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Short Communication

Leptin concentrations and the immune-mediated reduction of feed intake in sheep infected with the nematode *Trichostrongylus colubriformis*

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The hypothesis that increases in the concentration of the anorectic peptide leptin may be responsible for the immune-mediated reduction in feed intake (FI) during gastrointestinal parasitism in sheep was investigated. In a 2 × 2 × 2 factorial design, the first factor was age at the start of infection (5 months old *v.* 17 months old). The second factor was parasite infection (no infection *v.* administration of eighty L3 infective *Trichostrongylus colubriformis* larvae/kg live weight (LW) per d three times per week for 77 d). The third factor was immunosuppressive therapy with a corticosteroid (no therapy or weekly intramuscular injection of 40 mg methylprednisolone acetate/30 kg LW). Relative to their uninfected counterparts, a 20 % reduction in FI per unit LW (FI/LW; g DM/kg LW) was observed in infected non-suppressed 5-month-old lambs from 21 to 63 d post-infection ($P<0{\cdot}001$) but not in comparable17-month-old ewes or in corticosteroid-treated lambs or ewes ($P>0{\cdot}05$ for all), allowing the suggestion that the anorexia was a consequence of the developing immune response. The reduction in FI/LW in 5-month-old lambs was not associated with an increase in plasma leptin concentration. Furthermore, plasma leptin concentrations were greater in corticosteroid-treated animals ($P<0{\cdot}001$) and in 17-month-old animals ($P<0{\cdot}001$), none of which displayed an infection-induced reduction in FI/LW. Plasma leptin was positively correlated with carcass fat percentage in both 5-month-old ($P=0{\cdot}016$) and 17-month-old ($P<0{\cdot}001$) animals and did not appear to provide a direct feedback mechanism that restricted energy intake. The results do not support the hypothesis that an increase in circulating leptin is directly responsible for the immune-mediated anorexia in lambs during *T. colubriformis* infection.

Ovine nutrition: Leptin: Nematoda: Body composition: Anorexia

The reduction in feed intake (FI) during gastrointestinal nematode infections in livestock imposes a considerable nutritional and production penalty on the host. However, despite the importance of infection-induced anorexia to both the welfare and productivity of commercial livestock, the mechanism(s) involved are unclear. Recent investigations show that a 30 % reduction in FI that was observed in naive lambs during infection with the intestinal nematode *Trichostrongylus colubriformis* can be prevented by corticosteroid treatment[(1)]. This implicates component(s) associated with the developing immune response in the anorexia of infection in sheep[(1)]. In other species, pro-inflammatory cytokines involved in the acute-phase response have been implicated in the anorexia of infection[(2)]. Furthermore, the adipocyte-derived peptide leptin can stimulate pro-inflammatory cytokine production and the overall immune response[(3)]. Interestingly, leptin release can be blocked by the immunosuppressive glucocorticoid dexamethasone[(4)].

Therefore, our aim was to test the hypothesis that the primary cause of anorexia in *T. colubriformis*-infected lambs is a consequence of an immunologically mediated release of the anorectic peptide leptin which may be abated by corticosteroid-induced immunosuppression.

Experimental methods

Animals, treatments and leptin analysis

The experimental design, feeding and sampling of animals has been described previously[(1)]. The experiment was carried out with approval from, and in accordance with, the Lincoln University Animal Ethics Committee (authority LU39/01). Briefly, seventy-two Coopworth female sheep were used in a 2 × 2 × 2 factorial experiment with factors being age (5-month-old lambs maintained in a parasite-free environment since age 3 weeks or 17-month-old parasite-experienced ewes

Abbreviations: d.p.i., days post-infection; epg, eggs/g fresh faeces; FEC, faecal egg counts; FI, feed intake; IF, infected non-corticosteroid-treated group; IS, non-infected corticosteroid-treated group; ISIF, infected and corticosteroid-treated group; LW, live weight.

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removed from pasture 2 weeks before the start of the trial), parasite infection (no infection or trickle infection with the equivalent of eighty L3 infective *T. colubriformis* larvae/kg live weight (LW) per d) and corticosteroid treatment (no treatment or weekly subcutaneous injections of the corticosteroid methylprednisolone acetate (Depredone, 40 mg methylprednisolone acetate/ml; Jurox Pty Ltd, Rutherford, NSW, Australia) at a rate of 1 ml/30 kg LW). All animals were treated with anthelmintic at housing with 1 ml/5 kg LW of a combination drench (37·5 g levamisole/l and 23·8 g albendazole/l, Arrest; Ancare New Zealand Ltd, Auckland, New Zealand) to remove any resident parasite burden. This design produced four experimental groups within each age cohort: a non-infected non-corticosteroid-treated control group, an infected non-corticosteroid-treated group (IF), a non-infected corticosteroid-treated group (IS) and an infected and corticosteroid-treated group (ISIF).

