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Castration Modulates Leptin and its Receptor in the Hypothalamus from Male Pigs

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Abstract: The aim of the present study was to investigate the effects of castration on the gene expression of leptin and leptin receptor in the hypothalamus from male pigs. Sixteen pairs of pigs were used in our experiment. Each pair was composed of two male full sibs, one of which was castrated at 35 days old and the other remained intact. Eight pairs of pigs were slaughtered at 147 and 210 days old, respectively. Boars weighed more than barrows at 210 days old. However, barrows ate more feed and showed higher carcass fat weight, leaf fat weight and average back fat thickness at 147 and 210 days old. Higher serum leptin but lower testosterone concentration was found in castrated pigs as compared to intact pigs. Castration induced an increase in Ob mRNA in both subcutaneous fat and leaf fat tissue in male pigs. However, there were no differences in hypothalamic expression of Ob-Rb between barrows and boars. These results suggest that castration may influence leptin sensitivity in male pigs.

Key words: Castration, fat, hypothalamus, leptin, leptin receptor, pigs

INTRODUCTION

It is known that changes in sex steroid hormones and adipose tissue are closely interrelated (De Pergola, 2000; Pasquali, 2006). Several researchers have reported that the body weight, particularly the amounts of adipose tissue increased in castrated male pigs (Bonneau, 1998; Pauly *et al*., 2008, 2009). Various factors may involve in the increase of adiposity following castration but the mechanisms are still not clear.

Hormones secreted in proportion to body fat provide an important regulatory signal to the brain. Leptin, a hormone secreted by adipocytes has been identified as a lipostatic factor that regulates the amounts of body fat stores by reducing food intake and increasing energy expenditure through a negative feedback loop involving the hypothalamus (Hamann and Matthaei, 1996). Moreover, leptin mRNA and plasma leptin levels are closely associated with the body fat mass (Shan *et al*., 2008).

After puberty, testosterone further modulates leptin synthesis and secretion via sex steroid receptor-dependent transcriptional mechanisms (Machinal *et al*., 1999). In addition, testosterone has been shown to regulate both leptin mRNA expression and leptin production in cultured human fat cells (Wabitsch *et al*., 1997a). Leptin is secreted from adipose cells and is transported via blood to the hypothalamus where it binds to leptin receptor (Ob-Rs). There is evidence that the expression of leptin receptor was modulated by sex steroid hormones like estrogen (Kimura *et al*., 2002).

Thus, it is possible that lake of testosterone induced castration could regulate the expression of leptin and its receptor as part of its mechanism of action. Indeed, evidence indicates that the relative amount of androgens play an important role in determination of the brain's sensitivity to the catabolic actions of leptin (Clegg *et al*., 2006). The objective of this experiment was to test if castration modulated leptin and its receptor in male pigs. To address this, we investigated the expression of Ob mRNA in adipose tissue and serum leptin concentration as well as the expression levels of Ob-Rb in the hypothalamus from castrated and intact male pigs at two slaughter ages.

MATERIALS AND METHODS

Animal: Sixteen pairs of male Landrace x Yorkshire piglets were selected from 6 L according to a matched-pairs

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Table 1: Sequences for real time PCR primers

Target[a]	Primer sequences[b]	Genbank accession	Product size (bp)
β-actin	S: 5'-ACTGGGACGACATGGAGAAGA -3' AS: 5'-TTGGCTTTGGGGTTCAGG -3'	AY550069	114
Ob	S: 5'-GTCTTCCTTCCTGTTCCATCT C -3' AS: 5'-CAACCAGAATACACCGCATAA -3'	NM_213840	201
Ob-Rb	S: 5'-CTCGAATAGCTCATGGGAGATAGAA -3' AS: 5'-TGAGAAGGAAAGGTGTGGTGAA -3'	AF0924229	91

[a]Ob-Rb (leptin long isoform receptor), [b]S: Sense primer; AS: Antisense primer

design. Each pair was composed of two male full sibs with the similar body weight and one of the pigs from each pair was randomly selected and castrated at 35 days old. Pigs were group-penned and each group consisted of two pigs of each type. They had *ab libitum* access to commercially available grower and finisher phase diets and water until slaughter. Feed consumption was recorded by pens of pigs. Animals were managed under normal husbandry conditions and all experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of Zhejiang University.

