ISSN 0370-2731/99 Proceedings of the New Zealand Society of Animal Production 59: 279-281

Mouse 3T3-L1 preadipocyte cells as a bioassay for serum-borne adipogenic factors

B.A. VEENVLIET, R.P. LITTLEJOHN, J.M.SUTTIE AND T.E. BROAD

AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel.

ABSTRACT

This study of the development of adipose tissue in sheep set out to investigate whether culture medium containing serum from Coopworth lean and fat selection lines had differential effects on proliferation and differentiation of mouse 3T3-L1 preadipocytes. Using fetal calf serum (FCS) as a positive control, the effects of serum from lean and fat lines of sheep and from goat and horse were compared by analysing total cell numbers, and protein and triglyceride (TG) levels per unit DNA in the mouse cell cultures. Differentiation was induced in all serum treatments. Mean cell numbers in cultures up to 13 days after differentiation were higher for sheep serum than for the other species ($p<0.001$). There was no significant difference ($p>0.05$) in protein content between FCS and serum from lean and fat sheep lines. Mean TG levels were significantly higher ($p<0.001$) for FCS than for the lean and fat selection lines, which did not differ significantly from each other ($p>0.05$). Significant differences in either proliferation or differentiation were not detected between the fat and lean sheep.

Keywords: Mouse preadipocytes; sheep, horse, goat and FCS; differentiation; fat and lean selection lines.

INTRODUCTION

Overfatness is a widespread problem in the New Zealand sheep industry, presenting an image of conspicuous waste in the production of sheep meat and potentially poor consequences for the health of consumers. It can partly be attributed to genetic differences among breeds, crosses, and selected strains (Clarke *et al.*, 1996). The present study focussed on fat and lean selection lines of Coopworth sheep that have a difference in subcutaneous fat depth of 4 phenotypic standard deviations (Morris *et al.*, 1997). It is not known whether a blood borne factor is responsible for the differences in fat content in the adult. We report here the results of a study using the preadipocyte mouse 3T3-L1 cell line, which has previously been shown to undergo an adipose differentiation using sera from other species, fetal calf and cat (Kuri-Harcuch and Green, 1978), and human (Hauner and Löffler, 1986), to investigate whether putative blood-borne factors are responsible for the differences in fat levels between the fat and lean Coopworth selection lines of sheep. The approach was to use mouse 3T3-L1 preadipocytes as a bioassay system to detect any putative factors in sheep serum that may affect their proliferation and differentiation.

MATERIALS AND METHODS

Serum from goat, horse and fetal calf was purchased from Gibco BRL. Serum was collected from the five leanest and fattest rams (born 1994) from the respective selection lines (Morris *et al.*, 1997) based on the estimated breeding value for leanness (JC McEwan, AgResearch Invermay, personal communication). The serum was sterilized using a 0.2 mm filter (Sartorius) and stored at -20°C. Serum concentrations of 2.5%, 5% and 10% were pre-tested for potential inhibitory effects before a final concentration of 10% was chosen for all studies.

3T3-L1 fibroblasts were purchased from the American Type Culture Collection (ATCC) and cultured and harvested according to their recommendations. Culture media (Dulbecco's modified Eagles media (Gibco BRL,) containing 10% serum and 1% antibiotic-antimycotic) was changed in the cultures every 2-3 days pre- and post-confluence. At confluence, culture medium was supplemented with 1.7 mM insulin, 1 mM dexamethasone and 0.5 mM isobutyl-methyl-xanthine (IBMX) to initiate differentiation (day 0). Two days later, culture medium was changed with culture medium supplemented with 1.7 mM insulin. This method was based on concentrations used by Rubin *et al.,* (1977), Student *et al.,* (1980), Swick and Lane (1992) and Boney *et al.,* (1994). Cells were harvested and resuspended in 300 ml of phosphate buffered saline (PBS). Aliquots were taken for cell counting, TG, protein and DNA analysis. Cells in samples taken for protein and DNA analysis were lysed with 0.3N NaOH, 1% SDS. All samples were stored at -80°C.

