:1–8. [PubMed: 9704875]
- [133]. Koylu EO, Smith Y, Couceyro PR, Kuhar MJ. CART peptides colocalize with tyrosine hydroxylase neurons in rat locus coeruleus. *Synapse* 1999;31:309–11. [PubMed: 10051113]
- [134]. Brischoux F, Griffond B, Fellmann D, Risold PY. Early and transient ontogenetic expression of the cocaine- and amphetamine-regulated transcript peptide in the rat mesencephalon: correlation with tyrosine hydroxylase expression. *J Neurobiol* 2002;52:221–9. [PubMed: 12210105]
- [135]. Wierup N, Richards WG, Bannon AW, Kuhar MJ, Ahren B, Sundler F. CART knock out mice have impaired insulin secretion and glucose intolerance, altered beta cell morphology and increased body weight. *Regul Pept* 2005;129:203–11. [PubMed: 15927717]
- [136]. Broberger C. Hypothalamic cocaine- and amphetamine-regulated transcript (CART) neurons: histochemical relationship to thyrotropin-releasing hormone, melanin-concentrating hormone, orexin/hypocretin and neuropeptide Y. *Brain Res* 1999;848:101–13. [PubMed: 10612702]
- [137]. Menyhert J, Wittmann G, Lechan RM, Keller E, Liposits Z, Fekete C. Cocaine- and amphetamine-regulated transcript (CART) is colocalized with the orexigenic neuropeptide Y and agouti-related protein and absent from the anorexigenic alpha-melanocyte-stimulating hormone neurons in the infundibular nucleus of the human hypothalamus. *Endocrinology* 2007;148:4276–81. [PubMed: 17525122]
- [138]. Yamamoto Y, Ueta Y, Hara Y, Serino R, Nomura M, Shibuya I, Shirahata A, Yamashita H. Postnatal development of orexin/hypocretin in rats. *Brain Res Mol Brain Res* 2000;78:108–19. [PubMed: 10891590]
- [139]. Van Den Pol AN, Patrylo PR, Ghosh PK, Gao XB. Lateral hypothalamus: early developmental expression and response to hypocretin (orexin). *J Comp Neurol* 2001;433:349–63. [PubMed: 11298360]
- [140]. Brischoux F, Fellmann D, Risold PY. Ontogenetic development of the diencephalic MCH neurons: a hypothalamic 'MCH area' hypothesis. *Eur J Neurosci* 2001;13:1733–44. [PubMed: 11359525]
- [141]. Presse F, Hervieu G, Imaki T, Sawchenko PE, Vale W, Nahon JL. Rat melanin-concentrating hormone messenger ribonucleic acid expression: marked changes during development and after stress and glucocorticoid stimuli. *Endocrinology* 1992;131:1241–50. [PubMed: 1505462]
- [142]. Heisler LK, Cowley MA, Kishi T, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Zigman JM, Cone RD, Elmquist JK. Central serotonin and melanocortin pathways regulating energy homeostasis. *Ann N Y Acad Sci* 2003;994:169–74. [PubMed: 12851313]
- [143]. Heisler LK, Cowley MA, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Marcus JN, Holstege H, Lee CE, Cone RD, Elmquist JK. Activation of central melanocortin pathways by fenfluramine. *Science* 2002;297:609–11. [PubMed: 12142539]
- [144]. Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones Z, Liu HY, Zigman JM, Balthasar N, Kishi T, Lee CE, Aschkenasi CJ, Zhang CY, Yu J, Boss O, Mountjoy KG, Clifton PG, Lowell BB, Friedman JM, Horvath T, Butler AA, Elmquist JK, Cowley MA. Serotonin reciprocally

regulates melanocortin neurons to modulate food intake. *Neuron* 2006;51:239–49. [PubMed: 16846858]

