Sense and nonsense in metabolic control of reproduction

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An exciting synergistic interaction occurs among researchers working at the interface of reproductive biology and energy homeostasis. Reproductive biologists benefit from the theories, experimental designs, and methodologies used by experts on energy homeostasis while they bring context and meaning to the study of energy homeostasis. There is a growing recognition that identification of candidate genes for obesity is little more than meaningless reductionism unless those genes and their expression are placed in a developmental, environmental, and evolutionary context. Reproductive biology provides this context because metabolic energy is the most important factor that controls reproductive success and gonadal hormones affect energy intake, storage, and expenditure. Reproductive hormone secretion changes during development, and reproductive success is key to evolutionary adaptation, the process that most likely molded the mechanisms that control energy balance. It is likely that by viewing energy intake, storage, and expenditure in the context of reproductive success, we will gain insight into human obesity, eating disorders, diabetes, and other pathologies related to fuel homeostasis. This review emphasizes the metabolic hypothesis: a sensory system monitors the availability of oxidizable metabolic fuels and orchestrates behavioral motivation to optimize reproductive success in environments where energy availability fluctuates or is unpredictable.

Keywords: appetitive behavior, hoarding, metabolic hypothesis, motivation, nutritional infertility, sex behavior, vaginal scent marking

Research at the interface of energy balance and reproduction is gaining momentum as investigators in many different fields recognize the relevance of this topic to basic biology and clinical practice. According to a recent PubMed search (using the search keywords energy balance and reproduction) only 135 journal articles were published in the decade between 1980 and 1990, whereas 609 articles were published in the last decade. Eighty of the 135 articles published between 1980 and 1990 were concerned with lactation in dairy animals, whereas the 600 or more published in the last decade covered a broad range of topics including the many orexigenic and anorectic peptides that influence reproduction in a wide variety of organisms, including invertebrates and vertebrates, human and non-human primates, and males and females (Table 1). The recent momentum reflects an exciting synergy that arises from melding reproductive biology with neuroendocrinology of ingestive behavior. Reproductive biologists benefit from the theories, experimental designs, and methodologies used by experts in ingestive behavior and energy homeostasis. Reproductive biology and physiological ecology bring context and meaning to the study of ingestive behavior and energy metabolism. This current special issue of Frontiers in Translational Endocrinology illustrates that metabolic control of reproduction is now, on its own, an established field of basic biological research with the most exciting discoveries just around the corner. New investigators are beginning to recognize that by viewing energy metabolism in the context of reproductive success, we open a window into human obesity, eating disorders, diabetes, and other pathologies related to fuel homeostasis.

With so many new researchers entering the field, it might be useful to provide sign posts to the most fruitful avenues of research, as well as to potential hazards, wrong turns, and dead ends. In particular, new investigators need to know which hypotheses and assumptions are supported by a preponderance of evidence and which are largely unsupported. Unfortunately, some of the most often repeated ideas in this field happen to be as untestable as they are seductive. Future research will be centered on molecular mechanisms at the level of the gene, but will be meaningless without attention to the developmental, epigenetic, environmental, and evolutionary context.

This review will focus on the five ideas that are likely to facilitate progress in research at the interface of reproduction and energy homeostasis.

(1) We will emphasize experimental designs that incorporate varying degrees of metabolic challenge and behavioral options relevant to the natural habitats in which those behaviors evolved, i.e., habitats in which food is not available ad libitum and the experimental subjects have options to choose between reproductive and ingestive behaviors.

(2) We will illustrate the importance of measuring behavioral motivation by quantifying appetitive sex and ingestive behaviors, i.e., not only eating and copulation, but also behaviors that bring individuals in contact with food or potential mating partners, such as food hoarding and courtship.

(3) We will emphasize the metabolic hypothesis, the idea that neuroendocrine systems function to maintain fuel (not body fat or food intake) homeostasis.
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<th>Central peptides</th>
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<td>Agouti-related protein (AgRP)</td>
<td>Increases food intake and hoarding (Rossi et al., 1998; Day and Bartness, 2004)</td>
<td>Inhibits LH in the presence of E (Schioth et al., 2001), stimulates LH in males (Stanley et al., 1999)</td>
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<td>α-melanocyte stimulating hormone (α-MSH), MTII</td>
<td>Decreases food intake and food hoarding (Shimizu et al., 1989; Keen-Rhinehart and Bartness, 2007)</td>
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<td>Bombesin-like peptides</td>
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<td>β-endorphin</td>
<td>Increases food intake (McKay et al., 1981)</td>
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<td>Cocaine and amphetamine-regulated transcript (CART)</td>
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<td>Cholecystokinin (CCK)</td>
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<td>Simulates LH secretion (Kimura et al., 1983; Perera et al., 1993)</td>
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<td>Corticotropin releasing hormone (CRH)</td>
<td>Decreases food intake (Levine et al., 1983; Heinrichs and Richard, 1999) and food hoarding in rats (Cabacac and Richard, 1999)</td>
<td>Inhibits LH secretion and lordosis (Olster and Ferin, 1987) and sex behavior (Jones et al., 2002)</td>
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<td>Dopamine (DA)</td>
<td>Decreases food intake (Heffner et al., 1977), increases food hoarding (Kelley and Stinus, 1985; Borker and Mascarenhas, 1991), and reward (Wise, 2004)</td>
<td>Stimulates sexual arousal, motivation and reward (Meisel and Mullins, 2006)</td>
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<td>Galanin</td>
<td>Increases food intake (Kyrkouli et al., 1986; Krasnow et al., 2003)</td>
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<td>Galanin-like peptides</td>
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<td>Glucacon-like peptide (GLP-II)</td>
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<td>Gonadotropin releasing hormone (GnRH I or II)</td>
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<td>Stimulates LH secretion and sex behavior (Moss and McCann, 1975; Clarke and Cummins, 1982; Temple et al., 2003)</td>
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<td>Kisspeptin</td>
<td>Decreases food intake (Stengel et al., 2011)</td>
<td>Stimulates GnRH and LH secretion (Gottsch et al., 2004; Irwig et al., 2004)</td>
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<td>Melanin concentrating hormone (MCH)</td>
<td>Increases food intake (Presse et al., 1996)</td>
<td>Inhibits LH secretion (Tsukamura et al., 2000a)</td>
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<td>Motilin (peripheral) delete</td>
<td>Increases food intake in fasted rats (Garthwaite, 1965)</td>
<td>Inhibits LH secretion (Tsukamura et al., 2000b)</td>
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<td>Neuropeptide Y (NPY)</td>
<td>Increases food intake (Stanley and Leibowitz, 1984) and food hoarding (Dailey and Bartness, 2009)</td>
<td>Inhibits LH in the absence of, stimulates LH in the presence of estradiol (Crowley et al., 1985; Sahu et al., 1987), inhibits sex behavior (Ammar et al., 2000), Inhibits LH in the absence of, stimulates in the presence of estradiol (Pu et al., 1998)</td>
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<td>Orexin/hypocretin</td>
<td>Increases food intake (Sakurai et al., 1998)</td>
<td>Stimulates sex behavior (Whitman and Albers, 1995)</td>
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<td>Oxytocin</td>
<td>Decreases food intake (Olson et al., 1991)</td>
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<td>RFamide-related peptide-3 = Gonadotropin inhibiting hormone</td>
<td>Increases food intake (Tachibana et al., 2005; Johnson et al., 2007)</td>
<td>Stimulates LH secretion (Babu and Vijayan, 1983)</td>
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<td>Secretin (move to VIP)</td>
<td>Decreases food intake (Cheng et al., 2011)</td>
<td>Stimulates LH in the presence of estradiol (Coen and MacKinnon, 1979) Inhibits LH secretion in the absence of estradiol (Coen et al., 1980; Koh et al., 1984)</td>
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<td>Serotonin (5HT)</td>
<td>Decreases food intake (Blundell, 1977)</td>
<td>Stimulates LH secretion in ewes (Holmberg et al., 2001), inhibits LH secretion (potentially; Li et al., 2005; Nemoto et al., 2010), directly inhibits Leydig cell function (Rivier, 2008)</td>
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<td>Thyroptropin releasing hormone</td>
<td>Decreases food intake (Mijayan and McCann, 1977)</td>
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<td>Urocrtin</td>
<td>Decreases food intake (Spina et al., 1996)</td>
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<td>Vasopressin</td>
<td>Decreases food intake (Meyer et al., 1989)</td>
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(4) We will urge investigators to focus specifically on mechanisms that promote opportunistic overeating or hoarding and fuel storage in anticipation of the high energetic demands of reproduction.

