

## Energy balance and reproduction

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### Abstract

The physiological mechanisms that control energy balance are reciprocally linked to those that control reproduction, and together, these mechanisms optimize reproductive success under fluctuating metabolic conditions. Thus, it is difficult to understand the physiology of energy balance without understanding its link to reproductive success. The metabolic sensory stimuli, hormonal mediators and modulators, and central neuropeptides that control reproduction also influence energy balance. In general, those that increase ingestive behavior inhibit reproductive processes, with a few exceptions. Reproductive processes, including the hypothalamic–pituitary–gonadal (HPG) system and the mechanisms that control sex behavior are most proximally sensitive to the availability of oxidizable metabolic fuels. The role of hormones, such as insulin and leptin, are not understood, but there are two possible ways they might control food intake and reproduction. They either mediate the effects of energy metabolism on reproduction or they modulate the availability of metabolic fuels in the brain or periphery. This review examines the neural pathways from fuel detectors to the central effector system emphasizing the following points: first, metabolic stimuli can directly influence the effector systems independently from the hormones that bind to these central effector systems. For example, in some cases, excess energy storage in adipose tissue causes deficits in the pool of oxidizable fuels available for the reproductive system. Thus, in such cases, reproduction is inhibited despite a high body fat content and high plasma concentrations of hormones that are thought to stimulate reproductive processes. The deficit in fuels creates a primary sensory stimulus that is inhibitory to the reproductive system, despite high concentrations of hormones, such as insulin and leptin. Second, hormones might influence the central effector systems [including gonadotropin-releasing hormone (GnRH) secretion and sex behavior] indirectly by modulating the metabolic stimulus. Third, the critical neural circuitry involves extrahypothalamic sites, such as the caudal brain stem, and projections from the brain stem to the forebrain. Catecholamines, neuropeptide Y (NPY) and corticotropin-releasing hormone (CRH) are probably involved. Fourth, the metabolic stimuli and chemical messengers affect the motivation to engage in ingestive and sex behaviors instead of, or in addition to, affecting the ability to perform these behaviors. Finally, it is important to study these metabolic events and chemical messengers in a wider variety of species under natural or seminatural circumstances.

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### 1. Introduction

The link between energy balance and reproduction follows from the central unifying principle of biology, the synthetic theory of evolution. According to this theory, the physiological mechanisms that we observe in extant populations are the result of natural selection having acted on random genetic variation in the ancestral populations. Thus, the mechanisms that control energy intake, storage and expenditure exist because these mechanisms are to some degree heritable, allowed animals to survive to reproductive maturity, and conferred a reproductive advantage. Living cells require a continuous supply of fuels for biosynthesis and metabolism, but food availability and energetic

demands fluctuate in most habitats, and most organisms stop eating when they engage in other behaviors that perpetuate the species. When the digestive tract is empty, the body relies on fuels and nutrients from internal and external reservoirs (body fat and food hoards, respectively). During the early evolution of animals, the ability to store significant quantities of energy inside the body and mechanisms that inhibit ingestive behaviors must have allowed animals to engage in other activities that improved reproductive success. Mechanisms that counterbalance the energetically costly activities associated with parental care of altricial offspring are presumed to have conferred a reproductive advantage. For example, some species increase energy intake during a period of intense parental care, whereas others increase both energy intake and storage in anticipation of the birth of offspring. In many bird species, the energetic needs of the new offspring require increased

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foraging and eating by both parents in order for the parents to produce a sufficient supply of crop milk. In mammals, lactation and parental thermoregulatory behaviors are the most energetically costly behaviors in the female repertoire. Prior to the birth of offspring, females of some mammalian species overeat and store the excess fuel as lipids in adipose tissue, and these stored fuels are later diverted to the energetic demands of lactation. In other mammalian species, food intake remains low throughout pregnancy while food hoarding increases during pregnancy to be eaten during the energetically demanding period of lactation. Thus, the mechanisms that control energy balance are integrated with those that control reproduction (reviewed in Refs. [34, 35, 224, 264, 267]), and thus, it is difficult to understand the physiology of energy balance, and the tendency of our own species toward positive energy balance and excessive energy storage, without understanding its link to reproductive success.

The ability to monitor internal and external energy availability must be central to the link between reproduction and energy balance. This ability allows animals to prioritize their behavioral options according to fluctuations in energetic and reproductive conditions. For example, when food is plentiful and energy requirements are low, energy is available for all of the processes necessary for immediate survival, including protein biosynthesis, maintenance of ionic gradients, waste removal, thermogenesis, locomotion, foraging, ingestion and digestion. Energetic priorities are set to include long-term investments, such as growth, immune function and reproduction. Behaviors related to territorial defense, courtship, mating and parental care receive a high priority, and surplus energy is stored as lipids in adipose tissue or hoarded in the home, nest or burrow.

Metabolic signals, hormonal mediators and modulators and neuropeptides give expression to these priorities in at least two interrelated ways. First, they are permissive for the neuroendocrine events that control spermatogenesis, ovulatory cycles and fertility. Second, in many species, the same metabolic signals and chemical messengers that increase the motivation to engage in reproductive behaviors also attenuate the motivation to engage in foraging, hoarding and eating (reviewed in Refs. [224, 267]).

Conversely, when energy is scarce, the physiological mechanisms that partition energy will tend to favor those processes that ensure the survival of the individual over those processes that promote growth, longevity and reproduction. The physiological processes that promote foraging, hoarding and ingestive behavior receive priority over reproduction because reproductive processes are energetically expensive and can be delayed when the survival of the individual is in jeopardy (reviewed in Refs. [32–35]). For example, during seasons when food availability is low and thermoregulatory demands are high, members of some mammalian species become gonadally regressed and sexually inactive. Even in species that breed year round, reproductive processes are inhibited when food availability is low

or when increased energy demands are not met by compensatory food intake [34].

During these energetic challenges, animals are predisposed toward behaviors, such as eating, foraging and food hoarding, by a variety of metabolic sensory stimuli (e.g., decreased availability of glucose and its metabolites), peripheral hormones (e.g., low plasma concentrations of insulin and leptin) and central feeding-stimulatory circuits [e.g., circuits involving neuropeptide Y (NPY) and agouti-related protein (AgRP)]. The adaptive significance of the “feeding-stimulatory” circuits is related to survival (bringing metabolic fuels, other nutrients, water, salt and other minerals into the organism to maintain cell structure and function) insofar as survival is a prerequisite to reproductive success. More important from an evolutionary perspective, the neuropeptides that stimulate eating and foraging also enhance survival during energetic challenges by inhibiting the hypothalamic–pituitary–gonadal (HPG) system. Conversely, when food is plentiful and energy demands are low, those central circuits that inhibit eating tend to facilitate aspects of reproduction. Natural selection is expected to favor those animals that curtail foraging and eating to enhance their reproductive success (reviewed in Ref. [213]).

The timing of these alternating periods of reproductive activity and quiescence differ according to species, but as a general rule, most organisms are more predisposed toward reproduction when energy is plentiful than when energy is scarce. In some species, territoriality, aggression, courtship and mating take place year round between meals, whereas in others, reproductive activities and ingestive behavior alternate within a breeding season in which energy availability is high and energy demands are low. In still other species, the energy intake and storage season precedes the breeding season. For example, in elephant seals and emperor penguins, a period of massive food ingestion and storage is followed by a 3-month period of fasting, competition for mates and breeding [9, 80, 102]. One important exception to this rule is the infertility in certain types of obesity that are due to pathologies of energy partitioning that lead to an excess storage of energy and deficits in the availability of fuels for intracellular oxidation. In such cases, infertility and overeating are the consequences of disproportionate energy storage (reviewed by Refs. [264, 267]).

Understanding the mechanisms that link energy balance to reproductive success will have clinical and agricultural benefits. It will aid in our understanding of rising obesity and associated diseases of the cardiovascular system as well as infertility and its physical and psychological sequelae. Women at both extremes of the body weight distribution and those with diabetes are at risk for various reproductive neuroendocrine disorders [153], and these disorders are typically accompanied by low circulating levels of ovarian steroids. Thus, research in this area has relevance for understanding nutritional infertility, amenorrhea, anovulation and diminished libido associated with eating disorders, such as anorexia nervosa, dieting or with

increased energy expenditure that is not offset by compensatory food intake. Low levels of ovarian steroids, especially estrogens, are associated with osteoporosis and possibly with impaired cognitive function, and therefore the appropriate design of military/athletic training and nutrition programs that optimize performance and minimize injuries requires a better understanding of sex differences in the neuroendocrine link to energy balance (reviewed in Ref. [186]). Furthermore, decreased appetite and food intake influence mortality associated with cancer, inflammatory and autoimmune diseases. In addition to its clinical relevance, this area of research is central to efforts to improve breeding and lactational performance in dairy and meat animals.

In the past 10 years, several hormones and neuropeptides have been purported to mediate the link between energy balance and reproduction. This work has been driven to a large extent by a medical/pharmaceutical approach to obesity, which emphasizes the search for a product that will bring body weight and adiposity into a hypothetical healthy and fashionable limit. In contrast, this review summarizes the same recent discoveries and integrates them with important data and theoretical concepts that have developed in physiology and behavioral neuroendocrinology over the previous 30 years. Some of these concepts include the following: (1) mechanisms that control energy balance and promote energy storage are linked to reproductive success, (2) mechanisms that control the motivation to engage in behaviors are at least partially distinct from those that control the performance of the behaviors, (3) a metabolic sensory system monitors fuel availability and acts directly on central effectors, (4) hormones can act directly on the central effectors or indirectly by changing the availability of metabolic fuels, and in turn changing the metabolic stimulus, and (5) central effectors are influenced by peripheral neural inputs to the caudal brain stem, and by detectors of metabolic fuel availability in the brain stem. In this review, energy balance and its inextricable link to reproduction are viewed within a “systems” approach, which includes the physiology and behavior of whole organisms and the habitats in which they evolved.

## 2. The loci of energetic effects on reproduction

### 2.1. Energetic effects on the HPG system

A vast array of chemical messengers and metabolic processes are involved in maintenance of energy balance. Consistent with the idea that energy-balancing mechanisms are related to reproductive success, most of these factors also influence reproductive processes, such as the HPG system. The HPG system as it functions when females are not energetically challenged is diagrammed in Fig. 1A. The master control of the HPG system lies within gonadotropin-releasing hormone (GnRH) neurons, the cell bodies of

which are located in the area that spans from the preoptic area (POA) to the arcuate nucleus (Arc) of the hypothalamus. The neurohormone GnRH has two modes of secretion. The pulse mode occurs during the follicular phase, when low concentrations of estradiol (E) have negative feedback effects on GnRH and LH secretion; that is, E limits GnRH and LH secretion to relatively low levels. The surge mode occurs during the periovulatory phase when high concentrations of E exert positive feedback effects on GnRH. The GnRH pulse generator is a little-understood oscillating neural circuit that results in the pulsatile secretion of GnRH from terminals in the median eminence into the pituitary portal system. Each pulse of GnRH leads to the release of a pulse of LH from leuteotrophs in the anterior pituitary [59]. FSH is also released. These LH and FSH pulses are critical for follicle development and steroid secretion. The rising levels of E have positive feedback on GnRH and LH, and these actions of E are required for the LH surge, which triggers ovulation (Fig. 1A).

Metabolic challenges, such as food deprivation, inhibit the HPG system at many levels (Fig. 1B and C). The primary locus, however, is the GnRH pulse generator, and these effects are similar in males and females (Fig. 1B, reviewed in Ref. [35]). Pulsatile LH secretion, follicle development and ovulation can be reinstated by pulsatile treatment with GnRH in food-deprived or food-restricted rats, sheep, pigs, cows, monkeys and women [12,32,49,50,70,83,136,162,184]. Metabolic challenges inhibit the HPG system in part by increasing the sensitivity to the negative feedback effects of E, and in part by steroid-independent effects. The role of steroid-negative feedback is discussed in detail under Sex hormones. In addition, metabolic challenges alter the GnRH, LH and FSH surge, independent of their effects on pulsatile LH secretion [67,116,167]. In male rats, GnRH synthesis is inhibited by food deprivation [104], and in female Syrian hamsters, food deprivation and other metabolic challenges decrease neural activation (as measured by FOS-like immunoreactivity) in GnRH-immunoreactive (IR) neurons [25]. In sheep, metabolic challenges, such as food deprivation or restriction, inhibit GnRH secretion into the pituitary portal circulation (reviewed by Refs. [82,119]). GnRH gene expression, immunoreactivity or content are either unchanged or increased by energetic challenges, perhaps reflecting inhibited GnRH release from neurons [75,121,166]. Inhibition of GnRH secretion leads to a cascade of inhibitory effects, including decreased gonadotropin secretion, retarded follicle development, inhibited synthesis of gonadal steroids and, in rodents and nonhuman primates, decreases in steroid-induced reproductive behaviors. Thus, deficits in most of these aspects of the HPG system can be traced to metabolic inhibition of GnRH secretion. GnRH transcription or translation might be expected to be an important locus of effect in birds and in mammalian species that are induced ovulators. In musk shrews, the inhibitory effects of food restriction on repro-

duction are rapidly reversed by refeeding [97,247,248]. Food restriction in female shrews leads to an increase in proGnRH-IR cells in the POA and greater GnRH-I content in the median eminence relative to ad-libitum-fed musk shrews that is reversed by 90 min of ad libitum feeding [247,248]. These results emphasize a role for direct effects of metabolic challenges on GnRH neurons.

