Identification of genes expressed differentially in subcutaneous and visceral fat of cattle, pig, and mouse

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Submitted 12 August 2004; accepted in final form 15 March 2005

Hishikawa, Daisuke, Yeon-Hee Hong, Sang-gun Roh, Hisae Miyahara, Yukihiiko Nishimura, Ai Tomimatsu, Hiroaki Tszutsuki, Chizu Gotoh, Masaaki Kuno, Ki-Choon Choi, Hong-gu Lee, Kwang-keun Cho, Hisashi Hidari, and Shinichi Sasaki. Identification of genes expressed differentially in subcutaneous and visceral fat of cattle, pig, and mouse. Physiol Genomics 21: 343–350, 2005. First published March 22, 2005; doi:10.1152/physiolgenomics.00184.2004.—The factors that control fat deposition in adipose tissues are poorly understood. It is known that visceral adipose tissues display a range of biochemical properties that distinguish them from adipose tissues of subcutaneous origin. However, we have little information on gene expression, either in relation to fat deposition or on interspecies variation in fat deposition. The first step in this study was to identify genes expressed in fat depot of cattle using the differential display RT-PCR method. Among the transcripts identified as having differential expression in the two adipose tissues were cell division cycle 4 homolog (CDC42), prefoldin-5, decorin, phosphate carrier, 12S ribosomal RNA gene, and kelch repeat and BTB domain containing 2 (Kbtbd2). In subsequent experiments, we determined the expression levels of these latter genes in the pig and in mice fed either a control or high-fat diet to compare the regulation of fat accumulation in other animal species. The levels of CDC42 and decorin mRNA were found to be higher in visceral adipose tissue than in subcutaneous adipose tissue in cattle, pig, and mice. However, the other genes studied did not show consistent expression patterns between the two tissues in cattle, pigs, and mice. Interestingly, all genes were upregulated in subcutaneous and/or visceral adipose tissues of mice fed the high-fat diet compared with the control diet. The data presented here extend our understanding of gene expression in fat depots and provide further proof that the mechanisms of fat accumulation differ significantly between animal species.

There are two types of adipose tissue, subcutaneous and visceral. Recent studies indicate that adipocytes in these two fat depots show differences in basal metabolic properties, for example, in regulating volume, lipid composition, and so on (22, 24). There is considerable current interest in visceral adipose tissue because of its relationship with various diseases such as cardiovascular disease, type 2 diabetes mellitus, hyperlipidemia, and syndrome X. There are a number of potential reasons why visceral adipose tissue may contribute to abnormalities in metabolism; among these are its anatomical site and pattern of venous drainage, and the presence of intrinsic and unique features of visceral adipocytes. The venous drainage of visceral adipose tissue is via the portal system, directly providing free fatty acid as a substrate for hepatic lipoprotein metabolism and glucose production (16, 22, 24). Additionally, in vitro studies using labeled tracers have demonstrated that visceral adipocytes have higher rates of lipid turnover than subcutaneous adipose tissue (19, 20).

Fat depot metabolism is also of importance in the commercial rearing of livestock such as cattle and pigs. One of the most important themes in the animal industry is the production of high quality meat at low cost. A better understanding of the specific accumulation mechanisms of fat depots should contribute to improved production efficiency in the animal industry.

Recently, gene expression profiles in normal and abnormal tissues have been produced using DNA chips, PCR subtractions, and mRNA differential display (5, 8, 9, 13, 28). Gene expression profiling has been used to search for factors that determine normal or abnormal differentiation mechanisms in adipocytes from ob/ob and db/db mice, and in 3T3-L1 preadipocytes (15, 17, 23). Despite the importance of understanding physiological differences between normal and abnormal fat depots, limited data are available to date. To help improve this situation, we have examined gene expression profiles in subcutaneous and visceral fat depots of cattle using differential display and reverse transcriptase-polymerase chain reaction (DDRT-PCR) analysis. This study identified a number of genes that showed different expression patterns in the two types of adipose tissue. The expression levels of some of these genes were subsequently investigated in adipose tissues of pigs and in control and high-fat-diet mice to investigate interspecies differences in fat depot metabolism.

