Relationship of plasma leptin concentration to intramuscular fat content in beef from crossbred Wagyu cattle

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Wegner, J., Huff, P., Xie, C. P., Schneider, F., Teuscher, F., Mir, P. S., Mir, Z., Kazala, E. C., Weselake, R. and Ender, K. 2001. **Relationship of plasma leptin concentration to intramuscular fat content in beef from crossbred Wagyu cattle.** Can. J. Anim. Sci. **81**: 451–457. Plasma leptin concentrations and beef cattle carcass characteristics in eight Continental Crossbred steers [0% Wagyu Cattle (WC)] were compared to crossbred cattle with 50 and 75% WC (eight steers each) genetic makeup to determine if a relationship exists between plasma leptin concentrations and intramuscular fat content (marbling) in beef cattle. Plasma leptin concentrations were measured at two stages of cattle growth, 16 and 4 wk prior to slaughter (WPS). Beef cattle characteristics including marbling score, ribeye area, i.m. total lipid content, and backfat depth were determined, and correlation coefficients obtained between these traits and leptin concentration at both sampling dates. Plasma leptin concentrations increased relative to the lipid content in the 24 steers based on the significant positive correlation observed between plasma leptin and total lipids (% wet weight) from both pars costalis diaphragmatis (p.c.d.)(16 WPS: $r = 0.69$, $P = 0.0004$; 4 WPS: $r = 0.35$, $P = 0.104$) and longissimus (16 WPS: $r = 0.59$, $P = 0.002$; 4 WPS: $r = 0.51$, $P = 0.011$) muscles. A trend was observed, however, at 4 WPS when the groups of varying Wagyu genetics were compared. Plasma leptin was positively correlated with muscle lipid content for the 0% Wagyu cattle (longissimus: $r = 0.62$, $P = 0.103$; *p.c.d.*: $r = 0.40$, $P = 0.410$)but there was almost no correlation in these parameters for the 50% WC (longissimus: $r = 0.11$, $P = 0.797$; p.c.d.: $r = 0.005$, $P = 0.990$). Plasma leptin concentration was negatively correlated with lipid content in the 75% WC (longissimus: $r = –0.60$, $P = 0.120$; p.c.d.: $r = –0.65$, $P = 0.164$). The results suggest that increasing Wagyu genetics negates any relationship between leptin concentrations and i.m. fat content in cattle.

Key words: Wagyu crossbred cattle, meat quality, intramuscular fat, marbling, leptin

Wegner, J., Huff, P., Xie, C. P., Schneider, F., Teuscher, F., Mir, P. S., Mir, Z., Kazala, E. C., Weselake, R. et Ender, K. 2001. **La relation du niveau de leptin de plasma à la teneur en graisse intramusculaire en bovins croisés de Wagyu**. Can. J. Anim. Sci. **81**: 451–457. Des niveaux de leptin de plasma et les caractéristiques de carcasse de bovins dans le métis continental (0% Wagyu (WC), 8 bouvillons) ont été comparés aux bovins croisés avec 50 ou 75% Wagyu (8 bouvillons chacun) pour déterminer si un rapport existe entre les concentrations de leptin de plasma et la teneur en graisse intramusculaire (marbrer) dans la viande bovins. Des niveaux de leptin de plasma ont été mesurés à deux étapes de croissance de bétail 16 et 4 semaines avant l'abattage (WPS). Des caractéristiques de bovins comprenant l'indice de marbrure, la zone de ribeye, le contenu total de lipide, et la profondeur de gras du dos ont été déterminées et des calculs de corrélation ont été exécutés. Les concentrations de leptin de plasma ont augmenté relativement au contenu de lipide car il y avait des corrélations positives significatives observées entre les lipides totaux (% de poids humide) et le leptin de plasma pour les pars costalis diaphragmatis (16 WPS: $r = 0.69$, $P = 0.0004$; 4 WPS: $r = 0.35$, $P = 0.104$) et les muscles longissimus (16 WPS: $r = 0.59$, $P = 0.002$; 4 WPS: $r = 0.51$, $P = 0.011$). On observe une tendance variable à 4 WPS quand les différentes croix sont comparées. Le leptin de plasma est franchement corrélé pour le 0% Wagyu (longissimus: $r = 0.62$, $P = 0.103$; p.c.d.: $r = 0.40$, $P = 0.410$), est presque zéro pour les bovins de 50% Wagyu (longissimus: $r = 0.11$, $P = 0.797$; p.c.d.: $r = 0.005$, $P = 0.990$), et démontre une corrélation négative pour les 75% WC (longissimus: $r = –0.60$, $P = 0.120$; p.c.d.: $r = –0.65$, $P = 0.164$). Les résultats suggèrent que cela la génétique croissante de Wagyu viole n'importe quel rapport entre les concentrations de leptin et la teneur en graisse intramusculaire dans les bétail.