Growth performance and body fatness: Eight pairs of pigs were randomly selected and slaughtered at 147 days old; the others were slaughtered at 210 days old. Before slaughter, all animals were fasted for 24 h. After slaughter, the carcasses were eviscerated according to standard commercial procedures. At 45 min postmortem, back fat thickness was measured using a ruler in the left side of the carcass at five locations (shoulder, thorax-waist, buttock, between the 13 and 14th rib and between the 6 and 7th rib). The carcass weight, carcass fat weight and leaf fat weight were recorded. Adipose tissue samples (subcutaneous fat and leaf fat tissue) were collected from the left side of the carcass. The boundaries used to dissect the hypothalamus were the cranial edge of the optic chiasm, the caudal edge of the mammillary bodies, and dorsally by the hypothalamic sulcus (Kojima *et al*., 2007). All tissues were collected within 30 min and frozen in liquid nitrogen, then stored at -70°C for isolation of total RNA and analysis of gene expression.

Serum hormones analysis: Blood samples were collected from each pig at slaughter during exsanguinations and than kept at 37°C for 2 h and centrifuged for 10 min at 3000×g at 4°C. Serum was collected and stored at -70°C for subsequent determination of hormones. Serum concentrations of testosterone and leptin were measured with commercial RIA kits (Beijing North Institute of Biotechnology, China).

Total RNA isolation and cDNA synthesis: Total RNA from subcutaneous and leaf fat adipose tissue and hypothalamus were isolated using Trizol reagent (Invitrogen) and chloroform according to the manufacturer's instructions. The RNA was run through a purification process using RNeasy Mini Kit (Qiagen). The quality and concentration of the RNA were determined by measuring the absorbance at 260 and 280 nm and RNA integrity was confirmed by agarose gel electrophoresis. For each sample, 1 μg of total RNA was used to synthesize the first cDNA using a MMLV-RT kit (Promega) according to the manufacturer's protocol.

Real time-PCR analysis of gene expression: Quantitative Real-Time PCR was carried out using the ABI StepOne Plus Real-Time PCR Detecting System (Applied Biosystems). Primers were designed to be an appropriate length and had optimal annealing temperature (Tm) of 60°C to increase precision of the qRT-PCR measurements (Table 1). Melt curve analysis was performed on all real time PCR reactions to confirm specificity and identify of the real time PCR products.

A Nontemplate Control (NTC) was run for every assay. A constant amount of cDNA (1 μL) was used for each real time RT-PCR measurement and three technical replicates were performed in duplicate for each gene. The amplifications were performed in 20 μL reaction mixtures containing 10.4 μL of SYBR Premix Ex Taq (2×) (Takara), 0.4 μL of each primer (10 μM), 7.8 μL distilled water and 1.0 μL cDNA. The cycling conditions consisted of 40 cycles of 95°C for 5 min, 60°C for 30 sec and 72°C for 20 sec. The PCR efficiencies for selected genes and β-actin were closed to 1. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real time RT-PCR data.

Statistical analysis: All statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Data for growth performance, body fatness, serum hormone concentrations and gene expression levels were analyzed using paired-samples T-test. All data were expressed as mean±SEM. The level of statistical significance was set at $p<0.05$ in all analyses.