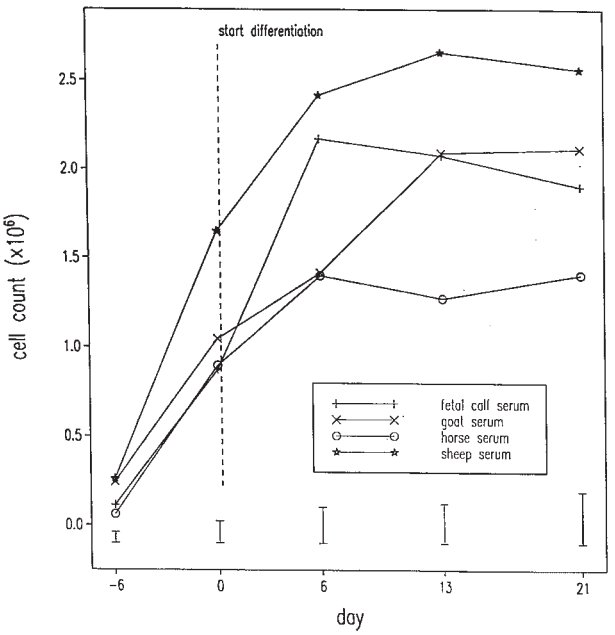
Cell number was determined using a Coulter counter (Coulter Electronics Ltd). The protein content of the lysed cells were measured by spectrophotometry using a Bichonic acid (BCA) protein assay kit (Pierce) with bovine serum albumin as a standard. The TG content of cells and of the sera used were measured by spectrophotometry using a Peridochrom Triglyceride GPO-PAP kit (Boehringer Mannheim) and measured against Precimat Glycerol as a control (Boehringer Mannheim). The DNA concentration of lysed cells was measured in a TKO 100 Mini-Fluorimeter (Hoefer Scientific Instruments) which was calibrated using calf thymus DNA at 1mg/ml in 10mM Tris-Cl, 50 mM EDTA. Insulin was assayed in the fetal calf, lean sheep and fat sheep serum at concentrations of <0, <0 and 8.8 ng/ml respectively by radioimmunoassay (Coat-a-count Kit, DPC California).

Data were analysed by analysis of variance, with the treatment structure given by population of cells, time relative to differentiation, and their interaction. Regression relationships were analysed by least squares.

RESULTS

Serum from sheep, and the other species tested, supported the growth of 3T3-L1 preadipocytes (Fig. 1). All the cultures reached confluence at about the same time after seeding, but those cultured in sheep serum attained a greater density at confluence (Fig. 1, day 0). There was a significant difference ($p>0.001$) between species of origin of serum on cell numbers up to 13 days after differentiation, with sheep (mean $2.7x10^6$ cells) greater than fetal calf (mean $2.1x10^6$ cells) and goat (mean $2.1 x10^6$ cells) which were greater than horse ($1.3 x10^6$ cells). The cell numbers for each treatment at day 21 after differentiation was similar to that at day 13.

FIGURE 1: Effect of serum from different species on cell number. Bars on the X-axis denote standard errors of the difference (sed).



All of the species sera tested supported differentiation of the mouse preadipocytes. Two days after differentiation was initiated, lipid droplets were seen in cells cultured in FCS, but were not seen until 5 days in all other sera tested. The amount of TG in cells cultured in FCS was consistently about 3-fold greater than that in other sera up to 21 days post-differentiation. No significant differences were detected in the lipid content of cells between the sheep, goat and horse sera treatments (data not shown).

There was no significant difference between sera from the sheep selection lines (Fig.2). The amount of cellular TG accumulated was about one third of that in cells grown in FCS (Fig. 2). Cultures untreated with insulin, dexamethasone and IBMX for 2 days, all failed to accumulate lipid with all of the sera tested. The mean cellular levels of protein (measured by the protein:DNA ratio) increased up to day 3 after differentiation in the fat and lean sheep serum and FCS, but decreased slightly by day 13 (Fig 3). There was no significant difference in protein levels among the treatments (Fig. 3).

FIGURE 2: Effect of serum from sheep selection lines and fetal calf on the TG:DNA ratio of cells during differentiation. Bars on the X-axis denote standard errors of the difference (sed).