(5) All of the above concepts will be discussed within the context of a distributed neural network with multiple, redundant function. We will argue that metabolic control of reproduction must include the hindbrain, midbrain and forebrain including the ventral premammillary nucleus. This is in sharp contrast to the typical focus on the arcuate nucleus of the hypothalamus (Arc).

The central unifying hypothesis is that neuroendocrine systems that control energy homeostasis optimize reproductive success in environments where energy fluctuates or is unpredictable. The testable predictions that emerge from this hypothesis are fundamentally different from those that follow from the idea that neuroendocrine systems exist to maintain body weight or adiposity at some particular level.

AN EVOLUTIONARY CONTEXT AND THE RELEVANCE OF A SEMI-NATURAL ENVIRONMENT

Metabolic control of reproduction is obscured in the laboratory when females are housed in isolation, in small cages, where locomotion is restricted, temperature is controlled, and where food availability is unlimited (Bronson, 1998). Under these conditions, there is more than enough energy available for all of the cellular and systemic processes including reproduction. Likewise, women in modern, westernized, industrial societies live surrounded by “foraging” opportunities that require very little energy expenditure. It is under these conditions of unlimited food availability that misconceptions developed about hormone effects on behavior. Two such misconceptions, for example, are that female sexual motivation in primates is emancipated from the effects of hormones, and that the main function of “satiety” peptides is to maintain stability in body weight. These naïve notions are shattered when animals are housed in semi-natural environments that mimic important aspects of their natural habitats that include a limited food supply, high energy demands, and the ability to choose between sex and ingestive behavior. When animals are studied under environmental conditions that approximate those in which the traits evolved, presumed “satiety” peptides increase sexual motivation and presumed “orexigenic” peptides promote vigilant foraging and food hoarding and opportunistic overeating. These effects are masked under conditions of ad libitum food intake and low energetic demand. Under these conditions, sex drive is already elevated, obscuring increases that might otherwise be induced by the release of anorectic peptides. Laboratory animals are perhaps in some ways more like modern humans in westernized societies in that, in both cases, the link between hormones, behavior, and environmental energy availability is obscured. An important function of these peptides, to orchestrate the appetites for food and sex, is revealed by studying laboratory animals under the energetically challenging conditions in which they evolved. As will be described in this review, laboratory rodents can be housed in a semi-natural burrow system that 1) requires that individuals expend energy on locomotion in order to gain access to food, and 2) provides opportunities to encounter potential mating partners during foraging expeditions. Under these conditions, the effects of hormones on motivation are revealed in sharp relief. Furthermore, fluctuation, rather than stability in ingestive behavior is the norm.

In contrast to the typical laboratory situation, food supplies in the natural habitats of most wild animals are not unlimited. Rather, in nature, food availability often fluctuates seasonally, with a nadir in the winter, dry, or rainy season depending on the geographic area. In addition, food availability varies unpredictably due to myriad factors including population density, inter and intra-species competition, famine, drought, storms, hurricane, tornadoes, floods, fires, global climate change, and diseases that destroy edible plants, crops, prey animals, and livestock (Bronson, 1989, 1998). Given the importance of environmental energy availability on reproductive function in members of every mammalian order, it is reasonable to hypothesize the mechanisms that control energy intake, storage, and expenditure serve to optimize reproductive success in environments where energy fluctuates (Bronson, 1989; Wade and Schneider, 1992; Schneider, 2006; Schneider et al., 2007). One prediction from this hypothesis is that the effects of ovarian hormones on ingestive and sex behavior will vary with the degree of energetic challenge, that is, the balance of energy supply and expenditure. This idea is inspired by the book, Mammalian Reproduction, which provides evidence for metabolic control of reproduction in females from representative species of every mammalian order and for the conclusion that energy availability is the most important factor that controls reproduction in female mammals (Bronson, 1989).

There are three important features to this perspective.

First, females anticipate the energetic demands of reproduction by eating more than their immediate energetic needs and storing the extra energy as body fat or as a food cache. All ingested macronutrients derived from food, including carbohydrates and fats, can be stored as triglycerides in adipocytes, so that later, these triglycerides can be hydrolyzed to release glycerol and free fatty acids for oxidation. During pregnancy and lactation, these fuels are mobilized for the growing conceptus and to produce milk for the offspring. In rats, progesterone (elevated during the luteal phase of the ovulatory cycle and pregnancy), in the presence of estradiol, promotes increases in food intake, and body fat storage, at least in the early phases of pregnancy (Wade, 1973; Trujillo et al., 2011). Models of mechanisms that affect ingestive behavior and body weight must incorporate the ability to anticipate future energy shortages.

Second, females anticipate opportunities for fertile matings by virtue of the fact that the same hormones that induce ovulation also stimulate sexual appetite while reducing the appetite for food. Neuroendocrine mechanisms stimulate sexual (rather than ingestive) motivation during the times of highest fertility, when mate searching, courtship, and copulatory activities are most likely to pay off in terms of genetic contributions to the next generation. The sequence of hormones necessary for ovulation is permissive for copulatory behavior and these same neuroendocrine mechanisms inhibit foraging, hoarding, and eating during the most fertile part of the cycle (Zucker, 1969, 1972; Wade and Zucker, 1970; Zucker et al., 1972; Wade and Gray, 1979; Wade and Schneider, 1992; Asarian and Geary, 2006; Klingerman et al., 2010;
Thus, models of ingestive behavior and physiology must include the choice between food and sex, fluctuations in ovarian hormones, and sex differences in ingestive and sex behavior responses to ovarian steroids.

Third, females have mechanisms that delay reproductive processes during energetic challenges and re-initiate reproductive activities when the energetic conditions improve. In species from rodents to primates, sexual motivation, sexual performance, puberty, birth intervals, hypothalamic gonadotropin releasing hormone (GnRH) secretion, luteinizing hormone (LH) secretion, and ovarian steroid synthesis and secretion are inhibited by energetic challenges (McClure, 1962; Kennedy and Mitra, 1963; Morin, 1975; Ronnekleiv et al., 1978; Bronson and Marsteller, 1985; Cameron et al., 1985; Foster and Olster, 1985; Armstrong and Britt, 1987; Bronson, 1987; Lively and Piacsek, 1988; Sprangers and Piacsek, 1988; Schneider and Wade, 1989; de Rider et al., 1990; Thomas et al., 1990; Cameron, 1996; Shahab et al., 1997, 2006; Ellison, 2001; Temple et al., 2002; Terry et al., 2005). When energetic challenges are so severe that they induce anestrous or inhibit the menstrual cycle, the primary locus of effect is the GnRH pulse generator, a diffusely located cell group in the medial basal hypothalamus. In support of this idea, pulsatile LH secretion, follicle development, and ovulation can be reinstated by treatment with pulses of GnRH at species-specific frequency and amplitude in food-deprived or -restricted rats, sheep, pigs, monkeys, and women (Nillius et al., 1975; Foster and Olster, 1985; Bronson, 1986; Day et al., 1986; Armstrong and Britt, 1987; Cameron and Nosbisch, 1991; Kile et al., 1991; Manning and Bronson, 1991).

Severe metabolic challenges can have effects at many levels (the gonad, pituitary, or hypothalamic GnRH generator, or the neural substrates that control sex behavior). Metabolic control of the GnRH pulse generator is the most widely studied. What about less severe metabolic challenges?

Fourth, and most recently, mild energetic challenges can have significant effects on reproductive and ingestive behavior long before there are effects on the mechanisms that govern steroid synthesis and secretion (Schneider, 2004; Schneider et al., 2007; Klinger et al., 2010). Furthermore, in order to observe the effects of mild energetic challenges on the reproductive system, it is necessary to house animals in semi-natural environments in which energy expenditure is high relative to energy supply and both food and mates are available simultaneously (Schneider et al., 2007; Klinger et al., 2010).

Attention to appetitive and consummatory aspects of behavior might shed light on so-called “feeding” hormones and neuropeptides which tend to stimulate ingestive behavior and inhibit aspects of the reproductive system, e.g., ghrelin, neuropeptide Y (NPY), and RF amide-peptide-3 (RFRP-3), also known as gonadotropin inhibiting hormone (GnIH), which tend to stimulate ingestive behavior and inhibit various aspects of the reproductive system (Table 1) (Clark et al., 1985; Guy et al., 1988; Kalra et al., 1988; Wren et al., 2000; Furuta et al., 2001; Johnson et al., 2007; Kriegsfeld et al., 2010; Shah and Nyby, 2010). It might also illuminate the functional significance of leptin, α-melanocortin stimulating hormone (α-MSH), kisspeptins, glucose-like peptide, cholecystokinin, and GnRH, which tend to decrease ingestive behavior and stimulate reproductive behavior and physiology (Table 1) (Gonzalez et al., 1993; Wade et al., 1997; Schneider et al., 1998; Ammar et al., 2006; Cragnolini et al., 2006; Kauffman and Risman, 2004a; Castellano et al., 2005, 2010; Kauffman et al., 2005; Fernandez-Fernandez et al., 2006; Crown et al., 2007; Millington, 2007). Given that many of these hormone–behavior systems were molded by natural selection in response to environmental energy availability, and that natural selection works via differential reproductive success, progress can be facilitated by attention to the influence of these hormone–behavior systems on reproductive success.