Materials and Methods

Animals. Bovine and porcine abdominal subcutaneous and visceral adipose tissues were sampled from 10 female Japanese Black cattle (18–24 mo of age) and 10 crossbred castrated male swine (body mass ~100 kg) at a local abattoir. Cattle were weaned at ~6 mo of age, placed on a standard “growing” diet until 9–10 mo of age, and then given free access to water and “concentrate” diet during a fattening period until they were 18–24 mo of age. The concentrate used in the fattening period contained 71% total digestible nutrients (TDN), 14% crude protein, 10% crude fiber, 10% crude ash, 2% crude fat, 0.3% phosphorus, and 0.3% calcium. Pigs were weaned at ~2.5 mo of age,
placed on a standard growing diet until 4 mo of age, and thereafter
given free access to water and fed a “finishing” diet during a fattening
period until their body weight reached 100 kg. The finishing diet
contained 77% TDN, 12% crude protein, 5% crude fiber, 7% crude
ash, 2% crude fat, 0.3% phosphorus, and 0.45% calcium. White
adipose tissues were rapidly separated from subcutaneous and visceral
(abdomen and ovaries) fat sites, immediately frozen in liquid nitrogen,
and stored at −80°C until RNA extraction. Three-week-old male
C57BL/6J mice were obtained from Charles River Japan. They were
housed individually in cages with wire-mesh bottoms at a temperature
of 20–22°C and a humidity of 50 to 60% under a 12:12-h light-dark
cycle. The animals had free access to water and chow (Oriental Yeast,
Chiba, Japan) containing 8.5% (wt/wt) fat, 43.7% carbohydrate, and
29.7% protein, with an energy content of 3.69 kcal/g, for an acclima-
tization period of 1 wk. The mice were then weighed and divided into
two groups of six with approximately equal mean body weights. One
group was fed the standard diet and the other received a high-fat diet
for 6 wk (4–10 wk of age). The high-fat diet was obtained from
Research Diet and contained 41% fat, 36% carbohydrate, and 23%
protein, with an energy content of 4.33 kcal/g; its fat source was the
same as that of the standard diet and it contained the same amounts of
protein and fiber as did the standard diet. The animals were weighed
every week. At the end of the experimental period, the mice were killed
by decapitation. White adipose tissues were rapidly separated from
subcutaneous and visceral (epididymal) fat sites, immediately frozen
in liquid nitrogen, and stored at −80°C until RNA extraction. All experiments were conducted in accordance with
the Shinshu University Guide for the Care and Use of Experimental
Animals and approved by an Institutional Review Board.

Total RNA extraction and DDRT-PCR. Total RNA was extracted
from pooled adipose tissues of Japanese Black cattle by the acid
guanidium thiocyanate-phenol-chloroform method (11) and was
subjected to DNase I to eliminate possible contamination with
genomic DNA. DDRT-PCR was performed between subcutaneous
and visceral adipose tissues using a Differential Display Kit (Takara,
Tokyo, Japan). We used 9 forward primers and 24 reverse primers for
DDRT-PCR amplification. In total, we used 216 forward and reverse
primers for subcutaneous and visceral fat, in agreement with the expression
patterns of DDRT-PCR, for cell division cycle 42 homolog
classified by ethidium bromide staining and analyzed with NIH image
software, where band intensity is expressed in pixels. Relative gene
expression was calculated as the ratio of band intensity of the cloned
gene to that of the β-actin. The amplified cDNAs were subcloned into
the pGEM-T easy vector, and the sequences were confirmed using an
automated DNA sequencer.

Statistical analysis. Data are presented as means ± SE of six or
seven animals. Comparisons were tested by ANOVA, followed by
Fisher’s protected least significant difference as a post hoc analysis.
Significance was set at P < 0.05.

RESULTS

Differential display and isolation of fat depot-related cDNA
fragments. We used a DDRT-PCR method to isolate genes
differentially expressed in subcutaneous and visceral adipose
tissues of Japanese Black cattle. Under standard differential
display (DD) conditions, and after analysis of the agarose gels,
we isolated 16 and 13 cDNA fragments highly expressed in
subcutaneous and visceral adipose tissues, respectively. We
selected and isolated bands that showed at least a twofold
difference in level of expression between subcutaneous or
visceral adipose tissues. Details of the 29 genes are given in
Table 1; we categorized the genes into eight functional groups
to aid interpretation of gene expression in these fat depots.

