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LEPTIN GENE AND ITS ROLE IN CATTLE- A REVIEW

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ABSTRACT

Interest in the bovine leptin gene has increased during the last few years. Leptin, a 16-kDa protein secreted from white adipocytes which is involved in regulation of feed intake, milk yield, energy balance, fertility, and immune functions in animals. The gene encoding leptin was identified by positional cloning and is the mutation leading to the profound obese phenotype of the ob/ob mouse. Exogenous administration of leptin in ob/ob mice leads to a significant improvement in reproductive and endocrine status as well as reduced food intake and weight loss. The frequency of polymorphism in leptin gene has been found to be very high in beef and dairy cattle. Some association studies have been carried out in beef, dairy and fertility traits of cattle like energy balance, feed intake, live weight, fat deposition, milk yield and protein yield. These associations will provide insight in to the underlying mechanism of leptin and polymorphism can be used for selection of beef and dairy cows.

DISCOVERY OF THE LEPTIN GENE

The ob gene was discovered in 1994 by positional cloning techniques (Zhang *et al.*, 1994). The 167 amino acid protein product of the ob gene was named leptin (derived from the Greek term 'leptos' means 'thin'). The leptin gene consists of three exons, of which the first exon is not transcribed into the leptin protein of 16 kDa. In mice, the first intron is more than 8 kb long and the second intron has a length of 1.6 kb (He *et al.*, 1995; Dela Brousse, *et al.*, 1996) (Figure 1).

Leptin has 67% sequence identity among species such as human, gorilla, chimpanzee, orangutan, rhesus monkey, dog, cow, pig, rat and mouse (Zhang *et al.* 1997). The human leptin gene is located at HSA7q31.3, the mouse leptin gene on MMU 6A3.3 and the bovine leptin gene maps to BTA 4q32 (Pfister- Genskow *et al.*, 1996).

To date several important transcription factor binding domains have been identified in the promoter region of leptin gene in human, rodents and ruminants. These include domain for CAAT/enhancer binding proteins (C/EBP) (Hwang *et al.*, 1996; Miller *et al.*, 1996; Taniguchi *et al.*, 2002), adipocyte determination differentiation dependent factor 1/sterol regulatory element binding protein 1 (ADD/SREBP1) (Kim *et al.*, 1998), peroxisome proliferators activated receptor γ (PPARγ) (Hollenberg *et al.*, 1997), hypoxia inducible factor 1 (HIF 1) (Grosfeld *et al.*, 2002; Meissner *et al.*, 2003), and Sp1 and Ip1 (Mason *et al.*, 1998). C/EBP is a transcription of most gene expressed in adipose tissue and for other gene involved in energy metabolism (Darlington *et al.*, 1995). Several groups (Hwang *et al.*, 1996; Mason *et al.*, 1998) mutated the C/EBP site of the leptin promoter in rat and mouse and demonstrated that this site was functional in regulating leptin gene expression. Taniguchi *et al.* (2002) showed the transcription study that C/EBP activities the promoter of the bovine leptin gene.

Nuclear magnetic resonance analysis of a crystalline form of leptin revealed that leptin is a four- helix protein A-B-C-D, which is similar to the structure of cytokine family (Zhang *et al.*, 1997) (Fig: 2). Leptin contains a single disulphide bond that links two cysteines within the C and D helices and this bond has been proven critical for the structural integrity and stability of leptin (Rock *et al.*, 1996). Leptin binds to its receptor at the inference of á- helices A and C (Hiroike *et al.*, 2000).

