



PHYSIOLOGICAL ROLE OF LEPTIN IN FARM ANIMALS

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ABSTRACT

Leptin is one of the most useful biomolecule to act as a marker for identifying high performing individuals leading to better adaptability and productivity. Leptin has a pleiotropic effect on regulating appetite, energy metabolism, growth, reproduction, body composition and immunity. Leptin is produced primarily in the adipocytes of white adipose tissue. It is also produced by brown adipose tissue (syncytiotrophoblasts) placenta, ovaries, skeletal muscle and stomach. Leptin circulates in blood in free form and bound to proteins. Leptin acts on receptors in the hypothalamus, where it inhibits hunger by counteracting the effects of neuropeptide Y, a potent hunger promoter secreted by cells in the gut and in the hypothalamus. Leptin also acts as a growth factor for many cell types, such as lymphocytes, cultured tracheal epithelial cells, lung squamous cells, pancreatic and embryonic cells. It has been found that recombinant murine leptin has stimulatory effect on the proliferation of cultured embryonic chicken muscle and liver cells. In cattle, leptin is expressed in the rumen, abomasums and duodenum before weaning but only in the duodenum after weaning. The ruminant mammary epithelial cells also synthesize leptin during pregnancy and during established lactation. Leptin and its receptor can be used as a marker for enhancing the productivity in livestock and are also potential candidates for marker assisted selection. Several studies support the concept that leptin could be one of the links between past and current nutritional factors, and short and long term physiological regulations. The possible role of leptin in fetal growth and development may enhance the productive efficiency of farm animals. Finally, the involvement of leptin in physiological regulation and adaptation to varied environment will focus to sustain the growth of livestock in the face of varied climatic situation. Therefore, it could be used as future biomolecule for enhancing productivity in farm animals.

KEYWORDS: Leptin, Satiety, Reproduction, Metabolism and growth.

INTRODUCTION

Livestock play a significant role by providing food security as well as income resource for less poor people in our country. It is necessary to enhance and sustain the production efficiency of livestock species in the changing face of population scenario to feed a large growing population. Therefore, it is important to adopt different strategies to increase productive efficiency of livestock (Agrawal *et al.*, 2008). Leptin is one of the most useful biomolecule to act as a marker for identifying high performing individuals leading to better adaptability and productivity. Leptin has a pleiotropic effect on regulating appetite, energy metabolism, growth, reproduction, body composition and immunity. It is mostly produced in the white adipose tissue and informs the central nervous system (CNS) about the total fat depot of the body. Leptin is also involved in regulation of nutritional status and reproductive function and also regulates fetal development in livestock. Plasma leptin level increases linearly with increases in body fat mass. Leptin reduces feed intake in rodents, ruminant and other livestock species and also plays a role in energy expenditure. The physiological properties support leptin as a strong candidate gene for evaluation of genetic polymorphisms, which has further been associated with growth, milk yield and other economic traits in different

livestock. The present review deals with the leptin structure and its implications in regulation of growth, reproduction, immunity and also enhancing livestock productivity. Leptin (from the Greek *leptos*, meaning thin) is a protein hormone with important effects in regulating body weight, metabolism and reproductive function. The protein is approximately ~16 kDa in mass and encoded by the obese (*ob*) gene. Leptin is a hormone also called as "satiety hormone" made by fat cells which regulates the amount of fat stored in the body. This happens by adjusting both the sensation of hunger and energy expenditures. Hunger is inhibited or satiety occurred when the amount of fat stored reaches a particular level. Leptin is then secreted and circulates through the body, eventually activating leptin receptors in the arcuate nucleus of the hypothalamus. Energy expenditure is increased both by the signal to the brain and directly via leptin receptors on peripheral targets. The effect of leptin is opposite to that of ghrelin, the "hunger hormone". Ghrelin receptors are on the same brain cells as leptin receptors, so these cells receive competing satiety and hunger signals (Brennan and Mantzoros 2006). Leptin and ghrelin, along with many other hormones, participate in the complex process of energy homeostasis. Leptin is expressed predominantly by adipocytes, which fits with the idea that body weight is sensed as the total mass of fat in the body.

