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Original Research Paper

COULD LEPTIN LEVELS PREDICT BODY WEIGHT IN BOARS?

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Summary: Leptin is a regulator of body weight homeostasis in mammals. Blood levels of leptin are in direct correlation with the amount of adipose tissue in the body. In the present study, leptin levels and body weight were monitored through postnatal development in young boars. Sixteen boars were followed from 6 to 22 weeks of age. There was no significant correlation between leptin levels and body weight at the same ages. However, strong positive correlation was found between leptin levels in 6 weeks old piglets and body weights during puberty between 16 and 22 weeks of age. Correlation was especially strong ($p<0.001$) between leptin levels at 6 weeks and body weight of boars at 22 weeks of age. The results of this study therefore suggest that leptin levels in young piglets might be used as a prediction for body weight and growth during first 6 months of age.

Key words: physiology; leptin-blood; body weight; swine

Introduction

Leptin is a peptide hormone, produced by white adipose tissue (1, 2). Leptin gene was first identified in ob/ob strain of mice, that carry mutations in leptin gene and are consequently, severely obese (3). A similar obese strain of mice, db/db mice, carries a mutation in leptin receptor gene, a member of cytokine receptors (4). Leptin levels in blood are in direct positive correlation with the amount of white adipose tissue in the mammalian body (1, 2). Leptin enters the hypothalamus, the main site of leptin action, through median eminence. Increased levels of leptin cause decreased neuropeptide Y and agouti related protein expression and increased levels of melanocortin in hypothalamus. This pattern of neuropeptides expression results in decreased feeding and increased energy expenditure, while reduced levels of leptin have opposite effects, resulting in increased feeding. This mechanisms thus ensure long term body weight homeostasis (reviewed in (5, 6). However, many other molecules such as cocaine and amphetamine related transcript (CART), orexins, corticotropine realizing factor (CRF), endorphins and others are also involved in either regulation of appetite or energy consumption, although their connection to leptin is less established (reviewed in (5, 6). Mechanisms regulating leptin expression in adipose tissue are not clear yet. Leptin is constitutively expressed in white adipocytes. However, leptin levels are also dependent on the levels of glucocorticoid hormones and sex steroids (7) and estrogens can directly stimulate leptin expression in adipose tissue both in vitro and in vivo (8, 9). Through postnatal development, leptin levels steadily rise before and during puberty (10, 11) and leptin is also thought to be a metabolic signal, permitting entry into puberty (12) and some studies in humans suggested correlation between postnatal energy stores and leptin levels and obesity later in life. In the present study, we report that leptin levels in piglets are also predictable for total body weight in pubertal boars, independent on the amount of back fat.

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Material and methods

Animals

Sixteen boars were kept in standard condition and fed regular chow at commercial pig farm Ihan. Every 2 weeks from 6 weeks of age to 22 weeks of age, all boars were weighed and had blood samples taken. Blood was collected from vena cava. Evacuated tubes with separation gel (Vacutainer®, Becton Dickinson, Heidelberg,

Table 1: Leptin levels at different ages from 6 weeks until puberty (mean ± S.E.)

Age (weeks)	Leptin levels (ng/ml)
6 weeks	1.51 ± 0.12
8 weeks	2.44 ± 0.12
10 weeks	1.04 ± 0.03
12 weeks	1.95 ± 0.05
14 weeks	1.91 ± 0.07
16 weeks	2.49 ± 0.12
18 weeks	2.22 ± 0.11
20 weeks	2.20 ± 0.21
22 weeks	3.26 ± 0.15