2.2. Energetic effects on reproductive behavior

The neural mechanisms that control sex behavior are affected by metabolic challenges in animals that do not have spontaneous ovulatory cycles. Musk shrews are reflex ovulators; ovulation and ovarian steroid secretion are induced by mating in this species. In the musk shrew, the

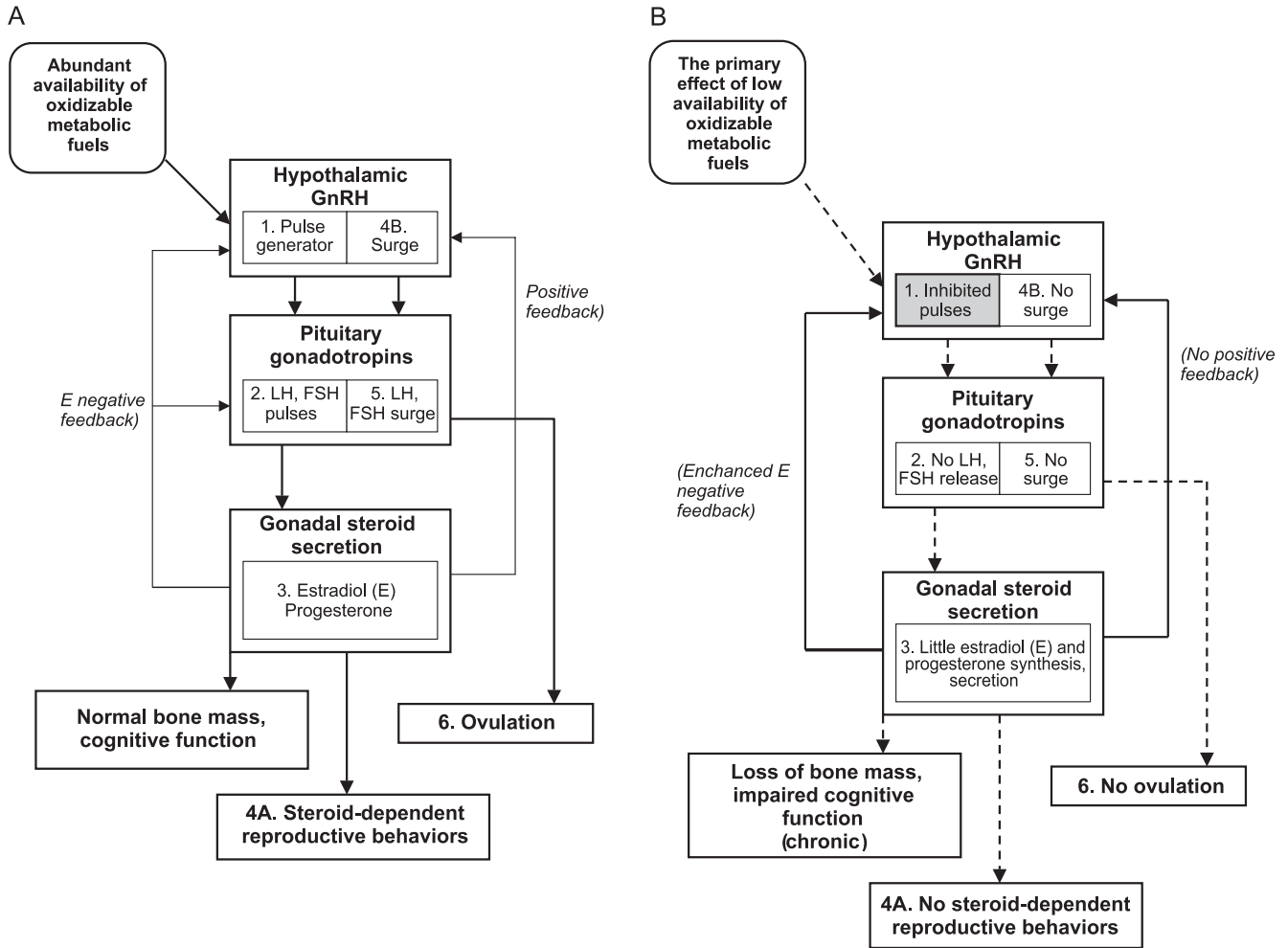


Fig. 1. (A) When there is an abundance of metabolic fuels, ovulatory cycles are characterized by the pulsatile secretion of GnRH released from the terminals of hypothalamic neurosecretory cells. Each pulse of GnRH is released into the pituitary portal circulation and stimulates the secretion of a pulse of luteinizing hormone (LH) from the anterior pituitary into the general circulation. Follicle development is stimulated by pulses of LH and FSH that bind to their respective receptors in the ovarian follicle. During follicular development (the follicular phase of the ovulatory cycle), the relatively low circulating concentrations of E have negative feedback effects on hypothalamic GnRH and pituitary gonadotropin secretion. E also has stimulatory effects on its own synthesis and secretion, and thus, E concentrations continue to rise in the systemic circulation. At higher concentrations, E has positive feedback effects on hypothalamic GnRH and pituitary gonadotropin secretion. E induces the LH surge, which in turn induces ovulation. (B) The primary effect of deficits in metabolic fuel availability is the inhibition of the hypothalamic GnRH pulse generator (1). This leads to a cascade of events, such as (2) inhibition of pituitary gonadotropin secretion, (3) inhibition of follicle development and the secretion of E and P. The effects of inhibited steroid secretion are (4a) inhibition of estrous behavior and (4b) inhibition of the LH surge. In women, when ovarian steroids remain chronically low, females experience loss of bone mass and perhaps even impaired cognitive function. We know that the GnRH pulse generator is the primary locus of effect because exogenous treatment with species-specific pulses of GnRH restores pituitary gonadotropin secretion, follicle development, ovarian steroid secretion and estrous behavior in food-deprived females (see text). (C) Deficits in metabolic fuel availability have direct effects on the reproductive system other than the GnRH pulse generator. These effects have been demonstrated by providing exogenous GnRH, or E and P to food-deprived or chronically food-restricted animals. For example, food-deprived, ovariectomized hamsters treated with E+P show estrous behavior with the same short latency, but remain in the lordosis posture for a significantly shorter duration than those hamsters fed ad libitum. Rats or sheep fed ad libitum show greater pituitary responsiveness to exogenously applied GnRH compared to food-deprived animals. In animals, such as the musk shrew, which do not show spontaneous ovulatory cycles and in which sex behavior is independent of E levels, female sex behavior is inhibited by food restriction. Sex behavior in food-restricted shrews is restored by treatment with GnRH-II, but not by treatment with GnRH-I (see text). \*One paradoxical effect of food deprivation or restriction is that pituitary LH surges in response to treatment with high doses of E are enhanced, rather than inhibited (see text and Refs. [160,240]).

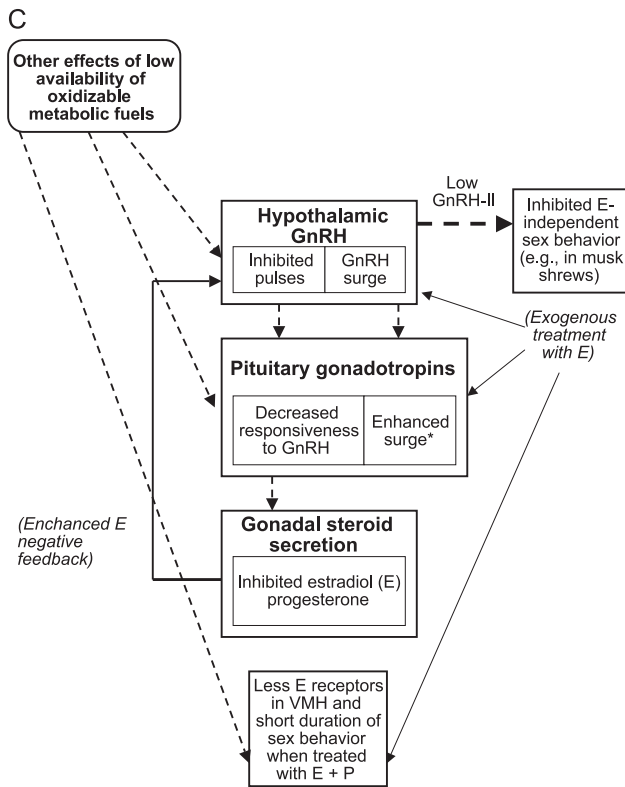


Fig. 1 (continued).

primary locus of effects on reproduction is on the neural mechanisms that control mating behavior. Sexual receptivity is inhibited in female musk shrews by a 48-h period of food restriction [97,247]. Recent evidence suggests that food-restriction-induced inhibition of sex behavior is mediated by a second form of GnRH called chicken GnRH-II. This form of GnRH was originally isolated in chickens and has more recently been found in marsupials, musk shrews, tree shrews, capybaras, monkeys and humans (reviewed Ref. [246]). Treatment with GnRH-II has little or no effect on LH secretion in fed animals; however, treatment with GnRH-II reverses the effects of food restriction on sex behavior in musk shrews [246]. Thus, it appears that GnRH-II might have a specific role in the rapid restoration of reproductive function after refeeding. It will be interesting to find whether GnRH-II plays this role in the rapid effects of refeeding on the sex behavior and the HPG system in a wider array of species.

In more commonly studied laboratory rodents, reproductive behaviors are dependent upon high concentrations of E and progesterone (P) synthesized and secreted from the ovarian follicle, which in turn is dependent upon pulsatile GnRH and gonadotropin secretion. Thus, inhibited HPG function precludes the display of steroid-induced sex behavior in these species. In addition, inhibitory effects of metabolic challenges on sex behavior can occur independent of the effects of these challenges on the HPG system even in species that show spontaneous ovulatory cycles

[72,77,78,148,187,188,203,233]. For example, in ovariectomized (OVX) Syrian hamsters brought into estrus with E and P treatment, the duration of lordosis is decreased by food deprivation [72]. Changes in lordosis duration might be related to the decrease in ER that is found in food-deprived hamsters, rats and mice in areas critical for estrous behavior, including the VMH [77,78,148,187,203].

### 2.3. Energetic effects on motivation vs. performance of ingestive and reproductive behavior

The metabolic signals, hormones and neuropeptides optimize reproductive success by prioritizing behavioral options, i.e., by changing the motivation to engage in either reproductive or ingestive behaviors. For example, in rats, food restriction increases the tendency to eat and decreases the tendency to engage in sex behavior. It is important to note that this process does not always include laboratory measures of behavioral performance, such as the amount of food eaten in a restricted time period. Brain mechanisms that control sex behavior and eating behavior do so by altering motivational aspects of the behavior as well as performance, and the neuroendocrine mechanisms that govern motivation are only partially representative of those that determine performance. For example, in some species, metabolic signals, peripheral hormones and central neuropeptides influence food procurement behaviors without necessarily influencing the amount of food ingested (reviewed by Ref. [213]). In Syrian hamsters, a period of food deprivation fails to influence subsequent food intake. In fact, hamsters rarely change meal size and frequency in response to a variety of stimuli that influence these parameters in rats [43,219,234], and yet, these same metabolic stimuli increase hunger motivation, as measured by the tendency to eat an unpalatable substance [71,219]. In nature, Syrian hamsters live in burrows where they are known to hoard large quantities of food. In the laboratory, when their nest boxes are connected to artificial burrows leading to an external food source, Syrian hamsters exhibit hoarding behavior. After a period of food deprivation, Syrian hamsters show significant increases in the amount of food hoarded, and treatment with leptin during food deprivation significantly attenuates the food-deprivation-induced increase in hoarding [43]. Similarly, in Siberian hamsters, hoarding behavior is increased by food deprivation [20] and by central treatment with AgRP [69], NPY or NPY agonists, and hoarding is decreased by treatment with NPY antagonists (D. Day and T. J. Bartness, personal communication, and reviewed in Ref. [213]). It would be interesting to examine whether the reverse is true; that is, when food is plentiful and energy demands are low, does the increased availability of energy, elevated concentrations of plasma leptin, and decreased central NPY secretion decrease the motivation to engage in hoarding and increase the motivation to engage in sex? These results emphasize that effects of leptin or other hormones and neuropeptides and metabolic sensory stimuli on the motiva-

tional aspects of sex behavior should be examined separately from the performance of the behaviors. Leptin treatment increases performance; that is, lordosis duration in OVX, steroid-primed hamsters fed ad libitum [265] and food deprivation-induced decreases in lordosis duration are prevented with NPY antagonists [133]. It would be interesting to examine the motivational aspects of sex behavior, such as courtship and solicitation. It is plausible that neuropeptides, hormones and metabolic signals do not universally influence the ingestion of food or the performance of sex, but rather, they influence the motivation to engage in species-specific

behaviors that ensure survival and reproductive success. So far, it appears that in hamsters, these factors influence food hoarding, which allows hamsters to ingest the food at a constant rate in the relative safety and more temperate environment of their burrow, and might also modulate fertility, sexual motivation and performance.

In summary, there is evidence that metabolic challenges inhibit reproduction at many levels, including the GnRH pulse generator (Fig. 1B), the GnRH, LH and FSH surges (Fig. 1C), and finally, by direct effects of metabolic fuel availability on the brain mechanisms that prioritize courtship, mating and ingestive behavior (Fig. 1C). This review will summarize evidence that metabolic and hormonal signals are detected in the caudal brain stem and periphery (Fig. 2). Peripheral neural signals from the vagus nerve are relayed in the nucleus of the solitary tract (NTS), and metabolic signals are detected in the area postrema (AP) or medial NTS and relayed to hypothalamic areas, such as the paraventricular nucleus (PVN), or to the POA via the parabrachial nucleus. From the PVN, neuronal projections are purported to influence GnRH secretion via contact with GnRH axons in the Arc. GnRH neurons also receive input from the anteroventral nucleus [105]. Other hormones and neuropeptides might act more directly in the hypothalamus to influence GnRH neurons in the Arc or POA. This neural pathway will be discussed in detail in the Central effectors section.

