Asian-Aust. J. Anim. Sci.
Vol. 21, No. 2 : 161 - 166
February 2008

www.ajas.info

The Body Weight-related Differences of Leptin and Neuropeptide Y (NPY) Gene Expression in Pigs*

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ABSTRACT : To determine if body weight change is directly related to altered leptin and neuropeptide Y (NPY) gene expression, we assessed adipose tissue weight, percent body fat, leptin and NPY mRNA levels and serum leptin concentration in pigs at weights of 1, 20, 40, 60, and 90 kg. The results indicated that the weight of adipose tissues and the percent body fat of pigs significantly increased and correlated with body weight (BW) from 1 to 90 kg ($p<0.01$). Serum leptin concentrations and leptin mRNA levels in omental adipose tissue (OAT) increased from 1 to 60 kg, and then decreased from 60 to 90 kg. At 60 kg, the serum leptin concentration and leptin mRNA level significantly increased by 33.5% ($p<0.01$) and 98.2% ($p<0.01$), respectively, as compared with the levels at 1 kg. At 60 kg, the amount of leptin mRNA in subcutaneous adipose tissue (SAT) was significantly higher than that of 1 and 40 kg animals ($p<0.05$). NPY gene expression in the hypothalamus also changed with BW and at 60 kg the NPY mRNA level significantly decreased by 54.0% ($p< 0.05$) as compared with that in 1 kg. Leptin mRNA in OAT was correlated with serum leptin concentrations ($r = 0.98$, $p<0.01$), body weight ($r = 0.82$, $p<0.05$) and percent body fat ($r = 0.81$, $p<0.05$). This is the first report of the developmental expression of leptin in porcine OAT, peritoneal adipose tissue (PAT) and SAT, and proves that the expression of leptin in OAT could reflect the levels of circulating leptin. These results provide some information for nutritional manipulation of leptin secretion which could lead to practical methods of controlling appetite and growth in farm animals, thereby regulating and improving efficiency of lean meat production and meat production quality. (**Key Words :** Fat Deposition, Gene Expression, Leptin, Neuropeptide Y, Pig)

INTRODUCTION

Leptin is the adipocyte-specific product of the obese gene and is synthesized and secreted predominantly by white adipocytes and relates to the feedback system that regulates long-term body fat weight and composition (Shin and Chung, 2007). Leptin have been respectively associated with stimulating and inhibiting appetite, and consequently influences fat deposition in animals through the control of appetite and energy expenditure (Morrison et al., 2001; Dai et al., 2007). Some studies have proved that leptin secretion is highly correlated with body fat mass in mice (Brockmann et al., 2000), in humans (Ahima and Flier, 2000), in beef cattle (Cheong et al., 2006), and ruminants (Delavaud et al., 2000). Leptin has many biologic roles in the pig, such as impacting energy metabolism, adiposity, the neuroendocrine axis, immunologic processes, and reproduction (Barb et al., 2001) and is perhaps linked to meat quality determinants such as marbling (Ji et al., 1998).

Neuropeptide Y (NPY) is a potent regulator of feeding, energy expenditure and fat storage (Barb and Barrett, 2005), which has been shown to be a target of leptin action in the central nervous system (Stephens, 1995). Previous study proved that hypothalamic NPY is responsive to changes in energy balance and blood leptin concentrations (Swart et al., 2001). However, there were no data about the developmental expression of leptin and NPY gene in pigs at different growth stages.

Therefore, the present study was conducted to investigate the developmental expression of leptin and NPY genes in pigs at different body weight (BW). The correlation between the expression of the two genes and adipose deposition as well as serum leptin concentration and BW were also studied to obtain information for regulating meat production quality.

* This work was supported by the National Basic Research Program of China (2004CB117506).

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Received May 11, 2007; Accepted July 28, 2007

Table 1. Specific primers for the leptin, neuropeptide Y, and β-actin genes

Gene	Accession number	Primer source	Oligonucleotide sequence	Amplification cycles	Annealing temp. (°C)	Length (bp)
Leptin	AF102856	Pig	5'-ATGAATTCATGCGCTGTGGACCCCTGTG-3' (sense primer) 5'-ATAAGCTTTCAGCAGCCAGGGCTGAGGT-3' (antisense primer)	29	60	504
NPY	AF264083	Pig	5'-GGTGTGCCTGTGTGCG-3' (sense primer) 5'-GGTCTTCGAGCCTAGTTCTG-3' (antisense primer)	31	58	224
β-actin	U07786	Pig	5' - CGGGACCTGACCGACTACCT - 3' (sense primer) 5' - GGCCGTGATCTCCTTCTGC - 3' (antisense primer)	25	58	411

