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BOVINE FAT DEPOTS DISCRIMINATE BY GENE EXPRESSION PATTERNS

N.J. Hudson[1], A. Reverter[1], D.W. Pethick[2], J.P. Siddell[3], P.L Greenwood[4] and Brian P. Dalrymple[1]

[1]CSIRO Animal, Food and Health Sciences, 306 Carmody Road, St. Lucia, Brisbane, QLD4067
[2] Murdoch University, Perth, WA 6150
[3] NSW Department of Primary Industries, Glen Innes Agricultural and Research Advisory Station, Glen Innes NSW2370
[4]NSW Department of Primary Industries Beef Industry Centre, University of New England, Armidale, NSW2351

SUMMARY

To improve our molecular understanding of bovine fat metabolism, global patterns of gene expression were explored in 5 fat depots: subcutaneous rump (SC), intermuscular (Inter), intramuscular (IMF), omental (Omen) and kidney (Kid). All depots share conserved co-expression gene sets relating to fundamental adipocyte cytoskeletal architecture, metabolism and inflammation. However, the various depots could clearly be discriminated from each other by gene expression. Differences in fatty acid saturation between SC and the other depots are reflected by differential expression of the *SCD* gene that encodes the Δ 9 desaturase enzyme. The fundamental lipogenic machinery such as the *ACACA* gene encoding the rate limiting synthetic enzyme acetyl coA carboxylase is expressed at lower levels in IMF. We also detected differences in expression consistent with divergent lipogenic fuel preferences. Across depots, the most differentially expressed (DE) genes align with those published in the literature for non-ruminants, illustrated by SC rump's highly divergent expression of *HOXA10* and *DLK1*. These genes are likely markers for populations of pre-adipocytes whose properties vary between depots.

INTRODUCTION

Deposition of marbling fat has a positive impact on sensory meat quality through enhanced flavour, juiciness and tenderness. Development of the non-edible fat depots, particularly subcutaneous fat (SC), is considered energetically and commercially wasteful. Therefore, a better understanding of fat depot biology contributes to the challenge of efficiently maintaining product quality in a resource-constrained world. Genetics and nutrition can alter percent intra muscular fat (IMF%) and fat depot distribution. However, IMF development remains an enigmatic trait. In cattle, there are few, if any, known causal mutations, phenotypic variation in IMF% explained by single nucleotide polymorphisms (SNP) is modest (Barendse *et al.* 2010) and the key precursor cell populations have not been unequivocally identified (Harper and Pethick 2004). Physiological differences between depots have been postulated. For example, IMF adipocytes are thought to have a lipogenic preference for glucose and lactate carbon while SC adipocytes prefer acetate (Smith *et al.* 2009). The expression research described here underpins gene and pathway discovery in *bovine* fat metabolism.

MATERIALS AND METHODS

In brief, 15 individual 250 day grain fed Angus, Hereford and Wagyu × Angus steers (n = 5 per breed) were slaughtered at ~26 months of age as part of a larger experiment detailed by (Greenwood *et al.* 2011). Fat depot samples were dissected from each carcass as soon as possible after slaughter from the *m. longissimus dorsi* (IMF), Inter, Omen, Kid and SC depots and snap frozen in liquid nitrogen. The *longissimus dorsi* muscle with IMF intact (LD) was also sampled. Total RNA was phenol chloroform extracted using Trizol (Invitrogen) following the

manufacturer's instructions. RNA yield and purity were determined using microphotometry and RNA integrity by agarose gel electrophoresis. RNA was submitted to the Ramaciotti Institute (Randwick, NSW, Australia) for hybridisation to the 4×44K one colour Agilent bovine array. Data was normalised using a previously described mixed-model approach (Reverter *et al.* 2005) and expressed as log2 values. The expression measurements represent mRNA abundance on a per unit total RNA basis.

Data-driven clustering. The expression profiles of 10,000 genes chosen at random were imported into Permut Matrix software (Caraux and Pinloche 2005). Global relationships between depots based on the molecular data were determined using unsupervised hierarchical clustering performed on columns for all tissues. LD muscle was included in this analysis for comparison.

Co-expression network. To gain insight into molecular relationships within and between fat depots, we used a co-expression approach to build the first exclusively fat depot-based *bovine* network. LD muscle samples were not included here. We filtered the normalised data to leave a manageable subset of genes that satisfied at least one of the following criteria: top 10% in terms of variability of expression; top 10% most abundant expression; annotated as either a transcription factor, cofactor, or chromatin remodeller by (Zhang *et al.* 2012). Significance of differences between genes across depots was determined by PCIT (Reverter and Chan 2008) followed by a hard threshold of 0.975.

RESULTS AND DISCUSSION

Data-driven clustering. Permut matrix software produced a dendrogram of relationships, interpretable from the top down. The text labels include breed and tissue. The first major split shows the LD was discriminated from the fat samples. The next split shows each fat depot could be clearly resolved (Figure 1A). IMF was awarded a unique branch within the fat tree. Inter and Omen were most closely related, followed by Kid then SC.

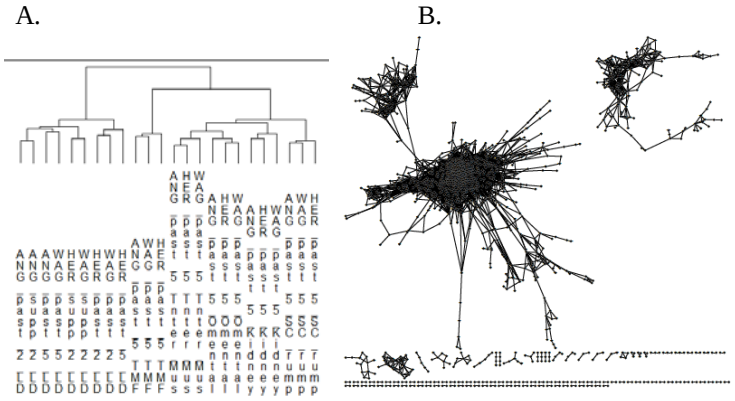

Figure 1. A. Hierarchical clustering of gene expression separated depots. B. Bovine fat depot co-expression network resolves into 3 major clusters reflecting cellular diversity.

