# Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats

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**Abstract** **The Otsuka Long-Evans Tokushima fatty (OLETF) rat is an animal model of type 2 diabetes, characterized by abdominal obesity, insulin resistance, hypertension, and dyslipidemia. To elucidate the underlying molecular mechanism of obesity and its related complications, we used representational difference analysis and identified the genes more abundantly and specifically expressed in the visceral adipose tissue (VAT) of obese OLETF rats compared with the diabetes-resistant counterpart, that is, Long-Evans Tokushima Otsuka (LETO) rats. By Northern blot analysis, we confirmed the differential expression of 13 genes, including 3 novel genes. The upregulated expression of well-characterized lipid metabolic enzymes, such as lipoprotein lipase, phosphoenolpyruvate carboxykinase, and cholesterol esterase, were observed in VAT of OLETF rats. We demonstrated the differential expression of secreted proteins in VAT of OLETF rats, such as thrombospondin 1 and contrapsin-like protease inhibitor. In contrast to lipid enzymes, the secreted proteins revealed exclusive mRNA expression and they were not detected in VAT of LETO rats. Furthermore, the novel genes OL-16 and OL-64 were also expressed specifically in VAT of OLETF rats and were absent in that of LETO rats and other tissues, including subdermal and brown adipose tissues. The C-terminal partial amino acid sequence of OL-64 revealed that it showed ~40% homology with $\alpha_1$-antitrypsin and it seemed to be a new member of the serine proteinase inhibitor (SERPIN) gene family. VAT of OLETF rats had a unique gene expression profile, and the accumulated VAT-specific known and novel secreted proteins may play a role(s) in the pathogenesis of obesity and its related complications.**—K. Hida, J. Wada, H. Zhang, K. Hiragushi, Y. Tsuchiyama, K. Shikata, and H. Makino. **Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. *J. Lipid Res.* 2000.** 41: **1615–1622.**

**Supplementary key words** obesity • OLETF rats • visceral adipose tissue • representational difference analysis • PCR-based subtraction method • molecular cloning

Obesity significantly increases the risk of death from cardiovascular diseases in humans. It has been reported that the relative risk associated with an increment of 1 in body mass index (BMI) was 1.10 for the mortality from cardiovascular diseases in 30- to 44-year-old men (1). The regional distribution of body fat is also recognized as a critical determinant for vascular complications. Although visceral adipose tissue is accurately assessed by computed tomography or magnetic resonance imaging, the accumulation of abdominal fat estimated from anthropometric variables, such as waist-to-hip ratio and waist circumference, is the major risk factor of cardiovascular diseases in both men and women (2, 3). It is now generally accepted that abdominal obesity is frequently associated with highly atherogenic metabolic complications, such as insulin resistance, hyperinsulinemia, hypertension, glucose intolerance, and dyslipidemia (4).

In addition to these epidemiological findings, the advance in molecular biology of adipocytes shows that visceral adipose tissue not only stores excess energy in the form of fat but also produces physiologically active substances (5). They are considered to be involved in the disease process of obesity-related metabolic disorders and subsequent atherosclerosis. For instance, obesity is associated with upregulated mRNA expression of tumor necrosis factor α (TNF-α) in adipose tissue of human and rodents, and TNF-α seems to be related to insulin resistance (6–8). The peroxisome proliferator-activated receptor γ (PPARγ) is expressed primarily in adipose tissues and the target of insulin sensitizer, that is, troglitazone. In obese Zucker rats, the administration of troglitazone did not change total

Abbreviations: BMI, body mass index; CAP, c-Cbl-associated protein; CE, cholesterol esterase; cDNA-RDA, representational difference analysis of cDNA; CPi, contrapsin-like protease inhibitor; DDRT-PCR, differential display reverse transcriptase-polymerase chain reaction; EMP-1, epithelial membrane protein 1; EST, expressed sequence tag; LETO rat, Long-Evans Tokushima Otsuka rat; LPL, lipoprotein lipase; NLS, nuclear localization signal; Nopp140, nucleolar phosphoprotein of 140 kDa; OLETF rat, Otsuka Long-Evans Tokushima fatty rat; PAI-1, plasminogen activator inhibitor 1; PEPCK, phospho*enol*pyruvate carboxykinase; PMP22, 22-kDa peripheral myelin protein; PPARγ, peroxisome proliferator-activated receptor γ; SERPIN, serine protease inhibitor; SH3, Src homology 3; SSC, saline sodium citrate; TGN, *trans*-Golgi network; TNF-α, tumor necrosis factor α; VAT, visceral adipose tissue.