Mots clés: Croises Wagyu, qualité de la viande, graisse intramusculaire, marbrant, leptin

Leptin is a small (16 kDa) hormone-protein secreted by white adipose tissue that regulates feed intake, energy expenditure, homeostatic body weight, and consequently influences fat deposition in both animals and humans (Houseknecht et al. 1998). Obese rodents and humans have been shown to have elevated concentrations of circulating leptin with the exception of animals in which the leptin gene is mutated (Mizuno et al. 1996; Mistry et al. 1997). Leptin resistance is also common in most obese humans and animals (Van Heek et al. 1997; Bjorbaek et al. 1998). Previous

Abbreviations: **i.m. fat**, intramuscular fat; **s.c. fat**, subcutaneous fat; **p.c.d.**, pars costalis diaphragmatis **WC**, Wagyu crossbred cattle; **WPS**, weeks prior to slaughter

studies have shown that leptin serves as an indicator of fat content (Bunger et al. 1999). 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 (Houseknecht et al. 1996; Friedman and Halaas 1998; Ahima and Flier 2000).

A detailed knowledge of the mechanism of adipose tissue development is crucial to the treatment of obesity in humans and manipulation of meat production in ruminants. 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 and Cross 1988; Wheeler et al. 1994). Although marbling may be a highly heritable trait in cattle (Shackelford et al. 1994), it is still unclear which gene(s) contribute to the deposition of i.m. fat. Reports have addressed the chromosomal locations to which marbling traits have been mapped (Zhang et al. 1994; Tartaglia et al. 1995; Stone et al. 1999). The ability to predict the degree of i.m. fat deposition in young cattle would greatly simplify breeding strategies in an attempt to produce high quality marbled beef.

The Wagyu breed of beef cattle has been widely known to deposit large amounts of i.m. fat within muscle tissue (Cameron et al. 1994; Zembayashi et al. 1995). Investigations of the marbling capability of cattle have included the Wagyu breed of cattle (Mir et al. 1999). The objective of this study was to determine if circulating plasma leptin relates to marbling and other carcass characteristics in Japanese Wagyu crossbred cattle.

MATERIALS AND METHODS

Animals and Diet

Crossbred Wagyu steers (n = 24), fed ad libitum, used in this study were 0 (Continental Crossbred), 50 (50/50 Wagyu/Angus or Simmental), and 75% (75/25 Wagyu/Angus, Holstein, or Simmental) Wagyu in genetic makeup. Animals were cared for according to the guidelines of the Canadian Council on Animal Care (1994). Calves were provided with a mixed backgrounding diet consisting of, on a dry matter basis, 35% barley grain and 65% barley silage containing supplemental protein and beef mineral mix. The finishing diet consisted of 79% rolled barley and 20% barley silage on a dry matter basis, with 1% mineral mix. Rearing and feeding is further described in Mir et al. (1999). Time of slaughter was determined by a combination of live weight (75% Wagyu > 460 kg; 50% Wagyu > 500 kg; Continental crossbred > 500 kg) and backfat thickness as detected by ultrasonography (>10 mm) (Park et al. 1994). Animals were slaughtered between the ages of 509 and 560 d in a commercial abattoir.