The hypothesis that putative anorectic and orexigenic peptides function to optimize reproductive success in environments where energy fluctuates or is unpredictable is relevant even for our own species. A look at modern, non-contracepting, non-industrialized societies shows that seasonal fluctuations and unpredictable scarcity in food availability have profound, measurable effects on reproductive success (Ellison, 2001). The link between reproduction and environmental energy is obvious in the !Kung, non-contracepting bush people who live in the Kalahari Desert where rainfall and hence food availability fluctuates dramatically within a year. Among the !Kung, the fluctuating food supply is coupled with a continuous need to expend energy because the workload is high throughout the year. !Kung women, for example, engage in miles of walking, carrying water, gathering firewood, harvesting, and cooking food, all while toting infants and toddlers. The !Kung show a dramatic loss of body weight during the “hungry season” and a dramatic drop in birth rate 9 months following the hungry season, suggesting that they only rarely ovulate during the time of low food production (van der Walt et al., 1978). Given that evolutionary change occurs via differential reproductive success, plus the clear effect of seasonal fluctuation in food availability on reproductive success in extant populations of humans, it is likely that fluctuations in food availability molded ingestive and reproductive traits in our own species during human history.

Furthermore, the effects of environmental energy availability on human reproductive function are not limited to rural, indigenous, tribal peoples. Starvation and food insecurity has impact on reproductive function today in many, if not all societies. In fact, most societies have members who expend more energy than they can acquire, and nutritional amenorrhea occurs in these subpopulations, either because they experience starvation in relation to poverty or because they voluntarily engage in exercise and limit their food intake (Loucks, 2003a,b; Loucks and Thuma, 2003; Rosetta and Mascie-Taylor, 2009). For example, a high incidence of delayed menarche, adult amenorrhea, decreased birth rates, and high infant mortality result from low food availability in non-contracepting populations in India and Bangladesh (Gopalan and Naidu, 1972; Chen et al., 1974). Birth intervals are longer and birth rates plummet along with low energy balance (energy intake and storage minus expenditure) in women from extant, diverse, subsistence gardening/farming cultures, including the Lese of the Congo’s Ituri Forest, the Tamang in the foothills of the Himalaya of central Nepal, and women who live in mountain valleys in rural Poland (Ellison, 2001). Not only are energetic effects on fertility present in the economically challenged people in every society, but these effects are seen in all strata of every society during famine (Chakravarty et al., 1982).
between energy availability and reproductive success in our own species come from extant, modern populations as well as populations descended from subsistence agricultural societies. Thus, when we hypothesize that the mechanisms that control energy balance serve to optimize reproductive success in environments where energy fluctuates, this likely applies to our ancestors and to modern human beings.

The idea that the energy balancing system optimizes reproductive success is, in some ways, similar to the so-called “thrifty gene” hypothesis (Neel, 1962, 1999), with important differences. According to Neel, fluctuations in energy availability during the early evolution of *Homo sapiens* have favored genotypes that code for metabolic efficiency, the ability to overeat and store excess energy in adipose tissue that would increase the chances of survival during famine (Neel, 1962, 1999). The idea is often invoked to explain the so-called obesity epidemic. Individuals predisposed toward body fat storage had selective advantage in Paleolithic times, whereas in the presence of modern food abundance, the same individuals become obese. Various reiterations of Neel’s ideas tend to be incomplete, and thus, in this review we emphasize three specific modifications. First, ingestive behavior, body weight, and body fat content are polygenic; many genes contribute to metabolic efficiency, body fat storage, and hunger, not just one “thrifty” gene. Second, survival during famine was not the sole function of the energy balancing system. The critical function was to modulate reproductive output according to environmental energy availability. Finally, overeating and obesity are not the inevitable outcome of one “thrifty” gene in an environment rich in food, but rather, the interaction of many genes with reproductive hormones, epigenetic factors, and environmental energy availability.

**APPETITIVE AND CONSUMMATORY BEHAVIORS**

Reproductive behavior is far more complex than the simple act of copulation, ingestive behavior is more than the act of eating food, and these complexities are important. The hormonal effects on reproduction are not limited to the hypothalamic–pituitary–gonadal (HPG) system, but extend to the brain mechanisms that control the hunger for food and sex. Survival and reproduction involves appetitive behaviors that bring animals in contact with food or mating partners (Sherrington, 1906; Craig, 1917). Appetitive sex behaviors, however, occur separated in time from lordosis and reflect sexual motivation but not necessarily the ability to perform the sex act (Sherrington, 1906; Craig, 1917; Lorenz, 1950; Johnston, 1974, 1977; Lisk et al., 1983; Everitt, 1990). These appetitive behaviors might include mate searching and assessment, competition, courtship, and the ability to attract a mate. Similarly, ingestive behavior is more than the amount of calories ingested or meal size, it is a multifaceted array of interrelated behaviors and physiological traits that include foraging, food hoarding, food defense, and diet preference.

The history of behavioral endocrinology contains hints that we have missed something important in our narrow focus on food intake and copulation. Primate research based on laboratory studies led to erroneous conclusions about hormonal effects on primate sex behavior. These conclusions were overturned when researchers such as Kim Wallen began studying primates in semi-natural environments wherein females were able to exercise volition in their sexual interactions. Contrary to the commonly held idea that female primates are emancipated from the effects of hormones on behavior, appetitive sex behaviors, such as male–female grooming and proximity to mating partners, are correlated with peri-ovulatory increases in circulating estradiol when primes are studied in a semi-natural habitat where they experience social risks and can exercise volition with regard to sex behavior. These effects on sex behavior in a semi-natural environment have been traced to circulating levels of estradiol (Wallen, 2000, 2001). Similar misconceptions about hormonal effects on human behavior result from the narrow focus on copulation of the pair, rather than on underlying sexual motivation of the individuals in question. The idea that women are somehow emancipated from the effects of ovarian hormones on sex behavior is supported only by the difficulty in showing repeatable, statistically significant correlations between menstrual fluctuations in ovarian hormones and the incidence of sexual intercourse in married women from modern, industrialized societies (with unlimited food intake and low energetic demands). In contrast, when the motivation of individual women is assessed, there are statistically significant associations between these measures of appetitive behavior and phases of the menstrual cycle in a growing number of studies (Stanislaw and Rice, 1988; Meuwissen and Over, 1992; Gangestad et al., 2002; Gangestad and Thornhill, 2008; Durante and Li, 2009). These examples from sexual behavior in primates all suggest that it is important to create a relevant context in the study of behavioral endocrinology.

A similar problem occurs in the study of human ingestive behavior. Researchers in industry and academia alike commonly operate under the assumption that women are emancipated from effects of their ovarian hormones on ingestive behaviors. The effect of phases of the menstrual cycle on food intake in women, i.e., a periovulatory nadir in food intake, is subtle and is statistically significant in most, but not all studies (Fessler, 2003). The periovulatory increase in locomotor activity is even more elusive in women (Fessler, 2003).

What would we find if sexual and ingestive motivation (not just food intake and the incidence of sexual intercourse) were measured in females with limited food availability and high energetic demands? Inspired by these questions, we have initiated a new line of research that manipulates energy availability and examines the effects of ovarian steroids on behavioral motivation (Klingerman et al., 2010, 2011a), and a recent example of this work is illustrated in the article by Klingerman et al. (2011b) in this issue.

The idea that any of these particular chemical messengers evolved to optimize reproductive success in environments where energy availability fluctuates is testable (i.e., it constitutes a hypotheses in which there is a realistic outcome that will refute the hypothesis). This hypothesis leads to the following testable predictions:

(a) The effects of the chemical messenger in question will vary when energy availability is manipulated. The effects of so-called orexigenic and anorectic peptides will be amplified in testing environments that mimic the habitats in which these neuroendocrine systems evolved, including the choice between food and sex, and the need to expend energy in order...
to acquire energy. Specifically, the greater the energetic challenges, the more putative orexigenic peptides inhibit sexual motivation, and directly or indirectly promote vigilant foraging, hoarding, and eating. The choice between food and sex is a prerequisite for observation of the phenomenon.