Confirmation of DDRT-PCR by RT-PCR in cattle. To con-
firm the differential expression of the genes, semiquantitative
RT-PCR, using specific primers, was performed. The primer
sequences, their target genes, and their product sizes are shown
in Table 2. These analyses were carried out in animals different
from those used for the DD experiment to ensure that the
observed differential expression was not due to a particular
genetic background or response of a single animal. RNA from
two to six cattle was used for this purpose. Of 29 initial
candidates, we confirmed differential expression between
subcutaneous and visceral fat, in agreement with the expression
patterns of DDRT-PCR, for cell division cycle 42 homolog
(CDC42), prepore-5, decorin, phosphatase carrier, 12S ribo-
sonal RNA gene, and kelch repeat and BTB domain contain-
ning-2 (Kbd2) (Figs. 2 and 3). Of the 29 genes initially
identified as showing apparent differential expression between
the two adipose tissues, 23 proved to be false positives.

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Semiquantitative RT-PCR analysis of the 29 candidate genes showed that there were no significant differences in expression of 23 of the genes between the two adipose tissues of cattle, pigs, and mice (data not shown).

Comparison of gene expression pattern in cattle, mouse, and pig. The expression patterns of the six differentially expressed genes described above were examined in subcutaneous and visceral adipose tissues of pigs and mice fed either the control or high-fat diet. The primers of the six genes used in our experiment were designed from conserved sequences of cattle, mouse, and pig genes. Mice fed a high-fat diet from age 4–10 wk showed a greater increase in body weight than those fed a control diet (Fig. 1). In addition, the amounts of subcutaneous and visceral fat in the animals fed the high-fat diet were ~1.5 and 2.5 times greater, respectively, than in mice fed the standard diet.

The levels of CDC42 mRNA were higher in visceral adipose tissue than in subcutaneous adipose tissue in cattle, pigs, and mice fed the control diet (Fig. 2). CDC42 mRNA levels were elevated significantly in both subcutaneous and visceral adipose tissues of mice fed the high-fat diet compared with those fed the standard diet (Fig. 2).

The levels of prefoldin-5 mRNA were significantly higher in subcutaneous adipose tissue than in visceral adipose tissue in cattle and mice fed the standard diet (Fig. 2). In mice fed the high-fat diet, prefoldin-5 mRNA levels were increased in both tissues (Fig. 2). The pattern of prefoldin-5 expression was different in pigs than in cattle and mice; in the pig, mRNA levels were higher in visceral adipose tissue than in subcutaneous adipose tissue (Fig. 2).

Decorin mRNA levels were higher in visceral adipose tissue than in subcutaneous adipose tissue in cattle, pigs, and mice fed the control diet (Fig. 2). Mice fed the high-fat diet showed a significant elevation of decorin mRNA in visceral adipose tissues (Fig. 2).

The expression of the phosphate carrier gene was significantly higher in subcutaneous adipose tissue than in visceral adipose tissue in cattle (Fig. 3). In contrast, in pigs and in mice fed the control diet, expression was significantly higher in visceral adipose tissue. Mice fed a high-fat diet had significantly increased levels of phosphate carrier gene mRNA levels in both adipose tissues (Fig. 3).

The expression pattern of 12S rRNA differed in the three species examined: in cattle, mRNA levels were significantly