### 3. A system for organizing the factors that control energy balance and reproduction

The list of chemical messengers and metabolic events that control food intake and reproduction has grown rapidly since the cloning of the *ob* (obese) gene in 1994 [284]. Section 3 will organize the factors into primary sensory stimuli (Section 3.1), hormonal mediators (Section 3.2), hormonal modulators (Section 3.3), and central effector systems (Section 3.4). In Section 3.1, *primary sensory stimuli* refer to extero- and intero-sensory signals that act on sensory detectors. Extero-sensory systems are those associated with taste, smell and texture of food, as well as social, temporal and spatial cues associated with food or opposite-sex conspecifics. Intero-sensory systems are related to stimuli, such as those detected by mechanoreceptors receptors in the stomach, osmotic and nutrient receptors in the intestine and liver, and to postabsorptive stimuli generated by changes in metabolic fuel oxidation. Examples of primary metabolic stimuli are those that arise from changes in the availability and oxidation of glucose and free fatty acids (FFAs), or from the ratio of adenosine triphosphate (ATP) to the other adenine nucleotides and inorganic phosphates [84–88]. In Sections 5.2 and 5.3, signals that arise from hormones are classified as *hormonal mediators or modulators*, not as primary sensory signals. As explained in Section 3.2, the secretion of some of these

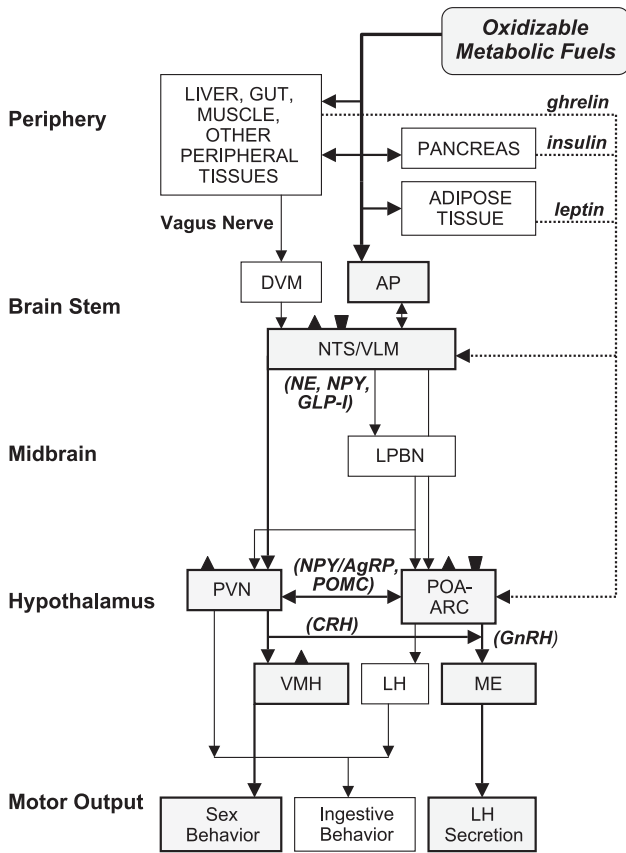


Fig. 2. Metabolic control of reproduction and ingestive behavior involves the central and peripheral nervous system and peripheral tissues. Bold-outlined boxes represent areas known to be important for metabolic control of reproduction. The primary sensory stimulus is the availability of oxidizable metabolic fuels, such as glucose and FFAs. These are detected in the caudal brain stem in areas, such as AP and reciprocally innervated NTS. In addition, it is possible that fuel availability is detected peripherally in tissues, such as the liver and gut, and these signals might be relayed to the brain via the dorsal motor nucleus of the vagus (DMV). Secretion of hormones, such as insulin and leptin, is stimulated by the influx of metabolic fuels into tissues, such as the pancreas and adipose tissue, respectively. Abbreviations: NE, norepinephrine; NPY, neuropeptide Y; CRF, corticotropin-releasing factor; GnRH, gonadotropin-releasing hormone; GLP-1, glucagon-like peptide; PVN, paraventricular nucleus hypothalamus; POA, preoptic area; LPBN, lateral parabrachial nucleus; ARC, arcuate nucleus; AgRP, agouti-related protein; VMH, ventromedial nucleus of the hypothalamus; VLM, ventrolateral medulla; LH, lateral hypothalamus; and ME, median eminence. ▼=Leptin and insulin receptors. ▲=Gonadal steroid receptors.

substances is linked to energy availability, and thus, the level of these hormones can provide information about the primary metabolic stimulus to central effector systems. Hormones inform the brain about the energetic status and reproductive state of the animal, and thereby enhance the occurrence of behavioral and metabolic adjustments that are appropriate for the environmental, reproductive and energetic conditions. For example, leptin secretion is related to adipocyte number and the flux of oxidizable metabolic fuels into adipocytes [144,268]. In most cases, the animal has the option to ignore the hormonal information if more urgent energetic needs arise related to survival and reproduction. As explained in Section 3.3, hormones can alter or modulate the metabolic stimulus. For example, some hormones alter the availability of oxidizable fuels (the primary metabolic sensory stimulus), and thus, have indirect effects on the central effector systems. For example, leptin increases energy expenditure, thermogenesis and fuel oxidation, and thus, has the capacity to influence food intake and reproduction indirectly by making more fuels available for oxidation, thereby changing the primary sensory metabolic stimulus [15,131,286]. Section 3.4 describes the *central effector systems* that are comprised of at least two putative eating-stimulatory and eating-inhibitory neural circuits that reside in the hypothalamus [224] and caudal brain stem [100,101]. The number of neuropeptides that influence food intake and reproduction is growing rapidly and will be discussed in the Central effectors section.

### 3.1. Primary metabolic sensory stimuli

Primary metabolic stimuli are generated by changes in the oxidation of metabolic fuels [84–88]. In addition, primary sensory stimuli arise from mechanical distention of the lumen, gut contractions and chemical changes within the lumen of the gut [230]. It is useful to distinguish these sensory events from endocrine events. One example of an endocrine event is the binding of a hormone, such as leptin, to the functional *Ob-Rb* receptor. In contrast to endocrine events, primary metabolic sensory stimuli result from the oxidation of metabolic substrates, such as glucose, FFAs and ketone bodies or from downstream metabolic sequelae of oxidation. For example, primary sensory signals might result from the intermediates of Krebs' cycle, electron transport of free energy that leads to the formation of ATP, changes in ATP content, or changes in the phosphorylation potential (the ratio of ATP to the other adenine nucleotides and inorganic phosphates) [87].

A metabolic sensory system that monitors energy availability and sends neural signals to the GnRH pulse generator accounts for the rapid response of the HPG system to the availability of metabolic fuels during food deprivation and refeeding. The effects of metabolic challenges on LH pulsatility occur far more rapidly than changes in body fat content in a wide variety of species [12,32,33,36,49,

50,61,229,243]. Changes in body fat content or in hormones secreted in response changes in body fat content cannot account for the increases in pulsatile LH secretion that occur within 60 min of refeeding [243]. For example, we found that estrous cycles are restored by 12–24 h of refeeding in food-deprived Syrian hamsters, and LH pulses are reinstated within an hour of refeeding in food-restricted ewes, and the restoration of reproductive function occurred prior to any increase in body fat content or in plasma leptin concentrations [27,212,243]. In contrast, the profile of metabolic fuels (ketone bodies, FFAs, triglycerides and glycerol) characteristic of the food-deprived animal is reversed within an hour of refeeding, as would be expected if a metabolic sensory system reinstated the HPG system [26]. We also found that in food-restricted ewes, pulsatile LH secretion was reinstated within an hour of refeeding and restored to the pulse frequency of fed ewes by 9 days of refeeding, despite the fact that adipose tissue and plasma leptin concentrations did not increase significantly above the levels of the food-restricted groups [243]. Together, these results show the GnRH pulse generator and sex behavior are influenced by the minute-to-minute availability of oxidizable metabolic fuels.

According to the metabolic hypothesis, body fat content, caloric intake and energy expenditure control reproductive function by acting through a common sensory stimulus, the general availability of oxidizable metabolic fuels. A 48-h period of food deprivation that occurs on Days 1 and 2 of the cycle during follicular development inhibits estrous cycles in lean, but not in fat Syrian hamsters [223]. Body fat can buffer against food deprivation by virtue of the fuels that can be hydrolyzed and mobilized from lipids. Factors that increase energy expenditure, such as cold exposure or prolonged exercise, can inhibit estrous cyclicity, but only when the increase in energy expenditure is not offset by utilization of oxidizable fuels mobilized from adipose tissue or by increased food intake. For example, increased exercise or prolonged housing at cold ambient temperatures inhibits estrous cycles in Syrian hamsters only when the increased energy expenditure is not offset by increased intake [193,222]. Similar results have been found in wild and laboratory mice [26,141,161]. Consistent with the results in rodents, in women, menstrual irregularities, amenorrhea, diminished sexual desire and activity, and infertility are common in athletes and dancers, but usually only in those who fail to increase their food intake to compensate for the unusual energetic demands of their training schedules. When the training schedule is relaxed, menstrual cycles resume without a significant increase in body fat content [2,74]. Exercise-induced inhibition of LH pulsatility is ameliorated by supplemental feeding to compensate for the energy expended in exercise regardless of body fat content [153]. Some investigators have posited that short-term signals result from fuel oxidation, while “long-term” signals come from body fat content. According to the metabolic hypothesis, instead of providing a long-term

signal, body fat provides a buffer against energetic challenges by virtue of the metabolic fuels stored in adipose tissue. When these stores are exhausted, a shortage of oxidizable fuels generates a signal that inhibits the HPG system. The HPG system is initiated whenever the availability of oxidizable fuels returns, prior to restoration of body fat content.

Little is known about the nature of the sensory detectors of fuel availability. However, the existence of this sensory system is revealed by the inhibition of reproduction and increase in food intake when specific metabolic pathways are inhibited. Research on the metabolic control of the sensory system that controls food intake led to several important principles that might apply to the sensory system that controls reproduction (reviewed by Refs. [85–89]). First, the sensory stimulus is generated by changes in the oxidation of metabolic fuels or in the metabolic sequelae of that oxidation, not to one particular kind of fuel. Second, the sensory stimulus interacts with an intracellular detector rather than a membrane receptor that detects circulating concentrations of metabolic substrates. Third, the stimulus can be detected peripherally and centrally. Finally, the effects of hormones, such as insulin and leptin, can occur by indirect influences on peripheral fuel oxidation. The effects of insulin and leptin on fuel oxidation and partitioning are discussed in the Hormonal modulators section.

Food intake can be increased and reproductive processes inhibited by treatment with agents that block specific metabolic pathways, such as glucose or FFA oxidation. For example, 2-deoxy-D-glucose (2DG) or 5-thio-glucose (5TG) are glucose analogs that inhibit glucose oxidation [37]. Systemic treatment with 2DG or 5TG induces robust increases in food intake in rats and other mammals (with only a few exceptions, one of which is Syrian hamsters) [90,142,185,198]. Treatment with high doses of insulin also increases food intake by inducing hypoglycemia in rats, hamsters and other mammals [94]. It is important to note that systemic insulin and 2DG stimulate food intake but have opposite effects on plasma glucose, demonstrating that food intake is responsive to decreased glucose oxidation or its metabolic sequelae, not concentrations of plasma glucose per se. Metabolic sensory control of reproduction is remarkably similar to metabolic sensory control of food intake. Systemic treatment with 2DG increases food intake in rats, inhibits estrous cycles in hamsters, and inhibits pulsatile LH secretion in rats and ewes, despite the fact that these treatments have negligible effects on body fat content [42,82,87,90,116,175,178,215,221,223]. Consistent with the idea that estrous cycles are inhibited by 2DG's competitive inhibition of glycolysis, the effects of 2DG on estrous cycles in Syrian hamsters can be overcome by pretreatment with an equal dose of glucose or fructose [216]. In this regard, it is notable that treatment with glucose prevents the fall in plasma leptin, however, treatment with fructose does not [242], and yet estrous cycles continue when fructose is provided.

Metabolic sensory detectors are sensitive to other metabolic stimuli in addition to those generated by changes in glucose oxidation. For example, food intake is increased and reproduction is inhibited by treatments that decrease FFA oxidation, such as methyl palmitoxirate (MP) or mercaptoacetate (MA) [48,90,140,200,210]. MP binds irreversibly to carnitine palmitoyltransferase I (CPT-I), the enzyme necessary for transport of long-chain FFAs into mitochondria [259]. MA, another inhibitor of FFA oxidation treatment, acts by blockade of mitochondrial acyl-CoA-dehydrogenases [22], and peripheral, but not central treatment with MA increases food intake [140,200,210]. Treatment with inhibitors of FFA oxidation is more effective in animals that are predisposed toward utilization of fat fuels. For example, MP treatment results in larger increases in food intake in rats that have been previously food deprived, or have been fed a high-fat diet, or have been treated with 2DG [90,91]. These studies illustrate that information related to the availability of specific metabolic substrates is integrated, perhaps at the intracellular or intramitochondrial level. It has been suggested that the sensory detectors are responsive to FFA oxidation per se (reviewed in Ref. [209]); however, other evidence indicates that inhibitors of FFA oxidation influence food intake because they decrease the general availability of metabolic fuels for the formation of ATP (reviewed in Refs. [87,89]).

Reproductive processes, like food intake, are also sensitive to changes in FFA oxidation, especially when animals are predisposed toward utilization of fat fuels. Fat hamsters do not show fasting-induced anestrus, but MP treatment inhibits estrous cyclicity in fat, fasted Syrian hamsters although MP fails to induce hyperphagia [219,221]. MP is more effective in inducing anestrus and in increasing food intake when given to food-deprived hamsters or to rats fed a high-fat diet [90,91,215,218,221,223]. Similarly, in OVX hamsters treated with E and P, lordosis duration and ER-IR in the VMH are decreased in hamsters treated simultaneously with 2DG and MP, but not with either drug alone [148]. These results are explained by the metabolic hypothesis. During fasting, caloric homeostasis is maintained by hydrolysis of triglycerides to FFAs and glycerol. FFAs are mobilized to various peripheral tissues where they are preferentially oxidized, thereby sparing glucose for oxidation in the central nervous system (CNS). According to the metabolic hypothesis, estrous cycles continue in fat, fasted hamsters because their large adipose tissue depots are a source of FFAs for cellular oxidation during food deprivation. In fat, MP-treated hamsters, MP blocks FFA oxidation, thereby neutralizing the advantage of a high body fat content. Similarly, in MP-treated hamsters fed ad libitum, normal estrous cycles continue because carbohydrates can be used as an alternative fuel source to maintain estrous cyclicity. When the utilization of both fuels is blocked by simultaneous treatment with MP and 2DG, estrous cycles are inhibited. The metabolic signal may be related to one or

several metabolic events, or to a common final metabolic event, such as the formation of ATP [87].

