

Genetic Regulation of Feed Intake and Energy Balance in Poultry¹

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ABSTRACT Intensive selection by poultry breeders over many generations for economically important production traits such as growth rate and meat production has been accompanied by significant changes in feed intake and energy balance. For example, the modern commercial broiler, selected for rapid growth and enhanced muscle mass, does not adequately regulate voluntary feed intake to achieve energy balance. When given unrestricted access to feed, broilers exhibit hyperphagia leading to an excessive accumulation of energy (fat) stores, making these birds prone to obesity and other health-related problems. Humoral and neural pathways have been identified and studied in mammals that link appetite and energy balance. A series of highly integrated regulatory mechanisms exists for both of these processes involving complex interactions between peripheral tis-

sues and the central nervous system. Within the central nervous system, the brainstem and the hypothalamus play critical roles in the regulation of feed intake and energy balance. Genes encoding key regulatory factors such as hormones, neuropeptides, receptors, enzymes, transcription factors, and binding/transport proteins constitute the molecular basis for regulatory systems that derive from integrated sensing, signaling, and metabolic pathways. However, we do not yet have a complete understanding of the genetic basis for this regulation in poultry. This review examines what is currently known about the regulation of feed intake and energy balance in poultry. A better understanding of the genes associated with controlling feed intake and energy balance and how their expression is regulated by nutritional and hormonal stimuli will offer new insights into current poultry breeding and management practices.

(Key words: appetite, energy balance, leptin, melanocortin system, genetic regulation)

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INTRODUCTION

The level of feed consumption is a basic and important factor that determines the rate of growth and body composition achieved by animals throughout their lifecycles. In animals, body weight is subject to homeostatic control mediated by adjustments in feed intake and energy expenditure (McMinn et al., 2000). Regulation of feed intake by the central nervous system and peripheral tissue mechanisms in poultry has been reviewed previously (Denbow, 1994; Kuenzel, 1994; Kuenzel et al., 1999).

Feed intake and energy balance in animals, however, have typically been studied separately as independently regulated functions. That approach is changing largely because of the recent discoveries of leptin and its receptor and their subsequent characterization as the molecular basis for the regulatory system linking the sensing of

peripheral energy stores with control of feed intake via the central nervous system (Friedman and Halaas, 1998). Leptin was originally identified as the product of the mouse *ob* gene that is produced predominantly in adipose tissue but is also produced by other tissues to a lesser extent (Zhang et al., 1994). It was found to play a role in the regulation of appetite, energy expenditure, and maintenance of body weight through its actions at specific hypothalamic sites as part of a negative feedback control system (Friedman and Halaas, 1998; Friedman, 2002). The signaling function of leptin was subsequently found to require the expression of specific leptin receptors (Tartaglia et al., 1995).

Because feeding behavior and the accumulation of energy stores are basic to the survival of all animals, it can logically be assumed that regulation of feed intake and energy balance in birds and mammals involves similar regulatory mechanisms, similar neural pathways, and

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Abbreviation Key: AgRP = agouti-related protein; CCK = cholecystokinin; GH = growth hormone; i.c.v. = central administration; LepR = leptin receptor; MCR = melanocortin receptors; α -MSH = α -melanocyte-stimulating hormone; NPY = neuropeptide Y; POMC = proopiomelanocortin; RT = reverse transcription; T₃ = thyroid hormone; UCP = uncoupling proteins.

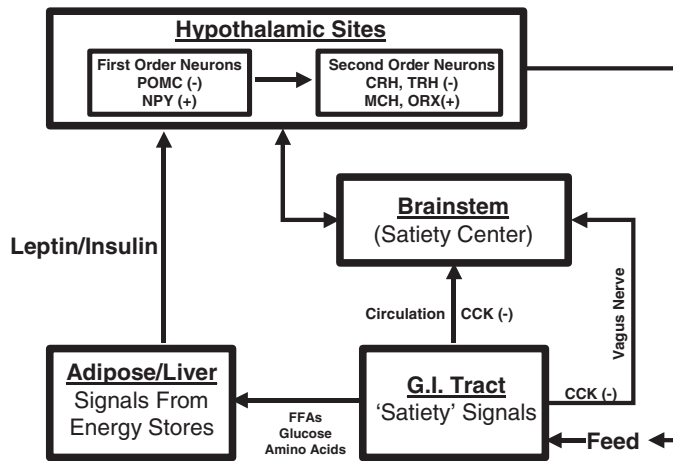


FIGURE 1. A model for the regulation of feed intake in poultry involving short-term satiety-based signaling (represented by cholecystokinin, CCK) and long-term sensing of energy stores (represented by leptin/insulin). The brainstem and hypothalamus play important roles in processing these signals and activating specific efferent pathways leading to adjustments in feed intake. The hypothalamus is proposed to contain two sets of peptidergic neural circuits: first order neurons expressing neuropeptide Y (NPY) or proopiomelanocortin (POMC) and second order neurons expressing genes for such neuropeptides as corticotropin releasing hormone (CRH), thyrotropin releasing hormone (TRH), melanin concentrating hormone (MCH), orexin (ORX), and others. Positive (+) and negative (-) effects on feed intake regulation of the various pathways are so noted.

similar neuroanatomical sites (Kuenzel, 1994; Kuenzel et al., 1999). In fact, much of what has been learned about the regulation of food intake and energy balance from mammalian studies may be directly applicable to poultry species. In general, the control of feed intake to achieve energy balance in birds most likely involves a highly conserved and complex process that incorporates a highly integrated system of redundant neural and humoral pathways. The purpose of this review is to bring together current findings and ideas concerning the regulation of feed intake and energy balance in poultry.