Table 1. cDNA fragments isolated by DDRT-PCR

<table>
<thead>
<tr>
<th>Clone No. (Size, bp)</th>
<th>Accession No.</th>
<th>Homolog Gene</th>
<th>Relative Ratio (Fold)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular signaling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44 (219)</td>
<td>NM_009861</td>
<td><em>Mus musculus</em> cell division cycle 42 homolog (Cdc42), mRNA</td>
<td>V (2.8)</td>
</tr>
<tr>
<td>37 (438)</td>
<td>NM_002624</td>
<td><em>Homo sapiens</em> prefoldin-5 (Pfdn5), transcript variant 1, mRNA</td>
<td>S (7.1)</td>
</tr>
<tr>
<td>160 (157)</td>
<td>NM_004543</td>
<td><em>Homo sapiens</em> nebulin (NEB), mRNA</td>
<td>S (3.1)</td>
</tr>
<tr>
<td>103 (160)</td>
<td>BC013396</td>
<td><em>Homo sapiens</em> titin, mRNA, complete cds</td>
<td>S (2.9)</td>
</tr>
<tr>
<td>Extracellular matrix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>73 (199)</td>
<td>AF491944</td>
<td><em>Homo sapiens</em> decorin (DCN) gene, complete cds</td>
<td>V (4.0)</td>
</tr>
<tr>
<td>Mitochondrial gene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>242 (315)</td>
<td>X77338</td>
<td><em>Bos taurus</em> gene for phosphate carrier</td>
<td>S (2.6)</td>
</tr>
<tr>
<td>33 (310)</td>
<td>AF036273</td>
<td><em>Bison bison</em> cytosome-homolog (cyth) gene, mitochondrial encoding mitochondrial protein, complete cds</td>
<td>S (3.7)</td>
</tr>
<tr>
<td>Ribosomal protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 (240)</td>
<td>AF304203</td>
<td><em>Sus scrofa</em> breed Swedish wild boar mitochondrion, partial genome</td>
<td>V (6.1)</td>
</tr>
<tr>
<td>44 (282)</td>
<td>AF493541</td>
<td><em>Sus scrofa</em> isolate NADH dehydrogenase subunit 2</td>
<td>S (2.4)</td>
</tr>
<tr>
<td>52 (240)</td>
<td>AY012145</td>
<td><em>Sus scrofa</em> 12S ribosomal RNA gene, partial sequence; and rRNA-Val gene, complete sequence</td>
<td>S (2.9)</td>
</tr>
<tr>
<td>Enzyme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81 (176)</td>
<td>BC007474</td>
<td><em>Mus musculus</em> stearyl-CoA desaturase 1, mRNA, complete cds</td>
<td>V (2.9)</td>
</tr>
<tr>
<td>10 (364)</td>
<td>NM_021549</td>
<td><em>Mus musculus</em> polynucleotide kinase 3′-phosphatase (Pskp), mRNA</td>
<td>V (4.3)</td>
</tr>
<tr>
<td>119 (128)</td>
<td>NM_174615</td>
<td><em>Bos taurus</em> superoxide dismutase 1, soluble (SOD1), mRNA</td>
<td>S (2.6)</td>
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<tr>
<td>Unknown function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 (178)</td>
<td>NM_009381</td>
<td><em>Mus musculus</em> thyroid hormone responsive SPOT14 homolog (Rattus) (Thrsp), mRNA</td>
<td>V (4.5)</td>
</tr>
<tr>
<td>239 (211)</td>
<td>AC092341</td>
<td><em>Homo sapiens</em> chromosome 16, clone RP11-250K13, complete sequence</td>
<td>S (2.2)</td>
</tr>
<tr>
<td>27 (569)</td>
<td>NM_145958</td>
<td><em>Mus musculus</em> kelch repeat and BTB (POZ) domain containing 2 (Kbtbd2), mRNA</td>
<td>S (3.8)</td>
</tr>
<tr>
<td>118 (251)</td>
<td>AK056582</td>
<td><em>Homo sapiens</em> cDNA FLJ32020 fis, clone NTONG100123</td>
<td>V (2.9)</td>
</tr>
<tr>
<td>198 (372)</td>
<td>NM_175154</td>
<td><em>Mus musculus</em> RIKEN cDNA 2310003P10 gene, mRNA</td>
<td>S (2.7)</td>
</tr>
<tr>
<td>19 (286)</td>
<td>AF199905</td>
<td><em>Homo sapiens</em> PRO2853 mRNA, complete cds</td>
<td>S (4.1)</td>
</tr>
<tr>
<td>170 (249)</td>
<td>BC017702</td>
<td><em>Homo sapiens</em> zinc finger, DHHC domain containing 7, mRNA</td>
<td>S (2.0)</td>
</tr>
<tr>
<td>ESTs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 (166)</td>
<td>CB430001</td>
<td>609272 MARC 6BOV <em>Bos taurus</em> cDNA 3′, mRNA sequence</td>
<td>V (4.3)</td>
</tr>
<tr>
<td>161 (194)</td>
<td>AV618165</td>
<td><em>Bos taurus</em> ovary fetus <em>Bos taurus</em> cDNA clone E10V015G11 3′, mRNA sequence</td>
<td>S (3.1)</td>
</tr>
<tr>
<td>90 (206)</td>
<td>CB532062</td>
<td>754938 MARC 6BOV <em>Bos taurus</em> cDNA 3′, mRNA sequence</td>
<td>S (2.0)</td>
</tr>
<tr>
<td>73 (196)</td>
<td>BF603401</td>
<td>270800 MARC 3BOV <em>Bos taurus</em> cDNA 3′, mRNA sequence</td>
<td>S (3.7)</td>
</tr>
<tr>
<td>147 (349)</td>
<td>CB535474</td>
<td>768900 MARC 6BOV <em>Bos taurus</em> cDNA 3′, mRNA sequence</td>
<td>V (2.9)</td>
</tr>
</tbody>
</table>