RESULTS AND DISCUSSION

Growth performance and body fatness: As shown in Table 2, boars were heavier than barrows at 210 days old ($p<0.05$) but no sex-related differences was found at 147 days old. Similarly, boar had a higher carcass weight at 210 days than barrows ($p<0.05$). Average feed intake

Table 2: Growth performance and body fatness

	147 days		210 days	
Factors	Castrate	Intact	Castrate	Intact
Body weight (kg)	85.38±0.07^{a}	84.00±0.07^{a}	120.6±0.07^{a}	133.29±0.07^{b}
Carcass weight (kg)	60.38±2.49^{a}	58.22±3.82^{a}	85.01±3.97^{a}	95.28±5.50^{b}
Average feed intake (kg day^{-1})	2.54±0.21^{a}	2.17±0.23^{b}	2.81±0.27^{a}	2.64±0.26^{b}
Fat weight (kg)	5.52±0.07^{A}	3.13±0.07^{B}	6.89±0.07^{A}	4.41±0.07^{b}
Leaf fat weight (kg)	0.40±0.04^{A}	0.19±0.02^{B}	0.75±0.14^{a}	0.47±0.11^{b}
Average back fat thickness (cm)	2.63±0.20^{A}	1.63±0.11^{B}	2.79±0.21^{A}	2.14±0.27^{B}

Data are expressed as mean±SEM of eight animals of each group; ^{ab}Means not sharing a common letter of the same age differ significantly ($p<0.05$); ^{AB}Means not sharing a common letter of the same age differ significantly ($p<0.01$)

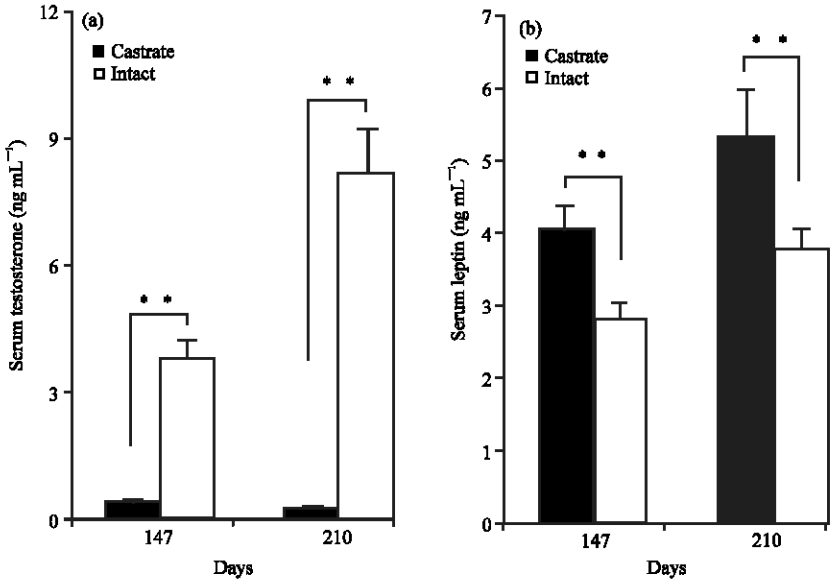

Fig. 1: Serum hormone levels (a) testosterone and (b) leptin in castrated (gray bars) and intact (white bars) male pigs at 147 and 210 days of age. All values are expressed as means±SEM, n = 6 per group, **$p<0.01$

was influenced by castration ($p<0.05$), boars consumed less food than barrows. Compared to boars, barrows had higher carcass fat weight, leaf fat content and Average Back Fat thicknesses (ABF) at 147 and 210 days.

Serum hormone concentrations: As shown in Fig. 1, castration treatment significantly reduced serum testosterone concentration in male pigs at both 147 and 210 days old ($p<0.01$) (Fig. 1a). Serum leptin concentration was higher in barrows compared with boars at both 147 and 210 days old ($p<0.01$) (Fig. 1b).

Gene expression for ob in adipose tissues: To determine whether the castration may modulate leptin expression, the influence of castration on adipose tissue Ob mRNA expression by quantitative real-time RT-PCR was studied. As is shown in Fig. 2, both Subcutaneous Fat tissue (SC) and Leaf Fat tissue (LF) Ob mRNA expression levels were up-regulated by castration at 210 days old ($p<0.05$) (Fig. 2a, b).