A SYSTEM FOR REGULATING FEED INTAKE

Based on extensive investigations of mammalian species, it is clear that regulation of feed intake has two key components: one that involves the short-term control of feeding and one that involves long-term regulation of energy balance by the central nervous system (Woods et al., 1998; McMinn et al., 2000; Jensen, 2001; Berthoud, 2002; Blevins et al., 2002).

Current models for the regulation of feed intake include a central system that serves as the controller of feed intake. This central system comprises specific neural sites and circuits in the brainstem and hypothalamic regions that receive input from two major peripheral systems (Figure 1). A short-term system, also referred to as the 'peripheral satiety system,' transmits meal-related signals (e.g., the presence of feed or specific nutrients) primarily from the gastrointestinal tract to satiety centers located in the brain-

stem (Jensen, 2001). Satiety signals originating from the gut are relayed from the brainstem to the hypothalamus to activate neural pathways that modulate feed intake in the short-term (i.e., on a meal-to-meal basis). A long-term system provides information to the hypothalamus about the amount of energy stores (e.g., adipose tissue mass).

Long-term regulation of energy balance occurs via neural and neuroendocrine pathways activated in the hypothalamus in response to specific signaling molecules from the periphery. Signals from these afferent peripheral pathways are also integrated with satiety signaling pathways originating in the brainstem. The net result is a system that cumulatively regulates meal-to-meal feed intake along with long-term maintenance of energy (fat) storage to achieve energy homeostasis and, ultimately, to promote stability in body weight (Woods et al., 1998; McMinn et al., 2000; Jensen, 2001; Blevins et al., 2002; Berthoud, 2002).

Table 1 lists a number of molecules that have been identified and studied in poultry species with respect to their effects on appetite and energy balance. These signaling molecules function in peripheral and central sites to activate specific neural circuits that effect changes in feed intake as well as in energy metabolism. The signaling molecules listed in Table 1 have been studied in birds, either by injection into central nervous system or peripheral sites (Denbow, 1994; Kuenzel, 1994). In some cases, mammalian analogues have been tested, although some avian homologues have also been studied. Specific examples of some well-studied avian signaling molecules include neuropeptide Y (NPY), proopiomelanocortin (POMC) and its product α -melanocyte-stimulating hormone (α -MSH), cholecystokinin (CCK), and bombesin (Denbow, 1994; Kuenzel, 1994; Jensen, 2001).

For each of these signaling molecules to be active, specific receptors that recognize and bind them must be produced at sites of action. In the case of poultry, a number of these specific receptors have been identified and characterized at the gene or protein level in both peripheral and central tissue sites. Some examples include leptin receptor (Horev et al., 2000; Ohkubo et al., 2000), NPY receptors (Holmberg et al., 2002), melanocortin receptors (Takeuchi and Takahashi, 1998), and others. In general, the avian homologues for both signal and receptor molecules appear to be somewhat similar in structure and function to those characterized in mammalian species. However, a definitive assessment of similarities and differences, especially with respect to bioactivity, awaits more information to be obtained from ongoing efforts to identify and fully characterize the avian gene homologues that encode these important molecular species.

SHORT-TERM REGULATION OF FEED INTAKE

In animals, the drive to feed ensures that immediate energy and nutritional requirements are met from meal-to-meal, as feed is available for consumption. Appetite control is crucial to ensuring optimal nutrition and

achieving full potential for growth and development in poultry. Control of feed intake in the short term (i.e., meal to meal) involves hormonal and neural signals that originate primarily in the gut but also in the pancreas and liver. These satiety signals are generated in response to nutrient content and the physical presence of feed or specific feed components in the gut. The presence of feed in the gastrointestinal tract stimulates the release of a number of different peptides that control gut motility and secretion, as well as serve as satiety signals to the brain. Short-term regulation of feed intake thus involves a satiety response during feed consumption with satiety signals (peptides) originating in the gut transmitted to the brainstem via the activation of neural (vagal) afferent pathways or via secretion of signaling substances into the bloodstream (Figure 1).

