Gene Expression Profile Of Intramuscular And Subcutaneous Fat In Japanese Black And Holstein Steers

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Introduction

Intramuscular fat is an important quality factor in beef influencing palatability and juiciness (Wood *et al.* 1999). Consequently, accumulation of adipocytes in skeletal muscle is desired whereas subcutaneous and visceral fat depots are considered as "waste fat" and should be minimized (Gotoh *et al.* 2009). Depending on their location in the body, adipocytes have different properties (Rosen and MacDougald 2006). Adipocytes produce and secrete adipocytokines whereby adipose tissue functions as an endocrine and paracrine organ involved in several signaling pathways (Mohamed-Ali *et al.* 1998). However, the differential mechanisms regulating deposition and release of fat in intramuscular and other adipose depots like subcutaneous adipose tissue are not well understood (Bong *et al.* 2010). The aim of this study was to compare the gene expression profiles of intramuscular and subcutaneous adipose tissue in Japanese Black and Holstein steers fed a concentrate-rich diet.

Material and methods

Samples, RNA preparation, and microarray analysis. Intramuscular (M. longissimus) and subcutaneous adipose tissue was dissected from carcasses of 3 Japanese Black and 3 Holstein steers fed a concentrate-rich diet (Gotoh *et al.* 2009). Total RNA was isolated using TRIzol Reagent (Sigma, Taufkirchen, Germany) as described by the manufacturer. The fragmentation and labeling was performed with the GeneChip® Terminal Labeling Kit (Affymetrix, St. Clara, USA) according to the manufacturer's instructions. Five µg of total RNA per sample were used for preparation of antisense, biotinylated RNA for hybridization. Expression patterns were assessed using the GeneChip® Bovine Genome Array (Affymetrix, St. Clara, USA).

RT Real-time PCR. The iScriptTM cDNA Synthesis Kit (Bio-Rad Laboratories GmbH, Munich, Germany) was used to synthesize cDNA from 100 ng of total RNA from each sample according to the manufacturer's instructions. The mRNA abundance for the genes listed in Table 1 was determined as described by Xu *et al.* (2009).

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Statistical analyses. The data were processed with Affymetrix GeneChip® Operating Software (GCOS) and Affymetrix® Expression Console™ Software. A total of 24,013 probe sets was analyzed per sample. The correction for multiple testing was applied (False Discovery Rate, FDR, q-values). Between breed and between tissue comparisons for single genes were done with Fisher's LSD test. Regulated pathways were identified with Ingenuity Pathway Analyses (IPA) software (Ingenuity Systems, Redwood City, USA).

Results and discussion

Analysis of the gene expression in intramuscular fat (IMF) of the M. longissimus and subcutaneous fat (SCF) revealed only few differently expressed genes between Japanese Black and Holstein steers fed a carbohydrate-rich diet (166 and 105 probe sets, respectively). After correction for multiple testing, there remained only 79 and 66 genes different between the breeds in IMF and SCF, respectively ($q < 0.30$). Pathway analysis was performed with genes exhibiting a 2-fold up- or down-regulation ($p < 0.05$). In IMF, significantly regulated networks of genes were found belonging to the functional clusters "Cell Cycle" and "Drug Metabolism, Lipid Metabolism, Small Molecule Biochemistry" (Figure 1A). However, these networks are characterized by mostly indirect interactions with only few differently expressed genes at the periphery. The breed differences found in SCF referred predominantly to an increased immunological response of the adipocytes of Japanese Black steers (data not shown). In both tissues, the networks contained only genes up-regulated in Japanese Black cattle. The few genes with down-regulation (IMF: 7, SCF: 20) could not be assigned to functional networks.

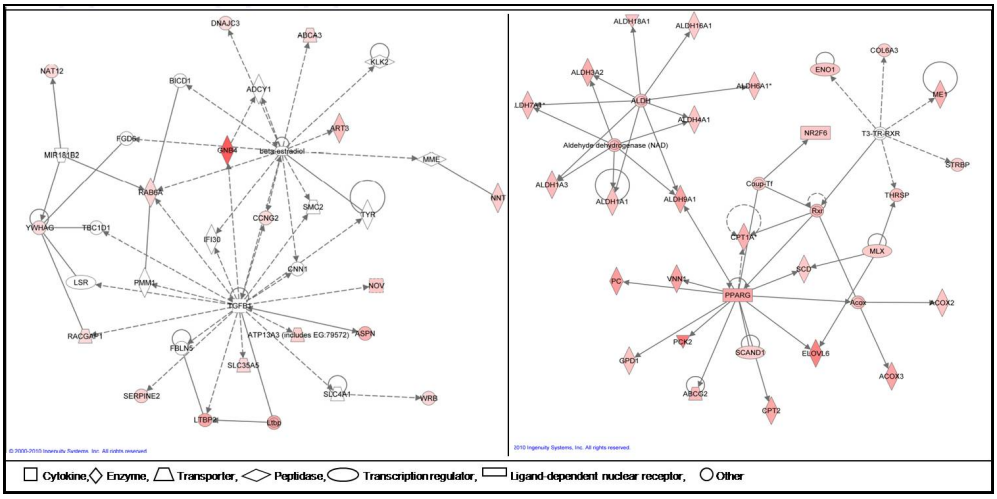

Figure 1: Examples for networks of regulated genes. (**A**): Different in IMF between breeds. (**B**): Different between IMF and SCF independent of breed.
(Dashed line: indirect interaction; Solid line: direct interaction, Red: up-regulated in JB [left], in IMF [right])