MATERIAS AND METHODS

Animals and experimental designs

All of the experiments were done according to the guidelines for animal experiments at the National Institute of Animal Health. Total of thirty female pigs (Duroc× Landrace×Yorkshire) weighed at 1, 20, 40, 60 and 90 kg (n = 6/BW) were selected randomly and euthanized under anaesthesia after a 12 h fast and *ad libitum* access to water. The ingredient and chemical compositions for the diets fed to the pigs (20-90 kg) were: DE 14.46 MJ/kg, crude protein 19.50%, calcium 0.80%, phosphorus 0.66%. Blood samples (10-ml) were collected for determination of plasma concentrations of leptin. Omental adipose tissue (OAT), subcutaneous adipose tissue (SAT) and peritoneal adipose tissue (PAT) in right-half carcasses and the hypothalamus were collected, and rapidly frozen in liquid nitrogen, then stored at -80°C until RNA analysis for determination of leptin and NPY gene expression. Left-half carcasses were weighted after the head, hooves, tail, viscera (except the kidney) were removed. The OAT, SAT and PAT in left-half carcasses were collected and weighed for the percent body fat.

Adiposity deposition and serum leptin concentration

The adipose deposition was determined by the percent body weight expressed as the sum of the weight of PAT, SAT and OAT divided by body weight ×100 (Nogalska and Swierczynski, 2001). Serum leptin concentrations were determined in duplicate with a commercially available radio-immunoassay procedure (Linco Research, Missouri) using an RIA kit (Multi Species RIA Kit, Linco Research, St. Charles, MO) (Qian et al., 1999).

RNA extraction and reverse transcription

Total RNA was isolated from the adipose tissues and hypothalamus using Trizol Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted RNA was dissolved in ultra pure water and the purity and concentration of total RNA were measured by a spectrophotometer at 260 nm and 280 nm. Ratios of absorption (260/280 nm) of all samples were between 1.8 and 2.0. Two micrograms of isolated RNA were used for reverse transcription according to the method of previous study (Wang et al., 2005).

PCR assay

Oligonucleotide primers (ShangHai Sangon Biological Engineering Technology and Service Company) specific for porcine leptin, NPY and β-actin were based on known sequences deposited in Genbank (Table 1). The appropriate number of cycles, optimum PCR primer concentration, Mg^{2+} concentration and annealing temperature that would result in linear amplification of each transcript were determined by a preliminary experiment (data not shown). PCR was performed in a 50 μl reaction volume containing 2.0 μl tissue specific cDNA, 5.0 μl 10× reaction buffer, 3.0 μl $MgCl_2$ (25 mM), 0.5 μl *Taq* DNA polymerase (2 U/μl) (Promega, Madison, USA), 1.0 μl dNTP (10 mM), 1.0 μl sense primer (20 μM), 1.0 μl antisense primer (20 μM), 36.5 μl Nuclease-Free Water. All subsequent amplification reaction steps were performed using a GeneAmp® PCR System 9600 (Perkin-Elmer, Fremont, CA, USA).

The PCR profiles for letpin, NPY and β-actin consisted of denaturation at 94°C for 2 min, followed by appropriate number of cycles (Table 1) with denaturation at 94°C for 50 sec, annealing at the optimum temperature (Table 1) for 50 sec, and extension at 72°C for 50 sec, and a final extension at 72°C for 10 min. After amplification, 5 μl of each PCR product were analyzed by agarose gel electrophoresis (1.0%). The RT and PCR reactions of each sample were repeated 3 times.

To confirm that the amplified fragments were those predicted, the PCR products were cloned and sequenced.