(b) The “orchestration of motivation” implies effect on the choice between food and sex, because in natural environments animals do not live and forage in the absence of conspecifics and do not engage in sex in a separate enclosed space precluded from eating and foraging. Thus, sexual motivation, i.e., the appetitive aspects of behavioral choice, will be more sensitive to energetic challenges than the consummatory aspects of behavior. Food hoarding and the preference for spending time with males vs. food will be significantly affected by food restriction prior to significant changes in follicle development and estradiol secretion.

(c) Periovulatory increases in estradiol disinhibit mechanisms that control sexual motivation and behavior, thereby switching behavioral priorities from ingestive to sexual.

These hypotheses have been examined in female Syrian hamsters, rodents in which motivation (appetitive behavior) and ability (consummatory behavior) are easily measured, with respect to both ingestive and sex behavior. Sexual performance of the lordosis posture is a reflex triggered by male conspecific odors and tactile stimulation. These sensory cues are integrated in neural structures only when those neural structures are stimulated by periovulatory levels of estradiol and progesterone, which bind to their receptors, estradiol receptor-alpha (ER-α), and progestin receptor (PR). Lordosis reflects an unknown combination of both motivation and ability and consistently occurs on day 4 of the 4-day estrous cycle, known as proestrus in rats (Ciaccio and Lisk, 1971; Lisk et al., 1972; Steel, 1981). Motivation, in contrast to performance, can be measured in this species by counting the number of vaginal scent marks, or by measurement of the preference for sex vs. food. Vaginal scent marking increases gradually over days 1–3 of the Syrian hamster estrous cycle (known as diestrous 1 and 2 in rats) as circulating estradiol (but not progesterone) is rising. In addition, female hamsters decrease levels of agonist behavior toward males, and spend progressively more time in closer proximity to males as circulating levels of estradiol increase (Johnston, 1974, 1975). Hamster appetitive sex behaviors increase linearly with increasing levels of estradiol alone and are inhibited by progesterone (Ciaccio et al., 1979; Steel, 1981).

Hamsters are prodigious hoarders in the wild and in the laboratory. Metabolic challenges, such as food deprivation increase the appetitive ingestive behavior, food hoarding, but not the consummatory measure, food intake (Silverman and Zucker, 1976; Rowland, 1982). In their natural habitat, hamsters emerge from underground burrows for only 90 min per day and spend virtually every minute of this time hoarding food (Gattermann et al., 2008). These ecological observations suggest that the choice between hoarding and courtship has important consequences for reproductive success and evolution by natural selection. Thus, female hamsters were acclimated to a burrow system that included vertical tubes in a t-configuration leading in one direction to food and in the opposite direction to an adult male hamster. For 8 days prior to testing, they were either fed ad libitum or food-restricted to 75% of their ad libitum intake. At the onset of the dark period of the light–dark cycle, they were tested every day of the estrous cycle for their preference for males vs. food, food hoarding, and food intake (Klingerman et al., 2010).

According to our first hypothesis, we predicted that the effects of the anorectic hormone estradiol would differ according to the availability of metabolic energy, and that appetitive behaviors would be more sensitive than consummatory behaviors to energetic challenges. We first tested this by measuring ingestive and sexual motivation over the estrous cycle under two different energetic conditions: limited and unlimited food availability. In two other experiments we manipulated the available energy by increasing the demand for energy expenditure. We used two conditions in which hamsters must increase their energy expenditure: housing at cold ambient temperature (5˚C for at least 7 days before testing), and access to voluntary exercise (the hamsters were housed with running wheels). In all types of energetically challenged females, whether food-limited, cold-housed, or exercising), we predicted that there would be clear fluctuations in appetitive behaviors over the estrous cycle, with food preference at its nadir and sexual motivation at its peak on estrous cycle days 3 and 4. If our first hypothesis were correct, only energetically challenged females would fluctuate over the cycle in food hoarding and the preference for males vs. food, whereas females with unlimited energy availability would not fluctuate to the same degree. Our hypothesis would be refuted if females with unlimited energy availability did not differ from those with limited intake or increased energetic demands. If our second hypothesis were correct, energetically challenged females would differ from energy unchallenged females in appetitive (food hoarding and preference for spending time with males), but not consummatory behavior (food intake and the incidence of lordosis).

As hypothesized, only the food-limited, cold-housed, and wheel-running females varied significantly over the estrous cycle in appetitive sex behaviors. Those with unlimited energy availability showed consistently high preference for sex throughout the estrous cycle. For example, food-limited, cold-housed, and wheel-running females showed significant fluctuations in male preference ([time spent with males – time spent with food] divided by total time; Figure 1; Klingerman et al., 2010]). Females with unlimited food, housed at room temperature without access to running wheels varied little if any over the estrous cycle in male preference (i.e., they preferred to visit the males more than 75% of the time of the time; Figure 1). Similarly, the food-restricted and cold-housed females varied significantly over the estrous cycle in food hoarding (Figure 2). Food-limited and cold-housed females showed low levels of food hoarding and high levels of male preference on the night of high circulating concentrations of estradiol and ovulation, and high levels of hoarding and low levels of male preference on all other estrous cycle day (Figure 2), and yet the same females showed little fluctuation in food intake over the estrous cycle (Figure 3). Females with unlimited food supply, housed at warm temperatures and without access to running wheels showed little variation in food hoarding over the estrous cycle, and none of the groups showed dramatic changes in food intake. With regard to sex behavior, the response to energy deficit was similar, whether the deficit
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FIGURE 1 | Mean and SEM of male preference, calculated as the (time spent with the male minus the time spent with food) divided by the total time. Male preference is shown for females on either the periovulatory day (when circulating estradiol is high, black bars) or the postovulatory day when (circulating estradiol is low, white bars), in three experiments. Experiment 1 compared food-limited to food-unlimited females at 22˚C without access to wheels. Experiment 2 compared cold-housed (5˚C) to warm housed (22˚C) in females with unlimited food availability. Experiment 3 compared females housed with running wheels to those without running wheels at 22˚C with levels of food equal to those fed ad libitum without wheels. a = significantly different from ad libitum-fed at P < 0.01, b = significantly different from warm housed at P < 0.01, c = significantly different from no-wheel group at P < 0.001. Food-limited and unlimited groups adapted from Klingerman et al. (2010). Food availability and cold temperatures, but not by increases in voluntary wheel running. Females with wheels decreased their preference for males and yet hoarded very little food, perhaps providing a window into brain areas that partition energy for either sexual motivation or hunger for food.

FIGURE 2 | Mean and SEM of food hoarding shown for females on either the periovulatory day (when circulating estradiol is high, black bars) or the postovulatory day when (circulating estradiol is low, white bars), in two experiments. Experiment 1 compared food-limited to food-unlimited females at 22˚C. Experiment 2 compared cold-housed (5˚C) to warm housed (22˚C) in females with unlimited food availability. a = significantly different from ad libitum-fed at P < 0.001, b = significantly different from warm housed at P < 0.001. Food-limited and unlimited groups adapted from Klingerman et al. (2010).

was due to limited food availability, cold ambient temperature, or wheel running. Food hoarding, however, was increased by limited energy availability and cold temperatures, but not by increases in voluntary wheel running. Females with wheels decreased their preference for males and yet hoarded very little food, perhaps providing a window into brain areas that partition energy for either sexual motivation or hunger for food.

These experiments suggest that changes in energy intake and expenditure change the responsiveness or sensitivity to ovarian steroids. A follow-up experiment showed the same effects of limited energy availability when ovariectomized (Ovx) females were compared to females treated with the hormonal regimen typically used to induce lordosis in this species (estradiol 48 h and progesterone 6 h prior to testing). Four days of food restriction increased food hoarding and decreased male preference in Ovx + vehicle females relative to Ovx + estradiol and progesterone treated females (Klingerman et al., 2010). The effects are not attributable to changes in circulating levels of ovarian steroids, but to the response to those steroids (Klingerman et al., 2010). In other words, ovarian steroids had obvious measurable effects on appetitive behaviors in females with limited food availability, but these were attenuated in females fed ad libitum. Furthermore, at these short durations of mild food restriction, steroid-energy availability interaction was apparent only in appetitive, not consummatory behaviors, and they occurred in response to exogenous steroid treatment.

Together, these experiments are consistent with the idea that an important role of estradiol is to orchestrate the choice between ingestive and sex behavior under conditions where energy availability fluctuates and that this decision occurs at the level of behavioral motivation rather than performance. Furthermore these experiments show that the effects are not limited to food deprivation, but they extend to any situation in which overall availability of energy...
is limited, for example, when the need for energy expenditure is elevated relative to energy intake.