The expression of 8 genes proposed as markers for different IMF deposition in cattle or known as key factors for adipogenesis (Lee *et al.* 2008, Wang *et al.* 2009) was not different

between both breeds. These results were confirmed by RT Real-time PCR analysis (Table 1). Our data indicate only small differences in gene expression of adipocytes derived from the same depot between breeds, even when differing considerably in fatness traits. There are no marked differences in expression of gene related to lipid metabolism of adipocytes either from IMF or from SCF when the animals are fed the same diet. Despite low numbers of genes differentially expressed between both breeds, there are some genes with high differences in IMF (e.g. *GNB4* [guaninie nucleotide binding protein, beta polypeptide 4]: 9.7-fold increase in Japanese Black, Figure 1 A) and with largely unknown function in adipocytes. Furthermore, there are differences in genes which were not assigned to networks but which may mark yet unknown pathways.

In contrast to the breed comparison, the analysis of IMF and SCF in both breeds resulted in a list of 3,376 differently expressed genes of which 3,296 were up-regulated in SCF. Functional clustering of these genes involved several metabolic pathways like "Lipid Metabolism" and "Carbohydrate Metabolism" beside more general networks (e.g. "Cell Cycle", "Inflammatory Response"). One network of genes belonging to the category "Lipid Metabolism" is given in Figure 1B. A key regulator of adipocyte differentiation *PPARG* (peroxisome proliferator-activated receptor gamma) residing in the center of this network was already described to be different between adipose tissue depots but not between breeds (Huff *et al.* 2004). Compared to the networks obtained for the breed comparison, more direct relationships between the genes could be observed and the number of differently regulated genes was considerably higher. This is an indicator of substantial differences in the lipid metabolism between IMF and SCF at the time of slaughter.

Table 1: Expression of adipogenesis related genes in IMF of Holstein and Japanese Black steers

Breed	**Holstein**		**Japanese Black**	
Method	**Real-time PCR (AU)[1]**	**Microarray (lg signal)**	**Real-time PCR (AU)[1]**	**Microarray (lg signal)**
Gene				
PPARG	0.17 ± 0.04	8.50	0.21 ± 0.06	8.64
ADIPOQ	0.010 ± 0.003	8.95	0.017 ± 0.007	9.19
CEBPA	0.12 ± 0.04	9.99	0.09 ± 0.03	9.72
DLK1	0.003 ± 0.002	6.96	0.003 ± 0.001	6.88
LIPE	0.31 ± 0.130	11.91	0.27 ± 0.08	11.86
CEBPB	0.10 ± 0.04	9.89	0.08 ± 0.03	10.26
FABP4	18.44 ± 11.65	13.62	9.34 ± 3.28	13.55
ADFP	0.24 ± 0.04	8.16	0.61 ± 0.33	9.31

[1]Arbitrary Units: values relative to RPS18 expression

Recently, Bong *et al.* (2010) compared the expression profiles of bovine SCF and IMF in Korean native cattle by serial analysis of gene expression (SAGE). They identified 50 and 32 genes more than 2-fold up- and down-regulated in SCF compared to IMF, respectively. Compared to almost 3,400 genes with different expression in our study, the advantage of the global approach (Bovine Genome Array) is obvious. Most of the up-regulated genes in SCF found by Bong *et al.* (2010) were significantly increased also in our study (e.g. *ANXA5,*

SPARC, FTH1, and *DGAT2*). Although, none of these genes was involved in one of the derived functional networks, the observed accordance of the expression results obtained in different breeds indicates general differences in the transcriptional profile of adipocytes located in IMF and SCF, respectively. On the other hand, some of the genes identified as highly abundant in IMF by Bong *et al.* (2010) showed an opposite regulation in our sample (e.g. *NMT2*: 2.5-fold up-regulation vs. 2.0-fold down-regulation). This may be attributed to existing breed differences and/or different environmental influences. Chen *et al.* (2010) investigated the expression of lipogenic genes in redifferentiating visceral- and intramuscular-derived dedifferentiated progeny cells in the pig. They obtained different expression profiles between different depots. Despite the limited comparability of both investigations and species differences, this observation may be a further indicator for differences between different fat depots. There are unique developmental gene signatures in different adipose depots in mice (Yamamoto *et al.* 2010). Similarly, unique depot signatures were detected in cattle during growth in this study and by Bong *et al.* (2010).

Conclusion

We provide the first comprehensive comparison of the expression profiles of bovine intramuscular fat and subcutaneous fat in Japanese Black and Holstein steers. No different expression of proposed candidate genes was observed at this time point. Our results demonstrate that adipocytes exhibit a distinct expression profile dependent on their location. These differences are by far larger at slaughter than that between breeds regardless their IMF content and further fatness traits. This study provides a promising list of candidates for further investigations on the regulation of differential fat deposition.

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