Co-expression network. The co-expression network (Figure 1B) was visualised in Cytoscape (Shannon *et al.* 2003). We clustered using a Cytoscape algorithm called 'organic.' This shortens the path length between highly inter-connected genes producing visually coherent representations of gene interactions. Overall, it resolved into three major sub networks (Figure 1B) functionally enriched for 1) cytoskeletal architecture and metabolism (largest cluster; hypergeometric *P*-value = 7.57e-17) 2) inflammation (top left; *P* = 3.63e-18) and 3) peptidyl serine phosphorylation (top

right; *P* = 1.34e-4). These major clusters reflect the diversity of cell types which include macrophages and other immune cells in addition to adipocytes, pre-adipocytes and endothelial cells (Lee *et al.* 2013). Given the emerging link between inflammation and adiposity (Smorlesi *et al.* 2012), the inflammatory cluster is noteworthy. Molecules present in these modules display highly coordinated changes in expression across depots. The network contained several representatives from the three major gene sets previously identified (De Jager *et al.* 2013) representing triacylglyceride (TAG) synthesis (e.g. fatty acid binding protein 4, *FABP4*), fatty acid synthesis (e.g. fatty acid elongase 6, *ELOVL6*) and *PPARG* (e.g. acetyl coenzyme A synthetase, *ACSS2*).

In comparing SC (the most divergent depot) against the other depots we detected extreme DE of delta-like 1 homolog (*DLK1*) and homebox A10 (*HOXA10*) among others (Figure 2). In humans, it has previously been noted that genes regulating early development, including members of this family of phylogenetically ancient homeotic (*HOX*) genes, differ among undifferentiated pre-adipocytes between depots (Tchkonia *et al.* 2007). Similarly, *DLK1* has also been described as a marker for adipocyte progenitors (Shan *et al.* 2013). Gene expression clearly detects the presence of RNA diagnostic of skeletal muscle in our IMF sample. It is unclear to what extent the muscle RNA complicates the marbling adipocyte interpretation.

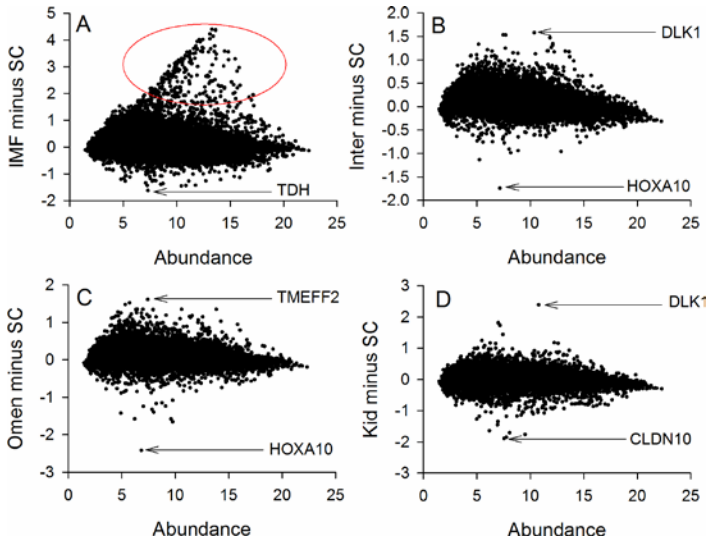

Figure 2. SC rump versus other fat depots. Oval highlights muscle derived transcripts

A targeted examination of enzymatic expression profiles across depots informed by known differences in tissue phenotypes relating to saturation (*SCD*), elongation (*ELOVL6*), TAG synthesis (*DGAT2*), synthetic capacity (*ACACA*), and acetate (*ACSS2*) and glucose (*MDH2*) fuel usage highlighted the following possible molecular drivers (Table 1). IMF displays lower expression of key lipogenic enzymes in line with, but to a lesser extent than, previous biochemical measurements made in cattle and pigs (Bonnet *et al.* 2007, Gardan *et al.* 2006).

Table 1. Log2 expression of genes encoding rate limiting enzymes of fatty acid composition.

Gene	Enzyme (EC#)	Probe	IMF	Inter	Kid	Omen	SC
SCD	Δ 9 desaturase (1.14.19.1)	A_73_P101286	10.49	11.15	11.03	11.01	11.76
ELOVL6	fatty acid elongase 6 (2.3.1.n8)	A_73_119372	10.88	11.44	11.43	11.42	11.54
ACACA	acetyl coA carboxylase (6.4.1.2)	A_73_P038926	6.90	7.45	7.41	7.27	7.74
DGAT2	diacylglycerol O-acyltransferase (2.3.1.20)	A_73_118582	15.86	16.40	16.42	16.34	16.72
ACSS2	acetyl coenzyme A synthetase (6.2.1.1)	A_73_P037091	13.10	13.77	13.71	13.62	14.01
MDH2	malate dehydrogenase 2 (1.1.1.37)	A_73_P422416	18.42	18.03	18.09	18.04	18.11

CONCLUSIONS

Biological similarity between fat depots is reflected by shared clusters of some highly co-expressed genes. Having said this, the 5 bovine depots can be clearly separated by global gene expression patterns, in a manner similar to other species. These depot-specific differences reflect, in part, the proportion and behaviour of populations of pre-adipocytes coupled with metabolic differences such as saturation and lipogenic fuel preference.

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