These results lead to additional testable hypothesis about the mechanisms by which estradiol orchestrates behavioral choice. For example, we hypothesize that during the early follicular phase of the estrous cycle when circulating estradiol levels are low, sexual motivation is tonically inhibited by one or a number of putative “orexigenic” peptides, such as ghrelin, GnIH, NPY, agouti-related protein (AgRP), endocannabinoids, or some combination of these. Furthermore, we hypothesize that periovulatory levels of estradiol disinhibit the effects of ghrelin, and/or other neuropeptides in order to couple reproductive motivation with fertility and inhibit food hoarding. These hypotheses lead to testable predictions about the effects of estradiol on neural activation in identified neurons, e.g., those that contain ghrelin or endocannabinoid receptors and secrete GnIH, NPY, or AgRP. Consistent with this idea, changes in neural activation in GnIH cells are more closely associated with appetitive than consummatory sex and ingestive behaviors (Klinger et al., 2011b).

These studies illustrate that, when studying the effects of energy availability, it is imperative to differentiate appetitive from consummatory behaviors. Appetitive behaviors are more sensitive, and occur prior to effects on gonadal steroids. By exclusive focus on food intake estrous cyclicity and pulsatile gonadotropin secretion, the effects of energy availability will be missed. Furthermore, it is well known that appetitive aspects of behavior often involve different brain areas and different hormonal stimulation (Ball and Balthazart, 2008), and these are often more relevant to human behavior. Most relevant to metabolic control of reproduction and ingestive behavior, cellular activation that corresponds with NPY/AgRP effects on food hoarding occurs in the subzona incerta and central nucleus of the amygdala, whereas cellular activation that corresponds to effects on food intake involve the typical activation of paraventricular nucleus of the hypothalamus (PVH), and other areas, but not the subzona incerta or central nucleus of the amygdala (Teubner et al., 2011). Food hoarding species, such as Siberian and Syrian hamsters should receive a great deal more attention in the future, now that it has been documented that our own species is more like hamsters than rats in that they do not show postfast hyperphagia to the same degree as laboratory rats and mice (Hetherington et al., 2000; Al-Hourani and Atoum, 2007; Levitsky and DeRosimo, 2010), but instead show significant changes in grocery shopping, i.e., carrying food from a source outside the home and storing it in their home prior to consumption (Dodd et al., 1977; Tom, 1983; Beneke and Davis, 1985; Beneke et al., 1988; Mela et al., 1996). These ideas are extensively covered in a recent, lucid review of the appetitive behavior, food hoarding, by Bartness et al. (2011).

SENSE AND NONSENSE, THE LIPOSTAT HYPOTHESIS

The above-mentioned results suggest that not all hormones and neuropeptides function to keep body weight within limits that we imagine to be healthy and fashionable. Rather, they function to respond to changes in environmental energy availability by modulation of reproductive and ingestive behaviors. In nature, energetic demands differ in males and females, within groups of males and females, over seasons, and over the entire lifespan.

This is important because translational research programs are often built upon the idea that “normal” individuals have a healthy “set point” for body weight and adiposity, whereas overweight and obese individuals do not. The “lipostat” hypothesis purports that factors secreted from adipose tissue dictate the level of food intake in service of maintaining this set point in adiposity. A modern version of the lipostat hypothesis purports that factors such as leptin are secreted from fat cells in proportion to overall body adiposity, induce satiety, and therefore decrease meal size. When extended to reproduction, the lipostat hypothesis is called the “critical body fat” hypothesis, which suggests that puberty is delayed or adult reproduction is inhibited when body fat content and levels of the lipostatic hormone falls below a particular threshold.

The lipostat, set point, and the critical body fat concepts are intuitively satisfying descriptions that are seldom tested directly but are often reinforced with circular reasoning. In contrast, science progresses by testing hypotheses that can conceivably be ruled out by a realistic experimental outcome (Popper, 1963). The set point model is refuted whenever an experimental group fails to defend a set point for body weight (e.g., due to a change in diet, reproductive cycle, photoperiod, or ambient temperature). Instead, any significant increase in body weight is interpreted as evidence for a “resetting” or “sliding” set point. Like the existence of God, the sliding set point hypothesis cannot be refuted. It is difficult to imagine how natural selection (based on individual survival and superior reproductive success) would favor defense of a set point in environments where food availability fluctuates or is unpredictable. When food is scarce and energy demands are high, the maintenance of a particular set point for body weight or adiposity should receive low priority compared to the break down of lipids in adipose tissue to usable metabolic fuels necessary for survival. Conversely, if females encounter a windfall of energy rich food, why abstain from overeating in order to preserve a putative set point for body weight when pregnancy and lactation are so energetically demanding and the food supply so unreliable? Why not eat heartily and store the excess energy as lipids in adipose tissue deposits especially designed to provide fuels for milk production that will feed your genetic contribution to the next generation? Females might develop obesity but would be more likely to get their genes into the next generation, particularly if the unhealthy consequences of obesity accrue after the reproductive years.

The set point is a seductively satisfying label or analogy that has been criticized because it terminates, rather than stimulates further investigation. To name a phenomenon is not, in and of itself, progress in understanding the phenomenon. The set point is used as an analogy, but that does not translate into a testable hypothesis. The lipostat and the set point idea are inspired by the engineers’ design of the mechanical thermostat. A homeowner’s thermostat contains a physical object, a thermometer, that can measure temperature and can be calibrated and set by the homeowner. The room temperature varies above or below the set point, and when it deviates too far from set point, the heating or air conditioning corrects the error. In animals, there is no physical object that corresponds to the thermostat. We have not identified the location and biochemical nature of the detectors for metabolic fuel availability, and we have no idea how such a set point for...
fuel availability or for body fat content might be set or calibrated. The set point theory has been repeatedly refuted on both empirical and theoretical grounds in numerous excellent reviews that are highly recommended (Wirtshafter and Davis, 1977; Van Itallie and Kissileff, 1996; Bronson and Manning, 1991; Bronson, 1998). Due to its intuitive and seductive nature, however, it is bound to come up whenever a new investigator enters the field of metabolic control of reproduction.

In contrast to the lipostat idea, the hypothesis that putative orexigenic and anorectic hormones function to optimize reproductive success in environments where energy availability fluctuates leads to testable hypotheses. It is in line with data showing that putative satiety peptides ensure overeating when those peptides are low, but are often ineffective in curbing appetite when they are high (Ahima et al., 1996; Flier, 1998). The hypotheses are reminiscent of Optimal Foraging Theory, which has its origins in ecology and is based on the ubiquitous observation among wild animals that food intake decreases with increasing cost in terms of time and energy expenditure. The corollary is that energy intake increases when food is cheap, plentiful, and requires little energy to obtain and digest (Emlen, 1966; MacArthur, 1966). When animals encounter diets of different caloric density that can be consumed at a particular energetic cost, intake increases as cost decreases and as net calories increase, and this choice ultimately results in accumulation of body fat (Collier et al., 1972; Houston and McNamara, 1989). This effect is linear, and thus, any lipostatic explanation would have to include a new set point reached for every excess calorie consumed. Instead of stability, body weight displays remarkable plasticity that allows anticipation of future metabolic challenges and permits the learning and memory formation that occurs when postgestional cues reinforce sensory cues from food. It is important to embrace this fact of life, and study the mechanisms whereby cheap and calorically dense food elicits changes in metabolism that allow excess storage, rather than search for an elusive and possibly non-existent mechanism that is supposed to maintain one set point for body weight throughout adult lifespan.

Like body fat content, food intake is not held at a set point. For example, when laboratory chow is diluted with non-nutritive bulk, laboratory rodents do not maintain their food intake at a set point. Quite the contrary, they show a controlled increase in food intake in proportion to the caloric dilution, and reproductive function is related to a threshold of usable fuels, not a particular level of bulk intake (Adolph, 1947; Peterson and Baumgardt, 1971; Nance and Gorski, 1977; Louis-Sylvestre, 1987; Szymanski et al., 2009). In contrast, changing the vitamin, mineral or essential fatty acid content of food while keeping calories constant elicits little or no change in food intake (Adolph, 1947). Furthermore, when animals must exercise or expend energy to obtain food, or when animals must expend more energy to keep warm at cold ambient temperatures, animals increase their food intake to compensate for the extra energy expended (Kraly and Blass, 1976; Browne and Borer, 1978; Tsai et al., 1982; Barreness et al., 1984; Rowland, 1984; Louis-Sylvestre, 1987; Manning and Bronson, 1990; Schneider and Wade, 1990b). Females of many species will more than double their food intake during lactation to meet the energetic demands of milk production (Cripps and Williams, 1975; Fleming, 1976a,b, 1978; McLaughlin et al., 1983; Louis-Sylvestre, 1987; Woodside et al., 2000). Lactating female mice, Peromyscus leucopus, increase their food intake 230% when housed in cold ambient temperature compared to their preprogesterone food intake at laboratory temperatures (Perrigo, 1987).