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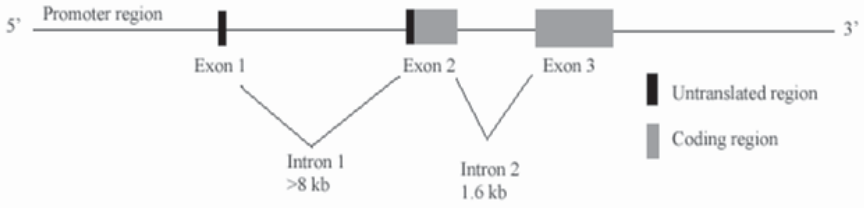

Figure 1. *Molecular organization of the leptin gene.*

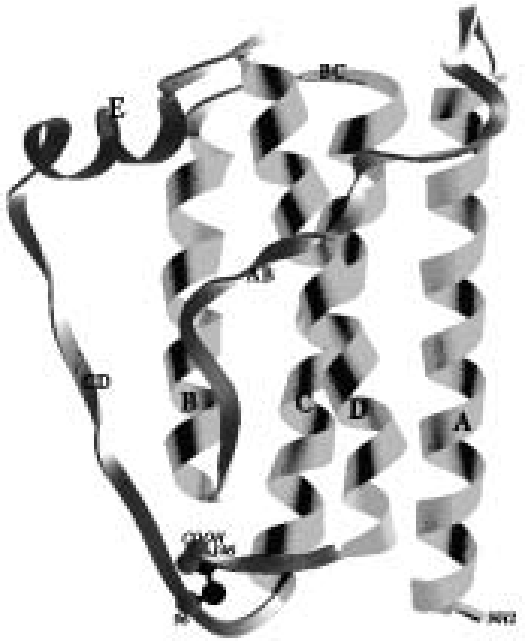

Fig 2. Four helical structure of leptin molecule

The first 21 amino acids of leptin functions as a single peptide and are cleaved off before the 146 amino acid protein is released into the blood as a circulating protein. In ob/ob mice, a cytocine to thymidine change at codon 105 changes the amino acid arginine into a stop codon that causes premature termination of transcription of the gene. This results in the synthesis of a truncated non-functional protein. Mutations in the leptin gene have also been identified in obese humans. Until now, only two obese families have been discovered; a Turkish family, having a missense mutation at codon 105 resulting in an arginine to tryptophan replacement (Strobel *et al.*, 1998; Ozata *et al.*, 1999) and a Pakistani family with a single nucleotide deletion at codon 133, resulting in a frameshift mutation (Montague *et al.*, 1997). Both mutations are rare and therefore probably not responsible for the majority of obese human.

PHYSIOLOGY OF LEPTIN

Within the central nervous system, the hypothalamus is the main site of leptin action

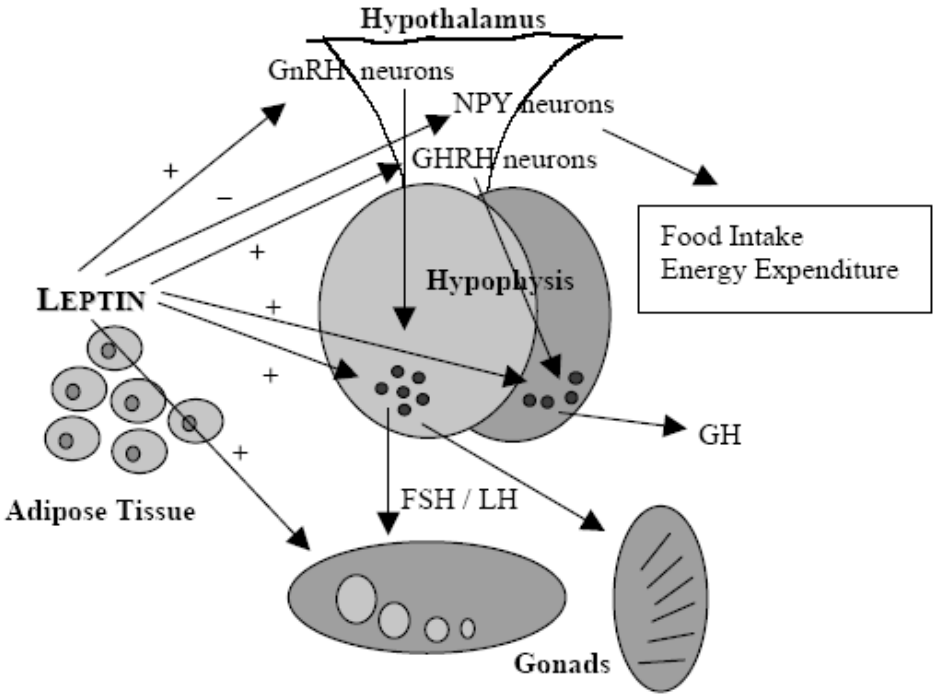

FIGURE 3. PATHWAYS OF LEPTIN.