- [145]. Xu Y, Jones JE, Kohno D, Williams KW, Lee CE, Choi MJ, Anderson JG, Heisler LK, Zigman JM, Lowell BB, Elmquist JK. 5-HT₂CRs expressed by pro-opiomelanocortin neurons regulate energy homeostasis. *Neuron* 2008;60:582–9. [PubMed: 19038216]
- [146]. Zhou L, Sutton GM, Rochford JJ, Semple RK, Lam DD, Oksanen LJ, Thornton-Jones ZD, Clifton PG, Yueh CY, Evans ML, McCrimmon RJ, Elmquist JK, Butler AA, Heisler LK. Serotonin 2C receptor agonists improve type 2 diabetes via melanocortin-4 receptor signaling pathways. *Cell Metab* 2007;6:398–405. [PubMed: 17983585]
- [147]. Hudson B, Ritter S. Hindbrain catecholamine neurons mediate consummatory responses to glucoprivation. *Physiol Behav* 2004;82:241–50. [PubMed: 15276785]
- [148]. Ritter RC, Epstein AN. Control of meal size by central noradrenergic action. *Proc Natl Acad Sci U S A* 1975;72:3740–3. [PubMed: 1059162]
- [149]. Ritter S, Bugarith K, Dinh TT. Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. *J Comp Neurol* 2001;432:197–216. [PubMed: 11241386]
- [150]. Ritter S, Dinh TT, Li AJ. Hindbrain catecholamine neurons control multiple glucoregulatory responses. *Physiol Behav* 2006;89:490–500. [PubMed: 16887153]
- [151]. Ritter S, Watts AG, Dinh TT, Sanchez-Watts G, Pedrow C. Immunotoxin lesion of hypothalamically projecting norepinephrine and epinephrine neurons differentially affects circadian and stressor-stimulated corticosterone secretion. *Endocrinology* 2003;144:1357–67. [PubMed: 12639919]
- [152]. Ritter S, Wise D, Stein L. Neurochemical regulation of feeding in the rat: facilitation by alpha-noradrenergic, but not dopaminergic, receptor stimulants. *J Comp Physiol Psychol* 1975;88:778–84. [PubMed: 239031]
- [153]. Taylor K, Lester E, Hudson B, Ritter S. Hypothalamic and hindbrain NPY, AGRP and NE increase consummatory feeding responses. *Physiol Behav* 2007;90:744–50. [PubMed: 17289093]
- [154]. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, Birnbaum S, Yanagisawa M, Elmquist JK, Nestler EJ, Zigman JM. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci* 2008;11:752–3. [PubMed: 18552842]
- [155]. Lidov HG, Molliver ME. Immunohistochemical study of the development of serotonergic neurons in the rat CNS. *Brain Res Bull* 1982;9:559–604. [PubMed: 6756556]
- [156]. Zhou FC, Sari Y, Zhang JK. Expression of serotonin transporter protein in developing rat brain. *Brain Res Dev Brain Res* 2000;119:33–45.
- [157]. Hansson SR, Mezey E, Hoffman BJ. Serotonin transporter messenger RNA in the developing rat brain: early expression in serotonergic neurons and transient expression in non-serotonergic neurons. *Neuroscience* 1998;83:1185–201. [PubMed: 9502257]
- [158]. Whitaker-Azmitia PM. Serotonin and brain development: role in human developmental diseases. *Brain Res Bull* 2001;56:479–85. [PubMed: 11750793]
- [159]. Lam DD, Przydzial MJ, Ridley SH, Yeo GS, Rochford JJ, O'Rahilly S, Heisler LK. Serotonin 5-HT₂C receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. *Endocrinology* 2008;149:1323–8. [PubMed: 18039773]
- [160]. Zhou L, Williams T, Lachey JL, Kishi T, Cowley MA, Heisler LK. Serotonergic pathways converge upon central melanocortin systems to regulate energy balance. *Peptides* 2005;26:1728–32. [PubMed: 15993514]
- [161]. Herlenius E, Lagercrantz H. Development of neurotransmitter systems during critical periods. *Exp Neurol* 2004;190(Suppl 1):S8–21. [PubMed: 15498537]
- [162]. Loizou LA. The postnatal ontogeny of monoamine-containing neurones in the central nervous system of the albino rat. *Brain Res* 1972;40:395–418. [PubMed: 4537284]
- [163]. Rinaman L. Ontogeny of hypothalamic-hindbrain feeding control circuits. *Dev Psychobiol* 2006;48:389–96. [PubMed: 16770760]
- [164]. Rinaman L. Postnatal development of catecholamine inputs to the paraventricular nucleus of the hypothalamus in rats. *J Comp Neurol* 2001;438:411–22. [PubMed: 11559897]

