

## Optimisation of *In Vitro* Conditions for the Differentiation of Primary Stromal Vascular Cells from Wagyu Cattle

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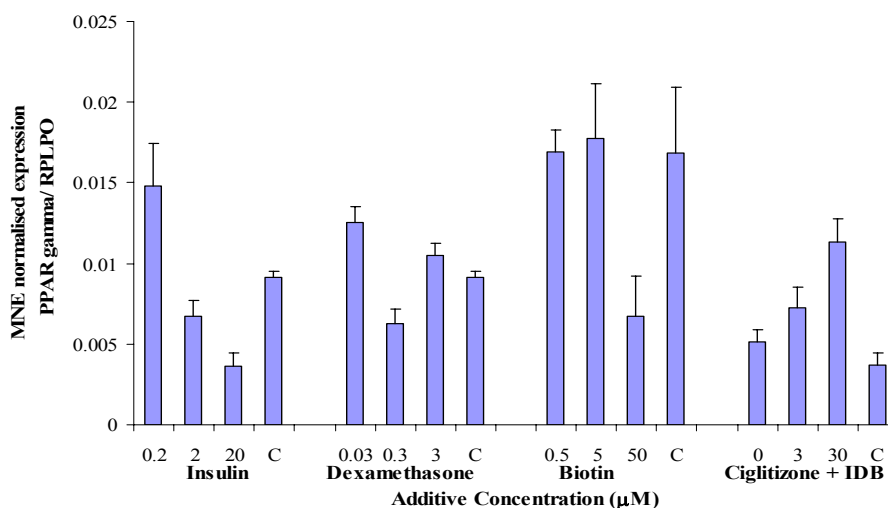
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Japanese and South Korean consumers have a strong preference for highly marbled beef (Thompson 2004). Therefore, understanding the mechanisms by which marbling is regulated may have a significant financial impact on the Australian beef industry. Together, intramuscular fat (IMF) and subcutaneous fat (SCF) determine the market suitability of a carcass for the export market and, consequently, the financial value to producers. *In vitro* cell metabolism provides a method to study adipogenesis and examine why animals differ in marbling scores. The aim of the present study was to establish optimal *in vitro* conditions for a bovine fat differentiation model. This work represents a preliminary study which will lead to further studies investigating differential regulation of primary bovine SCF and IMF stromal vascular (SV) cell differentiation.

Primary SV cells were isolated from SCF obtained from Wagyu cattle (n=3), at slaughter, and cultured in media comprised of DMEM/Ham's F12 media with 10% fetal calf serum + 2% antibiotics. SCF SV cells were then treated with a c-AMP agonist (0.5mM 3-Isobutyl-1-methylxanthine) and one of a combination of insulin, dexamethasone, biotin and ciglitizone, at different concentrations. The IMF SV cells were not used due to the complexity of developing techniques in both cell types. Total RNA was isolated from the control (untreated) and treated cells and the mRNA abundance of the adipogenic differentiation factor, peroxisome proliferator activated receptor  $\gamma$  (*PPAR* $\gamma$ ) determined by quantitative RT-PCR (qRT-PCR). Data was normalised to house keeping gene RPLP0 and MNE normalised expression determined by qgene software (Muller *et al*, 2002).



**Figure 1. Expression of PPAR $\gamma$  mRNA in response to increasing concentrations of insulin, dexamethasone, biotin and ciglitizone in bovine SCF SV cells *in vitro*. Control (C) is basal media containing IBMX; IDB is insulin, dexamethasone and biotin.**

In the present study, the optimal concentration for insulin was 0.2 $\mu$ M. The expression of *PPAR* $\gamma$  did not correspond to different concentrations of dexamethasone. Further experiments are needed to determine the optimal concentration for dexamethasone. Biotin did not appear to have any effect at lower concentrations (0.5 and 5 $\mu$ M), and an inhibitory effect at the highest concentration (50 $\mu$ M). The optimal concentration of ciglitizone in the presence of insulin, dexamethasone and biotin was determined to be 30 $\mu$ M. In conclusion, we have tested a number of conditions necessary for the differentiation of bovine SCF SV cells. The response of IMF SV cells to the optimised conditions requires validation.

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Muller, P. Y., Janovjak, H., Miserez, A.R., and Dobbie, Z. (2002). *Biotechniques*, **32**:1372.

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