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PLASMA LEPTIN LEVELS AND INTRAMUSCULAR FAT CONTENT IN BEEF FROM CROSSBREED JAPANESE CATTLE

Jochen Wegner[1], Changping Xie[1], Phillip Huff[1], Falk Schneider[1], Priya S. Mir[2], Zahiruddin Mir[2], E. Chris Kazala[3], Fred J. Lozeman[3], Randall J. Weselake[3] and Klaus Ender[1]

[1]Research Institute for Biology of Farm Animals, D-18196 Dummerstorf, Germany;
[2]Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada T1J 4B1;
[3]Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4

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Background:
Leptin is a hormone secreted by adipocytes that regulates feed intake and energy expenditure and consequently influences the fat deposition in animals and humans. Obese rodents and humans have been shown to have elevated levels of circulating leptin with the exception of animals in which the leptin gene is mutated. Leptin resistance is common in most obese humans and animals with leptin serving as an indicator of fat content. The regulation of leptin is integrated into a broad regulatory network including other hormones and cytokines. Peripheral hormones, including insulin and glucocorticoids, stimulate the expression of leptin. The general biological role of leptin has been reviewed thoroughly (Friedman and Halaas, 1996; Houseknecht et al., 1998). A detailed knowledge of the mechanism of fat tissue development is crucial to the treatment of obesity in humans and manipulation of meat production in livestock. Leptin may play a role in the regulation of regional fat distribution. The deposition of intramuscular (i.m.) fat, termed marbling in meat science, is considered to be positively related to meat quality (Savell et al. 1988; Wheeler et al. 1994). Although marbling may be a highly heritable trait in cattle (Shackelford et al. 1994), it is still unclear which genes contribute to the deposition of i.m. fat. Recent reports have addressed the chromosomal locations to which marbling traits have been mapped (Zhang et al., 1994; Tartaglia et al., 1995). The Wagyu breed of beef cattle is distinguished by a high amount of i.m. fat and thus good meat quality. It is widely included in animal breeding programs designed to improve the marbling capability of cattle.

Objectives:
The objective of this study was to investigate the association between plasma leptin levels and fat content in Wagyu crossbred Japanese cattle. With the elucidation of the relationship between leptin levels and i.m. fat content, the role of leptin in marbling will be further understood.

Material and Methods:
Plasma was obtained from 24 crossbred Wagyu steers, fed *ad libitum*, on two occasions: once while on a low barley grain (35% of dry matter) diet, 16 weeks prior to slaughter, and again while on a high-energy finishing diet, 4 weeks prior to slaughter. The finishing diet consisted of 80% rolled barley and 20% barley silage on a dry matter basis, with 1% mineral mix (Mir et al., 1999). All animals were maintained on the same diet and reared in group pens, as described for year 2 in Mir et al., (1999). Numerous measures of carcass fatness were obtained after slaughter, including subcutaneous (s.c.) fat depth, marbling score, solvent-extractable fat, and a calculation of lean meat yield. The lipid content of the 10^{th} to 12^{th} rib *longissimus* muscle samples was obtained via the soxhlet extraction method using petroleum ether as the solvent, and determined gravimetrically after evaporating the extracting solvent. Blood for the leptin assay was collected with K_3EDTA-coated evacuated tubes, and held on ice for approximately 1 to 2 hours. Blood samples were subsequently spun at 1700 x g for 15 minutes and plasma was transferred to fresh tubes. Plasma samples were then stored frozen at -80°C, thawed once to aliquot plasma for transfer, and frozen immediately at -80°C before being shipped to Germany on dry ice. The concentrations of leptin were analyzed from 0.5 mL bovine plasma by the commercially available multi-species I^{125} radioimmunoassay (Linco Research, Inc., St. Louis, cat. XL-85K). This assay was developed for use in human research and contains human leptin as the standard. The Linco assay has been validated for use with bovine serum (Minton et al., 1998). Data were analysed using The SAS-System for Windows v. 6.12. Significance was calculated using a paired t-test.