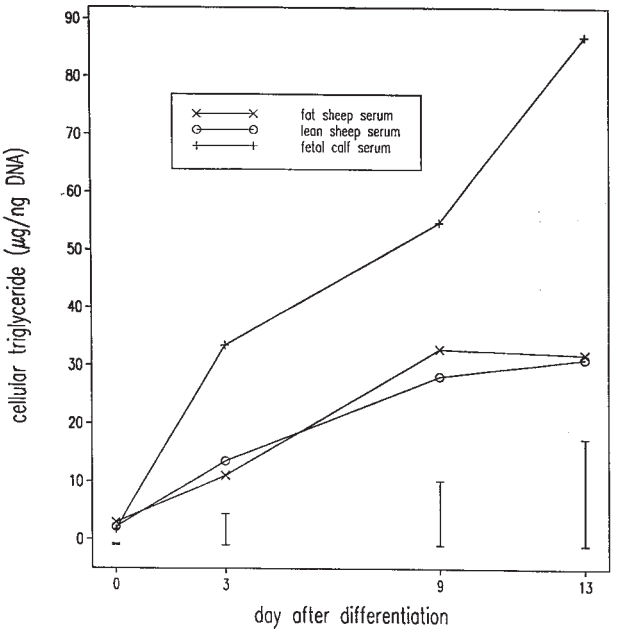
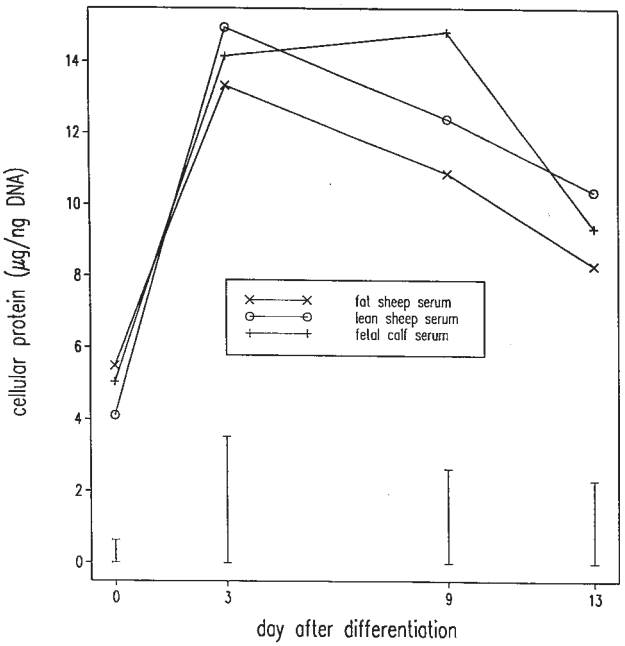


FIGURE 3: Effect of serum from sheep selection lines and fetal calf on the protein:DNA ratio of cells during differentiation. Bars on the X-axis denote standard errors of the difference (sed).



Cellular TG is produced by both endogenous synthesis and uptake of exogenous lipid. A significant regression relationship ($P<0.01$) was found between 21 day cellular TG level and serum TG from the 4 species used in Fig. 1. This suggests there may be a dose response effect between serum TG and the level of adipose development attained in the cultures.

DISCUSSION

This study has clearly shown that the growth and adipose conversion of the mouse 3T3-L1 preadipocyte cell line was supported by the serum of sheep, horse and goat and that this *in vitro* system is a suitable model for testing for adipogenic factors in these species. No significant difference was detected between the fat and lean selection lines in their ability to support an adipose conversion of mouse preadipocytes (Fig. 2). However, substantially less TG was accumulated in the cells cultured with the serum of the fat and lean lines of sheep than in FCS (Fig. 2). FCS has been noted to have a greater adipogenic potential than newborn calf serum and cat serum (Kuri-Harcuch and Green, 1978) and human serum (Hauner and Löffler, 1986). The lipid accumulated in the cell comprises that from endogenous synthesis plus that from the uptake of exogenous lipid. We were unable to quantify the relative contributions of these sources to the total accumulated. Preliminary studies have shown that increasing the concentration of glucose (a substrate for lipid biosynthesis) in the culture medium 4-fold had no effect on the time course or magnitude of the TG accumulation (data not shown). This suggests that endogenous synthesis was not the main pathway of lipid accumulation. However an approximately linear relationship was noted between the TG concentration in the serum used and the magnitude of lipid accumulation detected.

