THE OBESE GENE AND VOLUNTARY FEED INTAKE IN ADULT FEMALE PIGS.

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SUMMARY

Leptin is thought to play a major role in the control of voluntary food intake. Twenty non-pregnant and non lactating Largewhite x Landrace sows of approximately 110 kg were randomly allocated into 2 groups and received either a high protein (HP, 15.6% crude protein and 14.5 MJ DE/kg) or low protein (LP, 5.8% crude protein and 14.6 MJ DE/kg) diet over a period of 114 days. The resulting fat and lean groups and 2 different diets were used in a 2x2 factorial arrangement to measure VFI for 14 days after completion of the first period (114 days). Blood and fat were sampled throughout the 114 days and analysed for *ob* gene mRNA and leptin in plasma. The body composition of the pigs was manipulated successfully and there was a significant increase in leptin concentration over time but there was no affect of fat % on *ob* mRNA levels, leptin levels or voluntary food intake.

Keywords: Obese gene, Leptin, Voluntary food intake, Pigs

INTRODUCTION

According to the lipostatic theory, voluntary feed intake (VFI) should decrease in response to increased fat deposition to achieve energy balance. However, there are reports that are not consistent with this theory (Nakavisut *et al.* 1997; Steele *et al.* 1982). In contrast, feed intake in lactating sows has been shown to be associated with an accumulation of body fat (Mullan and Williams, 1989; Weldon *et al.* , 1994; Revell *et al.*, 1993). That is, the fatter the sow at the end of gestation the less she will eat during lactation. It is possible that physiological state affects the relationship between fat composition and VFI and may explain these contrasting results. Moreover, *ob* gene expression has always been higher in obese humans when compared to normal humans at the same age. The increase in expression of the *ob* gene due to increasing fat composition is evident across most species investigated. However, these previous studies did not investigate a link between the *ob* gene expression and VFI. This experiment was designed to assess the relative expression of the obese gene and VFI of fat and lean sows that are not lactating or pregnant. The hypothesis to be tested in this experiment is that *ob* gene expression varies with adipose tissue deposition and there is a corresponding variation in VFI.

MATERIALS AND METHODS

Feeding trial After an acclimatisation period of 30 days, 20 non-pregnant and non lactating Largewhite x Landrace sows of approximately 110 kg were randomly allocated into 2 groups of 10 and received either a high protein (HP, 15.6 % crude protein and 14.5 MJ DE/kg) or low protein (LP, 5.8 % crude protein and 14.6 MJ DE/kg) diet over a period of 114 days. Dietary treatments were randomly allocated to the pigs. The resulting fat and lean groups and 2 different diets were used in a 2x2 factorial arrangement to measure VFI for 14 days after completion of the first period (114 days). These diets and feeding regimes, adapted from Revell *et al* (1993), were used to obtain pigs of

similar weight but of different body fat composition at the end of the dietary treatment. The pigs were weighed and back fat thickness was measured at P2 position using an ultrasonic device (Meritronics) every 2 weeks throughout the experiment.

Measurement of VFI and body composition of pigs. During the VFI period, pigs from fat and lean groups were allowed *ad lib* feeding on either the LP or HP diet 3 times a day at 0900, 1400 and 2000. Feed was offered to ensure that there was more than enough feed every feeding time. The amount of feed offered was recorded and leftovers were removed and measured at 2030 every day for 14 days. The body fat content of pigs (g/kg liveweight) was estimated from backfat thickness using the equation derived by Mullan (1991) for a similar genotype to that used in this experiment. The weight of lean tissue was calculated by difference between live weight and the weight of body fat, assuming that lean and fat represent 95% of the total body weight.

Blood and fat sample collections. On day -1 (1 day before the commencement of dietary treatments), day 55 and day 110 of the treatment, blood samples were collected from the jugular vein and fat samples were collected from the subcutaneous adipose tissue at the P2 position. Blood samples were transferred into tubes containing 0.1 ml Heparin and centrifuged at 3500 rpm for 15 minutes. Plasma was decanted to a fresh tube and frozen in a -20°C freezer until analysis for leptin. Fat samples were immediately wrapped with aluminium foil, immersed in liquid nitrogen and then transferred to a -86°C freezer prior to extraction of total RNA.

Measurement of obese gene expression. Total RNA was extracted from fat samples using RNAzol^{TM}. Co-amplification of the *ob* mRNA and β actin mRNA was performed by quantitative RT-PCR (Gause and Adamovicz, 1995). The β actin gene, a housekeeping gene, is expressed constitutively by every cell and as such can be used as an internal standard for quantitative RT-PCR. The *ob* mRNA was the target to be quantified while the β actin mRNA was used as an endogenous control. The primers used in RT-PCR to detect *ob* mRNA were the same as those used previously (Nakavisut *et al.* 1997). The primers used for detecting β actin mRNA produced a 477 bp product. A Multi-Species Leptin Radioimmunoassay (RIA) Kit (Linco) was used to quantitate leptin protein in plasma. Assayzap (Biosofts) was used to analyse the results.

RESULTS

Growth and body composition over the dietary treatment period. Pigs were of different body composition and of similar live weights at the end of the 114 day dietary treatment. Pigs in the fat group had 40% higher fat content than the lean group ($p<0.001$). Body fat accounted for 86% of the body weight gain in the fat group and only 56% of the gain in the lean group ($p<0.001$). It was estimated that the fat pigs had approximately 21 kg more body fat and 16 kg less lean than their lean counterparts ($p<0.001$). One of the pigs in the lean group was removed from the experiment due to arthritis and consequently data from 9 pigs from the lean group were analysed.