*S indicates higher expression in subcutaneous adipose tissue compared with visceral adipose tissue with relative ratio (S/V); V indicates higher expression in visceral adipose tissue compared with subcutaneous adipose tissue with relative ratio (V/S); DDRT-PCR, differential display RT-PCR.
higher in subcutaneous adipose tissue; in pigs, the two adipose tissues showed similar levels of expression; in mice fed the control diet, mRNA levels were significantly higher in visceral adipose tissue (Fig. 3). Both adipose tissues showed significant upregulation of 12S rRNA in mice fed the high-fat diet (Fig. 3). The level of Kbtbd2 mRNA was higher in subcutaneous adipose tissue than in visceral adipose tissue in cattle (Fig. 3). In contrast, in both pigs and in mice fed the control diet, Kbtbd2 expression was higher in visceral adipose tissue than in subcutaneous adipose tissue. The levels of Kbtbd2 were elevated significantly in subcutaneous adipose tissue in mice fed the high-fat diet (Fig. 3).

DISCUSSION

We have identified six genes that are differentially expressed in subcutaneous and visceral adipose tissues of cattle and furthermore shown that these genes also show differential expression patterns in pigs and mice. Comparison of the patterns of expression of genes belonging to different functional groups suggests that many factors control where fat is deposited. Our data can be interpreted in two ways. First, the regulation of fat accumulation in individual depots is clearly different for genes involved in different metabolic processes, for example in the cell matrix, mitochondria, and signal transduction. Second, gene expression patterns vary between different species, suggesting that control of the metabolic processes of fat depot development is likely to be species specific. Various factors have been implicated in fat depot development: location of fat depot (e.g., visceral vs. subcutaneous), regulation of the regenerative capacity of individual depots, and regulation of metabolic status by paracrine and autocrine signals generated by different cell types present in a particular depot. Although visceral obesity appears to be associated with increased morbidity, the basis of this association is not clear. In cattle, visceral adipose tissues dramatically increase after 10 mo of age despite the absence of any physiological abnormality. In pigs, subcutaneous fat rapidly accumulates after 3 mo of age. This comparison suggests that, not only are there differences in the accumulation of fat over the whole body in cattle, pigs, and mice, but fat accumulation in each adipose tissue is controlled by species-specific regulatory mechanisms.

CDC42 is a member of the Rho GTPase family. Rho proteins containing CDC42 act as molecular switches to control cellular processes by cycling between the active GTP-bound and inactive GTP-bound states. CDC42 mRNA level does not change during differentiation of 3T3-L1 preadipocytes to adipocytes (25). Moreover, a recent study has demonstrated an important role for CDC42 as a novel signaling molecule in the insulin action pathway leading to glucose transporter-4 translocation and stimulation of glucose trans-

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Sequence (5’-3’)</th>
<th>Size, bp</th>
<th>PCR Condition, Cycle, Annealing Temperature</th>
<th>Relative Expression*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdc42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>AAAAGTTGGATACAAAAACT</td>
<td>414</td>
<td>30 cycle, 55°C</td>
<td>V</td>
</tr>
<tr>
<td>Antisense</td>
<td>CTCTGGAGTAATAGGCTTCT</td>
<td></td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>Prefoldin-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>CTGACGAGTTCTATGATGGTC</td>
<td>223</td>
<td>28 cycle, 55°C</td>
<td>S</td>
</tr>
<tr>
<td>Antisense</td>
<td>GAAATTTTCGGATCACCTTTTT</td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Decorin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>GGAACGAGTTTCTACTGAGAA</td>
<td>451</td>
<td>33 cycle, 50°C</td>
<td>V</td>
</tr>
<tr>
<td>Antisense</td>
<td>AGCTCTGTTGTTGACTCAAAGTT</td>
<td></td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>12S rRNA gene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>GGTCTACATGATTACACCMAAA</td>
<td>449</td>
<td>33 cycle, 55°C</td>
<td>S</td>
</tr>
<tr>
<td>Antisense</td>
<td>ACCATATACTACACCCTGGA</td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Phosphate carrier</td>
<td></td>
<td></td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Sense</td>
<td>TTAATGCTATTCTACAGTACAG</td>
<td>429</td>
<td>30 cycle, 55°C</td>
<td>S</td>
</tr>
<tr>
<td>Antisense</td>
<td>TGACTAGCTAATGCTGACATGA</td>
<td></td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>Kbtbd2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense</td>
<td>GAAAAACACAAAAACAAATC</td>
<td>449</td>
<td>30 cycle, 52°C</td>
<td>S</td>
</tr>
<tr>
<td>Antisense</td>
<td>ACCCTCCTAATATGAAAAAT</td>
<td></td>
<td></td>
<td>V</td>
</tr>
<tr>
<td>β-Actin</td>
<td></td>
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<tr>
<td>Sense</td>
<td>ACCGTGAGAAGATGACTCAG</td>
<td>456</td>
<td>28 cycle, 55°C</td>
<td>V</td>
</tr>
<tr>
<td>Antisense</td>
<td>GAAAGAAGTCTCGAGAGGACC</td>
<td></td>
<td></td>
<td>V</td>
</tr>
</tbody>
</table>