The role of glucose and FFA oxidation differ depending on the species and its life history. For example, musk shrews have an exceptionally high metabolic rate. They eat almost constantly during their active phase, and they store far less body fat than mice, rats and hamsters. In musk shrews, mating behavior is inhibited by food restriction and restored by only 3 h of ad libitum feeding of a laboratory diet. The effects of refeeding are blocked by treatment with either 2DG or MA, and neither glucose alone nor fat alone are as effective as laboratory chow in restoration of mating [249]. These results suggest that shrews which store very little energy in the form of body fat, are particularly susceptible to the overall availability of metabolic fuels, and are ultrasensitive to deficits in any one type of fuel whether it be fat, carbohydrate or protein [249].

In the studies described above, pharmacological inhibitors of metabolic fuel oxidation are used as tools to tease apart the roles of different metabolic pathways. Two relatively new drugs used to study food intake include the synthesized inhibitor of fatty acid synthase (FAS), C75 and the naturally occurring FAS inhibitor, cerulenin. Both intracerebroventricular and systemic treatment with these drugs produce profound decreases in food intake and loss of body fat in rats and mice [152,158], and thus might be expected to stimulate reproductive processes. I am not aware of studies of the effects of C75 and cerulenin on reproduction, but these drugs might be worth further exploration. The mechanism and specificity of action of these agents have not been elucidated. Initial reports suggest that they inhibit the action of FAS and thereby increase concentrations of malonyl CoA, and from this, it was inferred that high concentrations of malonyl CoA would inhibit CPT-I and thus inhibit the transport of FFAs into the mitochondria and block FFA oxidation [152]. Loss of body fat in the face of inhibited FFA oxidation is paradoxical, and upon closer examination, it was shown that C75 actually increases the activity of CPT-I and peripheral FFA oxidation, despite causing increases in malonyl CoA [250]. Other investigators have suggested that these drugs inhibit food intake because they increase central glucose utilization [276]. These drugs will be of more use if the mechanism of action is reconciled with the paradoxical effects on energy balance. One limitation of all these experiments using inhibitors of metabolic pathways (including MP, MA, 2DG and C75) is that it is difficult to demonstrate that the effects of these pharmacological treatments are within the physiological range that would occur between meals and during natural energetic challenges.

With regard to systemic treatment with drugs, such as MP and 2DG, it might be suggested that the effects are so dramatic as to directly inhibit the GnRH neurons or the VMH mechanisms that control lordosis. This possibility can be ruled out because the effects of systemic 2DG and insulin treatment on reproduction and food intake are blocked by small lesions of brain nuclei located at a substantial distance

from the hypothalamus. AP lesions block the inhibitory effects of systemic 2DG or high doses of insulin on estrous cycles in hamsters, ER-IR in VMH and Arc, lordosis induced by exogenous hormone treatment in OVX hamsters, and LH pulses in rats [53,148,188,228]. Similarly, AP lesions attenuate increases in food intake induced by treatment with 2DG [63,201]. Consistent with the idea that decreased glucose oxidation is detected in the caudal brain stem, slow microinfusion of 2DG into the fourth ventricle in rats increases food intake, elicits the sympathoadrenal hyperglycemic response, and inhibits pulsatile LH secretion [175,199]. Neural activation in the AP/NTS is increased with metabolic challenges that induce anestrus (e.g., food deprivation and 2DG treatment), but not with metabolic challenges that do not induce anestrus (e.g., MP treatment in fed hamsters) [214]. Microinfusion of 0.5 M 5TG into the fourth ventricle at a rate of 1  $\mu$ l/h over Days 1 and 2 of the estrous cycle inhibits ovulation and sex behavior in Syrian hamsters fed ad libitum [285]. However, higher doses were required to induce anestrus than were required to induce a sympathoadrenal hyperglycemic response, suggesting that these two responses are dissociable. Other studies in our laboratory have shown that food deprivation-induced anestrus occurs in adrenalectomized animals (discussed in the Adrenal hormones section). Furthermore, in rats, leptin treatment eliminated the 2DG-induced increases in concentrations of corticosterone, and yet, this leptin treatment failed to prevent 2DG-induced inhibition of LH secretion [180]. These results confirm that reproductive processes are controlled by changes in metabolic fuel availability that are independent of deficits in plasma leptin concentration and increases in adrenal corticosterone. Collectively, these data provide evidence for a neural circuitry whereby information about fuel availability is detected or integrated in the brain stem and influences glucose homeostatic mechanisms, food intake and the HPG system. The sensory stimulus, while related to glucose oxidation, might actually be generated by changes in the availability of other substrates in intracellular and mitochondrial metabolism.

Vagotomy and capsaicin treatment have been used to demonstrate a role for peripheral detectors of fuel oxidation and their afferent connections via the vagus nerve in control of food intake [200,201]. In rats, vagally carried signals might play a role in inhibition of the estrous cycle by a metabolic challenge. Fasting-induced suppression of pulsatile LH secretion in rats, as well as increases in PVN ER-IR in rats and increases in medial POA ER-IR in hamsters, are reversed by total subdiaphragmatic vagotomy [46,77,148]. Evidence for vagal mediation of metabolic signals for reproduction depends on the species, the metabolic stimulus and the level of the HPG system that is observed. In Syrian hamsters, anestrus is not induced by treatment with 2,5-anhydro-D-mannitol, the fructose analog that increases food intake by reducing hepatic energy status in rats [211]. Furthermore, total bilateral subdiaphragmatic vagotomy fails to prevent anestrus, reduced lordosis duration, and

reduced ER-IR in the VMH induced by food deprivation or metabolic inhibitors [148,216,218]. Although the role of the vagus is not supported in all experiments, participation of the vagus might be important depending upon the species, the type of metabolic stimulus and the locus of effect on reproduction. It is possible that the inhibition and reinitiation of the HPG system are separate mechanisms mediated by a different metabolic sensory stimuli.

In summary, peripheral and central metabolic signals influence ingestive behavior and reproduction. When fluctuations in metabolic fuel availability alter the secretion of hormones that in turn alters ingestive behavior and reproduction, the hormones act as mediators between fuel availability and behavior. When the hormones affect metabolic fuel availability and oxidation which in turn alters behavior, hormones act as modulators of the metabolic stimulus. As discussed subsequently, a variety of hormones and central neuropeptides that affect ingestive behavior and reproductive function have significant effects on energy expenditure and partitioning (e.g., thermogenesis, cardiac output, metabolic rate, glucose and FFA oxidation, and triglyceride synthesis). Thus, it is often difficult to discriminate between the *direct* effects of hormones and neuropeptides on the central circuits that control behavior and the *indirect* effects on fuel availability and oxidation.

In the Central effectors section, a neural circuit will be described whereby metabolic signals can be detected in the AP and medial NTS, and metabolic signals detected in the periphery can reach the NTS via the vagus nerve. From these caudal brain stem areas, catecholaminergic and perhaps other types of neurons can bring information about fuel availability to the PVN, which in turn has projections to GnRH neurons (Fig. 2).

### 3.2. Hormonal mediators

Hormones are purported to serve as mediators between the metabolic sensory events and the brain mechanisms that control energy balance and reproduction when their concentrations in the blood and binding to their receptors reflect either the amount of stored energy or the availability of oxidizable metabolic fuels. It is hypothesized that the hormone crosses into the brain and binds to receptors, thereby informing the brain about the availability of internal energy. Circulating hormone concentrations are also associated with particular seasons and reproductive states, and thus, they can modulate the disposition of metabolic fuels and prioritize behaviors to optimize reproductive success.

Some general principles about hormone–behavior relationships apply to the hormones in this review. First, they are not triggers for behavior. Rather, they provide a milieu or context that changes the probability that a particular behavior will occur by modulating metabolic stimuli, altering the perception of sensory stimuli, or by acting at various nodes of integration in the CNS. Second, the motivation or drive to perform the behavior can occur in the absence of hormones.

Third, hormones can become coadapted during evolution to control a variety of behaviors and physiological processes that are important for survival to reproductive maturity and reproductive success. Fourth, different hormones serve the same function in different species, whereas the same hormone can play a different role in different species.

How do we know when a particular hormone controls a particular behavior? First, under natural circumstances in outbred nonmutant animals, circulating concentrations of hormones should rise or fall prior to the change in behavior. Second, replacement of the hormone (at doses within the physiological range) should reverse the effects of removal of the hormone on the frequency of occurrence of the behavior in the appropriate environmental context. Third, hormone binding to its own receptor must be necessary and sufficient for expression of the behavior within the normal environmental context. For example, in animals in which the source of the hormone has been removed, receptor-specific agonists applied to brain areas that contain those receptors should increase the incidence of the behavior in animals tested in the appropriate environmental context and with the appropriate social stimuli. Antagonists to those particular receptors should significantly diminish the incidence of the behavior when the hormone is infused systemically at doses within the physiological range, or in animals that have high endogenous levels of the hormone.

#### 3.2.1. Sex hormones

Sex hormones illustrate the contrast between hormonal modulators and hormonal triggers. Male mating behavior in response to an estrous female develops at the time of puberty concomitant with a natural rise in endogenous androgens. In laboratory rodents, male mating behavior is inhibited by castration, restored by treatment with testosterone and inhibited by antagonists to the androgen receptor implanted into the medial preoptic area (mPOA) of castrated laboratory rodents treated with testosterone. Male-typical plasma concentrations of testosterone do not *trigger* male copulatory behavior. Rather, male-typical plasma concentrations of testosterone increase the probability that a male will perform courtship and mating behaviors in the presence of a sexually motivated female, and in the absence of predators, competitors, energetic challenges or other life-threatening environmental factors. Furthermore, the sex drive can exist and be expressed without hormonal stimulation. For example, copulatory behavior continues even after gonadectomy in sexually experienced individuals of many different species, and gonadal hormones are dissociated from sex behavior in other species. Sex hormones also serve to illustrate the idea that hormones can become coadapted during evolution to control a variety of behaviors and physiological processes that are important for survival to reproductive maturity and reproductive success. For example, in rodents and other species, ovarian secretion of estrogen is necessary for the LH surge and ovulation. Ovarian estrogen and P have become coadapted during

evolution to increase the probability that mating occurs during the time when fertilization of the ovum is likely to be successful. Sex hormones also serve to illustrate the related notion that species differ in the hormones that control the behavior. In some species, E and P are critical for female-typical sex behavior, while in others; androgens or glucocorticoids are critical for the synchronization of sex behavior and ovulation, such as in musk shrews. Similarly, male-typical sex behavior is influenced by estrogens, androgens or P, or by none of these steroids, depending on the species. During evolution, P has been coadapted to facilitate male-typical behavior in certain species that lack testosterone. P has the potential to influence male sex behavior in all mammals, including *Homo sapiens* [66]. Attention to a diverse array of species has been essential to understanding how our behavior and physiology can be influenced by hormones, neuropeptides, neuromodulators and various drugs that act on the endocrine system.

Similarly, the reproductive hormones have been coadapted to modulate ingestive behavior, energy storage and energy expenditure in service of survival and reproductive success. Hormones increase the probability of eating when fuels are needed, but the drive exists apart from hormones. The motivation to eat can be elicited directly by changes in fuel availability without changes in sex hormones. The hormones differ from species to species. In at least some species, the hormones of pregnancy and lactation have been coadapted to facilitate increases in food intake. For example, in most species, circulating levels of P rise dramatically during pregnancy while E levels rise to a lesser extent. In OVX female rats, treatment with moderate levels of E decreases whereas treatment with high levels of P increases food intake (reviewed by Ref. [264]). In these species, it appears that the hormones of pregnancy can increase energy intake and storage in preparation for the energetic demands of lactation. Lactation, in contrast, is characterized by high levels of the anterior pituitary hormone, prolactin. Not surprisingly, prolactin treatment induces a dose-dependent increase in food intake in OVX rats primed with E [95,96]. Changes in levels of these hormones, however, do not fully account for the changes in food intake. The increases in food intake during lactation are of a greater magnitude than those induced by exogenous hormone treatment. These hormones influence food intake when microinfused directly into the brain, and yet, no one would argue that they are either “triggers” or “primary sensory signals” for energy availability and oxidation. Rather, they inform the brain about the reproductive state of the animal, and enhance the occurrence of behavioral and metabolic adjustments that are appropriate for that phase of reproduction in that particular species. During lactation, when vast amounts of energy are diverted toward milk production, powerful interoceptive signals probably arise directly from changes in energy status in the mother’s CNS or periphery, and these are more likely to account for the dramatic increases in food intake. The hormones of pregnancy act in concert with or accentuate the

primary metabolic sensory signals that arise from the energetic demands of reproduction. Moreover, the metabolic sensory signals and the hormonal mediators affect not only food intake, but also the utilization and partitioning of stored energy, and there are species differences in the way these hormones modulate food intake, storage and expenditure (reviewed by Ref. [264]). During pregnancy, female rats, mice and some women increase energy intake in excess of the demands of the growing conceptus, thereby bolstering their own maternal energy stores in anticipation of the energetic demands of lactation. In contrast, females of other species, such as Syrian hamsters, do not increase food intake during pregnancy. Rather, they continue to eat the same amount of food as nonpregnant hamsters and mobilize their own fat stores to meet the demands of the growing conceptus [263]. During pregnancy, Siberian hamsters also fail to increase food intake and, as a consequence, lose internal energy stores (body fat) [220], but at the same time increase food hoarding, an appetitive behavior that increases external energy stores [19]. Unlike rats and other species, female hamsters enter lactation in a state of internal negative energy balance, and meet the energetic demands of lactation by increasing food intake, thereby taking advantage of their externally stored food. Females of other species, such as Grey seals, do not eat during lactation. During this period of intense energy expenditure, they repartition their energy to draw upon their maternal fat stores and direct energy toward milk production (reviewed by Ref. [264]). These examples illustrate that different species use a variety of energetic strategies, employing different hormonal signals, with different patterns of intake, storage and expenditure, to meet their energetic requirements for reproductive success.