Statistical analysis

Band intensities of the PCR products in agarose gels were quantified using Image Master VDS software (Amersham Pharmacia Biotech, Uppsala, Sweden). Mean expression levels of leptin and NPY were normalized against β-actin levels and presented in integrated optical

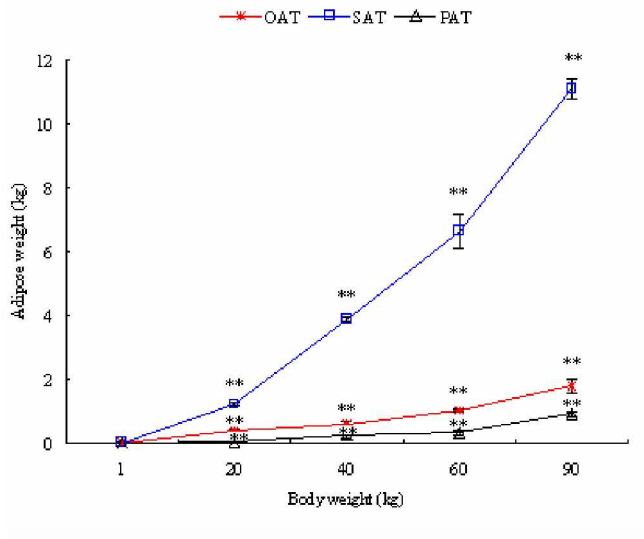

Figure 1. Subcutaneous adipose tissue (SAT), peritoneal adipose tissue (PAT) and omental adipose tissue (OAT) weight of pig at weights 1, 20, 40, 60 and 90 kg. Each data represents the mean of six individual pigs±SEM. ** p<0.01.

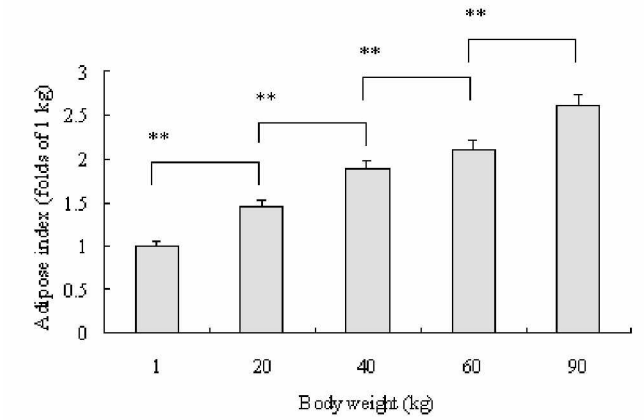

Figure 2. Percent body fat of pigs at weights 1, 20, 40, 60 and 90 kg. Results expressed as folds of 1 kg. Each column represents the mean of six individual pigs±SEM. ** p<0.01.

density. The mRNA levels of leptin in adipose tissues and NPY in hypothalamus at different BW were compared on the basis of leptin/β-actin and NPY/β-actin ratios. Means and standard errors of the means (SEM) were calculated. The statistical significance of difference between groups was assessed by one-way ANOVA procedure (SAS Institue, 1989), followed by Duncan's multiple-range test. Difference between the groups and correlations were considered as significant when p<0.05.

RESULTS

Adipose deposition and serum leptin concentration

The weight of OAT, SAT and PAT of pigs significantly increased with BW from 1 kg to 90 kg (p<0.01, Figure 1).

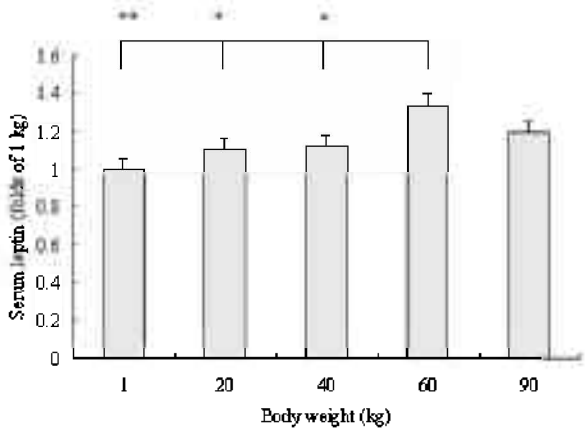

Figure 3. Serum leptin concentration of pigs at weights 1, 20, 40, 60 and 90 kg. Results expressed as folds of 1 kg. Each column represents the mean of six individual pigs±SEM. * p<0.05, ** p<0.01.