* indicates higher expression in subcutaneous adipose tissue compared with visceral adipose tissue, V indicates higher expression in visceral adipose tissue compared with subcutaneous adipose tissue, and S = V indicates no difference between subcutaneous and visceral adipose tissues. CDC42, cell division cycle 42 homolog; Kbtbd2, kelch repeat and BTB (POZ) domain containing 2.

Fig. 1. Growth curves of mice fed either a control or a high-fat diet. Data are means ± SE of values from 6 mice.
port. In addition, it was found that CDC42 is downstream of Gq/11 in that signaling system and lies upstream of phosphatidylinositol 3-kinase and PKC (30). The high expression level of CDC42 in visceral fat of cattle, pig, and mice and in high-fat diet induction of mice may indicate a difference of insulin action in fat depots and in high-fat diet induction. The importance of CDC42 action in specific fat depots remains to be explored, but the data suggest complex effects dependent on the interplay of circulating insulin signaling and CDC42 expression. In addition, the pattern of expression of CDC42 is similar in cattle, pigs, and mice in showing higher expression levels in visceral adipose tissue than in subcutaneous adipose tissue; this consistency of expression pattern may indicate that CDC42 is more involved with the development of visceral than subcutaneous fat.

Prefoldin is a recently discovered chaperone protein that functions by directing unfolded target proteins to cytosolic chaperonin. Prefoldin binds to nascent actin during its biosynthesis and may thereby block the irreversible agglutination of actin (31). The relative expression of prefoldin-5 was different in subcutaneous and visceral adipose tissues of pigs compared with cattle and mice. It may indicate that, during fat accumulation, the cytoskeleton differs in the two fat depots in animal species and is further changed by high-fat diet induction. Because the extracellular matrix is linked to the nucleus by cytoskeletal fibers that facilitate hormonal signal transduction (4), during adipose tissue enlargement, structural changes take place that may affect cytoskeleton and extracellular matrix protein expression. With regard to this, our previous results showed that cytoskeletal nonmuscle-type cofilin is differentially expressed in visceral fat and may play a role in lipid accumulation (10). Although the function of prefoldin-5 is still unclear, the differential expression of prefoldin-5 in fat depots may indicate that the cytoskeleton can affect cell morphology and may also be a factor in the etiology of interspecies differences.
We also found that decorin expression is higher in visceral adipose tissue than in subcutaneous adipose tissue of cattle, pigs, and mice. Decorin is a proteoglycan and is present in the extracellular matrix. Proteoglycans have been suggested to play important roles in the morphogenesis of many organs. A recent study suggested that alteration in the expression level of extracellular matrix proteins may contribute to the development of obesity-associated adipose tissue growth (7). The relatively high level of expression of decorin in visceral adipose tissue and after induction by a high-fat diet may contribute to the proliferation and development of adipose tissue. It may suggest that the extracellular matrix is changed in individual fat depots, with fat accumulation being different depending on the environment of each fat depot in the whole body.

Both the phosphate carrier and 12S ribosomal RNA genes are mitochondrial genes. Mitochondria generate most of the ATP used by cells to drive reactions that require an input of free energy. The phosphate carrier gene catalyzes the transport of inorganic phosphate across the inner mitochondrial membrane into the matrix compartment for the oxidative phosphorylation of ADP to ATP (14). The 12S ribosomal RNA gene codes an essential part of the decoding site of the ribosome and a subunit association crucial for either RNA-protein or RNA-RNA interactions (18, 34). The differential expression of these mitochondrial genes in subcutaneous and visceral fat depots, between animal species, and after high-fat diet are indicative of differences in mitochondrial function presumably linked to differences in energy requirements.