For the purpose of the present study, six out of the nine animals from each group were chosen based on sequential tag numbers to provide a representative subsample of the treatment groups. Blood samples were taken every 2 weeks from −7 days post-infection (d.p.i.) using jugular venepuncture into a 10 ml vacutube (Becton Dickinson, VACUTAINER Systems, Rutherford, NJ, USA) and immediately placed at 4°C. After centrifugation at 1200 ***g*** for 20 min plasma was separated and stored at −20°C. Plasma leptin concentration was assayed in duplicate using a disequilibrium double antibody bovine RIA previously validated in ovine plasma[5]. The assay is based on a primary rabbit antibody raised against recombinant bovine leptin and a secondary goat antibody raised against rabbit γ-globulin. Recombinant bovine leptin was used for iodination and standards. The assay has a sensitivity of 0·5 ng/ml and a range of 0·5–20 ng/ml. Samples were assayed in duplicate in a volume of 100 μl and re-assayed in duplicates in a volume of 50 μl when the concentration exceeded 15 ng/ml. Analysis of the complete set of samples involved four assays. The inter- and intra-assay CV for these four assays were less than 6 and 7 %, respectively.

Diets, body composition and faecal egg counts

Fresh water and a pelleted complete ruminant ration formulated to supply 10·1 and 8·5 MJ metabolisable energy and 101 and 68 g metabolisable protein/kg DM for diets offered to lambs and ewes, respectively, were offered daily. The nutrient content of the diets was intended to supply sufficient energy in addition to 50 g metabolisable protein in excess of maintenance requirement[6] when daily DM consumption was 3·5 and 3 % of LW for 5-month-old and 17-month-old animals, respectively. This level of excess metabolisable protein has previously been shown to be sufficient to allow the development of immunological responses in lambs of a similar age and infected with the same species of nematode as used in the present study[7]. Individual feed refusals were collected and weighed weekly. Subsamples of feed offered and refused were taken at the same time for the determination of DM percentage after drying for 72 h at 90°C.

Weekly faecal samples were taken directly from the rectum from −6 d.p.i. and the concentration of nematode eggs in the faeces (faecal egg counts; FEC) determined by the modified McMaster technique[8] and expressed as eggs/g fresh faeces (epg) with a sensitivity of 100 epg.

Live weights were measured weekly, with FI expressed relative to LW (relative FI; FI/LW; g DM/kg LW per d) to facilitate comparison between age groups. In addition, fasted LW was measured at 77 d.p.i. to aid the estimation of body composition by *in vivo* X-ray computed tomography. Carcass composition was estimated by comparison of the composition of three reference slices taken from each animal at the thoracic vertebrae 8, lumbar vertebrae 5 and ischium with estimates of whole carcass composition by the calvaleri procedure as described previously[1]. Carcass fat percentage was calculated by dividing the estimated weight of adipose tissue in the carcass by the estimated carcass weight.

Statistical analysis

Data were analysed as a $2 \times 2 \times 2$ factorial design using the GENSTAT statistical package (version 10, 2007; Lawes Agricultural Trust, VSN International Ltd, Hemel Hempstead, Herts, UK) with factors being age, infection and corticosteroid treatment. For all measurements, the main effects and interactions were investigated. Plasma leptin was square root transformed and FEC log10 $(n + 1)$ transformed before analysis to remove skewness with back-transformed means (n^2 and $10^n - 1$, respectively) presented. Only data from infected animals were included for the analysis of FEC; consequently infection was removed as a factor. Plasma leptin concentration, FI/LW and FEC underwent sequential comparison of ante-dependence structures for repeated measures before being analysed by restricted maximum likelihood with time added as a factor. The relationship between leptin concentration at 70 d.p.i. and carcass fat percentage at 77 d.p.i. was analysed using regression analysis. Alternative regression models were tested, with simple linear regression found to account for the greatest variation. The effects of age, infection and corticosteroid treatment on the regression models were examined using linear regression with groups.

Results

The parasitological and production data for this subset were similar to the data reported for the entire dataset[1].

Faecal egg counts

A patent infection was achieved in all infected animals with the exception of IF ewes. Overall, there was an age × corticosteroid treatment × time interaction ($P < 0{\cdot}001$), reflecting an increase in FEC of all corticosteroid-treated animals to greater than 3000 epg by the conclusion of the study. In comparison, mean FEC of 5-month-old IF lambs peaked at 1200 epg at 42 d.p.i. before declining to less than 50 epg by 63 d.p.i., while 17-month-old IF ewes maintained mean FEC at 50 epg or less throughout the entire study.