Two types of signals produced by the gastrointestinal tract have been proposed: those that stimulate feeding behavior such as ghrelin and those that inhibit it such as CCK and bombesin (Woods et al., 1998; McMinn et al., 2000; Jensen, 2001; Blevins et al., 2002). Examples of both types of satiety signals have been reported in poultry species. The recently discovered peptide ghrelin has been reported to stimulate feeding in mammals (Wren et al., 2000). Ghrelin is produced by chicken proventriculus, and it may modulate feeding behavior in addition to functioning as a releasing factor for growth hormone (GH) through the GH secretagogue receptor (Furuse et al., 2001; Ahmed and Harvey, 2002; Kaiya et al., 2002; Saito et al., 2002). Interestingly, ghrelin was shown to inhibit feed intake when administered centrally (icv) to chickens (Furuse et al., 2001; Saito et al., 2002). This seemingly contradictory effect may actually reflect its potent GH-releasing capability (Ahmed and Harvey, 2002), because GH administration or signaling through the GH

secretagogue receptor inhibits feeding in chickens (Rosebrough et al., 1991; Saito et al., 2002). However, it may also indicate a genuine species difference in the function of this gut-derived peptide.

Cholecystokinin, a potent inhibitor of feeding, has been well studied in birds as has bombesin and its related peptides (Denbow, 1994; Kuenzel, 1994; Jensen, 2001). Not only does CCK stimulate gastric emptying and the release of pancreatic enzymes to aid in the digestion of feed, but it also functions as a satiety signal to the brainstem and is capable of depressing appetite and, hence, the drive to feed.

Generally, these signals act locally to effect changes in gut secretions and motility as well as acting on afferent fibers of the vagus nerve that innervate the gut, liver, and pancreas and connect with the brainstem satiety center. Their effects are relatively short-lived, and components of the signaling system are found in the gut and brain. In addition, CCK, ghrelin, bombesin, and other peptide satiety signals are also released into the bloodstream where they can find their way into the central nervous system to activate specific neural pathways that affect appetite. Because these signals are only active for short periods, they are effective in regulating meal size but are not capable of producing long-term changes in energy balance or body weight.

LONG-TERM REGULATION OF ENERGY BALANCE

In mammals, experimental findings suggest that body energy stored in the form of adipose tissue is tightly regulated, much like the way in which blood pressure is rigorously maintained (McMinn et al., 2000). Adaptive changes in feed intake and energy expenditure result in

TABLE 1. Candidate signaling molecules involved in the regulation of appetite and energy balance in poultry

Anorexigenic/catabolic	Orexigenic/anabolic
Leptin ¹	Neuropeptide Y (NPY) ¹
α -Melanocyte stimulating hormone (α -MSH) ¹	Agouti-related peptide (AGRP) ¹
Corticotrophin releasing hormone (CRH, CRF)	Melanin concentrating hormone (MCH)
Thyroid releasing hormone (TRH)	Orexins/hypocretins ³
Cholecystokinin (CCK)	Peptide YY (PYY)
Bombesin/gastrin releasing peptide (GRP)	Pancreatic polypeptide (PP)
Ghrelin ²	Galanin
Somatostatin (SIRF)	β -Endorphin
Gastrin	Dynorphin
Calcitonin gene-related peptide (CGRP)	Growth hormone releasing hormone (GHRH)
Urotensin I/urocortin	Norepinephrine
Neurotensin	
Glucagon/glucagon-like peptide 1 (GLP-1)	
Serotonin	
Dopamine	
Epinephrine	

¹These molecules are particularly important in long-term regulation of energy (adipose tissue) stores in mammals (Woods et al., 1998).

²Because ghrelin administered i.c.v. inhibits feed intake in chickens (Furuse et al., 2001; Saito et al., 2002), it is included under the anorexigenic/catabolic category despite its opposite (orexigenic/anabolic) activity observed in mammals.

³Although orexin-A and -B did not stimulate feed intake in chickens (Furuse et al., 1999), they are included under the orexigenic/anabolic category based on their actions in mammals.

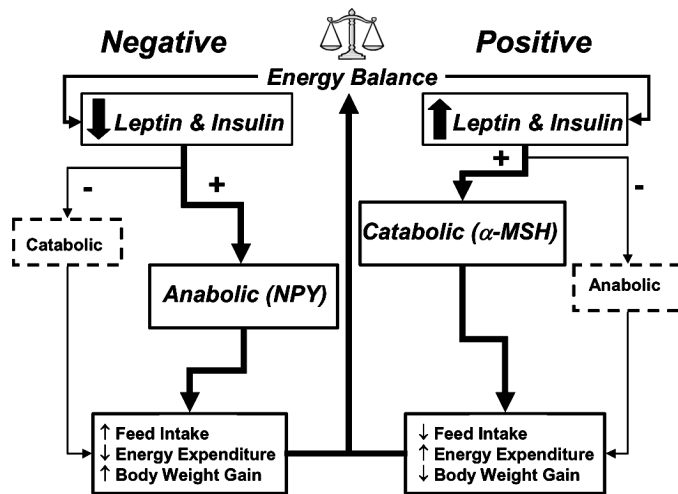


FIGURE 2. A model for the long-term regulation of energy balance and body weight (adapted from Woods et al., 1998). This model portrays the responses of two specific sets of hypothalamic neural circuits, designated as anabolic (NPY) or catabolic (α -MSH), in response to changes in energy status. The net effect is to produce the appropriate changes in feed intake and energy expenditure to bring the animal back into a state of energy balance. Circulating levels of the hormones leptin and insulin represent signals to the hypothalamus that determine the level of activity in the anabolic and catabolic pathways. Acting together, these important negative feedback circuits help ensure stability in body weight over the long term.

homeostasis of body energy stores. In addition to meeting immediate energy demands, feed intake can be adjusted to ensure that energy and nutrients are stored in anticipation of periods of high demand or periods of feed shortage.