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weight of white adipose tissue but increased number of small adipocytes. As a result, troglitazone normalized the expression level of TNF-α and also insulin resistance (9, 10). In addition to these molecules, plasminogen activator inhibitor 1 (PAI-1) mRNA is also upregulated in visceral fat in obese human subjects and it is implicated in the development of vascular diseases (11).

These lines of evidence led us to isolate the genes upregulated during fat cell hypertrophy in visceral adipose tissue, and that may play a role in the pathogenesis of obesity and its related complications in type 2 diabetes. As our experimental model, diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats and their diabetes-resistant counterparts, Long-Evans Tokushima Otsuka (LETO) rats, were used. Although body fat distribution has not been shown to correlate with obesity-related complications in rodents, OLETF rats are the most suitable animal model for our research because they are characterized by visceral fat obesity, insulin resistance, hyperinsulinemia, hyperglycemia, and hypertension (12). Although the impairment of insulin secretion was observed in OLETF rats at the age of 40 weeks, hyperinsulinemia and insulin resistance preceded pancreatic β cell dysfunction at 16 and 24 weeks of age (13, 14). To identify genes, the polymerase chain reaction (PCR)-based subtraction method, representational difference analysis of cDNA (cDNA-RDA), was used. This is a sensitive and efficient method compared with conventional subtractive cloning or differential display RT-PCR (DDRT-PCR) and we isolated novel genes that are involved in organ development and various pathological states (15–17). To efficiently isolate the genes upregulated in fat cell hypertrophy we compared gene expression in OLETF rats at 6 months and in LETO rats at 8 weeks, because LETO rats have a minimal volume of omental fat and reveal no insulin resistance (12). In this study, we newly isolated known and novel genes that are not reported to be upregulated in visceral adipose tissue of obese OLETF rats. In addition, some of the genes revealed visceral adipose tissue-specific expression and they were not detected in subdermal and brown adipose tissue by Northern blot analysis.

## MATERIALS AND METHODS

### Animals

Eight-week-old male Otsuka Long-Evans Tokushima fatty (OLETF) rats (n = 5), and sex- and age-matched Long-Evans Tokushima Otsuka (LETO) rats (n = 5), were obtained from the Otsuka Pharmaceutical Tokushima Research Institute (Tokushima, Japan). OLETF rats were allowed access to standard chow and water. Visceral adipose tissues, that is, omental adipose tissues, were harvested from 6-month-old OLETF rats and 8-week-old LETO rats, snap-frozen in liquid nitrogen, and subjected to total RNA extraction. OLETF rats at 6 months yielded 5 to 8 g of visceral adipose tissue per animal, while 0.5 to 1 g of visceral adipose tissue was obtained from LETO rats at 8 weeks. Various tissues, including brown and subdermal adipose tissues, were also harvested from 6-month-old OLETF rats.

### Representational difference analysis of cDNA

Total RNAs were extracted from visceral adipose tissue of OLETF and LETO rats by the guanidinium isothiocyanate-CsCl ultracentrifugation method (18). The mRNAs of visceral adipose tissue were isolated with oligo(dT) cellulose, using a FastTrack 2.0 kit (InVitrogen, San Diego, CA). The cDNA-RDA procedure, that is, the PCR-based subtraction method, was carried out as previously described (15–17). In brief, double-stranded cDNAs were synthesized from 2 μg of mRNA of visceral adipose tissues. They were then digested with *Dpn*II, adaptor-linker ligated, and PCR amplified by adaptor primers to generate representative amplicons (R-amplicons). The R-amplicon of OLETF rats served as a tester and that of LETO rats served as a driver to drive the subtraction process and obtain the subtracted cDNAs, which were differentially expressed in visceral adipose tissue in OLETF rats. In addition, the R-amplicon of LETO rats was used as a tester and the reverse-subtracted cDNA product was also obtained.