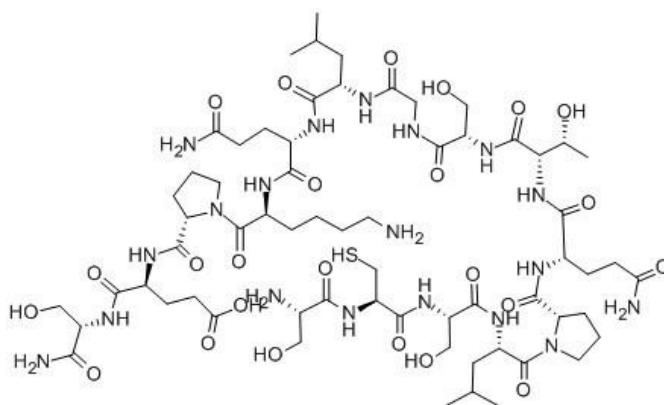
Smaller amounts of leptin are also secreted by cells in the epithelium of the stomach and in the placenta. Leptin receptors are highly expressed in areas of the hypothalamus known to be important in regulating body weight, as well as in T lymphocytes and vascular endothelial cells.

Although regulation of fat stores is deemed to be the primary function of leptin, it also plays a role in other physiological processes, as evidenced by its multiple sites of synthesis other than fat cells, and the multiple cell types beside hypothalamic cells which have leptin receptors

Leptin has multiple physiological effects and plays a pivotal role in the control of body growth, adaptability, immune function, angiogenesis, renal function, hematopoiesis and reproduction. Leptin also plays a key role in the regulation

of reproductive performance by stimulating GnRH, FSH and LH release. Leptin not only acts as an endocrine signal in brain and in different peripheral tissues in which leptin receptors are expressed, but also as an autocrine/paracrine signal within tissues where it is produced in rodents, human and ruminant. Zhang *et al.* (1994) positionally cloned the obese (ob) gene from a homozygous (ob-ob) mutant mouse with a thymine (T) for cytosine (C) substitution at nucleotide 105. The mutation changes an arginine codon (CGA) to a 'stop' codon (TGA), giving truncated 'obese protein' or 'leptin' that is destroyed within its source fat cell. Leptin proved to be the 'satiety factor' proposed by Coleman (1973).

Structure of Leptin



Leptin the 16 kDa molecular weight protein having 146 amino acids, is classified as a cytokine due to its structural similarity with leptin receptor and glycoprotein (gp) 130, a member of IL-6 family. Leptin is a four helix cytokine and contains a single disulfide linkage (cys 96, cys 146) connecting the clusture of differentiation (CD) loop to the carboxyl terminus.

Sites of synthesis

Leptin is produced primarily in the adipocytes of white adipose tissue. It is also produced by brown adipose tissue (syncytiotrophoblasts) placenta, ovaries, skeletal muscle, stomach (the lower part of the fundic glands), mammary epithelial cells, bone marrow, pituitary, liver, gastric chief cells and P/D1 cells (Bado *et al.*, 1998).

Physiologic variation

Leptin circulates in blood in free form and bound to proteins (Sinha *et al.*, 1996). Leptin levels vary exponentially, not linearly, with fat mass. Leptin levels in blood are higher between midnight and early morning, perhaps suppressing appetite during the night. The diurnal rhythm of blood leptin levels can be modified by meal-timing (Schoeller *et al.*, 1997).

In specific conditions

In animals, many instances are seen where leptin dissociates from the strict role of communicating nutritional status between body and brain and no longer correlates with body fat levels. Leptin level is decreased after short-term fasting (24–72 hours), even when changes in fat mass

are not observed (Chan *et al.*, 2003). Leptin plays a critical role in the adaptive response to starvation. Serum level of leptin is reduced by sleep deprivation. Leptin level is decreased by increases in testosterone levels and increased by increases in estrogen levels. Leptin level is chronically reduced by physical exercise training (de Salles *et al.*, 2010). Leptin level is increased by dexamethasone and insulin. Leptin levels are paradoxically increased in obesity.