The hypothalamus contains multiple peptidergic neuronal pathways that are involved in the regulation of feed intake and energy homeostasis. These pathways can be divided into two basic categories, anabolic and catabolic (Woods et al., 1998). Stimulation of one set (anabolic pathways) mediates a net increase in energy intake and storage, whereas stimulation of the other set (catabolic pathways) results in a net decrease in energy intake and storage. In mammals, changes in the circulating level of leptin and possibly insulin signal the hypothalamus to effect long-term changes in energy balance by activating or inhibiting specific anabolic and catabolic efferent pathways (Figure 2).

A considerable amount of experimental data has been collected concerning the expression, actions, and functional importance of leptin in a number of mammalian species. Although there is evidence that strongly suggests a conserved role for leptin and its receptor in regulating body weight and energy balance across a number of different mammalian species, considerably less is known about avian leptin and its functions.

To date, there have been two reports of the cloning and sequencing of a chicken leptin gene (Taouis et al., 1998; Ashwell et al., 1999). The deduced amino acid sequence shows high homology with mammalian leptin proteins. Peripheral (i.p.) and central (i.c.v.) administration of recombinant leptin protein to chickens reduced feed intake

in some trials (Denbow et al., 2000; Dridi et al., 2000; Taouis et al., 2001) or was without effect (Bungo et al., 1999). Leptin protein levels in plasma and tissue (liver and fat) samples from chickens have been analyzed using specific immunoassay techniques (Richards et al., 2000; Taouis et al., 2001).

Despite these findings, considerable doubt has been cast on the validity of the gene sequence reported for chicken leptin and on the analysis of leptin gene expression (Friedman-Einat et al., 1999). In fact, Friedman-Einat et al. (1999) and others using a variety of molecular techniques failed to find any evidence for the reported chicken leptin gene sequence in mRNA reverse transcribed from liver or fat tissue collected from several chicken strains, turkey, goose, or Japanese quail or in chicken genomic DNA samples. Furthermore, an initial report of mapping the chicken leptin gene to a microchromosome (Pitel et al., 1999) was later determined to be incorrect (Pitel et al., 2000). Therefore, in order to unequivocally establish the role of leptin as a signal of energy stores in birds, it is imperative that the entire leptin gene sequence and consistent leptin gene expression measurements in different tissues (especially liver and adipose tissue) be completed and fully verified for different poultry species. Only then will it be possible to ascribe a definitive role to the putative avian leptin hormone as a signal of peripheral energy stores to the central nervous system for long-term regulation of feed intake and energy balance in poultry.

In contrast to the controversy surrounding the chicken leptin gene, the leptin receptor gene has been clearly identified and characterized for both chickens and turkeys (Horev et al., 2000; Ohkubo et al., 2000; Richards et al., 2001). Based on the deduced amino acid sequence, it appears that the avian leptin receptor is quite similar to the mammalian receptor. To date only the 'long form' receptor has been characterized in birds. This form is capable of full signaling in response to bound leptin. Moreover, sequence analysis of the putative leptin-binding domain indicates that the avian leptin receptor is capable of binding mammalian leptin proteins (Richards et al., 2001). Similarly, the leptin-binding domain of the human leptin receptor has recently been shown to bind nonhuman leptin proteins, including recombinant chicken leptin (Sandowski et al., 2002). This may help explain the reported effectiveness of mammalian recombinant leptin proteins (viz., human and sheep) in reducing feed intake when administered to chickens (Denbow et al., 2000; Taouis et al., 2001). Based on these findings, it does appear that birds express a functional leptin receptor in both central nervous system and peripheral tissue sites (Horev et al., 2000; Ohkubo et al., 2000; Richards et al., 2001).

Chickens, like mammals, express similar genes encoding neuropeptides such as NPY and POMC (α -MSH is derived from proteolytic processing of the POMC precursor) that form anabolic and catabolic peptidergic effector circuits in the hypothalamus. The NPY gene has been cloned and sequenced in chickens and its localized expression in the brain determined (Blomqvist et al., 1992;

Wang et al., 2001). NPY gene expression in the brain responds to changes in energy status caused by fasting and feed restriction of chickens (Boswell et al., 1999). Moreover, NPY has been shown to be a potent orexigenic agent in chickens when administered centrally (Kuenzel and McMurtry, 1988). Specific NPY receptors (Y1 and Y5) have been reported to mediate NPY effects on feeding behavior in chickens (Holmberg et al., 2002). The POMC gene has been identified and sequenced in chickens, and it was shown to produce bioactive α -MSH that appears to play an important role in regulating feed intake in chickens (Takeuchi et al., 1999; Gerets et al., 2000; Kawakami et al., 2000). Central (i.c.v.) administration of α -MSH strongly inhibits feed intake in chickens (Kawakami et al., 2000). Not only are melanocortin receptors expressed in central sites, but they are also widely expressed in peripheral tissues of chickens (Takeuchi and Takahashi, 1998).