Most investigators study reproduction independent of food intake, and focus on the amount of food consumed in a particular time period. The motivational or appetitive aspects of ingestive behavior are also important, because the motivational mechanisms determine whether an animal will choose to engage in reproductive, ingestive or other behaviors.

The effects of energetic challenges on physiology and behavior are not always mediated by changing the secretion of sex hormones, but rather, by changing sensitivity to the hormone, probably by changing expression of the receptors for the hormone. Metabolic challenges inhibit ovulatory cycles and the HPG system in part by increasing hypothalamic sensitivity to the negative feedback effects of E. Food deprivation inhibits LH secretion in OVX rats treated with E, but not those treated with vehicle [45,177,181]. Most evidence suggests that E-negative feedback on GnRH occurs transsynaptically via neurons that project to GnRH or via glial cells, not via estrogen receptor- $\alpha$  (ER- $\alpha$ ) located on GnRH neurons themselves (reviewed by Ref. [111]). The essential E-negative feedback during energetic challenges might occur in the PVN of the hypothalamus, because microinfusion of E into the PVN accentuates fasting-induced suppression of pulsatile LH secretion in

OVX rats [181], and ER-IR is increased in the PVN in food-deprived rats [78] and the mPOA in Syrian hamsters [148]. These brain areas either contain GnRH neurons (mPOA) or contain nuclei that project to GnRH axons in the Arc, median eminence. Recent evidence suggests that estrogen might exert some effects on GnRH neurons directly through receptors for ER- $\beta$  [3,112,118]. GnRH neurons express ER- $\beta$  and protein at very low levels, and investigators have used these data to argue for and against a functional role for ER- $\beta$  [112,117,118,126,239]. However, ER- $\beta$  knockout (KO) mice exhibit subnormal fertility [139], suggesting that ER- $\beta$  plays at least some functional role in control of the HPG system. These effects might be through the well-known effect of ER binding on gene transcription, or through rapid nongenomic effects on intracellular signaling pathways. Estrogen treatment results in rapid phosphorylation of cAMP response element-binding protein (CREB) in GnRH neurons of wild-type mice, in ER- $\alpha$  KO mice, but not in ER- $\beta$  KO mice [3]. Phosphorylation of CREB is an index of changes in intracellular signaling. Thus, these results suggest that ER- $\beta$  might mediate the rapid effects of estrogen on GnRH [3]. The role of ER- $\beta$  in mediating energetic effects on steroid-negative feedback has not been studied to the best of my knowledge. Rapid nongenomic action of E via ER- $\beta$  would be consistent with the rapid effects of refeeding in restoration of the HPG system [243].

The neural mechanisms that control sex behavior are also affected by metabolic challenges and these effects are independent of the effects of metabolic challenges on the HPG system and gonadal steroid concentrations. As mentioned previously, OVX Syrian hamsters brought into estrus with E and P treatment, show decreased lordosis duration in response to food deprivation, treatment with metabolic inhibitors or insulin and housing at cold ambient temperatures [72,187]. Changes in lordosis duration in response to these metabolic challenges are consistently associated with a significant decrease in ER- $\alpha$  in the VMH [77,78,148,187,203].

### 3.2.2. *Leptin*

Leptin treatment decreases food intake, increases energy expenditure, fuel availability and oxidation and facilitates reproductive processes [15,60,68,81,108,227]. It has been widely hypothesized and also dogmatically stated that plasma leptin concentrations inform the brain about the level of body adiposity or the level of metabolic fuel availability. The evidence for leptin's functional role is largely circumstantial. Leptin has not passed the critical test as a satiety signal (that is, the satiating effects of leptin are not reversed by an antagonist that is specific to its receptor [39]). To the best of my knowledge, the effects of an antagonist to the leptin receptor on reproductive processes have not been examined. The evidence for, and against, a role for leptin in metabolic control of reproduction will be reviewed in separate sections.

*3.2.2.1. Evidence in support of a role for leptin.* Mice that are homozygous for a mutation in the gene that encodes leptin, the *ob* gene, are obese and infertile, and the obesity and infertility are reversed by leptin treatment [56]. At best, this suggests that at least some leptin must be in the organism, but does not elucidate the role of natural endogenous fluctuations in leptin. Systemic treatment with leptin prevents the effects of underfeeding on several aspects of reproduction in both lean and obese laboratory rodents [4–6,17,55,56,68,103,217,265]. In a variety of species, exogenous leptin treatment reverses the effects of food restriction or food deprivation on aspects of reproduction, including the onset of puberty [4,10,13,17,55], the length of the estrous cycle [56,217,225], the length of lactational diestrus [274], gonadotropin and gonadal steroid levels [6,281,282], and pulsatile LH secretion [81,109,179] in mice, rats, hamsters and nonhuman primates. Leptin treatments that produced plasma concentrations of total leptin estimated to be within the physiological range partially, but not fully, reverse the effects of food deprivation on E-induced LH and prolactin surges in rats [269]. These results demonstrate a clear effect of exogenously administered leptin and are consistent with a potential role for endogenous changes in leptin participation in metabolic control of reproduction.

A structural neural circuit has been identified that might be involved. It has been postulated that leptin's effects on the GnRH pulse generator or on the GnRH and LH surges occur via intermediate neurons that project from *Ob-Rb*-containing neurons [169,170] to other neurons that in turn project to GnRH neurons (reviewed in Ref. [111]). These pathways have been postulated to include neurons that secrete NPY, proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). Subcutaneous infusions of mouse leptin that maintain plasma levels of total leptin within the physiological range of rats prevent fasting-induced changes in neuroendocrine function and in NPY, POMC and CART mRNA levels in the Arc in rats [5]. These neuropeptides all have projections to areas that contain GnRH neurons or to other interneurons that project to the vicinity of GnRH neurons, as discussed in the Central effectors section. Based on these and other results, it has been widely postulated that a fall in leptin might be one factor that increases hunger and/or food intake and inhibits reproduction. Increases in plasma leptin concentrations are speculated to be a permissive signal for the onset of puberty [57] and to be the stimulus for the onset of reproductive function after a period of metabolic challenge has ended.

### *3.2.2.2. Evidence that fails to support a role for leptin.*

Despite adequate neuroanatomical structures necessary for leptin control of GnRH, the functional role of leptin remains controversial. First, to the best of our knowledge, an antagonist for the leptin receptor has not been used to demonstrate that leptin binding to its receptor is necessary and sufficient for any aspect of reproductive function. Intracerebroventricular treatment with an antibody to the

ob protein attenuates LH secretion [51]; however, it is not known whether the inhibitory effects of the antibody can be overcome by excess leptin. Thus, treatment with the antibody might have had nonspecific effects on LH secretion. Second, metabolic control of fertility occurs in the complete absence of leptin. For example, when the *ob/ob* mutation, which was maintained on the C57BL/6J inbred mouse strain, is placed on a different genetic background (e.g., C57BL/6J-BALB/cJ), the *ob/ob* mice are fertile despite a lack of leptin and morbid obesity and hyperglycemia/hyperinsulinemia [79,195]. Third, natural increases in plasma leptin concentration do not precede the restoration of fertility in food-restricted/repleted sheep, are not necessary for normal estrous cyclicity in intact hamsters, and are not necessary for normal lordosis duration in OVX hamsters brought into heat with ovarian steroid treatment. In ewes, LH pulses are seen within an hour after return to ad libitum feeding, return to the pulse rate of the fed ewes 9 days after refeeding, whereas plasma leptin concentrations and body fat content do not increase significantly within 10 days after refeeding [243]. Thus, although leptin treatment can reverse the effects of food restriction on LH pulsatility, natural endogenous increases in plasma leptin cannot account for the restoration of LH pulses after refeeding. Similar results are seen in food-deprived and refed Syrian hamsters. In this species, the 4-day estrous cycle is inhibited by a 48-h period, but not a 24-h period, of food deprivation. Hamsters refed for at least 12 h show normal estrous cycles, despite the fact that plasma leptin concentrations do not increase significantly above those of food-deprived hamsters at 3, 6, 12 or 24 h after the start of refeeding [27,212]. Thus, the normal estrous cycles of 24-h fasted hamsters cannot be explained by an increase in plasma leptin after refeeding. Similar results were shown with regard to the effects of food deprivation and leptin on steroid-induced estrous behavior in Syrian hamsters. In OVX hamsters treated with E and P, the duration of lordosis is shortened by food deprivation. Hamsters refed for at least 6 h prior to sex behavior tests show normal lordosis durations similar to those of fed hamsters, and yet, plasma leptin concentrations in these refed hamsters are not significantly higher than those of food-deprived hamsters at 6, 12 and 24 h after the start of refeeding [123]. Consistent with the idea that leptin synthesis and secretion is controlled by the availability of oxidizable metabolic fuels [144,268], it might be that the slow restoration of plasma leptin concentration in hamsters is due to the lack of postfast hyperphagia in Syrian hamsters [234]. The abovementioned results with ewes and hamsters were predicted based on a number of earlier studies in rats, ewes, monkeys and pigs, demonstrating that pulsatile LH secretion is inhibited by food restriction and restored within minutes or hours of refeeding, long before significant increases in body fat content [12,32,33,35,49,50,229]. Thus, although plasma leptin levels are an approximate reflection of fuel availability, natural changes in plasma leptin concentrations cannot account for metabolic control of reproduction. To-

gether, these results question the idea that above-threshold concentrations of plasma leptin are necessary for normal reproduction.

More work is needed to clarify the role of leptin in the normal physiology of energetically challenged animals. LH pulses might be increased without increases in plasma leptin concentrations if leptin transport across the blood–brain barrier is increased by food deprivation. Leptin transport across the blood–brain barrier is decreased by food deprivation and increased after refeeding [132], but is not altered by adrenalectomy, another stimulus that decreases plasma leptin concentrations. Still another possibility is that energetic challenges influence the sensitivity to leptin via changes in Ob-Rb, similar to the model proposed to explain increased steroid-negative feedback on the HPG system. These alternative hypotheses might explain how refeeding could increase leptin binding to central leptin receptors without a concomitant increase in plasma leptin concentrations. They also lead back to the inevitable question “What is the primary sensory stimulus for control of the blood–brain barrier or control of leptin receptors?” Entry of leptin into the brain or expression of leptin receptor must be controlled by some as yet unknown stimuli that result from refeeding in the absence of changes in adipocyte filling and in the absence of changes in plasma leptin concentrations. Still another possibility is that discrete pulses of leptin influence the HPG system [14,150], or that leptin is bound to a carrier protein that masks its functional concentrations in plasma. Alternatively, leptin levels might change within tissues at times when changes in plasma leptin are undetectable. In Syrian hamsters, leptin levels rise more rapidly within subcutaneous white adipose tissue (WAT) than in plasma in response to refeeding [27,212]. One possibility is that transcription and translation of the *ob* gene are rapidly increased by the availability of metabolic fuels in the perimetrial and epididymal WAT pads that are associated with the ovary and testes, respectively. Increased leptin secretion from tissues, such as the perimetrial WAT pad (which shares extensive blood supply with the gonads), might influence the estrous cycle and behavior by altering follicle development and steroid synthesis and secretion. However, a stimulatory action of leptin on steroid synthesis would not explain the rapid restoration of LH pulses upon refeeding because in refed animals, GnRH and LH pulses resume prior to changes in ovarian steroids. Within other peripheral tissues, such as brown adipose tissue, increases in concentrations of leptin might influence the brain via peripheral afferent neurons [16]. Neural pathways whereby changes in tissue concentrations of leptin might influence reproduction are unknown.

The preceding discussion relates to evidence demonstrating that plasma leptin concentrations above those seen in food-deprived anestrus animals are not *necessary* for normal reproductive function. Other data demonstrate that high plasma concentrations of leptin are not *sufficient* for normal estrous cycles. For example, treatment with leptin cannot

prevent food-deprivation-induced anestrus when leptin-induced increases in fuel oxidation are inhibited [217,225]. In addition, insulin treatment induces anestrus in hamsters limited to the food intake of saline-treated controls, although this treatment increases, rather than decreases body fat content and plasma leptin levels [28]. These data will be discussed further in the Hormonal modulators section.

It is clear that multiple signals control reproduction. Falling plasma leptin concentrations might generate inhibitory signals, whereas a facilitatory signal might be generated by rapid signals from re-alimentation (e.g., afferent neural signals from gut distension or a fall in ghrelin concentrations, or increases in metabolic fuel availability detected in the brain stem or received via neural signals from the vagus). It would not be surprising if the importance of leptin for reproduction varied with the age, species and whether there are other more pressing sensory inputs. Although levels of leptin are roughly correlated with adiposity in most species studied, and mutant mice without functional leptin are infertile (depending on the genetic background), the role of leptin in natural fluctuations in energy balance remains untested, and the role is expected to differ with species, age, season and reproductive phase.