MECHANISM OF ACTION

1. Central (hypothalamic)

Leptin acts on receptors in the hypothalamus, where it inhibits hunger by counteracting the effects of neuropeptide Y, a potent hunger promoter secreted by cells in the gut and in the hypothalamus. Leptin counteracting the effects of anandamide, another potent hunger promoter that binds to the same receptors as tetrahydrocannabinol (THC). Leptin promoting the synthesis of α -MSH, a hunger suppressant. This appetite inhibition is long-term, in contrast to the rapid inhibition of hunger by cholecystokinin (CCK). The absence of leptin (or its receptor) leads to uncontrolled hunger and resulting obesity. Fasting or following a very-low-calorie diet lowers leptin levels. Leptin levels change more when food intake decreases than when it increases. The dynamics of leptin due to an acute change in energy balance may be related to appetite and eventually to food intake rather than fat stores. It controls food intake and energy expenditure by acting on receptors in the mediobasal hypothalamus. Leptin

binds to neuropeptide Y (NPY) neurons in the arcuate nucleus in such a way as to decrease the activity of these neurons. Leptin signals to the hypothalamus which produces a feeling of satiety. Moreover, leptin signals may make it easier for people to resist the temptation of foods high in calories (Baicy *et al.*, 2007).

Leptin receptor activation inhibits neuropeptide Y (NPY) and agouti-related peptide (AgRP), and activates -melanocyte-stimulating hormone (-MSH). The NPY neurons are a key element in the regulation of hunger; small doses of NPY injected into the brains of experimental animals stimulates feeding, while selective destruction of the NPY neurons in mice causes them to become anorexic. Conversely, -MSH is an important mediator of satiety, and differences in the gene for the -MSH receptor are linked to obesity in humans.

Leptin is generally thought to enter the brain at the choroid plexus, where the intense expression of a form of leptin receptor molecule could act as a transport mechanism. Once leptin has bound to the Ob-Rb receptor, it activates the signal transducer and activator of transcription (STAT3), which is phosphorylated and travels to the nucleus to effect changes in gene expression. One of the main effects being the down-regulation of the expression of endocannabinoids, responsible for increasing hunger (Di Marzo., 2008). In response to leptin, receptor neurons have been shown to remodel themselves, changing the number and types of synapses that fire onto them. Increased levels of melatonin causes a down regulation of leptin. However melatonin also appears to increase leptin levels in the presence of insulin, therefore causing a decrease in appetite during sleeping. Partial sleep deprivation has also been associated with decreased leptin levels. Mice with type 1 diabetes treated with leptin or leptin plus insulin, compared to insulin alone had better metabolic profiles: blood sugar did not fluctuate as much; cholesterol levels decreased; less body fat formed.

2. Peripheral (non-hypothalamic)

Non-hypothalamic targets of leptin are referred to as peripheral targets, in contrast to the hypothalamic target which is the central target. Leptin receptors are found on a wide range of cell types. There is a different relative importance of central and peripheral leptin interactions under different physiologic states, and variations between species (Margetic *et al.*, 2002). In the periphery leptin is a modulator of energy expenditure, modulator between fetal and maternal metabolism, permissive factor in puberty, activator of immune cells, activator of beta islet cells, and a growth factor. Further, it interacts with other hormones and energy regulators: insulin, glucagon, insulin-like growth factor, growth hormone, glucocorticoids, cytokines, and metabolites.

VARIOUS PHYSIOLOGICAL EFFECTS OF LEPTIN

Circulatory system

The role of leptin/leptin receptors in modulation of T cell activity in immune system was shown in experimentation with mice. It modulates the immune

response to atherosclerosis, of which obesity is a predisposing factor. Exogenous leptin can promote angiogenesis by increasing vascular endothelial growth factor levels. Hyperleptinemia produced by infusion or adenoviral gene transfer decreases blood pressure in rats.