The agouti-related protein (AgRP) gene homologue has been identified, cloned, and sequenced in chickens, and expression of this naturally occurring antagonist of melanocortin action is reported to be widespread in central and peripheral tissues in chickens (Takeuchi et al., 2000). AgRP serves an antagonist of α -MSH in chickens, as it does in mammals, by binding to specific melanocortin receptor subtypes (MC3-R and MC4-R). AgRP is orexigenic in layer-type chickens, but not broilers, when administered intracerebroventricularly (Tachibana et al., 2001). Based on these observations, it was concluded that the MC4-R might function in the regulation of feed intake and energy balance in chickens, as this receptor subtype has been postulated to do in mammals (Tachibana et al., 2001).

METABOLIC PATHWAYS

The following two examples, one involving energy storage and the other energy expenditure, are included to demonstrate how metabolic pathways can be integrated with the actions of specific neuroendocrine pathways working through the central nervous system to coordinately regulate energy balance and achieve energy homeostasis. Peripheral tissues carry out the functions of flux, storage, mobilization, and utilization of fuels in organs under hormonal control with sympathetic and parasympathetic nervous system inputs (Berthoud, 2002). Circulating levels of metabolites (glucose, free fatty acids, and amino acids) might also serve as signals of energy or nutritional status. In this way, metabolic pathways and metabolites produced by them would be integrated into the regulation of feed intake and energy metabolism.

In birds, the major site of lipogenesis (i.e., *de novo* synthesis of triglycerides from glucose) is the liver (Hillgartner et al., 1995), with adipose tissue serving primarily as a repository for storing accumulated triglycerides (Figure 3). The genes encoding the enzymes involved in fatty acid synthesis are subject to regulation by the major metabolic hormones, insulin, glucagon, and thyroid hormone (T_3). Insulin and T_3 induce, whereas glucagon inhibits

expression of lipogenic genes (Hillgartner et al., 1995). Thus, in positive energy balance when the levels of circulating insulin and T_3 would be increased, lipogenic gene expression in liver is increased, leading to enhanced production of triglycerides and increased adipose tissue storage. Also, expression of the gene encoding the key transcription factor, sterol regulatory element binding protein 1c (SREBP-1), is regulated positively by insulin and T_3 and negatively by glucagon (Gondret et al., 2001).

This single transcription factor, which is highly expressed in livers of birds, regulates the expression of the major lipogenic enzyme genes producing a coordinated response in gene expression for this metabolic pathway (Gondret et al., 2001). In this way an entire metabolic pathway (e.g., lipogenesis) responds to changes in energy balance and, in doing so, directly determines changes in the size of adipose tissue mass that largely comprises stored triglycerides, the products of lipogenesis.

Changes in adipose tissue mass due to triglyceride accumulation would presumably influence leptin gene expression that would, in turn, affect feed intake and bring about appropriate adjustments in energy balance. Leptin gene expression in birds has been reported in liver and adipose tissue, with liver being the predominant site of expression (Taouis et al., 1998; Ashwell et al., 1999). This has been suggested to reflect the prominent role of the liver in lipogenic activity in birds and to represent a potential species difference in the regulatory pathway for long-term maintenance of energy balance in birds. Thus, a link would be established between metabolic and neuroendocrine pathways involved in feed intake and energy balance.

Heat production or thermogenesis is an important component of energy expenditure used by animals to maintain core body temperature. Nonshivering or adaptive thermogenesis is one method of generating heat. It involves uncoupling of oxidative phosphorylation in mitochondria. This is achieved by the actions of at least one member of a specific family of mitochondrial membrane transporter proteins termed uncoupling proteins (UCP) that dissipate the proton gradient that normally exists across the mitochondrial membrane (Himms-Hagen and Harper, 2001). This promotes the transport of protons into the mitochondrial matrix, resulting in its subsequent acidification. The ensuing increase in oxidation of substrates leads to production of energy in the form of heat with fatty acids being the predominant substrate utilized for thermogenesis (Himms-Hagen and Harper, 2001).