A particularly convincing argument for metabolic, rather than lipostatic control of food hoarding, comes from studies of Siberian hamsters fed a diet diluted with non-nutritive cellulose. Hamsters increase their food hoarding (but not their food intake) in proportion to the dilution and decrease their food hoarding in proportion to increases in caloric density. The change in food hoarding is immediate, and thus cannot be mediated by changes in body fat content, but is more likely controlled by postgestional cues that occur when the hamsters taste the diet just prior to hoarding (Wood and Bartness, 1996). Like internally stored energy, externally stored energy is flexible and varies with the energetic demands of the individual, its life history stage, and the environment. Rather than maintain a set point, food intake, food hoarding, body weight, and adiposity change dramatically in response to environmental cues (energy, ambient temperature, and photoperiod) in order to maintain the availability of oxidizable metabolic fuels for survival and reproduction (Friedman, 2008).

Contrary to commonly held dogma, stability in body weight is not a consistent feature in ad libitum-fed mammals, especially female mammals. The myth of stability in body weight is based on longitudinal studies, but is not supported by studies in which the same individuals are studied over many weeks. Far from the stability of body weight predicted by the lipostat hypothesis, body weight in animals with unlimited access to food increases steadily over time (Ahren et al., 1997). When genetically heterogeneous (outbred) strains of rats are singly housed with unlimited access to standard, laboratory chow, and monitored for more than 100 weeks, they fail to maintain “body weight homeostasis.” Instead they not only gain significantly more body weight and adiposity than food-limited rats, but they increase their body fat content from 6–7% at 6–7 weeks of age, to 25% at 14 weeks, to 36–42% at 106 weeks of age. They also develop significant hypertriglyceridemia and hypercholesterolemia relative to food-limited rats (Keenan et al., 2005). Male rats show the largest increase in body weight during the first year; females show the largest body weight gain in the second year after cessation of their estrous cycles. The study by Keenan et al. (2005) illustrates two points. First, rats in isolation and in a confined space do not self-regulate their intake, and do not avoid the negative consequences of obesity. Second, reproductive factors create differences in the propensity to store body fat. In females, the body weight gain was exaggerated after the postmenopausal decrease in ovarian steroid secretion, whereas in males, body weight gain occurred early in the life history cycle (Keenan et al., 2005). Thus, in addition to mechanisms that maintain fuel homeostasis for individual survival, there lies another layer of control that promotes internal fuel storage as body fat to anticipate the need to forego ingestive behavior in favor of reproductive behavior necessary for Darwinian fitness.

This brings us to the lipostatic hypothesis of reproduction popularized by Rose Frisch (Frisch and McArthur, 1974; Frisch, 1990), which purports that there is a critical level of body fat necessary for the initiation of puberty and menarche, the maintenance...
of adult menstrual cycles and fertility and for the restoration of reproductive function in animals that have become anestrous or hypogonadotrophic after food restriction or deprivation. Little evidence actually supports this hypothesis beyond the obvious correlation between body fat content and reproductive function; but correlation is not causation. Both body fat content and reproductive competence depend upon a third factor, the availability of oxidizable metabolic fuels, thus, body fat content and reproduction are correlated. When strong inference hypothesis testing is used as a direct test of the "critical body fat" hypothesis, the critical body fat hypothesis is refuted. For example, when females are rendered hypogonadotrophic by food deprivation and are then re-fed, the restoration of estrous cycles and pulsatile LH secretion occurs more rapidly than the recovery of body fat levels, and without significant increases in plasma leptin concentrations in sheep and hamsters (Schneider et al., 2000a; Jones and Lubbers, 2001; Szymanski et al., 2007). Finally, most animals respond rapidly to changes in fuel availability, too rapidly for the changes in reproduction to be due to the slow process of lipid accumulation (Bronson, 1986, 1998, 2000; Armstrong and Britt, 1987). Thus, contrary to the critical body fat hypothesis, LH pulses can be restored whenever overall metabolic fuel availability increases, even when body fat levels and levels of plasma leptin concentrations lag behind and remain at the same level as hypogonadal food-restricted females.

**SENSE AND NONSENSE, THE METABOLIC HYPOTHESIS**

"The simplest way in which this lipostasis could be achieved is by sensitivity to the concentration of circulating metabolites."

(Kennedy, 1953)

Gerald Kennedy is often credited with coining the word *lipostat*, which, over time, became associated with the set point hypothesis. It is important to note, however, that Kennedy’s idea of a fat-derived signal came not from circulating hormones or neural signals from adipose tissue, but rather from circulating metabolites associated with either lipolysis or lipogenesis (Kennedy, 1953). Furthermore, he proposed that the ability to sense metabolic fuel availability was critical for the control of food intake and reproductive development. Kennedy’s lipostat was more in line with the critical body fat hypothesis, which, over time, became associated with the set point hypothesis. The above-mentioned research was focused on changes in the periphery. More recent research is focused on CNS fatty acid oxidation and synthesis, and the bulk of this work is concerned with ingestive behavior. Prior to diving into this field of research, it is important to examine the role of glucose sensing, as well as the idea that food intake and reproduction are controlled by the availability of ATP, or the ratio of ATP:AMP (Friedman, 2008). Glucose and fatty acid oxidation do not occur independently, but rather they are interrelated. The availability of glucose and the ratio of ATP to AMP, for example, determine the extent to which cells engage in fatty acid oxidation. This is important because translational research aimed at one metabolic pathway will have to account for compensatory coordinated changes in the other, and detection of these different fuels may take place in different parts of the brain.

Cellular detectors of glucose availability important for control of estrous cycles have been localized to the brainstem, just as those for food intake, adrenal glucocorticoid secretion, and counterregulatory responses to glucoprivation are localized in the brainstem (Ritter et al., 2011). There are “glucose-sensitive” cells in many brain areas involved in other diverse functions (including neuroprotection, circadian rhythms, and reward, to name a few; Levin et al., 2004), but those outside the hindbrain require more than 10-fold higher concentrations and 100-fold higher volume of inhibitors of glucose oxidation for significant effects on ingestive behavior (Ritter et al., 2011). Ingestive behavior is stimulated (Smith and Epstein, 1969; Ritter, 1986), and reproductive processes are inhibited by treatments that block glucose oxidation given systemically (Schneider and Wade, 1989) or into the third or fourth ventricle (Ritter et al., 1981; Murahashi et al., 1996), and the effects of glucoprivation are prevented by lesions of the AP in the caudal most part of the hindbrain (Ritter and Taylor, 1990; Schneider and Zhu, 1994; Panicker et al., 1998). For both ingestive behavior and reproduction, catecholaminergic projections from...
hindbrain to the PVH are necessary for the effects of glucopriva-
tion (Ritter and Calingasan, 1994; l’Anson et al., 2003b; Bugarith et al., 2005).

For the HPG system and sex behavior, we are likely to find that brainstem structures are far more sensitive to changes in glucose availability than are forebrain structures. This is fore-
shadowed by work on ingestive behavior. Microinjections of an inhibitor of glucose utilization into hundreds of brainstem areas in the NTS increased food intake, whereas only a few did so when administered to hypothalamic areas (reviewed by Ritter et al., 2011). Small (200 nl volumes of 12–24 g per animal) of glucoprivic agents microinjected unilaterally into discrete hindbrain regions increase food intake and initiated counterregulatory responses. The same doses and even higher doses are not effective when injected into hypothalamic sites or even into the fourth ventri-
cle (Ritter et al., 2000). Furthermore, small microinjections of glucoprivic agents that increase food intake also increase mRNA for the orexigenic peptide NPY. The hyperphagia and increase in NPY mRNA are significantly decreased by immunotoxotoxic destruc-
tion of the catecholaminergic/NPYergic cells that originate in the hindbrain. This is not true in the hypothalamus. In con-
trast to brainstem lesions, NPY-saporin-induced lesions of Arc NPY neurons do not impair glucoprivic feeding or hyperglycemic responses (Ritter et al., 2006). Careful mapping of metabolic stim-
uli that affect food intake strongly suggests that the important signals are detected in the caudal brainstem, and this type of mapping should be done for metabolic control of reproduction. These brainstem structures and projections to the PVH are not required for the effects of food deprivation on food intake and reproduction, only for changes elicited by glucoprivation. Thus, glucoprivic control cannot explain all effects of natural energetic challenges on food intake and reproduction. However, the parallels between glucoprivic control of food intake and glucoprivic control of estrous cycles are striking and worth remembering when trying to unravel the effects of intracellular fuel metabolism on reproduction.