Numerous carcass characteristic measurements were obtained after slaughter as described in Mir et al. (1999). Our study included ribeye area (cm^2), subcutaneous (s.c.) backfat depth (mm), average s.c. fat depth (mm) at three carcass sites, marbling score, as well as solvent-extractable fat from longissimus and pars costalis diaphragmatis (p.c.d.) muscles. 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. Total lipids from the p.c.d muscle were extracted using a hexane-isopropanol solvent system (Hara and Radin 1978).

Blood Collection and Transfer

Blood samples were obtained on two occasions: once while cattle were on a low barley grain diet (35% of dry matter), 16 wk prior to slaughter (WPS), and when animals were on the high-energy finishing diet, 4 WPS. Animals were bled in the morning between 10.00 and 12.00 for the 16 and the 4 WPS samplings. Animals were not fed on either day until after being bled and weighed. Blood for the leptin protein assay was collected with K_3EDTA-coated evacuated tubes [lavender Vacutainer® (VWR Canlab, Missisauga, ON, Canada)], and held on ice for approximately 1 to 2 h. Blood samples were subsequently centrifuged at 1700 × g for 15 min at 4°C and plasma was transferred to fresh tubes. Plasma samples were stored frozen at –80°C, thawed once to transfer plasma, and frozen at –80°C before being shipped to the German Research Institute on dry ice.

Leptin Protein Assay

Plasma leptin concentration of each crossbred animal was analyzed from 0.5 mL of bovine plasma using the commercially available multi-species ^{125}I -radioimmunoassay (RIA) kit (Linco Research, Inc., St. Louis, MO, USA) 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). Though specific RIAs against leptin have been developed for cattle (Chilliard et al. 1998; Ehrhardt et al. 2000), and for sheep (Blache et al. 2000; Delevaud et al. 2000), none are currently commercially available. Since the relative response of plasma leptin to different Wagyu genetics is under investigation, the values obtained using the Linco kit are valid (Y. Chilliard, personal communication, INRA-Theix, St-Genes-Champanelle, France).

Statistical Analysis

Data were analyzed using The SAS System for Windows v. 6.12 (SAS Institute, Inc. 1995). Significance was calculated using a pair-wise t-test. The experimental units were steers of three crosses. The statistical aim was to test for differences between the crosses and for association between plasma leptin protein concentrations, at two different ages, and multiple carcass characteristics. Plasma leptin values were submitted to a one-way repeated measures ANOVA test to determine if concentrations at the two different sampling dates were significantly different. Data were submitted to a simple linear correlation analysis (Pearson product moment correlation) using SAS. Analysis was performed for correlations between leptin plasma concentrations and the carcass characteristics. Correlations were performed within the individual crosses, in cattle containing any Wagyu genetics (50 and 75%), and within all cattle (0, 50, and 75% Wagyu).

Table 1. Carcass characteristics and plasma leptin concentrations from Wagyu crossbred cattle

	0%[z] (a)	50% (b)	75% (c)	Significance[y]
Slaughter weight (kg)	538.9 ± 18.8 (53.1)	531.9 ± 11.7 (33.0)	510.0 ± 6.0 (16.9)	None
Ribeye area (cm^2)	78.4 ± 5.13 (14.5)[q]	80.6 ± 2.80 (7.93)	83.1 ± 3.30 (9.34)	None
s.c. backfat (mm)[x]	10.8 ± 1.29 (3.65)	16.1 ± 1.47 (4.16)	12.5 ± 1.00 (2.83)	**ab; †bc;
s.c. fat (mm)[w]	11.9 ± 1.08 (3.04)	17.8 ± 1.73 (4.89)	14.6 ± 1.03 (2.92)	**ab;
Marbling score[v]	8.4 ± 0.18 (0.52)	6.9 ± 0.48 (1.36)	7.1 ± 0.40 (1.13)	*ab; *ac;
longissimus i.m. TL (% wet weight)[u]	4.2 ± 0.48 (1.37)	6.3 ± 0.67 (1.89)	6.8 ± 0.39 (1.11)	**ab; **ac;
p.c.d. i.m. TL (% wet weight)[t]	9.78 ± 1.38 (3.90)	18.02 ± 1.97 (5.58)	17.60 ± 2.78 (6.81)	†ab; ***ac;
Leptin[s] (ng/mL) 16 WPS[r]	3.15 ± 0.21 (0.60)	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;