with respect to controlling food intake and energy expenditure. Numerous studies have evaluated the localization of leptin receptor messenger RNA (mRNA) within the hypothalamus of several species including humans (Considine *et al.*, 1996), rodents (Brogan *et al.*, 2000; Garcia *et al.*, 2000; Seeber *et al.*, 2002) and ruminants (Dyer *et al.*, 1997; Ren *et al.*, 2002). The hypothalamus transduces leptin signals into neural responses, which cause alterations in food intake (Tang-Christensen *et al.*, 1999). Figure 4 presents several pathways of leptin. Neuropeptide Y (NPY) seems to be important for regulation of food intake. Leptin inhibits the signaling of NPY and thus inhibits food intake (Jang *et al.*, 2000). Furthermore, mice deficient (*ob/ob*) or unresponsive (*db/db*) to leptin, are characterized by increased levels of NPY mRNA. Direct administration of leptin reversed these changes in *ob/ob* mice (Ahima *et al.*, 2000). Leptin administration also stimulated the production of the gonadotrophins LH and FSH (lutenizing hormone and follicle stimulating hormone) from the hypophysis via GnRH-neurons (gonadotrophin-releasing hormone) neurons in the hypothalamus (Woller *et al.*, 2001; Watanobe, 2002; Amstalden *et al.*, 2003).

ROLE OF LEPTIN IN NUTRITIONAL STATUS AND REPRODUCTIVE FUNCTION

1. Leptin, Food Intake and Body Weight

Infusion of leptin caused a rapid decrease in food intake and body weight in *ob/ob* mice (Campfield *et al.*, 1995), wild-type mice (Halaas *et al.*, 1995), monkeys (Tang-

Christensen et al., 1999) and pigs (Barb et al., 1998). When *ob/ob* mice were pair-fed with leptin-treated *ob/ob* animals and thus received the same amount of food, they lost 30% less weight than the leptin treated *ob/ob* mice. This data suggested that besides its effect on food intake via hypothalamic NPY neurons, leptin could also play an important role in regulating fat mobilization (Halaas *et al.*, 1995). The satiety effects of leptin were also observed in ruminants by administration of recombinant human leptin in ewes for 3 days. This treatment caused a decrease in voluntary dry matter intake to approximately a third of the normal intake (Henry *et al.*, 1999). However, these effects were lost when sheep were underfed and leptin was administered (Henry *et al.*, 2001; Morrison *et al.*, 2001). This indicates that another signal blocks the effect of leptin on feed intake when the body condition is in a negative energy balance. Acute or long-term changes in food composition or food restriction caused changes in plasma leptin in ruminants. In pregnant ewes and adult rams, the concentration of plasma leptin increased within 48 hours respectively 5 days after increasing the dietary intake from low to high (Blache *et al.*, 2000; Thomas *et al.*, 2001). Complete food deprivation caused a rapid fall in plasma leptin (Marie *et al.*, 2001) and long-term food restriction decreased plasma leptin concentration in sheep (Delavaud *et al.*, 2000; Ehrhardt *et al.*, 2000; Morrison *et al.*, 2001). Acute changes in plasma leptin were the result of changes in leptin mRNA expression in adipose tissue. Food deprivation reduced the level of adipose tissue leptin mRNA in cows (Amstalden *et al.*, 2000).

2. Leptin and Adiposity

There are two definitions of adiposity, the state of being fat (body fatness) or the excessive accumulation of lipids in a site or organ (obesity). In this article, the first definition is used for the term adiposity. In rodents and human plasma concentrations of leptin are positively correlated with adiposity (Maffei *et al.*, 1995; Bunger *et al.*, 1999). Similar relationships have been observed between body fatness and leptin in growing and lactating ruminants (Blache *et al.*, 2000; Chilliard *et al.*, 2000; Thomas *et al.*, 2001). A linear relationship was demonstrated in well-fed late lactating dairy cows between plasma leptin and body condition score (BCS) (Ehrhardt *et al.*, 2000). However, other studies in ruminants showed that plasma leptin is more related to the size of adipocytes (fat cells) than to body condition score (BCS) (Delavaud *et al.*, 2002).