Feed intake

Relative feed intakes (FI/LW; kg DM/d per kg LW) displayed an age × infection × corticosteroid treatment ($P < 0{\cdot}001$) and an age × corticosteroid treatment × time interaction

($P=0{\cdot}03$), reflecting a 20 % reduction in FI/LW in non-suppressed 5-month-old infected animals (IF) relative to their uninfected controls between 21 d.p.i. and 63 d.p.i. but not in corticosteroid-treated 5-month-old infected animals (ISIF) or in either of the infected 17-month-old ewe treatments.

Plasma leptin

Mean plasma leptin concentrations displayed an age × infection × corticosteroid treatment interaction ($P<0{\cdot}001$) and an age × corticosteroid treatment × time interaction ($P=0{\cdot}003$). These reflected a gradual increase in mean plasma leptin in 5-month-old animals from 3·0 (95 % CI 2·86, 3·09) ng/ml at −7 d.p.i. to 4·8 (95 % CI 3·69, 6·12), 3·7 (95 % CI 2·1, 4·51), 6·5 (95 % CI 4·61, 8·60) and 7·0 (95 % CI 4·67, 9·80) ng/ml at 70 d.p.i. for control, IF, IS and ISIF, respectively, and in 17-month-old animals from 3·7 (95 % CI 3·53, 3·79) ng/ml at −7 d.p.i. to 10·0 (95 % CI 8·32, 14·78) and 9·5 (6·49, 13·15) ng/ml at 70 d.p.i. for control and IF, respectively. In slight contrast, 17-month-old IS and ISIF animals had similar leptin concentrations at −7 d.p.i. and 7 d.p.i. to their non-corticosteroid-treated counterparts (control or IF) before displaying an increase in plasma leptin from 5·6 (95 % CI 5·25, 7·96) and 5·6 (95 % CI 4·51, 6·25) ng/ml at 7 d.p.i. to 16·5 (95 % CI 14·45, 24·09) and 11·9 (95 % CI 10·06, 17·64) ng/ml for IS and ISIF, respectively. Leptin concentrations for 17-month-old IS and ISIF animals continued to increase to 19·7 (95 % CI 16·36, 27·88) and 19·4 (95 % CI 15·37, 27·31) ng/ml for IS and ISIF, respectively, at 70 d.p.i.

Carcass composition and plasma leptin

The relationship between carcass fat percentage at 77 d.p.i. and plasma leptin concentration (Fig. 1) at 70 d.p.i. for 5-month-old animals differed from that observed for 17-month-old animals ($P=0{\cdot}003$). Treatment (corticosteroid and/or infection) had no effect on the relationship between carcass fat percentage and plasma leptin for animals of either age group ($P>0{\cdot}05$ for all).

Discussion

The results do not support the hypothesis that the immune-mediated anorexia experienced by sheep during gastrointestinal parasite infections can be attributed to changes in circulating leptin concentrations. The 0·20 proportional reduction in FI/LW in IF lambs compared with control lambs between 21 and 63 d.p.i. that was not exhibited in either IF ewes (compared with control ewes) with immunological competence to limit FEC or in infected but corticosteroid-treated animals (ISIF compared with IS) of either age was interpreted as a possible consequence of the actions of cytokines elicited during the immune response of the previously inexperienced host[1]. Although the anorectic effects of such cytokines have not yet been elucidated in sheep, the consistently lower leptin concentration in IF lambs compared with all other groups suggests that the reduction in FI experienced by IF animals was unlikely to be influenced by plasma leptin concentrations. A possible role for leptin in anorexia during gastrointestinal infections in sheep was first suggested by workers[9] who, without comparison with uninfected controls, observed a temporary increase in serum leptin associated with a decrease in FI 5–10 d.p.i. with the abomasal parasite *Teladorsagia circumcincta* followed by a reduction in leptin and restoration of appetite at 20–27 d.p.i. Although the authors conceded that the relationship between plasma leptin concentration and FI was at best tentative, they did hypothesise that increased leptin may be the result of parasite-induced increases in blood gastrin concentrations, which, in turn, down-regulate the synthesis of the potent stimulator of FI neuropeptide-Y. However, this hypothesis cannot explain the reduction in FI commonly observed during infections with intestinal dwelling nematodes and which would not be expected to cause an increase in serum gastrin. In one of the few studies comparing serum leptin concentrations of infected with uninfected lambs[10], the authors reported greater serum leptin concentrations in both Scottish Blackface and Suffolk × Greyface lambs infected with *T. circumcincta* but an infection-induced reduction in FI only in animals of the