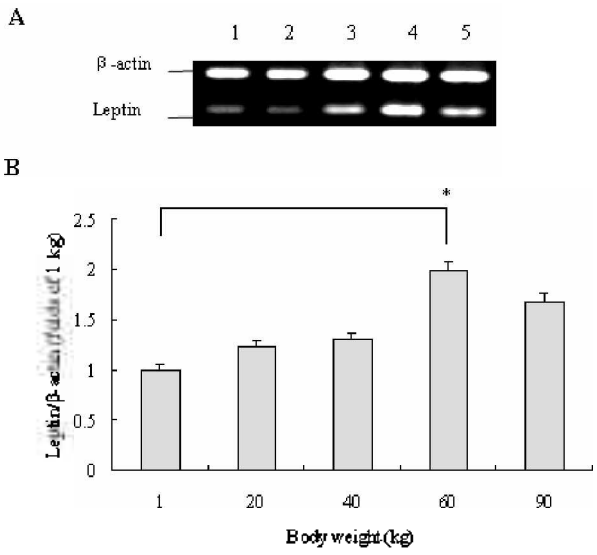

Figure 4. Leptin gene expression of pig in omental adipose tissue (OAT) of pigs at weights 1, 20, 40, 60 and 90 kg. (A) Electrophoresis results of RT-PCR for leptin and β-actin in OAT. Lane 1: 1 kg; lane 2: 20 kg; lane 3: 40 kg; lane 4: 60 kg; lane 5: 90 kg. (B) The Integrated Optical Density (IOD) ratio of each band of leptin and β-actin of pigs. Densitometric analysis of the porcine leptin were normalized to β-actin and shown as leptin/β-actin ratios. Results expressed as folds of 1 kg. Each column represents the mean of six individual pigs±SEM. * p<0.05.

Subsequently the percent body fat significantly increased with BW between 1 and 90 kg (p<0.01, Figure 2). Correlation analysis showed that there was a strong positive correlation between percent body fat and BW (r = 0.99, p< 0.01) from 1 to 90 kg. We also found that serum leptin concentration changed with BW and significantly increased (p<0.05) from 1 to 60 kg (Figure 3). At 60 kg, the serum leptin concentration increased about 33.5% (p<0.01) compared to the level of 1 kg. Additionally, strong

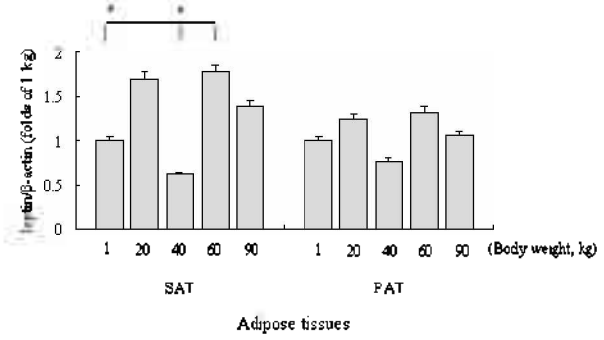

Figure 5. The relative expression of leptin in subcutaneous adipose tissue (SAT) and peritoneal adipose tissue (PAT). Densitometric analysis of the porcine leptin were normalized to β-actin and shown as leptin/β-actin ratios. Results expressed as folds of 1 kg. Each column represents the mean of six individual pigs±SEM. * $p<0.05$.

correlations between serum leptin concentration and percent body fat ($r = 0.88$, $p<0.05$), serum leptin concentration and BW ($r = 0.94$, $p<0.05$) in pigs between 1 and 60 kg were found.

Expression of leptin

Different mRNA levels of leptin gene in OAT at different BW were found (Figure 4). The relative abundance of leptin mRNA increased steadily from 1 to 60 kg, increasing by 98.2% ($p<0.05$) as compared with 1 kg, and then decreased from 60 to 90 kg (Figure 4B). Correlation analysis found that there was a significant positive correlation between the relative abundance of leptin mRNA in OAT and serum leptin concentrations ($r = 0.98$, $p<0.01$) during growth from 1 to 90 kg of BW (Figure 3). The amount of OAT leptin mRNA level was correlated with both the BW ($r = 0.82$, $p<0.05$) and percent body fat ($r = 0.81$, $p<0.05$).

The expression of leptin in SAT and PAT was also changed with BW (Figure 5). At 60 kg, the amount of leptin mRNA in SAT was significantly higher than that of 1 and 40 kg animals ($p<0.05$). The expression of leptin in PAT had no significant changes with the animals' growth. In addition, the relative expression of leptin mRNA in the SAT snd PAT was not correlated with the body weight or the serum leptin concentrations (data not shown).

Expression of NPY

NPY gene expression also changed with BW (Figure 6). At 60 kg, the gene expression of NPY was the lowest than the other stages we studied in this experiment, significantly decreased about 54.0% ($p<0.05$) as compared with the expression at 1 kg (Figure 6B). From 1 to 60 kg, an inverse correlations between NPY mRNA levels and and serum leptin concentrations ($r = 0.81$, $p<0.05$) was found.