[z]The values 0, 50, and 75% represent the percent of the breed Wagyu in the samples.
[y]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).
[x]s.c. backfat = subcutaneous fat depth on the back of the cattle.
[w]s.c. fat = subcutaneous fat depth at three separate sites.
[v]Canadian Grading Agency marbling scores based on an inverse 10-point scale with a score of 1 indicating very abundant marbling and a score of 10 indicating a carcass devoid of marbling.
[u]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.
[t]i.m. TL (% wet weight) = the lipid content from pars costalis diaphragmatis muscle samples was obtained using a hexane-isopropanol solvent system (Hara and Radin 1978).
[s]Leptin was obtained from bovine plasma.
[r]WPS = weeks prior to slaughter.
[q]The values listed represent the mean of $n = 8$ samples including ± standard error of the mean and (standard deviation).

RESULTS

Animals used in this study were a subset of the herd studied by Mir et al. (1999). Carcass traits and plasma leptin concentrations are summarized in Table 1. Slaughter weights of the animals from all crosses were not different from each other ($P > 0.05$). Ribeye area, a measure of muscle growth, was also not affected by either cattle type ($P > 0.05$). Differences were detected between the 0% Wagyu and 50% Wagyu crossbred cattle (WC) for both the s.c. backfat ($P < 0.01$) and s.c. fat ($P < 0.01$) measurements, and in the s.c. backfat between 50 and 75% WC cattle ($P < 0.10$) demonstrating that the 50% WC had the greatest level of backfat deposition. The deposition of i.m. lipid within both the longissimus and p.c.d. muscles demonstrated differences (various *P* values, see Table 1) between the 0% WC and both 50 and 75% WC .

Plasma leptin concentrations at the two different sampling dates were different ($P < 0.001$) from one another and were therefore treated separately in the analysis. Leptin values increased 1.2-, 1.7-, and 1.9-fold for the 0%, 50%, and 75% WC, respectively, over the course of the study.

Correlation analysis (Figs. 1 and 2) demonstrate that during the backgrounding phase plasma leptin concentrations were positively correlated with both total lipids from the longissimus muscle ($r = 0.59$, $P = 0.002$, Fig. 1 Ia) and also with the p.c.d. muscle ($r = 0.69$, $P = 0.0004$, Fig. 1 IIa). Plasma leptin was also shown to be positively associated with total lipids from the longissimus muscle ($r = 0.51$, $P = 0.011$, Fig. 1 Ib) and the p.c.d. muscle ($r = 0.35$, $P = 0.104$, Fig. 1 IIb) during the finishing phase of cattle growth. No correlations were observed between leptin concentrations at either sampling date and s.c. backfat depth or average s.c. fat depth ($P > 0.05$).

Correlations between leptin concentration and lipid content of longissimus muscle and p.c.d. at 4 WPS are depicted for 0%, 50%, and 75% WC in Fig. 2. In 0% WC, leptin concentration was positively correlated (longissimus: $r = 0.62$, $P = 0.103$; p.c.d.: $r = 0.40$, $P = 0.410$) with lipid content. There was almost no correlation (longissimus: $r = 0.11$, $P = 0.797$; p.c.d.: $r = 0.005$, $P = 0.990$) between leptin concentration and lipid content in 50% WC. In 75% WC, however, there was a negative correlation (longissimus: $r = –0.60$, $P = 0.120$; p.c.d.: $r = –0.65$, $P = 0.164$) between leptin concentration and lipid content

DISCUSSION

Multiple studies have been done to analyze and predict trends between cattle carcass characteristics and the deposition of i.m. fat in beef cattle. These studies include the analysis of lipid deposition enzymes (Middleton et al. 1999), the use of image analysis (Kuchida et al. 2000.), ultrasound predictions (Herring et al. 1998; Hassen et al. 1999), as well as correlation studies of fatty acid composition (Kazala et al. 1999). The current study outlines circulating plasma leptin concentrations from WC and their respective correlation to i.m. fat deposition.