### 3.2.3. Pancreatic hormones

The importance of the pancreatic hormones, insulin and glucagon, in control of the HPG system is still controversial. As discussed in the previous section, high doses of insulin influence reproductive processes indirectly by diverting metabolic fuels into storage. Systemic insulin treatment at doses sufficient to cause hypoglycemia inhibits LH secretion and estrous cyclicity, while at the same time it increases food intake, body fat content and plasma leptin content [28,266]. Conversely, low-dose intracerebroventricular insulin treatment tends to decrease food intake and facilitate pulsatile LH secretion [11,21,73,171]. In male sheep, streptozotocin-induced diabetes reduces the frequency of LH pulses [41], but this can be reinstated by intracerebroventricular treatment with insulin at doses that affect neither peripheral insulin nor glucose concentrations [245]. Mice that have a functional disruption of the insulin receptor gene when it is expressed in the brain, but not the periphery, show hypothalamic hypogonadism. The effects on food intake and on LH levels are exaggerated in females [38]. Thus, a total absence of functional insulin is incompatible with LH pulsatility and fertility. One possibility that has not been explored is whether the permissive effects of central insulin are due to direct action of insulin on brain circuitry for the GnRH pulse generator, or are secondary to the effects of insulin on glucose uptake in the brain. In male rhesus monkeys, meal-induced increases in insulin are not necessary for the meal-induced increases in pulsatile LH secretion [271]. This might represent a species difference in the role of insulin; however, to the best of our knowledge, similar experiments have

not been performed to examine the role of meal-induced increases in insulin in other species.

### 3.2.4. Ghrelin and CCK

Orexigenic peptides released from the gut tend to inhibit GnRH and LH secretion. Secretion of ghrelin, a 28 amino acid peptide synthesized in the stomach [138], is inhibited by meals and increases gradually during intermeal intervals. Systemic treatment with ghrelin induces hyperphagia and obesity [256,277]. Ghrelin might act on brain mechanisms that increase food intake and suppress pulsatile LH secretion because intracerebroventricular or microinjection of ghrelin into the PVN increases food intake in intact rats and inhibits pulsatile LH secretion in OVX rats [92,168,182]. Ghrelin is present in the brain as well as the gut, and thus, ghrelin has been purported to act as a central neuropeptide on the hypothalamic mechanisms that control food intake and reproduction. Conversely, other peptides tend to facilitate GnRH and LH secretion. Intravenous injection of CCK decreased food intake and stimulates GnRH release in male monkeys [189], while the gut peptide motilin decreases food intake and inhibits pulsatile LH secretion in rats [258]. The role of peripheral hormones in hunger and satiety is controversial at best. However, these data are consistent with the idea that when fuels are in short supply, a number of hormonal factors might conspire (either directly or indirectly) to increase food procurement and to conserve energy for the processes necessary for immediate survival. It is also important to note that these peptides have not met the criteria to qualify as necessary and sufficient for changes in food intake and reproduction; that is, the effects of these hormones have not been prevented consistently by peripherally administered antagonists that are specific to their receptors, to the best of my knowledge.

### 3.2.5. Adrenal hormones

The glucocorticoids, cortisol and corticosterone, as well as adrenal catecholamines, are secreted in response to food deprivation, generalized stress and cerebral metabolic emergency, and these hormones have inhibitory effects of reproductive processes. Thus, it might be hypothesized that adrenal hormones mediate the effects on metabolic challenges on the HPG system. Most evidence fails to support a critical role for adrenal steroids. The effects of glucoprivation (2DG treatment) on pulsatile LH secretion are not mediated via adrenal corticosterone. 2DG-induced decreases in LH secretion are not eliminated by leptin treatments that prevent 2DG-induced increases in plasma corticosterone [180]. In Syrian hamsters, neither adrenalectomy nor treatment with a glucocorticoid antagonist precludes the effects of food deprivation on estrous cyclicity, ruling out adrenal steroids and catecholamines as critical factors in metabolic control of estrous cyclicity in hamsters [29]. Furthermore, estrous cycles are inhibited by treatment with high doses of insulin in hamsters that are not allowed to show insulin-induced hyperphagia, but estrous cycles are not inhibited by

the same insulin treatment when hamsters are allowed to overeat. Both of these groups show cortisol concentrations higher than those of hamsters that show food-deprivation-induced anestrus. Hypercortisolemia is not sufficient to induce anestrus in the insulin-treated hamsters fed ad libitum [28,29]. In contrast, insulin-induced inhibition of LH secretion in rats is prevented by adrenal demedullation [47], suggesting that this particular metabolic challenge is mediated by adrenal catecholamines in at least one species.

### 3.3. Hormonal modulators

The previous section focused on the ability of hormones to act as mediators between energy balance and reproductive processes. This section examines a different role for hormones. Hormones, such as insulin and leptin, might influence reproduction by virtue of the ability of these hormones to modulate the intracellular availability and oxidation of glucose and FFAs.

#### 3.3.1. Leptin as a modulator

It is most often assumed that leptin acts directly on hypothalamic mechanisms that control the HPG system. It is important to note, however, that leptin, whether administered peripherally or centrally, dramatically up-regulates energy expenditure, thermogenesis, and fuel oxidation, and thus, has the capacity to influence food intake and reproduction indirectly by making more fuels available for oxidation, thereby changing the primary sensory metabolic stimulus [15,54,131,204,261,270,286]. In the Hormonal mediators section, I noted that natural endogenous changes in plasma leptin levels cannot explain rapid changes in reproductive status. However, exogenous treatment with leptin prevents the effects of food deprivation in a wide variety of species. It is possible that some or all of these effects of exogenous leptin occur via the effects of leptin on intracellular fuel oxidation. In support of this hypothesis, fasting-induced anestrus in Syrian hamsters is not significantly attenuated by either intraperitoneal or intracerebroventricular leptin treatment when each injection of leptin is preceded by an injection of the metabolic inhibitors 2DG or MP, although these doses of MP and 2DG did not induce anestrus in hamsters fed ad libitum [217,225]. These results might reflect an interaction between leptin and metabolic inhibitors at the level of intracellular fuel oxidation, although interactions at other levels are possible. A few studies suggest that leptin increases the availability and oxidation of glucose [131,172], whereas 2DG treatment has the opposite effect [37]. There is now a great deal of evidence consistent with the idea that leptin increases the intracellular availability and oxidation of FFAs. FFAs can be made available for oxidation by (1) decreasing de novo FFA synthesis, (2) decreasing intracellular FFA esterification to triglycerides, (3) increasing breakdown of triglycerides and FFA oxidation and (4) FFA exportation to nonadipose tissues. Leptin treatment of adipocytes decreases de novo

FFA synthesis, increases intracellular FFA esterification to triglycerides, increases triglyceride breakdown and FFA oxidation, and increases FFA exportation to nonadipocytes [270]. The net result is a 30% increase in the net efflux of FFAs from adipocytes, which is thought to prevent oxidation within adipocytes and promote FFA oxidation in nonadipocytes [270]. This is thought to occur via inhibition of acetyl CoA decarboxylase and consequent disinhibition of CPT-I [40,173,286]. It is interesting to note that the inhibitor of FFA oxidation that inhibits estrous cycles in fat, food-deprived hamsters (MP treatment) has the opposite effects, irreversibly binding CPT-I and preventing transport of FFAs into the mitochondria [259]. Because MP does not reach the brain in appreciable quantities, it might be suggested that the interaction between leptin and MP occurs intracellularly in ovary, liver or muscle where FFA oxidation is important for fuel homeostasis during fasting.

If leptin improves reproductive function by virtue of its ability to increase fuel oxidation, it would be predicted that leptin would fail to improve reproductive function when fuels are no longer available. In support of this idea, treatment with leptin can fully reverse the effects of fasting on puberty in rats restricted to 80% of their ad libitum food intake, but not in rats restricted even further to 70% of their ad libitum intake [57]. Similarly, in OVX hamsters brought into estrus by E and P treatment, leptin increases the duration of lordosis in ad-libitum-fed, but not in food-deprived, hamsters [265]. Food deprivation might limit the ability of leptin to facilitate sex behavior via increases in energy availability and oxidation. Furthermore, leptin treatment can shorten the duration of lactational diestrus in rats exposed to acute food deprivation, but not in rats exposed to prolonged chronic food restriction [274,275], consistent with the idea that leptin cannot facilitate reproduction if there is a deficit of fuel availability. Similarly, leptin treatment can *attenuate* but cannot fully overcome the effects of food deprivation on the E-induced LH and prolactin surges in rats [269]. In transgenic skinny mice that overexpress leptin, reproductive maturity is reached at an earlier age than in wild-type mice. However, these mice have extremely high levels of energy expenditure, thermogenesis and fuel oxidation and have virtually no body fat. Consistent with the idea that leptin facilitates reproduction via increases in fuel availability and oxidation, the reproductive system of transgenic skinny mice fails rapidly after puberty is attained, several months earlier than in wild-type mice with normal leptin expression [283]. Mice that lack a functional leptin gene (*ob/ob* mice) are obese and infertile. However, the obesity is prevented when *ob/ob* mutants have another mutation in the gene that encodes stearoyl-CoA desaturase-1 (SCD-1) [62], an enzyme that is critical for biosynthesis of the monounsaturated fats palmitoleate and oleate from saturated fatty acids. Mutations in the SCD-1 gene result in upregulation of energy expenditure and FFA oxidation in *ob/ob* mice, which can ameliorate the obesity in animals that lack a functional *ob* protein [62]. It will be

interesting to find if the SCD-1 mutation also reverses the infertility in *ob/ob* mice. It is possible that infertility in *ob/ob* mice is related to a deficit in FFA oxidation resulting from a predisposition toward triglyceride synthesis and storage. Leptin is important for preventing triglyceride synthesis and promoting intracellular glucose and FFA oxidation (reviewed by Ref. [260]). Lack of leptin in the *ob/ob* mutant might influence the reproductive system by decreasing the intracellular availability and oxidation of fuels. If so, it would be predicted that the double mutant (*ob/ob*, SCD-/SCD-), which has elevated energy metabolism and FFA oxidation, would also be fertile, despite a lack of functional leptin.

### 3.3.2. Insulin treatment as a modulator

The notion that infertility results from excess energy storage (as occurs in some types of obesity) and the associated deficit in metabolic fuel availability and oxidation is illustrated by experiments that employ high-dose insulin treatment as a tool to shunt endogenous metabolic fuels out of circulation in to tissues where they are stored. Systemic insulin treatment at high doses increases food intake and promotes fat storage in mammalian species, including Syrian hamsters. Insulin treatment inhibits estrous cycles in Syrian hamsters that are limited to the food intake of the saline-treated control hamsters, but the same dose of insulin fails to inhibit estrous cycles when the hamsters are allowed to overeat to compensate for increased energy storage [266]. Thus, the effects of insulin are to promote obesity and inhibit estrous cyclicity. The inhibitory effects of insulin are not direct effects of insulin on reproduction, because the hamsters treated with insulin and fed ad libitum show normal estrous cycles. Insulin only inhibits reproduction in hamsters that are not allowed to overeat in response to insulin treatment.

The adipostatic hypothesis, the idea that reproduction is controlled by changes in some hormonal mediator of body fat content, predicts that both the ad-libitum-fed and the food-limited, insulin-treated hamsters would show normal estrous cycles because both groups *gain* body fat and both have high concentrations of plasma leptin [28]. To the contrary, insulin inhibits estrous cyclicity only in the food-limited hamsters while causing both groups to gain body fat and elevated concentrations of leptin [28]. This phenomenon is not limited to Syrian hamsters, because in female rats and ewes, pulsatile LH secretion is inhibited by insulin infusion, but not when the metabolic effects of insulin are offset by simultaneous glucose infusion [61,107,202].

It is interesting to note that insulin-treated hamsters are infertile despite their high body fat content and plasma leptin concentrations. This experimental model illustrates how infertility can accompany certain types of obesity. If the obesity and hyperphagia result from a predisposition toward energy storage that precludes sufficient availability of fuels for intracellular oxidation, this would be expected to create a deficit in metabolic fuel availability that would generate stimuli that are inhibitory for the HPG system (reviewed in

Refs. [264,267]). For example, the infertility in *ob/ob* mice that lack functional leptin might be reversed by treatments that increase fuel oxidation. In support of this idea, both intracerebroventricular and peripheral leptin treatment increases fuel oxidation [54,131,270,286].

Together, the results of experiments that have examined insulin and leptin emphasize the idea that hormones can influence reproduction and energy balance indirectly, via effects on the metabolic sensory stimulus. In this sense, they are modulators of the metabolic stimulus, rather than mediators between the level of internal energy and the central effectors.

### 3.4. Central effectors

Hormones and metabolic sensory stimuli influence energy balance and reproduction by acting on effector systems in the brain stem and hypothalamus. The central effector for control of the HPG system and estrous cyclicity is the circuit of GnRH neurons located in the medial basal hypothalamus (including the Arc), the anterior hypothalamus and POA. The effectors for female sex behavior are less well defined but probably include the VMH, PVN and mPOA. The effectors for food intake are even more diffuse and include the areas mentioned for control of female sex behavior, plus the lateral hypothalamus, dorsomedial hypothalamus and other areas. Metabolic sensory information reaches these hypothalamic areas involved in the HPG system, sex and ingestive behavior via the caudal brain stem. Hormones can influence central effectors via modulation of the metabolic stimulus, or by direct action on neurons in the hypothalamic areas [224].

#### 3.4.1. Gonadotropin-releasing hormone

Most evidence indicates that metabolic sensory signals and hormones influence GnRH neurons indirectly through other peripheral and central pathways. In hamsters, AP/mNTS lesions prevent the inhibitory effects of systemic glucoprivation (2DG treatment) or hypoglycemia (insulin treatment) on estrous cycles [187,188,228]. Thus, systemic deficits in the availability of oxidizable glucose are not detected directly by GnRH neurons, but rather, these deficits are detected in the periphery or caudal brain stem and relayed to forebrain GnRH neurons. In rats and hamsters, most of the GnRH perikarya are in the AH, POA and anterior Arc, brain areas rostral to those rich in functional leptin receptors (Ob-Rb) and insulin receptors, and thus, it is unlikely that GnRH secretion is directly modulated by leptin and insulin. Receptors for ER- $\alpha$ , NPY, leptin and insulin have not been reported in GnRH cell bodies from whole animal preparations, to the best of my knowledge, despite well-known effects of treatment with these substances on GnRH secretion. It has been noted that leptin receptor mRNA is found on GT1-7 cells in culture using gene amplification, and GT1-7 cells secrete GnRH in response to leptin addition to the media [157]. However, sampling

GT1-7 cells from an *in vitro* culture is probably not representative of the sparsely distributed GnRH cells *in vivo*. To the best of our knowledge, Ob-Rb receptors on GnRH-IR neurons are not found *in vivo*. Thus, it seems more likely that hypothalamic and extrahypothalamic areas rich in receptors such as ER- $\alpha$  and Ob-Rb project to areas that contain GnRH neurons (reviewed by Ref. [111]).