The kelch motif is an ancient and evolutionarily widespread sequence motif of 44–56 amino acids in length (27). In general, kelch-repeat β-propellers are involved in protein-protein interactions; however, the modest sequence identity between kelch motifs, the diversity of domain architectures, and the partial information on this protein family in any single species all present difficulties for developing a coherent view of the kelch-repeat domain and kelch-repeat protein families (1, 27, 33). Kbtdb2 also has a BTB/POZ domain characteristic of a protein-protein interaction interface (3). The BTB domain is
known to have various functions: repression of transcriptional activity, punctate localization of the protein in the nucleus, and interaction with components of the histone deacetylase complex (2). The biological function of Kbtdb2 in adipose tissue remains to be determined, although there is the intriguing possibility of changes in protein-protein interactions in the development of fat depots.

Interestingly, the six differentially expressed genes isolated from cattle in our study are upregulated in subcutaneous and/or visceral adipose tissues of mice fed a high-fat diet. With the exception of prefoldin-5, the genes were highly expressed in the visceral adipose tissues of mice fed a control diet. This observation shows that many genes with different biochemical functions can influence the development of adipose tissue fat depots. However, these six genes were not changed during adipocyte differentiation of 3T3-L1 cells and of bovine and porcine primary preadipocytes (data not shown). We suggest that the process of fat accumulation in individual depots is not related to adipocyte differentiation from preadipocytes, even though such differentiation is still occurring during fat accumulation. Therefore, the upregulation of the expression of these six genes by the high-fat diet indicates that they may be involved both with the development of adipose tissues and with fat accumulation. Furthermore, these six genes differentially expressed in regional fat depots may contribute to the regional differences in the development of the each adipose tissue. There is a need to obtain a more detailed picture of how the many cell types present in different fat depots of each animal (e.g., adult adipocytes, preadipocytes, stem/progenitor cells, tissue macrophages, neurons, and endothelial cells) interact with each other and sense and respond to the metabolic and inflammatory status of the entire organism.

It is well known that nutritional state is one of the important factors on gene expression profiles (12, 29, 32). Recent studies have demonstrated that expression of genes related to adipocyte differentiation and lipid metabolism is regulated by nutritional status; the pattern of development of adipose tissue can be altered by variations in nutrition (6, 21, 26, 32). Livestock such as cattle and pigs are commonly fed according to a feeding program in which the diet varies at different stages of the animals’ development. Although expression of the six genes identified here can be altered by changes in the nutritional conditions, such as diets either high or low in energy and protein, the patterns of differential gene expression in regional fat depots were consistent in cattle and pigs raised using a standard feeding program. The main objective of the present study was to determine which genes typically show differential expression in different fat depots of cattle and pigs that had been raised under the standard conditions used in the livestock industry. Clearly, the next stage of this investigation will be to characterize how changes in nutritional status influence the growth performances of cattle and pigs.

In this study, we found 29 genes (from 8 functional groups) that appeared to show differential expression in fat depots of Japanese Black cattle. Six of these genes were confirmed as being differentially expressed and were studied in detail in cattle, pigs, and mice fed either a standard or a high-fat diet. Subcutaneous and visceral fat tissues are thought to display marked differences in both basal and stimulated lipolysis or lipogenesis after differentiation of preadipocytes to adipocytes. Further studies have to be performed examining the metabolic properties of each type of fat tissue to determine whether there are differences between subcutaneous and visceral adipose tissues. The question arises whether regional, not completely specified, regulatory mechanisms account for these different findings. As mentioned above, characteristic patterns of maturation and proliferation of adipocytes can be found at every adipose tissue depot. However, specific biomarkers of changes in cellular physiology and metabolism brought on by accumulation of fat in an individual depot that are truly associated with the development of adipose tissues of animal species are clearly needed. Our gene expression profiles indicate that adipose tissues can show characteristic biochemical differences and that these differences may vary between species. Such information contributes to our understanding of the metabolic processes involved in the formation of fat depots.

GRANTS

This work was partly supported by Grant-in-Aid No. 15780178 to S.-G. Roh and No. 15580246 to S. Sasaki for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

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