A high abundance of plasma leptin is associated with greater adiposity in pigs (Robert et al. 1998; Spurlock et al. 1998; Owens et al. 1999) and other mammals (Lissner et al. 1999; Phillips et al. 1999; Timtchenko et al. 1999). In cattle, Chilliard et al. (1998) have shown that leptin is positively correlated to adipocyte volume in both Holstein and Charolais breeds, with the correlation slightly higher in overfed ($r = 0.73$, $P = 0.0028$) than in underfed ($r = 0.68$, $P = 0.0021$) cattle. It is well known that Wagyu cattle deposit high amounts of i.m. fat as compared to other beef cattle (May et al. 1993) and are, therefore, included in breeding programs that aim to improve this trait Plasma leptin concentrations in our study increased in relation to the percentage of Wagyu in the cattle. We found no differences

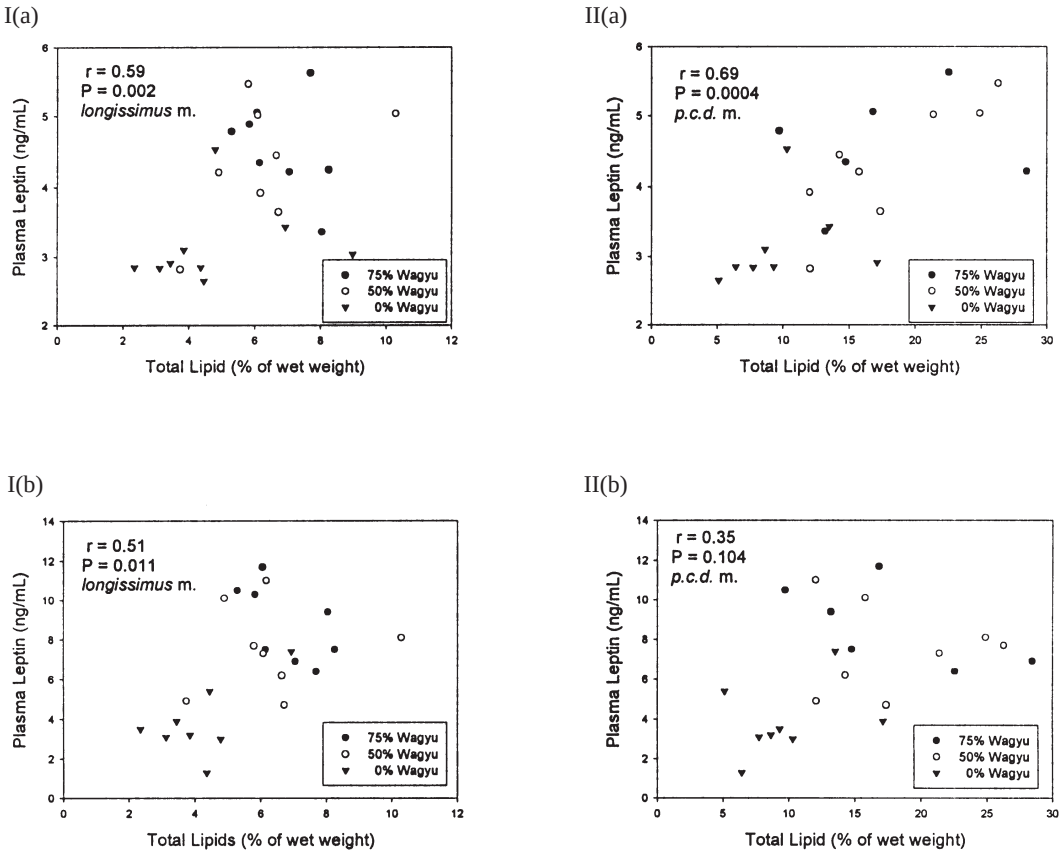

Fig. 1. Relationship between circulating plasma leptin concentration and lipid content of longissimus muscle and p.c.d. in crossbred WC (n = 24). (a) 16 WPS (b) 4 WPS. Simple correlation coefficients and probability levels are shown.

between the 50 and 75% WC in all fat characteristics but differences were detected in plasma leptin concentrations. The 75% WC in our study were slaughtered before their s.c and i.m. fat depots were fully developed at the age of 18 mo. Indeed, Nishimura et al. (1999) demonstrated that at 20 mo of age in Japanese Black cattle the longissimus i.m. fat depots are filled to less than 40% of their capacity.