Fetal lung

In fetal lung, leptin is induced in the alveolar interstitial fibroblasts ("lipofibroblasts") by the action of PTHrP secreted by formative alveolar epithelium (endoderm) under moderate stretch. The leptin from the mesenchyme, in turn, acts back on the epithelium at the leptin receptor carried in the alveolar type II pneumocytes and induces surfactant expression, which is one of the main functions of these type II pneumocytes (Torday and Rehan, 2006).

Bone

Leptin's ability to regulate bone mass was first recognized in 2000. Leptin can affect bone metabolism via direct signaling from the brain. Leptin decreases cancellous bone, but increases cortical bone. This "cortical- cancellous dichotomy" may represent a mechanism for enlarging bone size, and thus bone resistance, to cope with increased body weight (Hamrick and Ferrari 2008). Bone metabolism can be regulated by central sympathetic outflow, since sympathetic pathways innervate bone tissue. A number of brain-signaling molecules (neuropeptides and neurotransmitters) have been found in bone, including adrenaline, noradrenalin, serotonin, calcitonin gene related peptide, vasoactive intestinal peptide and neuropeptide Y. Leptin binds to its receptors in the hypothalamus, where it acts through the sympathetic nervous system to regulate bone metabolism. Leptin may also act directly on bone metabolism via a balance between energy intake and the IGF-I pathway (Martin *et al.*, 2007). There is potential for treatment of diseases of bone formation such as impaired fracture healing with leptin (Roszer *et al.*, 2014).

Brain

Leptin receptors are expressed not only in the hypothalamus but also in other brain regions, particularly in the hippocampus. Thus some leptin receptors in the brain are classified as central (hypothalamic) and some as peripheral (non-hypothalamic). Deficiency of leptin has been shown to alter brain proteins and neuronal functions of obese mice which can be restored by leptin injection. In humans, low circulating plasma leptin has been associated with cognitive changes associated with anorexia, depression, and HIV (Lieb *et al.*, 2009).

Immune system

Factors that acutely affect leptin levels are also factors that influence other markers of inflammation, *e.g.*, testosterone, sleep, emotional stress, caloric restriction, and body fat levels. While it is well-established that leptin is involved in the regulation of the inflammatory response, it has been further theorized that leptin's role as an inflammatory marker is to respond specifically to adipose-derived inflammatory cytokines.

In terms of both structure and function, leptin resembles to IL-6 and is a member of the cytokine superfamily. Circulating leptin seems to affect the HPA axis, suggesting a

role for leptin in stress response. Elevated leptin concentrations are associated with elevated white blood cell counts in both sexes. Along with chronic inflammation, chronically elevated leptin levels are also associated with obesity, overeating, and inflammation-related diseases, including hypertension, metabolic syndrome, and cardiovascular disease. However, while leptin is associated with body fat mass, the size of individual fat cells, and the act of overeating. When high caloric intake overtaxes fat cells' ability to grow larger or increase in number in step with caloric intake, the ensuing stress response leads to inflammation at the cellular level and ectopic fat storage, i.e., the unhealthy storage of body fat within internal organs, arteries, and/or muscle. The insulin increase in response to the caloric load provokes a dose-dependent rise in leptin, an effect potentiated by high cortisol levels. (This insulin-leptin relationship is notably similar to insulin's effect on the increase of IL-6 gene expression and secretion from preadipocytes in a time- and dose-dependent manner.) Furthermore, plasma leptin concentrations have been observed to gradually increase when acipimox is administered to prevent lipolysis, concurrent hypocaloric dieting and weight loss not with standing. Such findings appear to demonstrate high caloric loads in excess of fat cells' storage rate capacities lead to stress responses that induce an increase in leptin, which then operates as an adipose-derived inflammation stopgap signaling for the cessation of food intake so as to prevent adipose-derived inflammation from reaching elevated levels. This response may then protect against the harmful process of ectopic fat storage, which perhaps explains the connection between chronically elevated leptin levels and ectopic fat storage in obese individuals (Oswal and Yeo 2010).