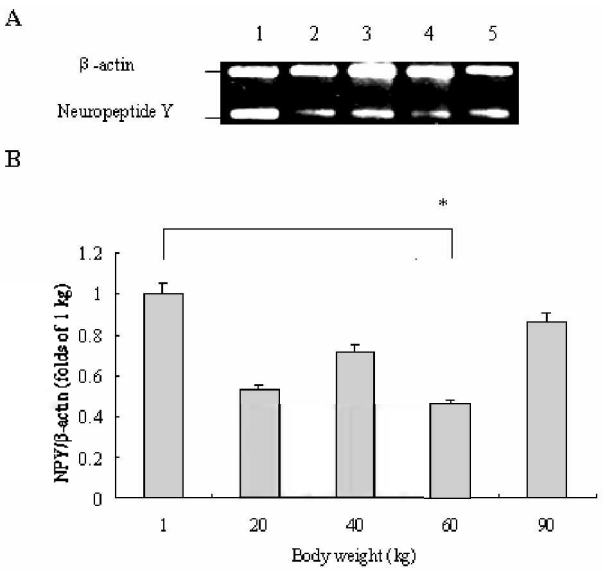

Figure 6. Neuropeptide Y (NPY) gene expression of pigs in hypothalamus of pigs at weights 1, 20, 40, 60 and 90 kg. (A) Electrophoresis results (six repetitions) of RT-PCR for NPY and β-actin in hypothalamus. Lane 1: 1 kg; lane 2: 20 kg; lane 3: 40 kg; lane 4: 60 kg; lane 5: 90 kg. (B) The Integrated Optical Density (IOD) ratio of each band of NPY and β-actin of pigs. Densitometric analysis of the porcine NPY were normalized to β-actin and shown as NPY/β-actin ratios. Results expressed as folds of 1 kg. Each column represents the mean of six individual pigs±SEM. * $p<0.05$.

DISCUSSION

Aging is associated with increased adiposity in humans and animals. Previous study had proved that the fat content of the carcass was significantly correlated with animal age (Souza et al., 2004). Schwartz (1998) showed that aging in mammals is often associated with a relative increase in body adiposity and body weight. Consistent with the previous studies, in the current study, we founded that the weight of SAT, PAT, OAT significantly increased with BW, and percent body fat in pigs significantly increased and correlated with BW between 1 and 90 kg.

Leptin is an afferent signal molecule that interacts with the appetite and satiety centers in the brain to regulate body weight (Meier, 1995), to influence energy intake, energy expenditure and hormonal function (Campfield et al., 1997), consequently influences fat deposition in animals and humans (Morrison et al., 2001; Dai et al., 2007). This allows leptin to serve as a marker for body fat stores. Previous studies showed that adipose tissue leptin mRNA level increased with age in rats (Nogalska and Swierczynski, 2001; Nogalska et al., 2003). In pigs, Chen et al. (2000) showed that leptin mRNA in adipose tissue increased with morphological development. In the present study, we found

that the relative expression of leptin in in OAT was correlated with both the body weight ($r = 0.82$, $p<0.05$) and percent body fat ($r = 0.81$, $p<0.05$). However, the relative expression of leptin in the SAT and PAT was not correlated with both the body weight and percent body fat. These results indicated that the gene expression of leptin in OAT could serve as a signal for body fat of the pigs.

Leptin is secreted from adipose tissue, circulates in the blood and functions as a hormonal sensing mechanism for fat deposition and BW homeostasis (Friedman and Halaas, 1998; Barb et al., 2001a). Serum leptin levels are changed with animals growth (Blum et al., 1997). Nogalska et al. (2003) found that serum leptin concentration was much lower in young than in old animals and other studies have proved serum leptin concentration increased with age in rat (Li et al., 1998; Nogalska and Swierczynski, 2001), in human (Garcia-Mayor et al., 1997) and in prepuberal gilts (Barb et al., 2001b). In addition, leptin secretion is highly correlated with body fat mass in mice (Brockmann et al., 2000), in humans (Ahima and Flier, 2000), in monkey (Mann et al., 2000) and in ruminants (Delavaud et al., 2000). Our present results indicated that serum leptin concentration increased with BW from 1 to 60 kg. The serum leptin concentration significantly correlated with both the percent body fat ($r = 0.88$, $p<0.05$) and BW ($r = 0.94$, $p<0.05$) in pigs from 1 to 60 kg. Consisted with the previous study (Nogalska and Swierczynski, 2001), we also found a positive correlation between the relative mRNA levels of leptin in OAT and serum leptin concentrations ($r = 0.98$, $p<0.01$) in pigs from 1 to 90 kg. However, the relative expression of leptin in the SAT and PAT was not correlated with serum leptin concentrations. These results indicated that serum leptin could play an important role in fat deposition in pigs and can be used as a signal of body weight and adipose deposition. The expression of leptin in OAT could reflect the levels of the circulating leptin, because there was a significant correlation between serum leptin level and leptin mRNA expression in OAT.