#### 3.4.2. Catecholamines

Research on control of food intake has led to discovery of a circuit by which changes in glucose availability and oxidation are detected in the AP and NTS and travel via noradrenergic projections to forebrain areas involved in ingestive behavior and the HPG system (Fig. 2). The role of the AP/NTS in detection of deficits in the availability of oxidizable glucose was described in Section 3.1. The AP has reciprocal connections to the NTS. Brain stem catecholamine cell bodies located in A2 area of the NTS, project entirely in the rostral direction, and many of them project to the PVN [208]. Norepinephrine (NE) is increased in the PVN in response to 2DG treatments that inhibit pulsatile LH secretion [176], and the inhibition of LH secretion by 2DG treatment or food deprivation are prevented by infusion of an NE synthesis inhibitor into the PVN [156,176]. These NE projections that emanate from the NTS are necessary for the effects of glucoprivation on food intake and reproduction. Injections of the immunotoxin saporin, conjugated to an antibody against dopamine- $\beta$ -hydroxylase (DSAP) that selectively targets catecholamines, prevent 2DG-induced increases in food intake [197] and 2DG-induced increases in the length of the estrous cycle [120]. These DSAP lesions severely reduced tyrosine hydroxylase immunoreactivity, a marker for catecholaminergic cells in the caudal brain stem, including the AP and NTS [120,197]. These results provide strong evidence for mediation of glucoprivic effects on reproduction and food intake by catecholaminergic projections from the brain stem.

Inhibition of LH secretion via these catecholaminergic projections might be mediated by up-regulation of PVN ER- $\alpha$  levels. For example, PVN ER levels increase significantly in response to noradrenergic input from the NTS to the PVN [156,181]. In addition, microinfusion of E into the A2 region of the NTS accentuates food-deprivation-induced suppression of pulsatile LH secretion in OVX rats [181]. Thus, E might also influence the catecholaminergic projections to the PVN by binding in the NTS [181]. From the NE/ NPY terminals in the PVN, it is hypothesized that CRH neurons project to GnRH axons in the Arc/median eminence, or to other GnRH-controlling neurons in the medial basal hypothalamus, POA or AH [147,151,155,156, 181,190,253]. GnRH-containing neurons also receive projections from the brain stem [52].

#### 3.4.3. Neuropeptide Y

NPY secretion increases in response to metabolic challenges, such as food deprivation and increased energy

expenditure [127,145]. NPY secretion is elevated during metabolic challenges that inhibit pulsatile LH secretion [127,145], and NPY levels are decreased by treatments that ameliorate metabolic deficit and reinstate HPG function [129]. NPY immunoreactivity is found in catecholaminergic projections to the PVN [208], and there are projections from PVN to the Arc median eminence [253] where these neurons might interact with GnRH neurons either pre- or postsynaptically. In addition, there are direct NPY projections to the POA where there are GnRH neurons [149], and thus, the neural circuitry exists to support NPYergic communication between metabolic fuel detectors in the brain stem and forebrain areas involved in GnRH secretion. In addition, food intake and various aspects of reproduction have been purported to be controlled by the well-known NPY/AgRP and POMC/CART pathway from the Arc to the PVN [24,30,31,65,110,146,205,255]. Some Arc neurons contain both leptin (Ob-Rb) receptors and NPY immunoreactivity (reviewed in Ref. [169]), and the effects of leptin on obesity are attenuated in mice lacking the functional NPY peptide [76]. Thus, these neurons are in position to influence energy balance, GnRH secretion and sex behavior. For example, NPY treatment increases CRH gene expression [241]; NPY neurons project to CRH neurons [147,151]; and CRH neurons are known to make contact with GnRH cells in rats [155].

NPY has multiple functions with regard to the HPG system and GnRH secretion. NPY treatment has different effects on the HPG system depending on the steroid milieu, the level of NPY secretion, and the brain area involved. For example, in fed animals, NPY is essential for the LH surge, which normally occurs in response to prolonged elevated concentrations of E (Fig. 1). However, NPY is inhibitory to the pulsatile mode of LH secretion (Fig. 1). Food-deprivation-induced increases in NPY secretion inhibit LH secretion, particularly when circulating levels of E are low (low circulating levels of E are characteristic of the follicular phase of the ovulatory cycle, the phase characterized by small, regular pulses of LH, Fig. 1). Infusion of NPY into the third ventricle inhibits LH pulse frequency and amplitude in OVX rats, and this effect is reversed by GnRH treatment [165]. Chronic third ventricular NPY treatment also inhibits the HPG system in intact male rats [191]. In males, the effects of NPY on pulsatile LH secretion are most likely mediated by the Y5 receptor because agonists that bind to the Y5 receptor inhibit LH pulses, and antagonists to the Y5 receptor prevent the effects of NPY treatment [196]. The Y5 receptor is also implicated in control of LH secretion in female rats. In food-restricted, lactating, anovulatory rats, elevated NPY-IR in the Arc persists for 10 days after return to *ad libitum* feeding. In keeping with a role for NPY in prolonged suppression of the HPG system by food restriction, plasma LH is inhibited in food-restricted rats during lactation, and LH does not increase to fed levels until 10 days after the return to *ad libitum* feeding [1]. Treatment with a Y5 receptor agonist lengthens the duration of

lactational diestrus, while treatment with a Y5 receptor antagonist attenuates the effects of fasting on the duration of lactational diestrus [254].

NPY influences sex behavior as well as the HPG system. For example, intracerebroventricular treatment with NPY antisera attenuates food intake and body weight gain, and increases the display of sex behavior in obese Zucker female rats [164]. NPY agonists increase food intake and decrease lordosis duration in OVX Syrian hamsters brought into estrus with ovarian steroid treatment [64]. The effects of NPY treatment on lordosis duration appear to be mediated by Y2 receptors, while the effects of NPY treatment on food intake in rats and Syrian hamsters are most likely mediated by Y5 receptors, with some influence also exerted via Y1 receptors [64,128,196]. In Syrian hamsters, the critical Y2 receptors appear to be located in the area of the caudal mPOA-AH-PVN continuum, as infusion of the Y2/Y5 agonist, peptide YY3-36, into these areas inhibits lordosis duration in OVX females brought into estrus with injections of E and P [133]. These are not areas that are commonly associated with sex behavior in rodents.

NPY/catecholaminergic projections from the brain stem to the PVN [208] might act on Y2 receptors in corticotropin-releasing hormone (CRH) neurons that project to other hypothalamic areas that control lordosis. The evidence for CRH effects on lordosis [125] is discussed in the Corticotropin-releasing hormone section.

The dual effects of NPY might allow individuals to take advantage of mating opportunities whenever ample fuels are available, and to forego mating in favor of foraging when oxidizable fuels are scarce. For example, in fed male rats, ingestion of a palatable sucrose solution is inhibited by the presence of a sexually receptive female. The converse is not true; that is, the sex behavior of fed males is not influenced by the presence of a bottle of sucrose [206]. In male rats treated with NPY, however, the latency to intromission and ejaculation is prolonged [58,192], and this effect is exaggerated by the presence of a bottle of sucrose [8]. NPY-treated males make more trips to and drink more from a bottle of sucrose than do saline-treated males, regardless of the presence of a sexually receptive female. The effects of NPY appear to influence the appetitive or motivational, rather than the consummatory aspects of both behaviors. It has been suggested that NPY treatment shifts attention toward eating and away from sex [8] without affecting erectile or ejaculatory function [58,130]. Similarly, NPY increases drinking from a bottle, but does not increase ingestion of a solution infused into the oral cavity [8,232]. The role of NPY in the inhibition of the HPG system, sex behavior and stimulation of food intake is seen in species as diverse as rodents, fish and snakes [8,174,183]. Together, these data support the idea that neurotransmitters allow animals to set priorities by influencing the motivation to engage in particular behaviors. These data suggest that we might make more progress in understanding these central

effector systems by studying the motivational aspects of sex and ingestive behavior.

#### 3.4.4. *Cholecystokinin*

The gut peptide, CCK, is secreted as a neuropeptide in the CNS in regions containing gonadal steroid receptors and GnRH neurons, such as the mPOA [235]. GnRH-containing neurons in the mPOA are thought to receive projections from CCK fibers [235], and CCK implanted in this area has both stimulatory and inhibitory influences on LH or GnRH secretion [106,137,189]. In food-deprived monkeys, CCK does not appear to mediate the effects of refeeding on restoration of LH secretion [229].

CCK has been implicated in the control of sex behavior independent of its effects on the HPG system. Binding of CCK to CCK-A receptors is critical for E-induced lordosis behavior in rats [114]. In contrast, peripherally secreted CCK does not appear to mediate the effects of fasting on sex behavior in Syrian hamsters [124]. Progress in understanding the effects of various chemical messengers on sex behavior will be facilitated by examining both motivated (e.g., foraging, consumption of an unpalatable diet, hoarding and overcoming obstacles of performing operant tasks to obtain food) and consummatory aspects of behavior in species-specific social contexts.

#### 3.4.5. *Glucagon-like peptide*

Treatment with another gut peptide, GLP-1, increases the secretion of LH within 5 min of injection in male rats [23]. GLP-1, like CCK, decreases food intake and is also secreted in the CNS (reviewed by Ref. [262]). Furthermore, a 48-h fast decreased hypothalamic GLP-1 content compared to that of fed rats. CCK neurons in the AP and GLP-1 neurons in the caudal NTS project to the PVN [280], one area where it is thought that food deprivation increases sensitivity to steroid-negative feedback on the GnRH pulse generator.

#### 3.4.6. *Corticotropin-releasing hormone*

The effect of steroid binding in the PVN is thought to increase the secretion of CRH in neurons that project to the vicinity of GnRH neurons [155,156,257]. Central CRH treatment has inhibitory influences on LH secretion, and treatment with antagonists to CRH prevents the effects of fasting on LH pulses in OVX, steroid-treated rats [156]. CRH treatments inhibit GnRH pulse generator activity in monkeys, and neither adrenocorticotropic hormone (ACTH) nor glucocorticoids mediate these effects [278,279]. CRH is in excellent position to inhibit GnRH secretion by direct action on GnRH neurons [155,190]. It is difficult to fit the related peptide urocortin into this picture. In contrast to CRH treatment, treatment with urocortin enhances LH secretion while at the same dose it decreases food intake in ewes [115]. It will be helpful to have data on the role of urocortin in control of the HPG system in a wider variety of species.

CRH has been implicated in the inhibition of the HPG system and sex behavior under a variety of conditions,

including metabolic challenges. CRH is typically secreted under life-threatening conditions that require either a fight or flight response, at a time when neither eating nor sex are high priorities. Vertebrates respond to many emergency situations, such as habitat destruction or inclement weather, with a characteristic set of adaptations that include an increase in locomotor activity and suppression of reproductive behavior [273]. In a variety of stressful situations, CRH initiates the hypothalamic–pituitary–adrenal (HPA) system, which rapidly mobilizes oxidizable metabolic fuels, increases heart rate and blood circulation, and inhibits digestion and other long-term energetic investments, including the immune system and the reproductive system (reviewed by Ref. [207]). CRH is also anxiogenic and increases memory retrieval and social affiliations. The effects of CRH extend to the motivational aspects of ingestion. For example, CRH-induced decreases in food intake are accompanied by decreases in food hoarding in rats [44].

CRH inhibits the HPG axis by direct action on GnRH secretion, as mentioned previously [155,190], and this effect would have secondary effects on ovulation, estrous cyclicity and sex behavior. CRH can also influence behavior directly. CRH secretion increases in response to food deprivation, and CRH infusion into the mesencephalic central gray, Arc-VMH or mPOA inhibits sex behavior as well as food intake in both male and female rats [236–238]. In OVX, steroid-treated Syrian hamsters, intracerebroventricular infusion of CRH or urocortin decreases the duration of lordosis, whereas intracerebroventricular infusion of a CRH receptor antagonist prevents food-deprivation-induced and NPY-induced decreases in lordosis duration [125]. Activation of the HPA system was neither necessary nor sufficient for the effects of CRH agonists [125]. Although it is clear that CRH affects copulatory performance, we are not aware of studies that examine direct effects of CRH or the related peptide, urocortin, on sexual motivation.

#### 3.4.7. $\beta$ -Endorphin

The release of endogenous opiates, particularly  $\beta$ -endorphin, is another aspect of the stress response that inhibits sex behavior. In female obese Zucker rats, treatment with an opioid antagonist attenuated obesity and improved sexual performance [163]. In nonobese rats and in white-crowned sparrows, treatment with opiates or opioid agonists inhibits sex behavior, including motivational aspects of sex behavior, such as ear wiggling, presentations and courtship vocalizations [159], while treatment with opioid antagonists facilitates sex behavior [7,159]. In both rats and white-crowned sparrows, the inhibitory effects of CRH are blocked by simultaneous treatment with antisera to  $\beta$ -endorphin or to opiate antagonists and thus, it has been suggested that CRH inhibits sex behavior by increasing release of  $\beta$ -endorphin [159,238]. These effects of  $\beta$ -endorphin on sex behavior have not been replicated consistently across species, or across sex within species (e.g., Refs. [124,236,251,252]). It

is possible that some of these discrepancies are due to differences in the time of testing relative to the time of treatment [252]. More work is needed to determine whether these peptides are involved in the normal expression of sex behavior, or whether they mediate the effects of energetic challenges on sex behavior, or both.