Reproductive system

In ruminants, recombinant ovine leptin administration to fasted mature beef cows stimulates LH secretion. Leptin concentration in plasma has been shown as direct reflection of the amount of body fat and reproductive function and is affected by body weight and nutritional status (Martin *et al.*, 1994). In mice, and to a lesser extent in humans, leptin is required for male and female fertility. Ovulatory cycles in females are linked to energy balance and energy flux (how much energy is consumed and expended) much more than energy status (fat levels). When energy balance is highly negative or energy flux is very high, the ovarian cycle stops and females stop menstruating. Only if a female has an extremely low body fat percentage does energy status affect menstruation. Leptin levels outside an ideal range can have a negative effect on egg quality and outcome during in vitro fertilization. Leptin is involved in reproduction by stimulating gonadotropin-releasing hormone from the hypothalamus. The placenta produces leptin. Leptin levels rise during pregnancy and fall after childbirth. Leptin is also expressed in fetal membranes and the uterine tissue. Uterine contractions are inhibited by leptin. Leptin plays a role in hyperemesis gravidarum (severe morning sickness' of pregnancy). Immunoreactive leptin has been found in human breast milk; and leptin from mother's milk has been found in

the blood of suckling infant animals (Casabiell *et al.*, 1997). Leptin and its receptor can be used as a genetic marker for enhancing the productivity in livestock and are also potential candidates for marker assisted selection (Agrawal *et al.*, 2008). Molecular genetics techniques are currently available that allow direct genotyping for candidate genes using PCR. Leptin is a hormone predominantly secreted from white adipose tissue and performs important roles in controlling body weight, milk production, feed intake, immune function and reproduction. As the hormone leptin is involved in regulation of nutritional status and reproductive function, this hormone is an interesting protein to investigate during the periparturient period in dairy cattle. Leptin binds receptor mainly localized on Neuropeptid – Y – neurons, which also appear in hypothalamus to play a key role in the integration of feeding behaviour with internal signals of body energy status. In dairy cattle, the increase in milk yield has been accompanied by more negative energy balance during early lactation and a decrease in fertility. Leptin hormone concentrations were high during late pregnancy and declined to a lowest point at parturition. This indicates that the fall in circulating leptin levels towards and during lactation is due to the energetic costs of milk production (Liefers *et al.*, 2005). In cattle, leptin is expressed in the rumen, abomasums and duodenum before weaning but only in the duodenum after weaning. The ruminant mammary epithelial cells also synthesize leptin during pregnancy and during established lactation. Several polymorphisms in the leptin gene have been associated with milk performance, increased perinatal mortality in dairy calf birth and weaning weights in beef and dairy and reproductive performance in dairy cattle. (Sanchez-Garrido and Tena-Sempere 2013) High levels of leptin, as usually observed in obese females, can trigger neuroendocrine cascade resulting in early menarche. Changes in leptin level and leptin mRNA expression were associated with puberty onset in cattle and pigs (Barb and Kraeling, 2004). noted leptin increased in heifers going through puberty but saw no distinct peripubertal leptin rise in heifers fed to gain at 0.5, 0.8 or 1.1 kg/day.

Effects on Growth and Energy Balance

Leptin regulates feed intake, energy metabolism and body composition in both human and livestock. Leptin plays a critical role in regulating body weight and growth in mammals. Leptin also acts as a growth factor for many cell types, such as lymphocytes, cultured tracheal epithelial cells, lung squamous cells, pancreatic and embryonic cells. It has been found that recombinant murine leptin has stimulatory effect on the proliferation of cultured embryonic chicken muscle and liver cells. Leptin has direct effect on proliferation, differentiation, mineralization, and to induce prolonged life span of human primary osteoblasts by inhibiting apoptosis (Gordeladze *et al.*, 2002). Leptin also helps in wound healing and reverse the atrophied morphology of wound margins into a well-organized hyperproliferative epithelium. Furthermore, it has been observed that topically administered leptin accelerated normal wound-healing conditions in wild type mice. Leptin

acts as unique nutritional signal to the growth axis as high leptin levels were observed to inhibit the feed intake through binding to specific receptor in hypothalamus.