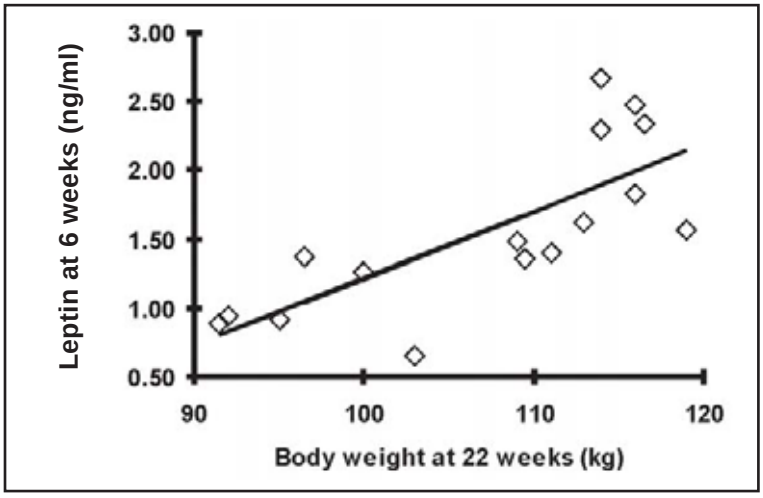

Diagram 1: Line fit plot for linear regression analysis to compare leptin levels at 6 weeks and body weight at 22 weeks

Germany) and anticoagulant were used for sample collection. Blood was centrufuged at 1000 rpm for 5 minutes and plasma was removed and stored at -20oC until used. All animal work was approved by Veterinary commission of Slovenia. At 16, 20 and 22 weeks, back fat thickness was measured using ultrasound device Krautkramer FGM2 (Agfa gevaert N.V., Mortsel, Belgium) at two points in lumbal region about 3 cm laterally from the spine. Average of two measurements was used for statistical analyses.

Hormone measurement

Serum levels of leptin were measured by Multi-species leptin RIA kit (LINCO Research, Inc., St. Charles, MO, USA), validated for porcine leptin, following original instructions of manufacturer (http://www.lincoresearch.com/products/xl-85k.html). For low values (mean 3.5 ng/ml) intra- and inter-assay coefficients were 7.71% and 8.72% respectively, for high values (mean 28.47 ng/ml) they were 8.73% and 5.71%, respectively.

Statistical analyses

Data (hormone levels, body weights and back fat thickness) were subjected to statistical analyses using SigmaStat software (Jandel scientific, Erkrath, Germany). Pearson correlation coefficient and linear regression was calculated to predict correlations between leptin levels, body weights and back fat thickness.

Results

Boars' body weight increased linearly from 6 weeks of age as expected. The mean body weight at the beginning of experiment was 11.59 ± 0.37 kg (mean ± S.E.) and at the conclusion of experiment 107.32 ± 2.16 kg (mean ± S.E.).
Leptin levels at 6 weeks of age were 1.51 ± 0.12 ng/ml (mean ± S.E.). Thereafter, the leptin levels steadily increased to reach the levels 3.29 ± 0.15 ng/ml (mean ± S.E.) at 22 weeks of age (Table 1). Thickness of back fat was measured at 16, 20 and 22 weeks of age. Thickness increased from 7.70 ± 1.25 mm at 16 weeks to 13.73 ± 2.81 mm at 22

Table 2: Pearson correlation coefficients between leptin levels at 6 weeks of age and body weights (BW) from 12 to 22weeks (* p<0.05, ** p<0.01, *** p<0.001)

Body weight	Leptin(6 weeks; ng/ml)
6 weeks	R = 0.1466
8 weeks	R = 0.4985
10 weeks	R = 0.4919
12 weeks	R = 0.5379*
14 weeks	R = 0.6116*
16 weeks	R = 0.7059**
18 weeks	R = 0.7551***

weeks (mean ± S.E.). Interestingly, statistical analyses did not show statistically significant correlation between leptin levels and measured back fat thickness at 16, 20 and 22 weeks. Similarly, there was no significant correlation between body weight and leptin levels at particular ages.

However, when all the data was subjected to correlation analyses, strong correlation was found between leptin levels at 6 weeks of age and body weights from 16 weeks until the end of experiment at 22 weeks of age (Table 1). Linear regression analyses confirmed these results and showed very strong correlation between leptin levels at 6 weeks of age and body weight at 22 weeks (Diagram 1). However, correlation between leptin levels at 8 weeks and body weight at 15 to 22 weeks disappeared and there was no correlation between body weight and leptin levels at any other age.