Regression analysis demonstrated a positive correlation between total lipids from the longissimus muscle in the WC with plasma leptin at 16 WPS. Positive correlations were also observed between leptin at 16 WPS and the p.c.d. muscle. Positive correlations at 16 WPS were confirmed with similar correlations at 4 WPS. These results are in agreement with studies in ruminants (Chilliard et al. 1998; Minton et al. 1998), that demonstrated positive associations between body fatness and leptin concentration. Increases in lipid content, which translate into increases in plasma leptin during adipocyte hypertrophy, are in agreement with the positive relationship (r = 0.73, P < 0.01) demonstrated between plasma leptin and bovine adipocyte volume by Chilliard et al. (1998). This is also observed in humans and in rodents (Maffei et al. 1995) that are fed near their maintenance requirements. Considine et al. (1996) and Klein et al. (1996) suggested that the increase in plasma leptin is caused by the up-regulation in gene expression related to a greater number and/or size of fat cells as opposed to an abnormality in lipid clearance by leptin. In addition, we detected no correlations between subcutaneous fat depots and leptin concentrations. We hypothesize that leptin may serve as an indicator of i.m. fat deposition in cattle.

The various correlations shown in Fig. 2 demonstrate differences between the different WC for both the longissimus and p.c.d. muscles. The cattle containing no Wagyu genetics followed the trend observed in Fig. 1, with positive correlations between lipid content and plasma leptin. As the percentage of WC genetics increased though, the correlation coefficients became almost zero and subsequently negative. This report shows for the first time in crossbred Wagyu that plasma leptin is linked to the lipid content (marbling) of the muscle. Although leptin appears to be up-regulated as i.m. fat increases in 0% WC, the protein appears to be down-regulated in 75% WC. The contrasting relationship between leptin concentration and i.m. fat content in 0% WC versus 75% WC suggests that the physiology of i.m. fat deposition can be influenced by breed.

Though post-mortem processing treatments such as cold-chilling, mechanical tenderization, electrical stimulation, and the addition of enzymes, have been suggested as viable methods to improve the consistency of meat palatability, an accurate method of determining which carcasses will require such treatments is needed. The lack of cost-effective measurement methods has held back the use of genetics in the improvement of meat palatability. Plasma leptin could thus prove to be a viable indicator of i.m. fat depot levels and a potentially important evaluation method for post-mortem treatments.