Results and Discussion:
The ribeye area and the lean meat yield values indicated that steers containing a percentage of the breed Wagyu can produce an acceptable lean meat content within the age of 17 months. The degree of marbling ($P<0.05$) and the chemical fat content (based on a dry and wet weight) ($P<0.01$) of the *longissimus* was higher in 50% and 75% Wagyu steers than 0% Wagyu steers. The differences between the 50 and 75% Wagyu are not so clear. The 75% Wagyu cattle grew slower and did not mature as fast as the 50% Wagyu. Subsequently, the 75% Wagyu cattle were slaughtered before their intramuscular fat depots were mature. It was observed that the most significant effect ($P<0.001$) was in circulating leptin. Before finishing, the 0% Wagyu cattle contained 3.15 ng/ml and the 75% contained 4.57 ($P<0.001$). After finishing, it was found that the leptin levels in both Wagyu groups increased approximately 2-fold. Timtchenko et al. (1999) found that an increased fat storage in mouse lines selected for extremely high growth was associated with more than a 3-fold elevated leptin level in blood plasma. Minton et al. (1998) found that serum leptin in finishing heifers was positively correlated with carcass fatness. These results, in addition to our findings, support the concept that there is a relationship between circulating leptin and carcass fat in cattle genetically predisposed to good marbling. Further investigation could elucidate this relationship and its potential application in beef production.

Table 1. Wagyu cross-bred cattle analysis

	0 % [1] (a)	50 % (b)	75 % (c)	Significance [9]
Leptin[2](ng/ml) 16 WPS [3]	3.15 ± 0.21 (0.60)[4]	4.32 ± 0.30 (0.86)	4.57 ± 0.24 (0.68)	**ab; ***ac;
Leptin (ng/ml) 4 WPS	3.85 ± 0.65 (1.83)	7.50 ± 0.80 (2.26)	8.78 ± 0.69 (1.95)	***ab; ***ac; *bc;
Marbling Score [5]	8.4 ± 0.18 (0.52)	6.9 ± 0.48 (1.36)	7.1 ± 0.40 (1.13)	*ab; *ac;
s.c. Fat (mm) [6]	11.9 ± 1.08 (3.04)	17.8 ± 1.73 (4.89)	14.6 ± 1.03 (2.92)	**ab;
s.c. Backfat (mm) [7]	10.8 ± 1.29 (3.65)	16.1 ± 1.47 (4.16)	12.5 ± 1.00 (2.83)	**ab; bc;
i.m. TL (% dry weight) [8]	15.3 ± 1.57 (4.44)	21.8 ± 1.91 (5.42)	23.3 ± 1.10 (3.12)	**ab; **ac;
i.m. TL (% wet weight) [8]	4.2 ± 0.48 (1.37)	6.3 ± 0.67 (1.89)	6.8 ± 0.39 (1.11)	**ab; **ac;
Ribeye area (cm^2)	78.4 ± 5.13 (14.5)	80.6 ± 2.80 (7.93)	83.1 ± 3.30 (9.34)	none
Lean Meat Yield (%)	56.7 ± 1.48 (4.19)	53.4 ± 1.64 (4.65)	56.4 ± 1.21 (3.43)	*ab;

[1] The values 0, 50, and 75% represent the percent of the breed Wagyu in the samples.
[2] Leptin was obtained from bovine plasma.
[3] WPS = Weeks Prior to Slaughter.
[4] The values listed represent the mean of n=8 samples including ± standard error of the mean and (standard deviation).
[5] Canadian Grading Agency marbling scores based on an inverse ten point scale with a score of 1 indicating very abundant marbling and a score of 10 indicating a carcass devoid of marbling.
[6] s.c. Fat = Subcutaneous fat depth at 3 separate sites.
[7] s.c. Backfat = Subcutaneous fat depth on the back of the cattle.
[8] i.m. TL (% dry and wet weight) = The lipid content of the 10th to 12th rib *longissimus* muscle samples was obtained via the soxhlet extraction method using petroleum ether as the solvent, and determined gravimetrically after evaporating the extracting solvent.
[9] Significance = The letters a, b, c represent the 0, 50, 75 % content of the breed Wagyu, respectively, ($P < 0.10$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). (Paired t-test calculated using The SAS-System for Windows v. 6.12).

Conclusions:

Increasing the quantity of intramuscular fat while concurrently reducing the quantity of fat in other depots is one of the main goals of beef production. Among the numerous regulatory and metabolic molecules involved in marbling, leptin could prove useful as a target protein in the study of marbling.

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