We were unable to detect differences between the fat and lean selection lines of sheep in their capacity to affect the growth and proliferation of mouse preadipocytes. However, serum from genetically obese rodents was reported to be 4-10 times more active in promoting the adipose conversion of 3T3-L1 cells than that from their lean littermates (Loffler *et al.*, 1983) whereas Hauner and Löffler (1986) reported that adipogenic activity of serum from obese adult humans did not differ from that from non-obese subjects. The latter authors proposed that growth hormone may account for about half of the adipogenic potential of human serum and that the remainder was due to an unidentified low molecular weight protein. Differences in growth hormone levels have been reported between sheep fat and lean selection lines (Suttie *et al.*, 1993). However, the lack of difference noted here on this bioassay system suggests that serum borne factors, for example growth hormone and insulin, may not be contributing directly to the fatness differences between the selection lines. Other studies of the effect of human serum on rat preadipocyte cultures have failed to detect adipogenic factors (Sypniewska *et al.*, 1986). Our data agrees with these, suggesting it will not be productive to use this experimental system to further investigate differences between the sheep selection lines. Consistent with this proposal is the suggestion that the amount of lipid accumulated by these cells may reflect the level of circulating TG. A strong association between adipogenesis in rat preadipocytes and circulating TG levels in human serum was noted by (Sypniewska *et al.*, 1986). Thus, factors affecting TG synthesis and turnover may be more important in producing the differences in body composition of the selection lines than those affecting the proliferation and differentiation of adipocytes.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of John McEwan, AgResearch Invermay, Mosgiel, whose fat and lean selection lines of Coopworth sheep formed the basis of this study, and who calculated the EBVs by which we identified the five most extreme rams for serum collection; Dr Mary Thompson, Dept. Biochemistry, University of Otago, Dunedin, for advice about the differentiation of the 3T3-L1 cell line; and the NZ Foundation for Research, Science and Technology for funding this work.

REFERENCES

Boney, C.M.; Moats-Stats, B.M.; Stiles, A.D.; D'Ercol, A.J. 1994. Expression of insulin-like growth factor-1 (IGF-1) and IGF-binding proteins during adipogenesis. *Endocrinology* **135:** 157-162.

Clarke, J.N.; Morris, C.A.; Speck, P.A.; Upreti, G.C.; Nicoll, G.B. 1996. Genetic improvement of meat quality in sheep and cattle. *Proceedings of the New Zealand Society of Animal Production* **56:** 157-162.

Hauner, H.; Löffler, G. 1986. Adipogenic factors in human serum promote the adipose conversion of 3T3-L1 fibroblasts. *International Journal of Obesity* **10:** 323-330.

Kuri-Harcuch, W.; Green, H. 1978. Adipose conversion of 3T3-l1cells depends on a serum factor. *Proceedings of the National Academy of Science USA* **75:** 6107-6109.

Loffler, G.; Feick, P.; Hauner, H.; Herberg, L. 1983. An adipogenic serum factor in genetically obese rodents. *FEBS Letters* **153:** 179-182.

Morris, C.J.; McEwan, J.C.; Fennessy, P.F.; Bain, W.E.; Greer, G.J.; Hickey, S.M. 1997. Selection for high or low backfat depth in Coopworth sheep: juvenile traits. *Animal Science* **65:** 93-103.

Rubin, C.S.; Lai, E.; Rosen, O.R. 1977. Acquisition of increased hormone sensitivity during *in vitro* adipocyte development. *The Journal of Biological Chemistry* **252:** 806-817.

Student, A.K.; Hsu, R.Y.; Lane, M.D. 1980. Induction of fatty acid synthetase in differentiating 3T3-L1 preadipocytes. *The Journal of Biological Chemistry* **255:** 4745-4750.

Suttie, J.M.; Veenvliet, B.A.; Littlejohn, R.P.; Gluckman, P.D.; Corson, I.D.; Fennessy, P.F. 1993. Growth hormone pulsatility in ram lambs of genotypes selected for fatness or leanness. *Animal Production* **57:** 119-125.

Swick, A.; Lane, M.D. 1992. Identification of a transcriptional repressor down-regulated during preadipocyte differentiation. *Proceedings of the National Academy of Science USA* **89:** 7895-7899.

Sypniewska, G.; Karlsson, M.; Björntorp, P. 1986. Adipogenic activity in human plasma. Effects of feeding state and obesity. *International Journal of Obesity* **10:** 265-276.