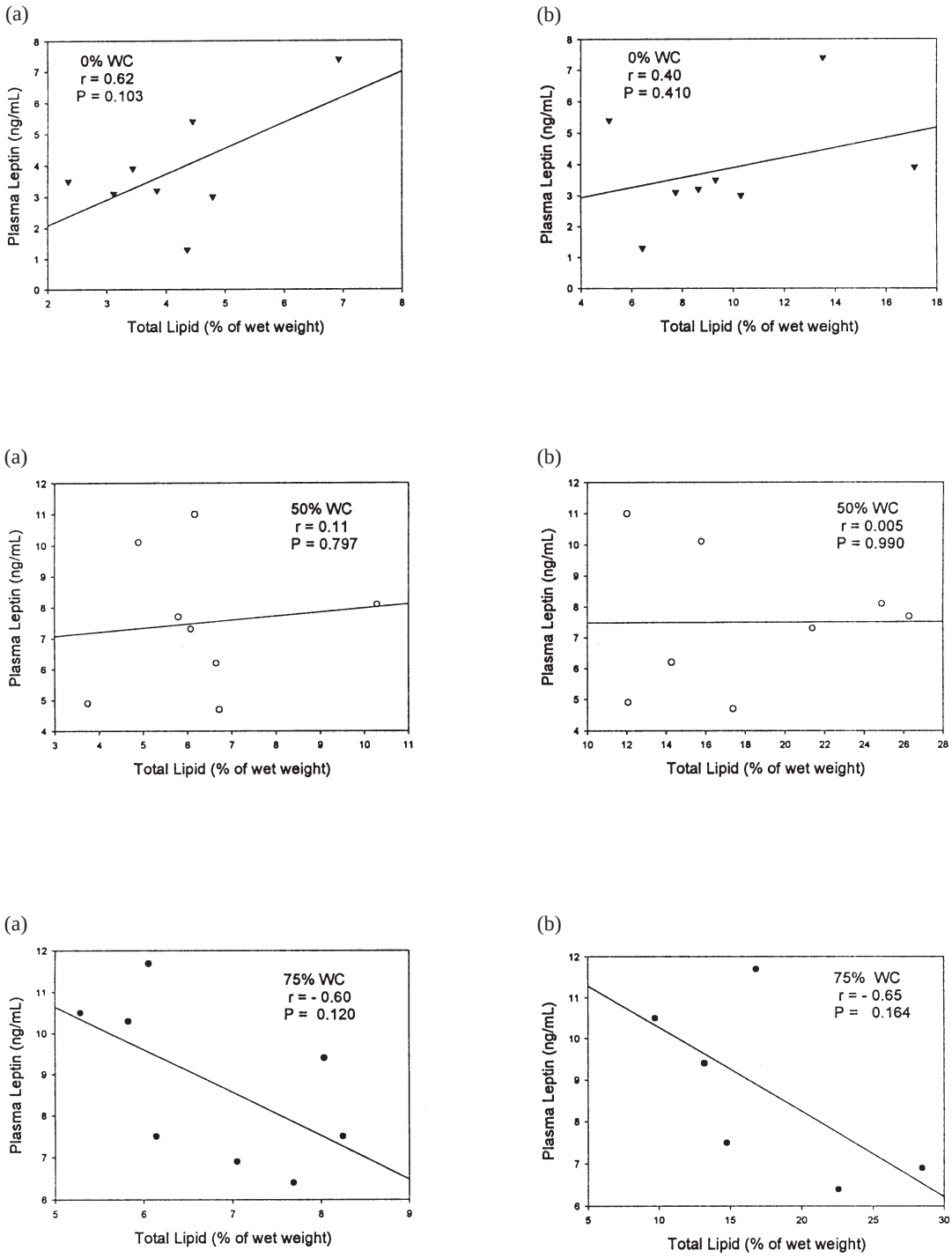

Fig. 2. Relationship between circulating plasma leptin concentration and lipid content of (a) longissimus muscle and (b) p.c.d. in cattle with various degrees of Wagyu genetics (n = 8 in each group) at 4 WPS. Simple correlation and probability levels are shown.

CONCLUSION

These results, in addition to other findings, support the concept that there is a relationship between circulating leptin and i.m. fat in cattle. Further investigation could elucidate this relationship and its potential application in beef production.

Leptin concentration varied with i.m. fat content in cattle, and the nature of the associations appeared to depend to some extent on breed. Further investigation may elucidate differences in the regulation of i.m. fat deposition as a function of breed. The link between lipid accumulation and plasma leptin may be part of a feedback loop that regulates food intake, thus influencing the performance of cattle and the lipid content of muscle tissue. Assessment of leptin plasma concentration in beef cattle may prove to be of diagnostic value in breeding programs aimed at enhancing the marbling trait.

ACKNOWLEDGEMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (DFG project WE 281/1-2) and a Germany–Canada Cooperation in Science and Technology (Agriculture Sector) Project 7/97 from the Bundesministerium für Ernährung, Landwirtschaft und Forsten, Germany, the Matching Grants Initiative from Agriculture and Agri-Food Canada, and the AgriTech International Corporation, Calgary, Alberta, Canada. The authors also acknowledge Karola Marquardt for technical assistance.

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