Expression of the obese gene and leptin detection. The total RNA extracted from fat tissue was of sufficient quality and quantity for quantitative RT-PCR analysis. Table 1 shows the quantitation analysis of the *ob* mRNA expression from the agarose gel using the Fluoroimager (Perkin-Elmer) and the program ImageQuant (Molecular Dynamics Inc.). The expression of the *ob* gene was found to increase with time and fat content, but the difference in expression between treatments was not

significant. However, the relative expression of the obese gene to β actin gene for the lean group was significantly higher than that for the fat group at day 110 of the dietary treatment (Table 1). The intraassay coefficient of variation for the RIA detection was 3.7% and the interassay CV was 5.1%. Leptin concentrations for the fat group had a tendency to be higher than the lean group across sampling times despite no statistical significance. The differences in leptin concentrations between fat and lean groups became greater with treatment time. There were approximately 0.2 ng/ml and 0.5 ng/ml higher leptin in fat pigs than lean counterparts at day 55 and day 110 while the average concentration of leptin for both groups was about the same before the treatment started (3.2 vs 3.1 ng/ml for Fat and Lean). However, there was no statistically significant difference in the plasma leptin concentration between treatment groups.

Table 1. Quantitative analysis of the expression of the *ob* gene a) in arbitrary unit and b) relative to β-actin

a) *ob* gene expression (arbitrary unit)

	Fat	Lean	SEM	p-value
day -1	0.19	0.20	0.012	0.676
day 55	0.27	0.23	0.167	0.081
day 110	0.48	0.46	0.015	0.518

b) *ob* gene expression relative to β actin

	Fat	Lean	SEM	p-value
day -1	0.52	0.46	0.043	0.380
day 55	0.57	0.61	0.027	0.261
day 110	0.61	0.67	0.014	0.007**

Voluntary feed intake. One of the pigs in the fat group offered HP diet was removed from the experiment due to arthritis and consequently data from only 4 pigs in the group was analysed. VFI was not affected by either body fat content or the protein content of the diet. The average VFI during the 14-day ad lib feeding period was 4.26 kg/day for fat pigs and 4.44 kg/day for lean pigs. Average VFI was 4.08 and 4.62 kg/day for pigs offered HP and LP. There was no significant interaction between the body fatness of the pigs and the protein content of the diets (P=0.147)

Correlation analysis. All quantitative variables including body weight at day 110, fat content, ob gene expression, ob gene expression relative to β-actin, leptin level and VFI measured in this experiment were analysed for correlations. Only 18 data for each variable were analysed since 2 pigs were terminated from the experiment. None of the variables studied was found to have a strong relationship to any other. VFI had a negative relationship with plasma leptin level, ob gene expression. Body fat content and leptin levels were associated with ob gene expression.

DISCUSSION

We were successful in using diets, different in their energy to protein ratios, to produce pigs of similar liveweights but significantly different body compositions. More specifically, we were able to produce pigs with a significantly different amount of fat. However, our results also do not support the hypothesis that the VFI of sows is influenced by the amount of body fat. There was no strong relationship among the measured variables, back fat thickness, *ob* gene mRNA levels, leptin levels and VFI. In addition, the findings were not consistent with the lipostatic theory, since VFI during the *ad lib* feeding period was independent from the body fat composition. Despite lean pigs having a

higher average VFI than their fat counterparts (4.44 vs 4.26 kg/day), it was not statistically significant because of a high variation between individuals within treatment groups (CV=19.2%). Steele *et al* (1982) also showed that feed intake did not differ between high fat, low fat or contemporary lines of pigs. In addition, Nakavisut *et al* (1997) reported that there was no affect of fat composition on the VFI and obese gene expression in young pigs that were growing rapidly. They suggested that it was possible that the obese gene or its receptor may not be functional in pigs at that physiological stage of development since the pig is attempting to grow as rapidly as possible. However, our results were not consistent with the results of Revell *et al* (1993) who showed that VFI in lactating sows was negatively associated with body fat composition at the end of gestation. This is surprising since the only difference between the two experiments was that we used sows that were not lactating. It is apparent from this, and the report of Nakavisut *et al* (1997), that physiological state influences the relationship between body fat composition and the control of VFI. Another reason for the inconsistency may be that the difference in body fat composition between treatment groups may not have been biologically significant in terms of the influence on VFI. However, the difference in body fat composition between fat and lean treatments in this experiment was virtually identical to the difference reported by Revell *et al* (1993).

The hypothesis that fat pigs would express higher levels of the ob gene than lean pigs was also not supported by our results. Fat pigs tended to express higher leptin mRNA and protein levels but the magnitude of the difference was not significant. In contrast, Ramsay *et al* (1998) reported that total RNA derived from genetically selected high fat pigs contained 113% higher ($P<0.05$) concentrations of obese mRNA than contemporary crossbred pigs. In addition, relative levels of porcine leptin from genetically obese swine were approximately 306% higher ($p<0.05$) than levels present in sera from contemporary crossbred swine. It is apparent from these results that there is a genetic basis for the control of leptin levels and there is the potential to select animals to influence VFI. Importantly, there is a need to examine the structure and regulation of the *ob* gene in different breeds to determine the reason for the differences in expression of the *ob* gene, rather than examine expression in animals that have been forced to change body composition through dietary manipulation.

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