3. Leptin and Insulin

Insulin is an important regulator of energy homeostasis. It stimulates glucose, free fatty acid and amino acid uptake by tissues. Therefore it is not surprising that circulating plasma insulin concentrations were positively correlated with leptin mRNA levels in adipose tissue and with circulating plasma leptin concentrations (Maffei *et al.*, 1995). Leroy et al. (1996) showed that adipose tissue cells expressed leptin mRNA *in vitro* and secreted leptin protein after administrating insulin to these cells. Kieffer et al. (1996) reported that leptin receptors were expressed in insulin producing beta-cells within the pancreatic islets, suggesting that leptin might influence insulin secretion through a direct action on these cells. However, results of further studies were conflicting and therefore no clear evidence for this hypothesis exists. Tanizawa et al. (1997) reported stimulation by leptin of insulin secretion whereas others found an inhibition (Kieffer *et al.*, 1997; Poitout *et al.*, 1998). Some studies did not find any influence of leptin on pancreatic cells (Leclercq-Meyer *et al.*, 1996; Leclercq-Meyer and Malaisse, 1998).

4. Leptin and Fertility

Infusion of leptin in *ob/ob* mice, which are infertile, increased serum LH concentrations, ovarian weight in females and elevated serum

levels of FSH, increased testicular weights, and elevated sperm counts in males (Barash *et al.*, 1996). Furthermore, repeated administration of leptin in female ob/ob mice results in ovulation, pregnancy and parturition after insemination (Chehab *et al.*, 1996). Consistent with this study, leptin infusion in the fasted male rhesus monkey, increased mean plasma levels of LH and FSH, LH pulse frequency and LH pulse amplitude (Finn *et al.*, 1998). Leptin administration stimulated GnRH producing neurons in the hypothalamus, but also directly stimulated the hypophysis to produce LH and FSH (Figure 3). In ruminants, recombinant ovine leptin administration to fasted mature beef cows stimulated LH secretion (Amstalden *et al.*, 2002), and in fasted ovariectomized dairy cows leptin affected LH secretion in a dose-dependent manner; the highest concentration leptin (20 ng/ml) did not affect LH secretion, whereas 0.2 and 2.0 ng/ml leptin stimulated LH secretion 141% and 122% respectively (Zieba *et al.*, 2003b). However, in ovariectomized food restricted ewes, and in well-fed and undernourished ewe lambs, intracerebroventricular infusions of recombinant ovine leptin did not affect plasma concentrations of LH or FSH, LH pulse frequency or amplitude (Henry *et al.*, 1999; Morrison *et al.*, 2001). Furthermore, Morrison et al. (2002) showed that intravenous administration of leptin did not affect LH secretion in growing prepubertal ewe lambs. Thus the specific effect of leptin on gonadotropin secretion may depend on age (prepubertal/mature), species (cow/sheep), concentration of administered leptin, and whether or not the animals were operated (ovariectomized/not-ovariectomized).

5. Leptin and the Onset of Puberty

It has been suggested that leptin is a regulator of puberty onset in mice (Ahima *et al.*, 1997; Chehab *et al.*, 1997), rats (Gruaz *et al.*, 1998) and humans (Matkovic *et al.*, 1997). In humans, leptin concentrations increased during puberty and only in males leptin concentrations decreased after puberty due to testosterone inhibition of leptin secretion (Horlick *et al.*, 2000). This also probably explains the gender differences in leptin secretion (Havel *et al.*, 1996; Rosenbaum *et al.*, 1996). Leptin administration advanced the pubertal onset in mice (Ahima *et al.*, 1997; Chehab *et al.*, 1997) and rats (Gruaz *et al.*, 1998). Leptin concentrations in dairy cattle increased linearly from 16 weeks before until the week of pubertal ovulation in yearling heifers (Garcia *et al.*, 2002). Block et al. (2003a) did not find this increase and suggested that leptin might be a permissive factor for the onset of pubertal ovulation when metabolic resources are adequate, but the secretion of leptin alone is not sufficient to initiate puberty.