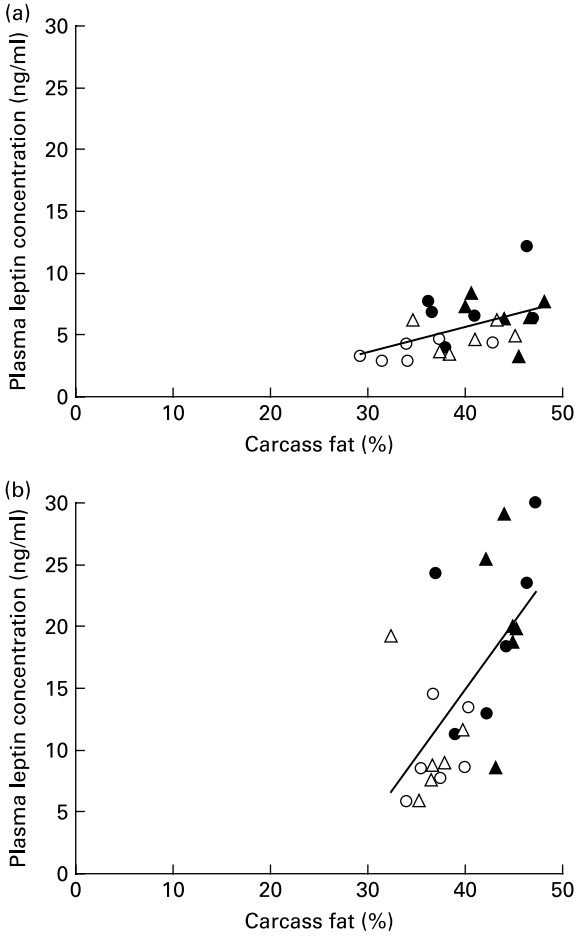

Fig. 1. Relationship between carcass fat percentage measured by X-ray computed tomography at 77 days post-infection (d.p.i.) and plasma leptin concentration (ng/ml) at 70 d.p.i. for 5-month-old lambs (a) and 17-month-old ewes (b). The animals were infected with (a) 2000 or (b) 3200 *Trichostrongylus colubriformis* larvae/d (○), similarly infected and treated with 1·3 mg corticosteroid/kg live weight (●), corticosteroid-treated only (▲) or remained as controls (△). (—), Trend line for all data points within each plot. For 5-month-old animals (a), $y=0{\cdot}204x-2{\cdot}55$; R^2 0·20 ($P=0{\cdot}016$). For 17-month-old ewes (b), $y=1{\cdot}085x-28{\cdot}43$; R^2 0·35 ($P=0{\cdot}001$).

latter breed. The authors concluded that leptin alone is unlikely to be responsible for the reduction in FI in parasitised lambs. Similar studies in Scottish Blackface and Greyface cross ewes have also reported no effect of infection with *T. circumcincta* on plasma leptin concentrations during the peri-parturient relaxation of immunity despite animals of both breeds exhibiting evidence of an infection-induced reduction in FI[11]. These results are in agreement with those of the present study that suggest that an increase in circulating leptin is not the cause of the immune-mediated reduction in FI in parasitised sheep.

Corticosteroid treatment resulted in greater plasma leptin concentrations in IS and ISIF animals of both ages compared with their control or IF counterparts. Leptin production of ovine adipose explants has been increased in the presence of the corticosteroid dexamethasone[12]. However, with the exception of a correlation between plasma cortisol and leptin concentrations in the sheep fetus during late gestation[13], this appears to be the first report of the effects of corticosteroids on leptin production in sheep *in vivo*. Support for the notion that corticosteroids up-regulated leptin production in the present study can be observed from the rapid increase in leptin concentration in corticosteroid-treated animals between 7 and 21 d.p.i. that would not be expected to be matched by adipose deposition. In contrast, however, the relationship between carcass fat percentage and plasma leptin was not altered by corticosteroid treatment in either age group (Fig. 1), which suggests that the greater plasma leptin concentration at 70 d.p.i. in IS and ISIF animals can, at least in part, be attributed to changes in adipose tissue mass. Furthermore, plasma leptin concentration appears to be more sensitive to changes in carcass fat percentage in 17-month-old than in 5-month-old animals. Nevertheless, the apparent lack of any anorectic effect of leptin observed in the present study supports previous suggestions that the primary role of leptin in ruminants is unlikely to be in the provision of a direct feedback mechanism which restricts energy intake[14].

In conclusion, these results do not support the role of leptin in the immune-mediated infection-induced anorexia observed during *T. colubriformis* infection in lambs.

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There are no conflicts of interest.

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