To date five UCP have been identified and characterized in mammals (Himms-Hagen and Harper, 2001), whereas a single UCP has been identified in birds (Raimbault et al., 2001; Vianna et al., 2001; Evock-Clover et al., 2002; Toyomizu et al., 2002). It appears that the avian UCP, which is expressed predominantly in skeletal muscle, most closely resembles mammalian UCP3 (Evock-Clover et al., 2002). Furthermore, it has been proposed that rather than an uncoupling function, UCP3 may in fact be involved with shuttling fatty acid anions across the inner mitochondrial membrane, and thus it might

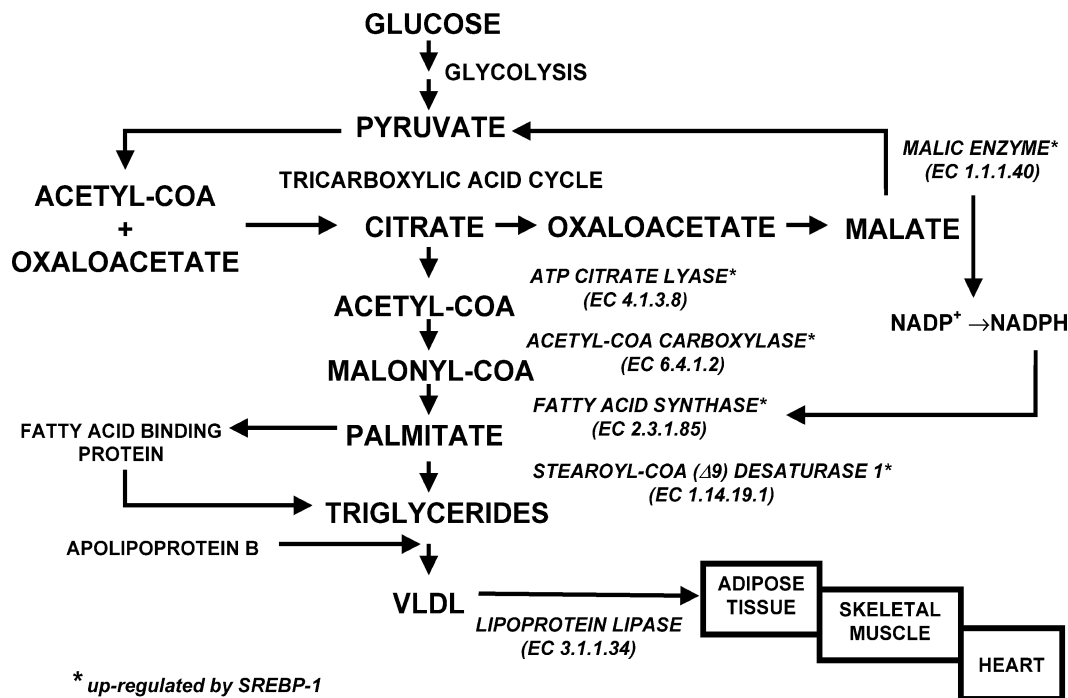


FIGURE 3. The lipogenic metabolic pathway responsible for the production of triglycerides from glucose. Specific enzymes (*italics*) are shown next to the steps in the reaction that they catalyze. Lipogenic enzyme genes that are up-regulated by the transcription factor sterol regulatory element binding protein 1 (SREBP-1) are marked with an asterisk.

play a role in fatty acid oxidation (Himms-Hagen and Harper, 2001; Evock-Clover et al., 2002). Because this process requires energy in the form of ATP, the net result of increased levels of UCP3 would be to increase energy expenditure.

Interestingly, leptin was shown in a recent report to accelerate cycling of fatty acids between triacylglycerol-bound (TAG) and free fatty acid (FA) states by simultaneous stimulation of lipolysis and reesterification (Reidy and Weber, 2002). This energy-requiring substrate cycle (TAG/FA) is commonly used for thermogenesis. Leptin brings about a shift in fuel utilization from carbohydrate to lipid because of a strong activation of substrate cycling, lipolysis, and fatty acid oxidation. This leads to the proposal of a new 'energy-wasting' role for leptin as a means to increase metabolic rate similar to what has been suggested for the action of uncoupling proteins. Although, there have been reports in mammals that UCP gene expression is increased by leptin, no effect of leptin on skeletal muscle UCP gene expression, either in negative energy (fasted) or positive energy (fed) balance, was found in chickens (Evock-Clover et al., 2002). However, glucagon and cold exposure have been shown to up-regulate the avian UCP gene expression in skeletal muscle (Raimbault et al., 2001; Toyomizu et al., 2002), whereas UCP gene expression level was negatively correlated with circulating insulin levels in fed and fasted chickens (Evock-Clover et al., 2002). Moreover, it has been suggested that UCP expression in skeletal muscle in mammals and birds responds positively to the level of circulating free fatty acids (Himms-Hagen and Harper, 2001; Evock-Clover et al., 2002). Clearly, more study is required to determine

what role UCP might play in achieving energy homeostasis in poultry.

AN INTEGRATED REGULATORY SYSTEM

The coordinate regulation of feed intake and energy balance involves integration of environmental cues (feed availability, photoperiod, and temperature) with internal physiological signals (hormone levels, metabolite levels, and energy stores). The brain plays a pivotal role in this process of integration. A distributed neural network for control of feed intake and energy balance has been proposed that involves a central processor (hypothalamus) and multiple negative feedback parallel processing loops (Woods et al., 1998; McMinn et al., 2000; Berthoud, 2002; Blevins et al., 2002).