6. Leptin and Pregnancy

From early to mid pregnancy circulating leptin levels increased and remained elevated until late pregnancy in humans (Mukherjea *et al.*, 1999), rat (Amico *et al.*, 1998; Garcia *et al.*, 2000), mice (Tomimatsu *et al.*, 1997) and sheep (Ehrhardt *et al.*, 2001; Forhead *et al.*, 2002). These elevations are due to an increase in adiposity as an increase in leptin mRNA expression in adipose tissue (Ehrhardt *et al.*, 2001). The increase in leptin concentration during pregnancy seems to be paradoxical as this is a period of increased nutritional demands, thus not a period in which the actions of leptin are expected to increase. However, as the concentration of circulating Ob-Re is increased during pregnancy, most leptin is present in the bound, not active form. This might induce a state of leptin resistance, or a change in leptin bioavailability (Mounzih *et al.*, 1998; Seeber *et al.*, 2002). In cows, no study has been performed yet to measure leptin levels during early and mid pregnancy, but leptin levels are high during late pregnancy (Block *et al.*, 2001).

7. Leptin and Lactation

During pregnancy leptin levels are high and they decline rapidly towards parturition. Eliminating the energetic costs of lactation by preventing milk delivery in rats and cows caused an increase in plasma leptin levels together with an increase in energy balance (Woodside *et al.*, 2000; Block *et al.*, 2001). This indicates that the fall in circulating leptin levels towards and during lactation is due to the energetic costs of milk production. The suckling stimulus itself did not appear to influence the decrease in leptin concentration (Brogan *et al.*, 1999). Pickavance *et al.* (1998) observed that the food intake-induced leptin increase was eliminated during lactation and they speculated that the hypoleptinemia may be an important factor promoting the hyperphagia of lactation. Overall, these data demonstrated that the onset of the negative energy balance is largely responsible for the declining leptin concentrations towards parturition and that the low leptin levels during lactation probably induce the hyperphagia of lactation.

Leptin gene polymorphisms in cattle

A sequence study of fragments of leptin and amyloid precursor protein (APP) genes in 22 diverse animals from the two subspecies *Bos taurus* and *Bos indicus* have been reported (Konfortnov *et al.*, 1999). The frequency of polymorphisms in the leptin gene was found 1 per 84 base pairs in exonic sequences whereas the APP gene had a frequency of 1 per 156 base pairs in exonic sequences. This indicates that the leptin exons have a high mutation frequency. Recently SNP concept has basically arisen from the recent need for very high densities of genetic marker for the studies of complex traits. SNPs can provide valuable data on association between specific genes or other DNA structures and phenotypes, or on population and genome dynamics.

Genetic differences in the leptin gene were first observed in mice; ob/ob mice lack functional leptin and are hyperphagic, obese, and infertile (Hamann and Matthaei, 1996). When leptin is administered, fertility is restored and body fat mass is reduced (Halaas *et al.*, 1995). The sterility of ob/ob female mice is caused by an insufficiency of hormones at the hypothalamic-hypophysis level rather than physical hindrance of excess adipose tissue (Chehab *et al.*, 1996). Polymorphisms in the human leptin gene were associated with low circulating leptin levels (Hager *et al.*, 1998), birth weight (Orbak *et al.* 2001) and obesity (Ohshiro *et al.*, 2000). Four polymorphisms in the porcine leptin gene were associated with fatness (Jiang and Gibson, 1999). The polymorphisms in the bovine leptin gene have been described by several workers (Pomp *et al.*, 1997; Fitzsimmons *et al.*, 1998; Haegeman *et al.*, 2000). An association with fat deposition with regards to leptin polymorphism in beef cattle has been reported (Fitzsimmons *et al.*, 1998). Lindersson *et al.* (1998) reported QTLs for milk production traits close to the leptin gene (82.8 cM) and also found QTLs for milk, fat, and protein yield at 65 and 85 cM, and for fat and protein percentage at 75 and 95 cM respectively.