- [165]. Gisel EG, Henning SJ. Appearance of glucoprivic control of feeding behavior in the developing rat. *Physiol Behav* 1980;24:313–8. [PubMed: 7375546]
- [166]. Williams CL, Blass EM. Development of postglucoprivic insulin-induced suckling and feeding in rats. *Am J Physiol* 1987;253:R121–7. [PubMed: 3300371]
- [167]. Leininger GM, Jo YH, Leshan RL, Louis GW, Yang H, Barrera JG, Wilson H, Opland DM, Faouzi MA, Gong Y, Jones JC, Rhodes CJ, Chua S Jr, Diano S, Horvath TL, Seeley RJ, Becker JB, Munzberg H, Myers MG Jr. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab* 2009;10:89–98. [PubMed: 19656487]
- [168]. Kelley AE, Baldo BA, Pratt WE. A proposed hypothalamic-thalamic-striatal axis for the integration of energy balance, arousal, and food reward. *J Comp Neurol* 2005;493:72–85. [PubMed: 16255002]
- [169]. Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav* 2005;86:773–95. [PubMed: 16289609]
- [170]. DiLeone RJ, Georgescu D, Nestler EJ. Lateral hypothalamic neuropeptides in reward and drug addiction. *Life Sci* 2003;73:759–68. [PubMed: 12801597]
- [171]. Nestler EJ. Is there a common molecular pathway for addiction? *Nat Neurosci* 2005;8:1445–9. [PubMed: 16251986]
- [172]. Antonopoulos J, Dori I, Dinopoulos A, Chiotelli M, Parnavelas JG. Postnatal development of the dopaminergic system of the striatum in the rat. *Neuroscience* 2002;110:245–56. [PubMed: 11958867]
- [173]. Naef L, Srivastava L, Gratton A, Hendrickson H, Owens SM, Walker CD. Maternal high fat diet during the perinatal period alters mesocorticolimbic dopamine in the adult rat offspring: reduction in the behavioral responses to repeated amphetamine administration. *Psychopharmacology (Berl)* 2008;197:83–94. [PubMed: 18004547]
- [174]. Ahima RS, Prabakaran D, Flier JS. Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. *J Clin Invest* 1998;101:1020–7. [PubMed: 9486972]
- [175]. Attig L, Solomon G, Ferezou J, Abdennebi-Najar L, Taouis M, Gertler A, Djiane J. Early postnatal leptin blockage leads to a long-term leptin resistance and susceptibility to diet-induced obesity in rats. *Int J Obes (Lond)* 2008;32:1153–60. [PubMed: 18379577]
- [176]. Davidowa H, Plagemann A. Different responses of ventromedial hypothalamic neurons to leptin in normal and early postnatally overfed rats. *Neurosci Lett* 2000;293:21–4. [PubMed: 11065128]
- [177]. Davidowa H, Plagemann A. Decreased inhibition by leptin of hypothalamic arcuate neurons in neonatally overfed young rats. *Neuroreport* 2000;11:2795–8. [PubMed: 10976965]
- [178]. Yura S, Itoh H, Sagawa N, Yamamoto H, Masuzaki H, Nakao K, Kawamura M, Takemura M, Kakui K, Ogawa Y, Fujii S. Role of premature leptin surge in obesity resulting from intrauterine undernutrition. *Cell Metab* 2005;1:371–8. [PubMed: 16054086]
- [179]. McCurdy CE, Bishop JM, Williams SM, Grayson BE, Smith MS, Friedman JE, Grove KL. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. *J Clin Invest* 2009;119:323–35. [PubMed: 19147984]
- [180]. Horvath TL. Synaptic plasticity in energy balance regulation. *Obesity (Silver Spring)* 2006;14 (Suppl 5):228S–233S. [PubMed: 17021372]
- [181]. Horvath TL. Synaptic plasticity mediating leptin's effect on metabolism. *Prog Brain Res* 2006;153:47–55. [PubMed: 16876567]
- [182]. Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 2004;304:110–5. [PubMed: 15064421]
- [183]. Gerozissis K. Brain insulin: regulation, mechanisms of action and functions. *Cell Mol Neurobiol* 2003;23:1–25. [PubMed: 12701881]
- [184]. Kozak R, Burlet A, Burlet C, Beck B. Dietary composition during fetal and neonatal life affects neuropeptide Y functioning in adult offspring. *Brain Res Dev Brain Res* 2000;125:75–82.
- [185]. Gupta A, Srinivasan M, Thamadilok S, Patel MS. Hypothalamic alterations in fetuses of high fat diet-fed obese female rats. *J Endocrinol* 2009;200:293–300. [PubMed: 19074472]