Another important line of research showed that food intake is not controlled by either glucose or free fatty acid availability, per se, but by the general availability of oxidizable fuels or a metabolic event in the final common pathway to ATP production, perhaps ATP content itself. This idea is supported by the results of experi-
ments in which pharmacological agents that reduce hepatic (liver) ATP increase food intake, and treatments that prevent depletion of hepatic ATP content also preclude increases in food intake (Raw-
son and Friedman, 1994; Rawson et al., 1994; Ji and Friedman, 1999; Ji et al., 2000). There is evidence that detectors of fatty acid oxidation and hepatic ATP content are in the periphery, most likely in the liver, because effects of fatty acids and of hepatic ATP content on food intake are blocked by treatments that disconnect the communication between the liver and brain via the vagus nerve (Ritter and Taylor, 1990; Tordoff et al., 1991). Metabolic inhibitors that decrease hepatic ATP status increase intracellular sodium and calcium concentrations in hepatocytes in vitro (Friedman et al., 2003; Rawson et al., 2003). Future research on metabolic control of food intake and reproduction will have to contend with the possi-
ability that changes in the brain have effects on these peripheral ATP detectors.

The metabolic hypothesis was introduced in the literature after the discovery that leptin, the protein product of the ob gene, decreased food intake and restored reproductive capabilities in obese, hyperphagic, infertile ob/ob mice (mouse homozygous for a mutation in the ob gene; Campfield et al., 1995; Halaas et al., 1995; Pellemounter et al., 1995). Most of the literature in the decade from 1995 to 2005 portrays leptin action as nothing more than endocrine signaling, with less attention to leptin’s effects on intracellular fuel oxidation or to the metabolic events that control leptin synthesis and secretion. However, some investigators gathered evidence from diverse sources, which together suggested that peripheral hormones like insulin and leptin act via intracellular fuel oxidation. M. I. Friedman pointed out that effects of insulin and leptin on digestive behavior . . .

“. . . is an indirect response to a shift in fuels from storage to oxidation, not a direct response to a satiety signal associated with the level of adiposity. Many experimental treatments that affect eating behavior, whether restricted to the central nervous system or not, alter peripheral metabolism. Because changes in fuel partitioning can affect food intake, knowing the metabolic consequences of such experimental manipula-
tions may be necessary to understand their effect on eating behavior.” (Friedman, 1998)

Inspired by conversations with Friedman, in the year 2000, J. E. Schneider et al. pointed out that . . .

“There are at least two possible ways that leptin might interact with the metabolic sensory system that controls reproduc-
tion. First, leptin synthesis and secretion in various tissues might be sensitive to metabolic fuel availability. . . . as a mediator of the metabolic signal. Second, leptin might affect reproduction indirectly by way of modulating the meta-

bolic signal that is known to influence reproduction. Leptin’s effects on reproduction coupled with its unique effects of fuel metabolism, i.e., it’s ability to promote in situ fuel oxidation without increasing the general availability of metabolic fuels in circulation, might provide new clues to the nature of the metabolic stimulus that controls reproduction.” (Schneider et al., 2000b)

Leptin decreases food intake and stimulates reproductive process in a wide variety of species (reviewed by Schneider, 2000), but contrary to the lipostatic hypothesis, leptin acts on estrous cycles by modulating the intracellular availability of oxidizable fuels. The first evidence for the notion that leptin modulates intracel-

lular fuel availability was demonstrated in vitro by the laboratories of Ungar and Rossetti (Rossetti et al., 1997; Shimabukuro et al., 1997; Wang et al., 1998). Does leptin modulate digestive behavior and/or reproduction by modulating the intracellular availability of glucose or free fatty acids?

Treatment with leptin prevents food deprivation-induced anestrus in Syrian hamsters, and, thus, follow-up experiments were designed to ask whether follow-up experiments show that leptin’s ability to prevent food deprivation-induced anestrus is dependent upon the ability to increase glucose and/or fatty acid oxidation (Schneider et al., 1998). Syrian hamsters were either food deprived or fed ad libitum and treated with doses of MP, the
inhibitor of free fatty acid oxidation, or 2-deoxy-D-glucose (2DG), an inhibitor of glucose utilization. Leptin was given either systemically or intracerebroventricularly. Doses of MP and 2DG were used that do not inhibit estrous cycles in ad libitum-fed females. The stimulatory effects of systemic and intracerebroventricular leptin on estrous cycles are blocked by treatments that blocked intracellular glucose or fatty acid oxidation (Figure 4; Schneider et al., 1998; Schneider and Zhou, 1999). This, to the best of our knowledge, was the first experiment to demonstrate the interaction between leptin and intracellular fuel oxidation on reproduction.

Whereas the above-mentioned experiments examined the effects of fatty acid oxidation, later work examined the effects of fatty acid synthesis. Generally, fatty acid synthesis is stimulated by excess fuel availability, i.e., when there is more than ample substrate availability for fuel oxidation and the formation of new ATP. Fatty acid oxidation occurs predominantly during fasting when the primary fuel available in the periphery is in the form of free fatty acids released from triacylglycerides in adipose tissue. If inhibition of fatty acid oxidation increases food intake and inhibits reproduction, does inhibition of fatty acid synthesis have the opposite effects? Inhibition of fatty acid synthase (FAS) in the brain and periphery decreases food intake (Loftus et al., 2000). FAS is the multienzyme protein that catalyzes the synthesis of fatty acids from the substrate malonyl-CoA under conditions of excess fuel availability. The discovery that food intake is inhibited by agents that inhibit the activity of FAS created a renewed interest in metabolic control of ingestive behavior focused on the effects of metabolic challenges (such as starvation and diabetes) and peripheral hormones such as leptin and ghrelin on enzymes and substrates involved in fatty acid synthesis in the brain.

The inhibitory effects of centrally administered FAS inhibitors on food intake was a surprise to many neuroscientists, given that glucose is the primary substrate oxidized in the CNS. It turns out that free fatty acids, and some particular amino acids also reach the brain. The same metabolic pathway for synthesis of fatty acids that functions in peripheral cells also exists in the CNS.

To review some of these basic pathways, fatty acids (i.e., LCFacyl-CoA, used in formation of triacylglycerides) are synthesized from malonyl-CoA (the reaction catalyzed by FAS). Malonyl-CoA is synthesized from acetyl-CoA [catalyzed by acetyl-CoA-carboxylase (ACC)]. ACC is inhibited by 5′-adenosine monophosphate-activated protein kinase (AMPK) as well as palmitate. AMPK is sensitive to energy availability, specifically the ratio of ATP to AMP. The formation of fatty acids (LCFacyl-CoA) for storage as triacylglycerides is an ATP-using process. Thus, stimulation of this pathway is appropriate under conditions of high energy availability.

There is a very large and confusing body of literature on the effects of fatty acid synthesis intermediates on control of food intake. Understanding these data requires that we step back and remember that these cerebroventricularly applied metabolic inhibitors might not mimic the endogenous events that control normal ingestion. Furthermore, these artificial CNS manipulations are likely to have effects on peripheral fuel metabolism that could, in turn, affect the behaviors in questions (Cha et al., 2005; Lam et al., 2005; Bartness et al., 2010; Bachman et al., 2002). Keeping in mind, the general outcome of these studies is that most factors that decrease food intake tend to decrease the CNS activity of AMPK, the nutrient-sensitive kinase that inhibits ACC. For example, ICV leptin, insulin, and GLP-I decrease the CNS activity of the enzyme that transports free fatty acids into mitochondria for oxidation in the periphery but is hypothesized to have a nutrient sensing function in brain. Factors that decrease food intake also increase the activity of brain-specific CPT-I, the enzyme that transports free fatty acids into mitochondria for oxidation in the periphery but is hypothesized to have a nutrient sensing function in brain. Factors that decrease food intake also increase mammalian target of rapamycin (mTOR), another nutrient-sensitive kinase involved in regulation of protein synthesis and energy balance. Conversely, factors that increase food intake tend to have the opposite effects in hypothalamic fatty acid synthesis and oxidation, i.e., activation of AMPK and CPT-I, and inhibition of ACC, malonyl-CoA, and mTOR (Minekoshi et al., 2002, 2008; Cota et al., 2006, 2008; Pocai et al., 2006; Proulx et al., 2008; Wolfgang et al., 2008).

The relative importance of each of these intermediates, the mechanisms involved, and their importance for ingestive behavior in brain vs. periphery, hypothalamus vs. brainstem is still in question. For example, agents that inhibit (e.g., leptin, Compound C) and stimulate (ghrelin, AICAR) the activity of AMPK in hypothalamus decrease and increase food intake respectively. However, these exogenously applied agents are likely to have myriad side effects. For example, transgenic knockout of AMPKα2 exclusively in NPY neurons results in a lean phenotype, whereas knockout of AMPKα2 in POMC neurons results in an obese phenotype, and both types of neurons with these knockouts show normal electrophysiological responses to leptin but are insensitive to glucose (Claret et al., 2007). The effects of energetic challenges and ghrelin on AMPK in liver and adipocytes are the opposite of that in liver and adipocytes.
brain, and thus, hypotheses about the role of AMPK and other intermediates in control of ingestive behavior must consider the whole organism. AMPK is an ancient and ubiquitous enzyme. Some specificity might be added by consideration of other proteins involved in fatty acid oxidation and mitochondrial respiration, such as uncoupling protein-2 (UCP-2). For example, studies that compare knockouts for UCP-2 with wild type mice show that effects of leptin and ghrelin depend upon a functional gene for UCP-2, whereas substrate-mediated effects of AMPK occur with or without a functional gene for UCP-2 (Andrews, 2011; Diano and Horvath, 2012).