Leptin controls body energy metabolism by reciprocally regulating AMP kinase in the hypothalamus and skeletal muscle. Leptin activates AMP kinase (AMPK) in skeletal muscle directly at the muscle level and indirectly through the hypothalamic-sympathetic nervous system. Leptin also inhibits food intake by suppressing AMPK activity in the hypothalamus. Reciprocal regulation of AMPK activity in the hypothalamus and skeletal muscle is necessary for the leptin's effect on energy metabolism. Leptin regulates food intake and controls how fats are distributed and stored in the body. Food deprivation (12 to 48 h) results in a rapid and drastic fall in leptin gene expression (Cusin *et al.*, 1995). Thus, leptin not only functions as an "adipostat" to signal the status of body energy store to the brain and perhaps other tissues, but also functions as a sensor of energy balance. Acute or long term changes in food composition or food restriction caused changes in the plasma leptin in ruminants. Leptin levels in blood are strongly correlated with the amount of adipose tissue accumulation, and food deprivation reduces the level of adipose tissue leptin mRNA in cows and sheep.

Therapeutic use

Leptin was approved in the United States in 2014 for use in congenital leptin deficiency and generalized lipodystrophy. An analog of human leptin metreleptin (trade name Myalept) was first approved in Japan in 2013, and in the United States (US) in February 2014. In the US it is indicated as a treatment for complications of leptin deficiency, and for the diabetes and hypertriglyceridemia associated with congenital or acquired generalized lipodystrophy (Chou and Perry, 2013). In nonobese diabetic mice with uncontrolled type 1 diabetes, leptin therapy alone or combined with low-dose insulin reverses the catabolic state through suppression of hyperglucagonemia. Additionally, it mimics the anabolic actions of insulin monotherapy and normalizes hemoglobin A1c with far less glucose variability. We show that leptin therapy, with insulin, normalizes the levels of hepatic intermediary metabolites in multiple chemical classes, including acylcarnitines, organic acids (tricarboxylic acid cycle intermediates), amino acids, and acyl CoAs. In contrast to insulin monotherapy. However, leptin lowers both lipogenic and cholesterologenic transcription factors and enzymes and reduces plasma and tissue lipids. The results imply that leptin administration may have multiple short- and long-term advantages over insulin monotherapy for type 1 diabetes.

A marker for enhancing productivity in livestock

Leptin and its receptor can be used as a marker for enhancing the productivity in livestock and are also potential candidates for marker assisted selection. Leptin and its receptor have been mapped in number of species. Numbers of microsatellite marker and single nucleotide polymorphism (SNP) have been identified for further use in selection programmes (Fitzsimmons *et al.*, 1998). The role of leptin on body growth, milk yield and other parameters has been

analysed in swine, cattle and buffalo. Polymorphism in the bovine leptin gene locus associated with genetic variation in energy balance, milk production, live weight and fertility trait have been reported by many researchers. These polymorphisms were associated with different growth stage, carcass composition, and fat characteristics. Similarly, polymorphism in exon 2 and intron 2 region of leptin gene has been associated with body growth in Indian goats. The leptin gene has also been cloned in mouse, rat, human, sheep, cattle, swine and chicken. Sequences of the human, mouse, rat and pig leptin receptor gene are known but only partial sequences of sheep, goat and cow leptin receptor have been reported.

CONCLUSION

Leptin may prove to be most useful to animal producers as a stimulant for growth as well as reproductive traits or as a molecular marker to genetically select high performing individuals for better productivity. Most importantly leptin will act as indicator of the animal's energy requirement for potential growth and survival. Several studies support the concept that leptin could be one of the links between past and current nutritional factors, and short and long term physiological regulations. The possible role of leptin in fetal growth and development may enhance the productive efficiency of farm animals. Finally, the involvement of leptin in physiological regulation and adaptation to varied environment will focus to sustain the growth of livestock in the face of varied climatic situation. Therefore, it could be used as future biomolecule for enhancing productivity in farm animals. The role of r-metHuLeptin as a new potentially useful medication that could be added to our therapeutic armamentarium is currently under intense investigation.

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