- [186]. Guo F, Jen KL. High-fat feeding during pregnancy and lactation affects offspring metabolism in rats. *Physiol Behav* 1995;57:681–6. [PubMed: 7777603]
- [187]. Walker CD, Naef L, d'Asti E, Long H, Xu Z, Moreau A, Azeddine B. Perinatal maternal fat intake affects metabolism and hippocampal function in the offspring: a potential role for leptin. *Ann N Y Acad Sci* 2008;1144:189–202. [PubMed: 19076377]
- [188]. Bayol SA, Simbi BH, Bertrand JA, Stickland NC. Offspring from mothers fed a 'junk food' diet in pregnancy and lactation exhibit exacerbated adiposity that is more pronounced in females. *J Physiol* 2008;586:3219–30. [PubMed: 18467362]
- [189]. Chang GQ, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity. *J Neurosci* 2008;28:12107–19. [PubMed: 19005075]
- [190]. Tamashiro KL, Terrillion CE, Hyun J, Koenig JI, Moran TH. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. *Diabetes* 2009;58:1116–25. [PubMed: 19188431]
- [191]. McMillen IC, Adam CL, Muhlhausler BS. Early origins of obesity: programming the appetite regulatory system. *J Physiol* 2005;565:9–17. [PubMed: 15705647]
- [192]. Plagemann A. Perinatal nutrition and hormone-dependent programming of food intake. *Horm Res* 2006;65(Suppl 3):83–9. [PubMed: 16612119]
- [193]. Plagemann A. Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. *Physiol Behav* 2005;86:661–8. [PubMed: 16280141]
- [194]. Plagemann A. A matter of insulin: developmental programming of body weight regulation. *J Matern Fetal Neonatal Med* 2008;21:143–8. [PubMed: 18297568]
- [195]. Plagemann A. 'Fetal programming' and 'functional teratogenesis': on epigenetic mechanisms and prevention of perinatally acquired lasting health risks. *J Perinat Med* 2004;32:297–305. [PubMed: 15346812]
- [196]. Patel MS, Srinivasan M. Metabolic programming: causes and consequences. *J Biol Chem* 2002;277:1629–32. [PubMed: 11698417]
- [197]. Patel MS, Srinivasan M, Aalinkeel R. Metabolic programming by nutrition during early development. *Indian J Exp Biol* 2000;38:849–55. [PubMed: 12561940]
- [198]. Patel MS, Srinivasan M, Laychock SG. Metabolic programming: Role of nutrition in the immediate postnatal life. *J Inherit Metab Dis* 2009;32:218–28. [PubMed: 19096914]
- [199]. Patel MS, Vadlamudi S, Johanning GL. Artificial rearing of rat pups: implications for nutrition research. *Annu Rev Nutr* 1994;14:21–40. [PubMed: 7946518]
- [200]. Patel MS, Vadlamudi SP, Johanning GL. Overview of pup in a cup model: hepatic lipogenesis in rats artificially reared on a high-carbohydrate formula. *J Nutr* 1993;123:373–7. [PubMed: 8429388]
- [201]. Faust IM, Johnson PR, Hirsch J. Long-term effects of early nutritional experience on the development of obesity in the rat. *J Nutr* 1980;110:2027–34. [PubMed: 7420206]
- [202]. Duff DA, Snell K. Effect of altered neonatal nutrition on the development of enzymes of lipid and carbohydrate metabolism in the rat. *J Nutr* 1982;112:1057–66. [PubMed: 6953166]
- [203]. Plagemann A, Heidrich I, Gotz F, Rohde W, Dörner G. Obesity and enhanced diabetes and cardiovascular risk in adult rats due to early postnatal overfeeding. *Exp Clin Endocrinol* 1992;99:154–8. [PubMed: 1526266]
- [204]. Zippel U, Heidel E, Plagemann A, Davidowa H. Action of CCK and 5-HT on lateral hypothalamic neurons depends on early postnatal nutrition. *Nutr Neurosci* 2001;4:143–52. [PubMed: 11842882]
- [205]. Davidowa H, Heidel E, Plagemann A. Differential involvement of dopamine D1 and D2 receptors and inhibition by dopamine of hypothalamic VMN neurons in early postnatally overfed juvenile rats. *Nutr Neurosci* 2002;5:27–36. [PubMed: 11929195]
- [206]. Davidowa H, Li Y, Plagemann A. Altered responses to orexigenic (AGRP, MCH) and anorexigenic (alpha-MSH, CART) neuropeptides of paraventricular hypothalamic neurons in early postnatally overfed rats. *Eur J Neurosci* 2003;18:613–21. [PubMed: 12911757]
- [207]. Davidowa H, Li Y, Plagemann A. Hypothalamic ventromedial and arcuate neurons of normal and postnatally overnourished rats differ in their responses to melanin-concentrating hormone. *Regul Pept* 2002;108:103–11. [PubMed: 12220733]