#### 3.4.8. Orexins

The orexins, also known as hypocretins, increase both food intake and energy expenditure by increasing general arousal and preventing a normal sleep period [154,272]. In addition, orexin stimulates LH secretion in OVX steroid-primed rats, and inhibits LH secretion in OVX, vehicle-treated rats or in OVX rats treated with low doses of E [93,194,244]. In the POA of sheep, approximately 30% of GnRH-IR cells are in close apposition to orexin-IR terminals projecting from the lateral hypothalamic/perifornical area [122]. Thus, orexin-containing cells of these brain areas project to GnRH neurons that could potentially participate in control of LH secretion.

#### 3.4.9. Melanocortins

One point of divergence between mechanisms that control food intake and those that control reproduction is that leptin influences food intake via melanocortinergic pathways, whereas the effects of leptin on LH pulsatility and estrous cyclicity appear to be independent of the melanocortin system [113,226]. For example, in *ob/ob* mice, intracerebroventricular treatment with the melanocortin receptor antagonist, SHU9119 reverses the effects of leptin on food intake, but not on gonadotropin levels or seminal vesicle weight [113]. Similarly, in Syrian hamsters, intracerebroventricular treatment with SHU9119 reverses the effects of leptin on food intake, but does not attenuate the ability of leptin treatment to reverse fasting-induced anestrus and does not induce anestrus in hamsters fed ad libitum [226]. Furthermore, the failure of SHU9119 to reverse the effects of leptin on fasting-induced anestrus was not explained by the hyperphagia in SHU9119-treated hamsters. SHU9119 treatment did not reverse the effects of leptin on estrous cyclicity even when the hamsters were limited to the food intake of the vehicle-treated controls [226]. Finally, mutant individuals (both mice and human beings) that lack MC4 receptor show moderate to severe obesity with no disturbance of the HPG systems and normal fertility (reviewed by Ref. [18]). However, treatment with  $\alpha$ -MSH decreases food intake and increases sex behavior in rats [99,231]. Treatment with the lateral hypothalamic hormone, melanin-concentrating hormone has stimulatory effects on LH release in rats [98].

## 4. Summary and conclusions

Central neuropeptides, sensory stimuli and hormonal mediators and modulators conspire to direct attention and

action toward behaviors that ensure survival under energetically challenging conditions and optimize reproductive success under energetically optimal conditions. During energetic challenges, deficits in the oxidizable fuels, changes in circulating hormone concentrations, and changes in secretion of neuropeptides all show the potential to initiate processes that (1) save energy by inhibiting reproductive behavior and the HPG system, and (2) ensure adequate energy availability by increasing the motivation to forage, hoard, and, in some cases, eat food. When food is plentiful, the primary sensory detectors send information informing the brain that the deficits in the availability of oxidizable fuels have been met. These signals have direct effects on central mechanisms that increase the likelihood of engaging in behaviors that perpetuate the species, such as courtship, mating and maternal behaviors. Thus, well-fed animals might be more likely to ignore palatable food in the presence of a sexually receptive or sexually proceptive opposite-sex conspecific. In addition, in some species, increases in peripheral hormones, such as leptin, decreases in peripheral hormones, such as ghrelin, and decreases in central neuropeptides, such as NPY, have the potential to increase the motivation to engage in behaviors that perpetuate the species. However, in some species, these hormonal changes appear to be too slow to account for changes in the neuroendocrine system that controls ovulation and sex behavior. Experiments on rats and mice have produced confusing data in which the consumptive aspects of ingestion are not affected by the same substances that influence the amount of food eaten. Attention to species that do not change food intake in response to energetic challenges has broadened our understanding of hormonal and central control of ingestive behavior. In such species, NPY and leptin influence hunger motivation and the amount of food hoarded to provide food in the safety of the burrow or nest, and these behaviors also inhibit sex behavior and the HPG system. In contrast, in species with a high metabolic rate, such as musk shrews, reproductive behavior is linked directly to metabolic fuel availability and has little or no association to body fat content or hormones secreted by adipocytes. Future research in this field should include attention to more diverse array of species under conditions that seek to replicate their natural habitats.

The similarity between these putative pathways for control of ingestive behavior and the pathway for control of reproduction is striking. It has become increasingly apparent that the study of ingestive behavior can be facilitated by attention to the methodologies and theoretical considerations from the field of reproduction and vice versa, as first suggested by Kennedy and Mitra [134,135] and further elucidated by Wade and Schneider [264]. At least one pathway, first identified to be essential for glucoprivic control of food intake in rats, is also essential for glucoprivic control of estrous cycles in rats. Fuel availability is detected in the brain stem. Decreases in the availability of oxidizable glucose that increase food intake in rats require an intact AP/

mNTS, and glucoprivic stimuli that inhibit estrous cycles and decrease lordosis duration in steroid-primed hamsters also require an intact AP/mNTS [63,187,188,201,228]. From the AP/mNTS, information is relayed via catecholaminergic neurons to the PVN. Noradrenergic cells originating in the AP and mNTS and dorsolateral medulla (DLM) project to PVN [208]. Increases in NE occur in the PVN in response to glucoprivic stimuli that increase food intake and inhibit estrous cycles [177], and glucoprivic effects on the HPG system are blocked by treatment with catecholamine synthesis inhibitors [177]. Cytotoxic lesions that selectively target catecholamines were shown to prevent glucoprivic effects on food intake [197] and estrous cycles [120], providing strong evidence that these noradrenergic pathways are functional in control of reproduction and food intake. Glucoprivic effects on this pathway occur independent of hormones, such as leptin and insulin, and thus, understanding of metabolic control of reproduction must include further studies of the sensory system that monitors fuel availability and controls reproduction.

The central effector systems include pathways that utilize CART [143], orexin [122] and MCH [98]. However, the peptides most strongly implicated in rodents are NPY and CRH. NPY projections from the brain stem have been implicated in control of food intake in rats [100] and estrous behavior in hamsters [133]. In addition, NPY and GLP-1 might affect food intake and reproduction via their brain stem projections to PVN [23,262,280]. Reproductive behavior and GnRH secretion might be affected via subsequent relays from PVN via CRH neurons [155,190]. Further control of GnRH secretion might also involve GABA and glutamate, which have long been purported to orchestrate pulsatile secretion of this hormone.

Hormones, such as leptin, insulin and glucocorticoids have the potential to carry information about the metabolic stimulus to the brain because these hormones increase with adiposity and the availability of metabolic fuels. Although a structural neural circuit whereby leptin might control reproduction has been mapped, the functional role of natural endogenous changes in circulating leptin has been difficult to demonstrate. For example, reproductive function is restored rapidly by refeeding, prior to detectable changes in body fat content and plasma leptin concentrations. Glucocorticoid concentrations are often correlated with energetic challenges and inhibition of reproduction, and virtually all obesities require intact adrenal glands. However, studies have shown that cortisol and corticosterone are not the causal link between low fuel availability and estrous cyclicity, or between low fuel availability and inhibited sex behavior. Glucocorticoids are secreted in response to metabolic challenges and other stresses, but high concentrations of these hormones are not necessary for the effects of metabolic challenges on reproductive function. In addition, hormones, such as insulin, leptin and glucocorticoids also modulate the metabolic stimulus by changing the uptake and oxidation of fuels. Thus, these hormones have the potential

to influence reproduction indirectly by altering the metabolic stimulus.

The link between energy balance and reproduction is relevant to understanding medical problems associated with dysregulation of energy balance (e.g., obesity). The feeding-inhibitory and feeding-stimulatory circuits that we have described have been successful for the perpetuation of species, including *H. sapiens*, but these mechanisms have been less successful at preventing obesity in modern times. The mean body weight and the incidence of obesity increased in most human populations when these populations were provided with ad libitum availability of highly palatable, calorically dense foods and were released from energetic challenges, such as the need for physical work to obtain food. The medical, social and economic consequences of obesity are severe and include cardiovascular disease and diabetes as well as myriad psychological problems. However, the preponderance of obesity is due in part to the adaptive advantage conferred by the ability to store body fat. The preponderance of evidence supports the theory that most existing traits arose because they conferred a reproductive advantage. Darwinian fitness is directly linked to the ability to bear offspring and raise them to reproductive maturity. Darwinian fitness is not a direct function of life expectancy and popular fashion. In western industrialized societies, life expectancy stretches far beyond the reproductive years. Our life span and quality of life is limited in large part due to the cardiovascular and other consequences of our obesity, but most offspring survive and reproduce whether their parents live to be 55 or 75 years of age. Thus, we can make an educated guess that our life span is only loosely associated with our Darwinian fitness. Infertility only occurs in extreme obesity, and does not occur in the vast majority of overweight individuals in the prime of their reproductive potential. It can be speculated that the ability to store energy as body fat might have been essential in our ancestors that experienced dramatic fluctuations in food supply, and that the reproductive consequences of body weight gain did not dramatically decrease fertility. Natural selection in human populations obviously has not precluded the propensity to store large amounts of body fat.

In the majority of cases, neither obesity nor low body weight can be explained by any one major dysfunction controlled by a gene or small group of genes. Body weight and adiposity are quantitative traits. Molecular analysis of quantitative trait loci has estimated that there are approximately 70 independent loci that influence body weight in mice, and thus there are likely to be many genes in which allelic variation can account for body weight differences in human beings [18]. It is not surprising then that single gene mutations account for only a tiny fraction of the overweight and obese population. Furthermore, environmental factors acting throughout development of the individual interact with these genetic factors. The wide range of body weight and adiposity and the relatively high degree of additive genetic variance in body weight and adiposity suggest that

natural selection has not acted intensely on any one particular morphological type, either lean or obese. The relationship between selection and additive genetic variance can be illustrated by comparing two traits that have been under varying degrees of selection. Body temperature is a quantitative trait that has been under intense natural selection during the evolution of mammals. Our species cannot survive and reproduce at a wide range of body temperatures, and thus additive genetic variation in body temperature is very low. Our species can, and does, survive and reproduce at a wide range of body weights and of adiposity and thus, selection has allowed for a great deal of additive genetic variation in this trait. It is not surprising then that we have not yet found a natural mechanism that controls food intake for the express purpose of constraining body weight and adiposity within a fashionable and healthy range that maximizes life expectancy in the face of calorie-dense, readily available food. Such a “healthy,” fashionable phenotype is observed, but it is not “normal” by evolutionary standards. This phenotype is simply part of a continuum of variation in adiposity that has been tolerated by natural selection during human evolution. The array of alleles that influence the feeding-inhibitory circuits have allowed organisms to engage in activities necessary for perpetuation of the species in the habitats in which these organisms evolved. In our species, it is likely that these habitats contained dramatic fluctuation energy availability, exacerbated by the energetic demands for migration, predator avoidance, hunting and agriculture. It is likely that the mechanisms that inhibit food intake came about in the ancestors of extant populations to optimize reproductive success under conditions in which the availability of, and demands for, metabolic fuels fluctuate.

The evolutionary basis of these energy-balancing mechanisms and their link to reproductive processes do not imply a fatalistic determinism toward the medical problems associated with obesity. To the contrary, knowledge of the adaptive significance of the feeding-stimulatory and feeding-inhibitory circuits and the effects of these circuits on reproduction should lead to realistic and testable hypotheses about the mechanisms that partition energy and prioritize behavioral options. It is more likely that we can understand the physiological mechanisms that underlie syndromes, such as obesity and anorexia, if we study the natural species-specific situations in which energy intake, storage and expenditure are controlled according to natural fluctuations in reproductive opportunities and energy supply and demand. Furthermore, this perspective emphasizes the role of reproductive hormones and neuropeptides. There are clear sexual dimorphisms in body fat content and adipose tissue distribution, and these dimorphisms might be brought about by hormone action during a critical period of development. In the past 20 years, there has been an alarming increase in presence of molecules that bind to steroid receptors in the environments of western industrialized nations and an alarming increase in obesity and fertility problems. A

biological perspective is essential to understanding the causes and cures. How might the ubiquitous presence of xenobiotic—and phytoestrogens and other compounds influence the developmental processes that underlie sex, fertility and body fat distribution? Chronic stress is known to increase adiposity (primarily via adrenal steroids) and inhibit fertility and sex behavior (most likely via increases in central CRH). It might be enlightening to explore the potential link between increases in obesity and factors that induce chronic stress, such as terrorism, war and the emphasis on violence by the news media. How do advertising strategies that promote consumption by diminishing self-esteem and provoking anxiety contribute to chronic stress in the general population? Are stress hormones acting on the mechanisms that increase the motivational aspects of eating as well as performance? For example, might we influence obesity by addressing the effects of stress and anxiety on the motivational aspects of ingestion, i.e., the urge to shop for food or for certain types of food? It is critical to understand the role of adrenal and gonadal steroid receptors in control of energy balance and sexual differentiation of the energy-balancing system. For biologists, this perspective suggests a variety of testable hypotheses concerning the mechanisms that control the consummatory and motivational aspects of sex behavior and ingestion in a natural or seminatural context in which the animals have choices between food and sex. More progress will be made if we keep in mind the following: (1) the primary metabolic stimulus can influence the effector systems independently from the hormones that bind to these central effector systems; (2) hormones often influence GnRH secretion, sex and ingestive behavior indirectly by modulating the metabolic stimulus; (3) the critical neural circuitry involves extrahypothalamic sites, such as the caudal brain stem that act on effector systems and involve hypothalamic neuropeptides, such as NPY and CRH; (4) the metabolic stimuli, hormonal mediators and modulators, and central effectors influence the motivation to engage in ingestive and sex behaviors instead of, or in addition to, affecting the ability to perform these behaviors; and (5) we can better understand the effects of these chemical messengers and metabolic events by examination of their effects in many diverse species under natural or seminatural circumstances.

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