Leptin acts on the brain and modulates the hypothalamic neuropeptide system to reduce food intake, increase energy expenditure, and alter endocrine activity (Barb et al., 2001b). NPY is the most effective neuropeptide in inducing feeding behavior and hyperphagia and has been shown to be a target of leptin action in the centural nervous system (Stephens et al., 1995; Cunningham et al., 1999). Hypothalamic NPY is responsive to changes in energy balance and serum leptin concentrations in rodents (Barb et al., 1999). Gruenewald et al. (1996) reported that NPY gene expression significantly decreased with age in rats. In the current study, we found that the mRNA levels of NPY in hypothalamus have the trends to decrease from 1 to 60 kg, and increased from 60 to 90 kg. Consisted with the previous study (Li et al., 1998), we also found that an inverse correlation between NPY mRNA levels and serum leptin concentrations, which suggests that NPY gene expression is responsive to serum leptin concentration.

IMPLICATION

In the current study, we first reported that the developmental expression of leptin in porcine OAT, PAT and SAT, and first demonstrated that the expression of leptin in OAT was the primary source of circulating leptin and could be serve as a signal for body fat of the pigs. These results would provide some information needed for the use of development of nutritional schemes to manipulate leptin and NPY secretion in regulating and improving meat production quality. However, the reason for higher serum leptin at a body weight of 60 kg but a lower level at 90 kg, the changes of the NPY expression in porcine hypothalamus and the effects of fasting on the porcine leptin and NPY gene expression will be studied in future.

REFERENCES

Ahima, R. S. and J. S. Flier. 2000. Leptin. Annu. Rev. Physiol. 62: 413-437.

Barb, C. R., J. B. Barrett, R. R. Kraeling and G. B. Rampacek. 1999. Role of leptin in modulating neuroendocrine function: A metabolic link between the brain-pituitary and adipose tissue. Reprod. Domest. Anim. 34(3-4):111-125.

Barb, C. R., G. J. Hausman and K. L. Houseknecht. 2001a. Biology of leptin in the pig. Domest. Anim. Endocrinol. 21(4): 297-317.

Barb, C. R., J. B. Barrett, R. R. Kraeling and G. B. Rampacek. 2001b. Serum leptin concentrations, luteinizing hormone and growth hormone secretion during feed and metabolic fuel restriction in the prepuberal gilt. Domest. Anim. Endocrinol. 20:47-63.

Barb, C. R. and J. B. Barrett. 2005. Neuropeptide Y modulates growth hormone but not luteinizing hormone secretion from prepuberal gilt anterior pituitary cells in culture. Domest. Anim. Endocrinol. 29(3):548-555.

Blum, W. F., P. Englaro, S. Hanitsch, A. Juul, N. T. Hertel, J. Muller, N. E. Skakkebaek, M. L. Heiman, M. Birkett, A. M. Attanasio, W. Kiess and W. Rascher. 1997. Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J. Clin. Endocrinol. Metab. 82:2904-2910.

Brockmann, G. A., J. Kratzsch, C. S Haley, U. Renne, M. Schwerin and S. Karle. 2000. Single QTL effects, epistasis, and pleiotropy account for two-thirds of the phenotypic F(2) variance of growth and obesity in DU6i×DBA/2 mice. Genome Res. 10:1941-1957.

Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos and P. Burn. 1997. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Sci. 269:546-549.

Chen, X. L., J. Lin, D. B. Hausman, R. J. Martin, R. G. Dean and G. J. Hausman. 2000. Alterations in fetal adipose tissue leptin expression correlate with the development of adipose tissue. Biol. Neonate 78(1):41-47.