- [208]. Davidowa H, Plagemann A. Hypothalamic neurons of postnatally overfed, overweight rats respond differentially to corticotropin-releasing hormones. *Neurosci Lett* 2004;371:64–8. [PubMed: 15500968]
- [209]. Davidowa H, Plagemann A. Insulin resistance of hypothalamic arcuate neurons in neonatally overfed rats. *Neuroreport* 2007;18:521–4. [PubMed: 17496815]
- [210]. Davidowa H, Plagemann A. Inhibition by insulin of hypothalamic VMN neurons in rats overweight due to postnatal overfeeding. *Neuroreport* 2001;12:3201–4. [PubMed: 11711856]
- [211]. Davidowa H, Ziska T, Plagemann A. Arcuate neurons of overweight rats differ in their responses to amylin from controls. *Neuroreport* 2004;15:2801–5. [PubMed: 15597058]
- [212]. Xiao XQ, Williams SM, Grayson BE, Glavas MM, Cowley MA, Smith MS, Grove KL. Excess weight gain during the early postnatal period is associated with permanent reprogramming of brown adipose tissue adaptive thermogenesis. *Endocrinology* 2007;148:4150–9. [PubMed: 17525123]
- [213]. Srinivasan M, Mitrani P, Sadhanandan G, Dodds C, Shbeir-ElDika S, Thamotharan S, Ghanim H, Dandona P, Devaskar SU, Patel MS. A high-carbohydrate diet in the immediate postnatal life of rats induces adaptations predisposing to adult-onset obesity. *J Endocrinol* 2008;197:565–74. [PubMed: 18492820]
- [214]. Srinivasan M, Dodds C, Ghanim H, Gao T, Ross PJ, Browne RW, Dandona P, Patel MS. Maternal obesity and fetal programming: effects of a high-carbohydrate nutritional modification in the immediate postnatal life of female rats. *Am J Physiol Endocrinol Metab* 2008;295:E895–903. [PubMed: 18682533]
- [215]. Breton C, Lukaszewski MA, Risold PY, Enache M, Guillemot J, Riviere G, Delahaye F, Lesage J, Dutriez-Casteloot I, Laborie C, Vieau D. Maternal prenatal undernutrition alters the response of POMC neurons to energy status variation in adult male rat offspring. *Am J Physiol Endocrinol Metab* 2009;296:E462–72. [PubMed: 19088253]
- [216]. Delahaye F, Breton C, Risold PY, Enache M, Dutriez-Casteloot I, Laborie C, Lesage J, Vieau D. Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. *Endocrinology* 2008;149:470–5. [PubMed: 18006626]
- [217]. Bellinger L, Lilley C, Langley-Evans SC. Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. *Br J Nutr* 2004;92:513–20. [PubMed: 15469656]
- [218]. Crnic LS, Chase HP. Models of infantile undernutrition in rats: effects on milk. *J Nutr* 1978;108:1755–60. [PubMed: 568655]
- [219]. Crnic LS. Models of infantile malnutrition in rats: effects on maternal behavior. *Dev Psychobiol* 1980;13:615–28. [PubMed: 6775995]
- [220]. Crnic LS, Chase HP. Models of infantile undernutrition in rats: effects on brain lipids. *Dev Neurosci* 1980;3:49–58. [PubMed: 7418629]
- [221]. Codo W, Carlini EA. Postnatal undernutrition in rats: attempts to develop alternative methods to food deprive pups without maternal behavioral alteration. *Dev Psychobiol* 1979;12:475–84. [PubMed: 488531]
- [222]. Nagy ZM, Porada KJ, Anderson JA. Undernutrition by rearing in large litters delays the development of reflexive, locomotor, and memory processes in mice. *J Comp Physiol Psychol* 1977;91:682–96. [PubMed: 559697]
- [223]. Simmons RA, Templeton LJ, Gertz SJ. Intrauterine growth retardation leads to the development of type 2 diabetes in the rat. *Diabetes* 2001;50:2279–86. [PubMed: 11574409]
- [224]. De Blasio MJ, Gatford KL, McMillen IC, Robinson JS, Owens JA. Placental restriction of fetal growth increases insulin action, growth, and adiposity in the young lamb. *Endocrinology* 2007;148:1350–8. [PubMed: 17110432]
- [225]. De Blasio MJ, Gatford KL, Robinson JS, Owens JA. Placental restriction of fetal growth reduces size at birth and alters postnatal growth, feeding activity, and adiposity in the young lamb. *Am J Physiol Regul Integr Comp Physiol* 2007;292:R875–86. [PubMed: 17023666]
- [226]. De Blasio MJ, Gatford KL, Robinson JS, Owens JA. Placental restriction alters circulating thyroid hormone in the young lamb postnatally. *Am J Physiol Regul Integr Comp Physiol* 2006;291:R1016–24. [PubMed: 16627695]