Discussion

Leptin is a central regulator of body weight in mammals. Secreted by adipose tissue into the circulation, it transmits signals about body fat deposits to the hypothalamus. Leptin levels are in many species strongly correlated with the amount of adipose tissue in the body (1, 2). In the hypothalamus, leptin is thought to regulate feeding and energy expenditure by regulating expression of several neuropeptides. The most important appears to be the neuropeptide Y/melanocortin systems (5, 6, 13)}. Besides regulating body weight homeostasis, leptin is thought to have several other functions. During postnatal period, leptin levels steadily rise (10, 11) and leptin appears to be one of the permissive factors allowing entry into puberty (12). Intriguing is also the difference between males and females in the levels of leptin that persist even after adjustment of data for adiposity (14, 15). The regulation of leptin expression in the adipocytes is not yet clear, although insulin, growth hormone, glucocorticoids and estradiol have all been implicated in the regulation of leptin gene expression (1). It is well documented that leptin levels are in direct positive correlation with the amount of white adipose tissue in many species. However, in our study, we failed to find such correlation at any studied age. One possible explanation is that boars differ from humans and rodents and there are possibly some other regulatory mechanisms that modulate levels of leptin secreted from white adipocytes. Perhaps more feasible explanation is that leptin levels and amount of adipose tissue are not directly correlated in fast growing animals, but would become correlated with the amount of adipose tissue in fully grown animals. However, in our study, we found that leptin levels in young boars are also strongly correlated with the body weight at around 6 months of age. This is intriguing, as it is difficult to explain, how leptin could act as a predictor for body weight several months later. One possible explanation is that animals with greater number of adipocytes would have higher levels of leptin (due to bigger number of adipocytes) already at early age, and higher number of adipocytes will ultimately result in higher body weight due to larger proportion of adipose tissue. However, the measurements of back fat thickness in studied boars did not show correlation between back fat and leptin levels at 22 weeks of age or correlation between leptin levels at 6 weeks and back fat thickness at either 16, 20 or 22 weeks. Another possible explanation could be connected to growth hormone. Houseknecht et al. (16) have reported that growth hormone in cattle upregulate leptin expression both in vivo and in vitro in the presence of insulin and dexamethasone. Therefore, higher levels of

leptin in young piglets could be at least partially due to higher intrinsic levels of growth hormone, and higher levels of growth hormone at early age could be responsible for bigger body weights in boars at 22 weeks of age. In conclusion, our study shows that leptin levels in 6 weeks old piglets are in strong correlation with body weights from 12 to 22 weeks and could be therefore potentially used in young animals as a marker for predicting pubertal body weight in boars.

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ALI RAVEN LEPTINA LAHKO NAPOVE TELESNO TEŽO MERJASCEV?

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Povzetek: Leptin je peptidni hormone, ki v sesalskem organizmu ureja telesno težo. Proizvajajo ga bele maščobne celice, njegova koncentracija v krvi pa je v neposrednem razmerju s količino bele tolšče. V pričujoči raziskavi smo ugotavljali povezanost med telesno težo in vrednostmi leptina v krvi mladih merjascev. Šestnajst merjascev smo spremljali od 6. do 22. tedna starosti. Meritve niso pokazale statistično značilnih povezav med vrednostmi leptina ter telesno težo v isti starosti. Ugotovili pa smo statistično zelo značilno povezavo med vrednostmi leptina pri mladih merjaščkih (6 tednov) in telesno težo pri merjascih v puberteti (od 16 do 22 tednov). Povezava je bila zelo močna med vrednostjo leptina pri 6 tednih in telesno težo pri 22 tednih ($p < 0.001$). Rezultati te raziskave tako kažejo na možno vlogo vrednosti leptina pri mladih merjaščkih kot pokazatelja rasti in telesne teže pri živalih v puberteti.

Ključne besede: fiziologija; leptin-kri; telesna teža; prašiči