The importance of taking a broad perspective that includes the whole organism (brain and periphery) is exemplified by the exaggerated diet-induced obesity that occurs in knockout mice that lack the functional gene for CPT-Ic (Wolfgang et al., 2008). This is not predicted by the theory that inhibition of CPT-Ic in brain decreases appetite, but is instead consistent with the idea that decreased fatty acid oxidation produces a deficit in fuels for oxidation that leads to peripheral mechanisms that conserve energy by inhibition of energy expenditure and promote fuel storage.

Given that food restriction tends to increase food intake and inhibit the HPG system and sex behavior, it might be expected that central inhibition of fatty acid oxidation might inhibit the reproductive system. Investigators have begun to explore the potential role of AMPK, mTOR, and glucokinase (GK) in metabolic control of reproduction. Central treatment with inhibitors of free fatty acid oxidation inhibit the HPG system (Sajapitak et al., 2008). Pulsatile LH secretion is suppressed in a dose-dependent manner by fourth ventricular treatment with an inhibitor of fatty acid oxidation in ovariectomized female rats that were either treated with estradiol or vehicle. These results suggest that central inhibition of free fatty acid oxidation inhibits the HPG system. In other studies, activation of hypothalamic mTOR reverses food restriction-induced inhibition of LH secretion (i.e., stimulated LH secretion). Furthermore, blockade of mTOR delayed reproductive maturation, prevented the restorative effects of leptin on LH secretion (Dieterich et al., 2010). Still other lines of research have focused on intermediate maturation, prevented the restorative effects of leptin on LH secretion (i.e., stimulated LH secretion). Furthermore, blockade of mTOR delayed reproductive maturation, prevented the restorative effects of leptin on LH secretion (Dieterich et al., 2010).

A final warning to new investigators would be to double check all assumptions about the importance of a neuropeptide, hormone, or brain area based on the number of published articles concerned with that topic. The vast majority of research on metabolic control of reproduction examines projections to and from the hypothalamus, and of those, most concern Arc NPY/AgRP, POMC, and more recently the Kisspeptin/GnIH system that includes the Arc, PVH, preoptic area, dorsomedial hypothalamus (DMH), and AVPV (Estrada et al., 2006; Franceschini et al., 2006; Kriegsfeld et al., 2006; Roa et al., 2006; Smith et al., 2008; Yeo and Herbst, 2011). In fact, a large body of elegant work in metabolic control of reproduction examines projections to and from the hypothalamus, and of those, most concern Arc NPY/AgRP, POMC, and more recently the Kisspeptin/GnIH system that includes the Arc, PVH, preoptic area, dorsomedial hypothalamus (DMH), and AVPV (Estrada et al., 2006; Franceschini et al., 2006; Kriegsfeld et al., 2006; Roa et al., 2006; Smith et al., 2008; Yeo and Herbst, 2011).
control of reproduction and ingestive behavior supports a widely distributed neural network that includes the caudal brain stem, midbrain, and forebrain (Schneider et al., 1993, 1995; Horn et al., 1999, 2001; Ritter et al., 2001; I’Anson et al., 2003b; la Fleur et al., 2003; Hudson and Ritter, 2004; Bugarith et al., 2005; Hayes et al., 2009; Skibicka and Grill, 2009). This work has been the subject of thoughtful and scholarly reviews (Grill and Kaplan, 1990, 2002; Grill, 2006; Friedman, 2008; Grill and Hayes, 2009). In the 1980s and 1990s, neuroanatomical characterization of POMC NPY cells located these molecules and their receptors in the brain stem as well as in other areas, and the notion of distributed neuroanatomical control of energy balance was well accepted (Sawchenko et al., 1985; Bronstein et al., 1992).

Agents that increase or decrease voluntary food intake do so when microinjected into the hindbrain, and, in many cases, these agents can have the same effects on passive intake in decerebrate animals (reviewed by Grill and Hayes, 2009). This applies to leptin (Grill et al., 2002; Harris et al., 2007). Furthermore, these hindbrain effects on food intake involve the activity of AMPK, and fail to occur when leptin-induced inhibition of AMPK is prevented (Hayes et al., 2009). Furthermore, the stimulatory effects of 2DG on food intake activate AMPK, and 2DG-induced hyperphagia does not occur without AMPK activation (Li et al., 2011). The main point for reproductive endocrinologists is that it would be a mistake to imagine that metabolic control of ingestive and reproductive behavior is restricted to the Arc or even the entire hypothalamus.

Both food intake and energy expenditure, and presumably energy expenditure for reproduction, are under the influence of the peptide systems in these widely distributed interconnected nuclei. For example, Skibicka and Grill (2009) measured food intake, core body temperature, heart rate, and spontaneous activity in rats that received two different picomolar doses of an MC4R agonist (MTII) into six different brain subnuclei distributed along the neuraxis. The MTII doses were titrated to be well below the threshold dose known to be effective when infused intracerebroventricularly. The results were unequivocal. In each case when the MTII infusion was demonstrated histologically to hit its intended target, five of the six brain areas, including the NTS and midbrain, showed increases in body temperature and heart rate as well as decreased food intake. The data confirm a growing body of data demonstrating that melanocortinergic effects on food intake and energy expenditure occur in the caudal brain stem (Grill et al., 1998; Williams et al., 2000, 2003; Grill and Kaplan, 2001, 2002).

The important implication for researchers interested in metabolic control of reproduction is that the brain is organized into a network of areas that display a great deal of redundancy of function, most of which impact aspects of energy expenditure as well as food intake. At this point, it should be obvious that a significant portion of total energy expenditure is allocated for reproduction. Investigators new to this field of research will no doubt come across published articles that employ “expression of a particular neuropeptide in the Arc” as evidence for the function of that peptide in control of ingestion. After reading this review, it should be obvious that an equally probably function is control of reproduction, as well as general activity, thermogenesis, body temperature, heart rate, and oxygen consumption.

**SUMMARY**

This review is intended to provide a foundation for future research at the interface of ingestive behavior and reproduction. At the interface of ingestive behavior and reproduction lies a metabolic sensory system that detects changes in fuel availability and initiates changes in motivation, appetitive sex, and ingestive behaviors, in addition to changes in the HPG system. The observed changes in behavior, hormones, and metabolic fuel partitioning are best understood within the metabolic hypothesis:

A sensory system monitors the availability of oxidizable metabolic fuels and orchestrates behavioral motivation to optimize reproductive success in environments where energy availability fluctuates or is unpredictable.

This hypothesis leads to testable predictions. For example, it predicts that orexigenic and anorectic hormones and neuropeptides will have different effects on ingestive and sex behavior depending on the energetic challenges faced by the animals. This prediction was realized with regard to the effects of estradiol on food hoarding and courtship in Syrian hamsters (Figures 1 and 2). It also predicts that animals housed alone, with unlimited food, with limited behavioral options are not the control group, but the experimental group. It predicts that behavioral motivation will be more sensitive than food intake or copulation (Figures 1–3), and that mechanisms that function to in short term choices between food and sex might not be capable of maintenance of long term stability in body fat content in environments where energy availability and energetic demands fluctuate.

There is now recognition that so-called lipostatic hormones, once thought to maintain a set point in body fat content, are actually modulators of metabolic fuel availability, more specifically, fuel oxidation, and synthesis. The challenge is to incorporate what is known about these mechanisms in fuel homeostasis to the demonstrated ability to engage in opportunistic overeating in anticipation of the high energetic demands of reproduction (as well as seasonal and unpredictable changes in energy availability).

Finally, all of the above concepts must be studied in the context of a distributed neural network with multiple, redundant function. More specifically, metabolic control of reproduction must include the hindbrain (AP/NTS), midbrain (e.g., the lateral parabrachial nucleus and VTA), and forebrain (e.g., hypothalamus and striatum), as well as areas such as the ventral premammillary nucleus (Donato et al., 2009) in sharp contrast to the more common exclusive focus on Arc. In fact, the PMV and medial amygdala are good candidates for mediation of metabolic control of motivation for ingestive and sex behavior (Donato et al., 2010).

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