A number polymorphism and its association with dairy and beef traits have been reported by different workers as presented in the Table 1. Liefers *et al.* (2002) genotyped a total of 623 cows for two RFLPs (RFLP1 and RFLP2) and one mocrosatellite BM1500 in the leptin gene locus at bovine chromosome 4 (Table 3) and an association study has performed on dairy traits. Because of the putative conformational change in the leptin molecule, the R4C polymorphism received a lot of attention by several research groups. Lagonigro *et al.*, (2003) did not find any association of R4C with dairy and beef traits (Holstein Friesian n=613; British Friesian, Aberdeen Angus, Hereford, Highland and

TABLE 1: Polymorphisms in the leptin gene and association with dairy and beef traits.

Polymorphism	Association	Beef/dairy	No. Of Animals	Reference
103	No	Beef/dairy	246	Lagonigro *et al.*,2003
252*	Feed intake	Beef/dairy	246	Lagonigro *et al.*, 2003
R4C*	Carcass traits	Beef	154	Buchanan *et al.*,2003
	No	Beef/dairy	246	Lagonigro *et al.*, 2003
	Milk yield/protein	Dairy	416	Buchanan et al., 2003
RFLP1	Milk yield/protein	Dairy	623	Liefers *et al.*, 2003
LEPSau3Al	Calving interval	Beef	149	Almeida *et al.*, 2003
LEPBsaAl	No	Beef	96	Almeida *et al.*, 2003
A59V*	No	Dairy	623	Liefers *et al.*, 2002
	No	Beef	100	Aimeida *et al.*2003
BM1500	Fat deposition	Beef	158	Fitzsimmons *et al.*, 1998
	No	Dairy	623	Liefers *et al.*, 2002
BsaAI-RFLP	No	dairy	403	Choudhary *et al.*, 2005

*polymorphism in exonic regions

Charolais n=245) which is in contrast with results of Buchanan *et al.* (2002, 2003) who found associations for carcass content in beef cattle (Angus, Charolais, Hereford, Simmental, n=154) and milk and protein yield in dairy cattle (Holstein, n=416). In study of Leifers *et al.* (2002) RFLP1 turned out to be associated with milk yield and found Heifers with the RFLP1, AB genotype produce 1.32 kg/day more milk and consume 0.73 kg/day more food compared with the RFLP1 AA genotype.

Almeida *et al.* (2003) studied the RFLP1 SNP for reproductive performance in beef cattle (Brangus Ibagé; 5/8 Aberdeen Angus x 3/8 Nelore) and did not find associations for weight at first calving or calving interval. In the study of Leifers *et al.* (2002), where no associations were found with A59V, no other studies were performed on A59V in dairy or beef cattle. Also for the BM1500 microsatellite found no association confirming the results of Lindersson *et al.* (1998) who also found no associations of BM1500 with milk, fat, and protein yields. Recently, Lagonigro *et al.* (2003) reported a new non-conservative mutation in exon 2 of the leptin gene. They used 5 different breeds (British Friesian n=49, Aberdeen Angus n=42, Hereford n=50, Highland n=48and Charolais n=56) and the frequency of the rare allele ranged from 9.4% (Highland) to 15.2% (Charolais). Frequencies of British Friesian, Aberdeen Angus and Hereford were 4.1, 3.6 and 12% respectively.

CONCLUSION

The leptin gene is important candidate gene which can be used to improve the reproduction and production of dairy/beef animals. The direct leptin treatment will not be feasible in livestock production unless affordable, potent analogs or delivery systems are developed. Until a, successful enhancement of reproductive function or manipulation of nutrient partitioning are more likely to be achieved through regulation of leptin production or sensitivity to leptin through nutritional or metabolic manipulation. Genetic polymorphisms in the intronic and exonic region of leptin gene reported are associated with productive and reproductive traits in cattle and this sort of genetic information can be used efficiently in breeding and management decisions.

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