Physiochemical interactions between specific neuropeptides and their receptors are important to understanding the regulation of feeding behavior, energy balance, and other processes such as reproduction and immune function. The genes encoding neuropeptides and their respective receptors, expressed in hypothalamic neurons as well as in peripheral tissues, are fundamental to creating a sensing and signaling network that forms the basis for regulation of feed intake and energy balance. It has been suggested that α -MSH acting through MC4-R serves as an important central mediator for leptin action on feed intake and energy balance (Forbes et al., 2001). Figure 4 depicts a system that is proposed to control energy balance in birds involving leptin signaling through the leptin receptor (LepR) and the response of POMC-expressing hypothalamic neurons that gives rise to α -MSH and

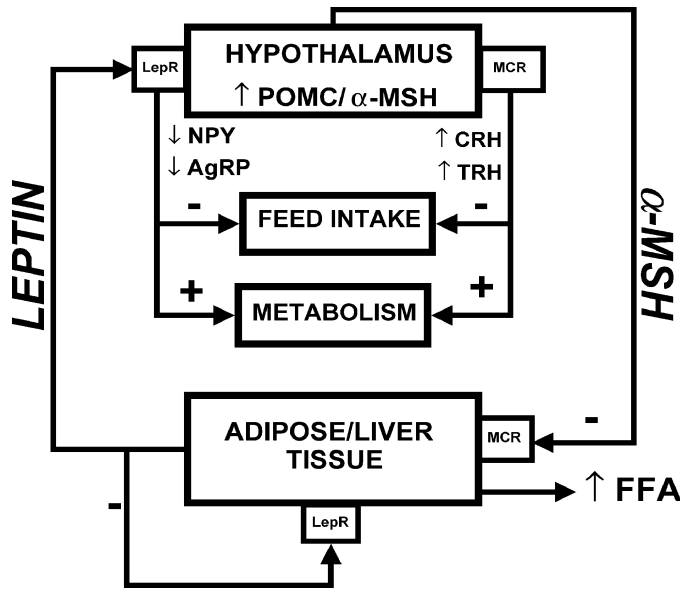


FIGURE 4. A proposed model for long-term regulation of feed intake and energy balance in poultry. Leptin is released into circulation in proportion to the size of energy stores. It is sensed in the hypothalamus by neurons that express the gene for leptin receptor (LepR). These neurons also express the gene for proopiomelanocortin (POMC), the precursor that gives rise to α -melanocyte stimulating hormone (α -MSH). Working through melanocortin receptors (MCR), α -MSH activates a series of neuropeptide pathways such as the corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TSH) genes that would work together to decrease feed intake and increase metabolic activity. In addition, α -MSH, secreted into the blood, can act directly on peripheral tissues to increase metabolic activity through MCR expressed in these tissues. Similarly, leptin acting through LepR suppresses the expression of anabolic neuropeptides such as neuropeptide Y (NPY) and agouti-related protein (AgRP) resulting in reduced feed intake and increased metabolic activity. Like α -MSH, leptin can also produce effects on peripheral tissues that express LepR. Thus, working together, leptin and the melanocortin system can effectively regulate feed intake and energy balance over the long term.

working through the melanocortin receptor (MCR) system.

In addition to the primary circuit (first order neurons, Figure 1), leptin and α -MSH can trigger additional neural pathways (second order neurons, Figure 1) that also affect feed intake and metabolic activity. For example, leptin represses expression of NPY and AgRP genes in hypothalamic neurons in mammals (Woods et al., 1998; McMinn et al., 2000; Blevins et al., 2002). These effects would repress anabolic actions of NPY and AgRP neural pathways, and the secondary pathways to which they connect, on feed intake and energy balance. Similarly, working through MCR, α -MSH, produced by stimulated POMC-expressing neurons, would activate additional neural pathways mediated by peptidergic neurons expressing corticotropin releasing hormone (CRH) and thyrotropin releasing hormone (TSH) genes that would work together to decrease feed intake and increase metabolic activity, in part, via the hypothalamus-pituitary-thyroid or hypothalamus-pituitary-adrenal axes (Woods et al., 1998; McMinn et al., 2000; Blevins et al., 2002). Thus, as plasma leptin levels rise in response to elevated energy stores, catabolic pathways are activated and anabolic pathways are re-

pressed. The opposite occurs in the face of depleted energy stores and lowered circulating leptin levels. Moreover, leptin and α -MSH may also act directly on peripheral tissues because their specific receptors (LepR and MCR) are widely expressed at these sites in chickens. The fact that additional downstream neural (second order neurons) and neuroendocrine pathways are activated following activation of the NPY/AgRP and POMC (first order neurons) pathways helps to explain, in part, the observed linkage of reproduction, immune system function, thermoregulation, bone metabolism, and other important physiological functions with energy status.