Cheong, H. S., D. H. Yoon, L. H. Kim, B. L. Park, E. R. Chung, H. J. Lee, I. C. Cheong, S. J. Oh and H. D. Shin. 2006. Leptin polymorphisms associated with carcass traits of meat in Korean cattle. Asian-Aust. J. Anim. Sci. 19(11):1529-1535.

Cunningham, M. J., D. K. Clifton and R. A. Steiner. 1999. Leptin's action on the reproductive axis: perspectives and mechanisms. Biol. Reprod. 60:216-222.

Dai, H. C., L. Q. Long, X. W. Zhang, W. M. Zhang and X. X. Wu. 2007. Cloning and expression of the duck leptin gene and the effect of leptin on food intake and fatty deposition in mice. Asian-Aust. J. Anim. Sci. 20(6):850-855.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler and G. Kann. 2000. Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519-526.

Friedman, J. M. and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature 395:763-770.

Garcia-Mayor, R. V., A. Andrade, M. Rios, M. Lage, C. Dieguez and F. F. Casanueva. 1997. Serum leptin levels in normal children: Relationship to age, gender, body mass index, pituitary-gonadal hormones, and pubertal stage. J. Clin. Endocrinol. Metab. 82:2849-2855.

Gruenewald, D. A., B. T. Marck and A. M. Matsumoto. 1996. Fasting-induced increases in food intake and neuropeptide Y gene expression are attenuated in aging male brown Norway rats. Endocrinol. 137:4460-4467.

Ji, S. Q., G. M. Wills, R. R. Scott and M. E. Spurlock. 1998. Partial cloning and expression of the bovine leptin gene. Anim. Biotechnol. 9(1):1-14.

Li, H., M. Matheny, N. Tumer and P. J. Scarpace. 1998. Ageing and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression. Am. J. Physiol. 275:405-401.

Mann, D. R., M. A. Akinbami, K. G. Gould and V. D. Castracane. 2000. A longitudinal sStudy of leptin during development in the male rhesus monkey: the effect of body composition and season on circulating leptin levels. Biol. Reprod. 62:285-291.

Meier, C. A. 1995. Advances in the understanding of the molecular basis of obesity. Eur. J. Endocrinol. 133:761-763.

Morrison, C. D., J. A. Daniel, B. J. Holmberg, J. Djiane, N. Raver, A. Gertler and D. H. Keisler. 2001. Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinol. 168:317-324.

Nogalska, A., A. Pankiewicz, E. Goyke and J. Sweirczynski. 2003. The age-related inverse relationship between ob and lipogenic enzymes genes expression in rat white adipose tissue. Exp. Gerontol. 38(4):415-422.

Nogalska, A. and J. Swierczynski. 2001. The age-related differences in obese and fatty acid synthase gene expression in white adipose tissue of rat. Biochim. Biophys. Acta 1533:73-80.

Qian, H., C. R. Barb, M. M. Compton, G. J. Hausman, M. J. Azain, R. R. Kraeling and C. A. Baile. 1999. Leptin mRNA expression and serum leptin concentrations as influenced by age, weight, and estradiol in pigs. Domest. Anim. Endocrinol. 16(2):135-143.

Schwartz, R. S. 1998: Obesity in the elderly (Ed. G. A. Bray, C. Bouchard and W. P. T. James). Handbook of obesity. New York: Marcel Dekker, Inc., 103-114.

Shin, S. C. and E. R. Chung. 2007. Association of SNP marker in the leptin gene with carcass and meat quality traits in Korean cattle. Asian-Aust. J. Anim. Sci. 20(1):1-6.

Souza, D. N., D. W. Pethick, F. R. Dunshea, D. Suster, J. R. Pluske and B. P. Mullan. 2004: The pattern if fat and lean muscle tissuedeposition differs in the different pork primal cuts of female pigs during the finisher growth phase. Livest. Prod. Sci. 91:1-8.

Stephens, T. W. 1995. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377:530-532.

Swart, I., J. M. Overton and T. A. Houpt, 2001. The effect of food deprivation and experimental diabetes on orexin and NPY mRNA levels. Peptides. 22:2175-2179.

Wang, Y. Z., Y. J. Tu, F. F Han, Z. R. Xu and J. H. Wang. 2005. Developmental gene expression of lactoferrin and effect of dietary iron on gene regulation of lactoferrin in mouse mammary gland. J. Dairy Sci. 88(6):2065-2071.