Gene expression for Ob-Rb in the hypothalamus: Figure 3 shows the levels of mRNA expression of Ob-Rb in the hypothalamus from barrows and boars. There was an inverse relationship between serum leptin and hypothalamus Ob-Rb mRNA expression, although hypothalamic Ob-Rb mRNA expression were not significantly different between castrated and intact male pigs at both 147 and 210 days old ($p>0.05$).

Previous studies have demonstrated that castration induced changes in body weight and body fatness (Sather *et al*., 1999; Latorre *et al*., 2003). In the study, castrated male pigs gained fat at both 147 and 210 days whereas the body weight is only slightly higher at 147 days. The reason could be due to the actions of testicular steroids. Testosterone increases muscle mass and protein synthesis in muscle and intact males have greater muscle mass and less fat than castrates (Bardin, 1996). Muscle mass constitutes approximately 40-50% of total body mass, so the intact males have a higher body weight due to the stimulation effect of testosterone on the

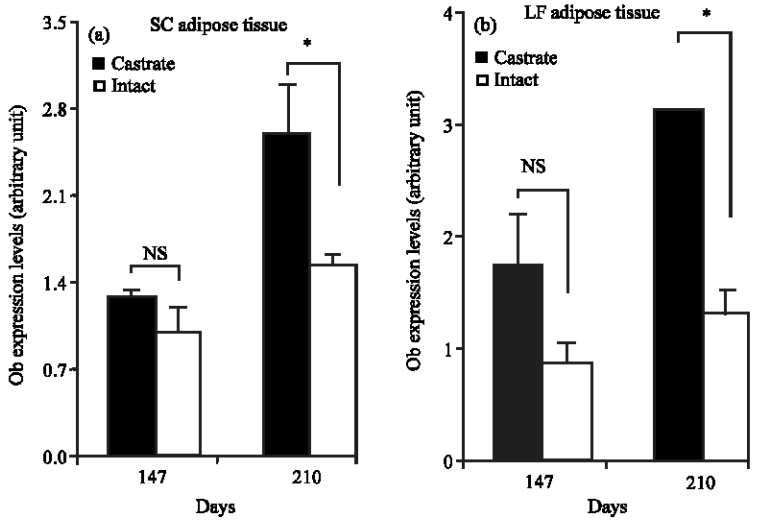

Fig. 2: Expression Ob mRNA in adipose tissue (a) subcutaneous fat tissue and (b) Leaf fat tissue in castrated (black bars) and intact (white bars) male pigs at 147 and 210 days of age. All values are expressed as means±SEM, n = 6 per group, *p<0.05, NS; p>0.05