It is not known if circulating insulin levels reflect adipose tissue size in birds as seems to be the case in mammals (Woods et al., 1998; McMinn et al., 2000; Blevins et al., 2002). Although insulin receptors have been identified in the central nervous system of chickens (Simon and Leroith, 1986), there are no reports of the effects of central administration of insulin on feed intakes of birds (Kuenzel, 1994). There is evidence for elevated circulating insulin levels in fed or feed-deprived chickens with lesions of the ventromedial hypothalamus, suggesting production of metabolic obesity (Sonoda, 1983). Transient changes in plasma glucose level do not appear to alter feed intake in chickens (Simon et al., 2000). Therefore, the role of insulin, if any, as an afferent signal for energy stores in poultry remains to be determined.

FUTURE RESEARCH DIRECTIONS

Clearly one of the highest research priorities is to continue to identify, sequence, and functionally characterize unique genes and their products involved in the regulation of feed intake and energy balance in poultry. Comparative genomics should prove to be quite useful in identifying and characterizing avian homologues of previously identified mammalian genes. The key will be to determine any true differences that might exist in gene structure, expression, and function in birds as compared to mammals. In addition, genomic methods are required to identify and characterize novel genes that might play important regulatory roles heretofore undiscovered. The availability and application of DNA array technologies and other screening tools specific for poultry genetic material will permit large numbers of expressed genes to be surveyed to identify those related to the regulation of feed intake and energy balance. It is also important to carefully characterize specific gene sequences obtained from different populations of birds in order to begin to identify the presence of genetic mutations such as single nucleotide polymorphisms (SNP) that may impact gene function. To date this has only been done to a very limited extent for the genes involved in regulating feed intake and energy balance in poultry. Clearly, many challenges already exist, and, certainly, leptin is a prime example of a gene that urgently needs further study to determine its true characteristics and functions specifically in poultry.

Analysis of the expression of the genes that code for the peptides and proteins that compose the basic compo-

nents of central and peripheral regulatory pathways is important, especially with respect to localized sites of expression and level of expression. Future studies are needed to determine specific sites of gene expression for the signaling substances and their respective receptor molecules. A variety of molecular techniques including reverse transcription (RT)-PCR, real-time RT-PCR, RT-PCR and capillary electrophoresis with laser-induced fluorescence detection (Richards and Poch, 2002), cDNA microarrays, and others are available to assess the expression of genes identified as important to the regulation of feed intake and energy balance in poultry. As DNA sequencing efforts continue to generate new and more specific avian sequence, gene expression analyses will continue to provide valuable information concerning gene function in poultry.

Newly emerging proteomic methodologies will be especially valuable in characterizing protein and peptide structures and identifying specific post-translational modifications in peptides and receptors that may be crucial to their normal functioning. Much of the evaluation of signaling proteins and peptides related to feed intake and energy balance to date has involved the effects of delivering various synthetic or recombinant homologues or analogs peripherally (i.p., i.v.) or centrally (i.c.v.) to birds and determining bioactivity. Unfortunately, conflicting results with respect to efficacy have sometimes been obtained, and it is difficult to ascertain if this is due to legitimate differences in activity or is related to discrepancies in the structures of the molecules being administered.

Rapidly developing proteomic tools will greatly aid in determining the native structures of signaling peptides and their receptors containing unique modifications that are responsible for bioactivity in poultry. Examples of such important modifications include fatty acid modification of ghrelin (Kaiya et al., 2002, Saito et al., 2002), phosphorylation of specific amino acid residues of the leptin receptor (Ohkubo et al., 2000; Horev et al., 2000), specific truncation or modification of peptides at the amino- or carboxyl-ends, and proteolytic processing of prohormone peptide precursors. Thus, it is also important to characterize signaling and receptor molecules at the protein or peptide level in addition to at the gene and mRNA levels.

Finally, specific lines of mice with spontaneous mutations in leptin (*ob/ob*) or leptin receptor (*db/db*) genes have proven to be highly valuable research tools for elucidating the regulatory mechanisms involved in controlling appetite and energy balance. Unfortunately, there are currently no such avian models. However, specific populations of birds (e.g., obese vs. lean, fast vs. slow growing, layers vs. broilers, etc.) developed through classical genetic selection techniques continue to provide a useful testing ground for discovery and evaluation of specific genes that might play a role in regulating feed intake and energy balance.

Transgenic animals have also proven to be invaluable in evaluating the effects of individual genes in mammals.

Knockout mouse models have been particularly useful in further defining the nature of the mechanisms regulating feed intake and energy homeostasis. A good example of this is the production of NPY knockout mice that display normal feed intake and energy balance phenotypes (Palmiter et al., 1998). This particular model highlights the redundancy in systems controlling feed intake and energy balance, and alternative pathways identified in such models represent potentially useful areas for future investigations in avian species. There has been recent discussion of using cellular and molecular biology methods to introduce specific genes into poultry by cloning to propagate desirable genotypes (Etches, 2001). The development of transgenic avian models specifically related to different aspects of the control of feed intake and energy balance would be most useful.

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