Figure 1. Body-weight regulation neurocircuitry in the rodent
Neuronal populations and projections in the ARH (A), LHA (B) PVH (C) midbrain and brainstem (D)

Figure 2. Postnatal development of AGRP and CART-ir in the LHA and ARH

AgRP fibers are shown in green and CART fibers and cells are shown in red. CART is developed in the LHA at birth whereas the neurons and projections within the ARH continue to mature rapidly until P16. AgRP and CART are never found to be colocalized but close appositions are made throughout the area. Images were captured with a 25X oil objective (1.25 NA).

Figure 3. Fluorescent Photomicrographs of AgRP and CART-ir in the LHA, ARH and PVH in the adult nonhuman primate

AgRP fibers are shown in green and CART fibers are shown in red. No colocalization is evident between fibers and neurons expressing AgRP and CART. Images were captured with a 25X oil objective (1.25 NA).

Figure 4. Postnatal development of Orexin and MCH-ir in the LHA of the rodent and NHP
 Orexin and MCH are two separate neuronal subtypes within the LHA. At P5, orexin and MCH neurons have robust expression of peptide. In the NHP, light staining exists at G100 and increases by G130, the beginning of the third trimester. Images were captured with a 25X oil objective (1.25 NA).

Figure 5. Postnatal development of Orexin and MCH-ir in the ARH of the rodent and NHP
 Orexin and MCH are two separate neuronal subtypes within the LHA. In the rodent and NHP, orexin fiber projections to the ARH develop in advance of MCH projections. In the rodent these projections develop postnatally whereas in the NHP they develop in utero. Even at P15, these projections are not to the level of the adult. Images were captured with a 25X oil objective (1.25 NA).