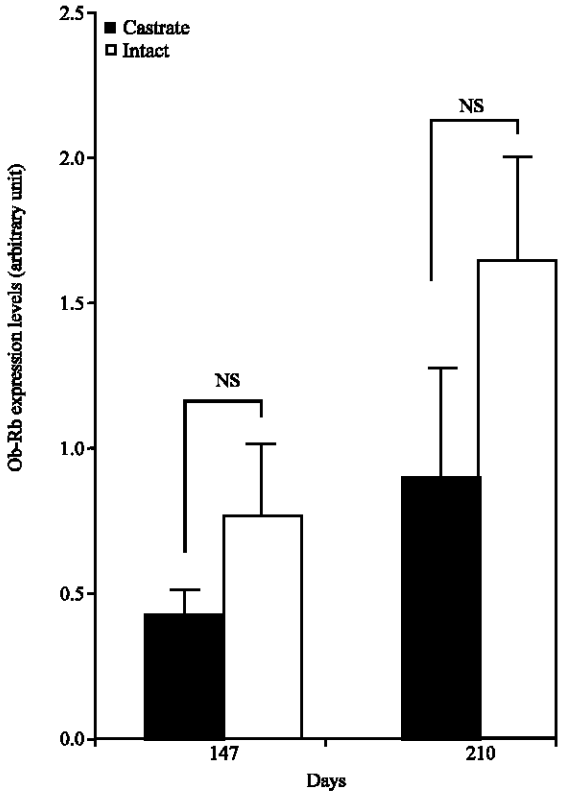

Fig. 3: Expression of the Ob-Rb in the hypothalamus of castrated (black bars) and intact (white bars) male pigs at 147 and 210 days of age. All values are expressed as means±SEM, n = 6 per group, NS; p>0.05

muscle growth after 147 days, even though castrated males gained more fat over the period. The results were consistent with those reported by others (Knudson *et al*., 1985; Bonneau, 1998; Zeng *et al*., 2002). Bauer *et al*. (2009) reported that testosterone influenced negatively feed intake. An increased average daily feed intake in castrated male pigs was found which is consistent to those reported by others (Sather *et al*., 1999; Latorre *et al*., 2003; Pauly *et al*., 2008).

The study demonstrated that castration of male pigs leads to a significant increase of serum leptin levels. In other words, the lack of testosterone is responsible for the increase in the serum leptin levels in castrated male pigs. This agrees with the inverse correlation between serum testosterone and leptin in men which was observed previously (Luukkaa *et al*., 1998). Similarly, Baltaci *et al*. (2006) found castration procedure significantly increases plasma leptin in rats and testosterone administration also inhibits the increase caused by castration in leptin levels. Thus, it can conclude that testosterone may down-regulation serum leptin.

It observed that the levels of adipose (SC and LF) Ob mRNA in castrated male pigs increased compared to intact males. The results are consistent with those of Wabitsch *et al*. (1997b) who showed that testosterone decrease Ob mRNA in human fat cells. The observations are also partly consist to those found in rats where study have demonstrated that castration induced an increase in Ob gene expression in perirenal adipose tissue and inversely a decrease in femoral subcutaneous adipose tissue (Machinal *et al*., 1999). These observations suggest that castration modulates adipose Ob mRNA

expression. The effects of testosterone seem to be mediated through the adipocyte androgen receptors (Dieudonne *et al*., 1995) because cyproterone acetate, apotent antagonist of androgen receptors, prevents the negative influence of androgens on Ob gene expression.

Leptin receptor transmits the satiety effect of leptin to the central nervous system. Although several splice variants of the leptin receptor are known, Ob-Rb is the critical variant for regulating energy balance (Chen *et al*., 1996). In fact, it found that the hypothalamic Ob-Rb mRNA expression was comparable in castrated and intact male pigs, consistent with the findings of Clegg *et al*. (2006) who observed no differences in hypothalamic Ob-Rb expression between male and castrated male rats although, serum leptin was higher in castrated male rats. In addition, Anukulkitch *et al*. (2007) also found no significant difference in hypothalamic mRNA expression of Ob-Rb between castrated and intact male rams at different ages. If the proteins levels of Ob-Rb were also comparable between barrows and boars, it is possible that the lack of testosterone induced by castration results in less sensitivity to leptin in male pigs. Similarly, Ainslie *et al*. (2001) reported that impaired central leptin sensitivity was caused by ovariectomy operations in rats.

CONCLUSION

The present study investigated the effect of castration on modulating of leptin and leptin receptor in the hypothalamus from male pigs. Although, changes in gene expression at the level of transcript do not necessarily imply changes at the level of protein function, the results gave a good indication of the effect that castration may have on modulating of leptin and leptin receptor in male pigs. Adipose Ob mRNA expression and serum leptin concentration were significantly increased by castration. However, the effect of castration on mRNA expressions of Ob-Rb in the hypothalamus was found to be minor in male pigs. Finally, The results indicated that the lack of testosterone induced by castration may decrease leptin sensitivity in male pigs.

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