### Cloning and sequence analysis of differential products

The subtracted final product was subcloned into the pCR2.1-TOPO vector (InVitrogen). After transformation into the TOP10 bacterial host (InVitrogen), colonies were randomly picked and ~100 plasmid clones with inserts were purified with a plasmid purification system (Qiagen, Hilden, Germany). The sequence reaction was carried out by the dye terminator method and analyzed by automated sequencer (ABI Prism; Perkin-Elmer, Foster City, CA). Ninety-six nonoverlapping clones were selected. The homology search of isolated genes was performed by the online-based BLAST program through the nonredundant and expressed sequence tag (EST) database at the National Center of Biotechnology Information (NCBI). The multiple sequence alignment was performed with CLUSTALW, available at the ExPASy molecular biology server (http://www.expasy.ch/tools/).

### Screening of upregulated genes by DNA nylon filter array

To further screen the differentially expressed clones, we used a DNA nylon filter array system, with a PCR select differential screening kit (Clontech, Palo Alto, CA). First, the inserts of the 96 nonoverlapping clones were PCR amplified by M13 forward and reverse primers. The PCR products were mixed with equal amounts of 0.6 N NaOH and arrayed onto four separate Hybond N+ nylon membranes (Amersham, Arlington Heights, IL). The membranes were neutralized with 0.5 M Tris-HCl (pH 7.5) and baked for 2 h at 70°C in an oven. R-amplicons of OLETF rats (tester) and LETO rats (driver), subtracted cDNA, and reverse-subtracted cDNA were radiolabeled with [α-$^{32}$P]dCTP (Amersham) and hybridized with the four membranes, respectively. The signal of each clone observed on the tester and subtracted cDNA probe-hybridized membranes was compared with that of the driver and reverse-subtracted cDNA probe-hybridized membranes, respectively. The clones, which revealed high signals in membranes hybridized with the tester and the subtracted cDNA probes, were selected and subjected to the following Northern blot analyses.

### Northern blot analysis

Two micrograms of mRNA extracted from visceral adipose tissue of LETO and OLETF rats was subjected to 2.2 M formaldehyde-1% agarose gel electrophoresis and capillary transferred to the Hybond N+ nylon membranes (Amersham). As size marker, 20 μg of total RNA was loaded. Plasmid clones were digested with *Eco*RI, and the inserts were purified by QIAEX kit (Qiagen), and radiolabeled with [α-$^{32}$P]dCTP, using the random primer labeling system (Amersham). The membranes were hybridized with radiolabeled cDNA probes ($1 \times 10^6$ cpm/ml) at 42°C in 50% formamide, 5× SSC (saline sodium citrate), 1× Denhardt's

solution, 50 mM sodium phosphate (pH 7.0), and salmon sperm DNA at 200 μg/ml for 24 h. Filters were washed under high-stringency conditions (four times at room temperature for 15 min in 1× SSC-0.1% SDS, followed by two times at 50°C in 0.1× SSC-0.1% SDS).

To investigate the tissue distribution of known and novel genes upregulated in visceral adipose tissue of OLETF rats, Northern blot analysis of various tissues, including brain, heart, lung, spleen, kidney, small intestine, muscle, brown adipose tissue, and subdermal adipose tissue of OLETF rats, was also performed. β-Actin served as an internal control.

## RESULTS

### Screening of upregulated genes in visceral adipose tissue of OLETF rats

The agarose gel electrophoresis of cDNAs derived from visceral adipose tissue of OLETF and LETO rats appeared to smear, ranging from 0.5 to 10 kb. After restriction enzyme digestion with *Dpn*II, DNAs were ligated to adaptor-linkers and the R-amplicons prepared. They were then subjected to cDNA-RDA and the subtracted final DNA product was subcloned into pCR2.1-TOPO vector and ~100 plasmid clones were isolated and sequenced. Overlapping clones were eliminated and the final 96 clones selected. The inserts of cDNA clones were dot-blotted on nylon membranes. Nine clones strongly hybridized with radiolabeled OLETF (tester) probe compared with LETO (driver) probe (**Fig. 1**, left panels). The rest of the clones showed minimal signals as background and differential expression was not confirmed. Although this method is widely used, there is one major disadvantage: Only cDNA molecules corresponding to highly abundant RNAs, that is, mRNAs that constitute more than 0.2% of the total cDNA in the R-amplicon, will produce detectable hybridization signals (16). Thus, most of the clones were considered to be relatively low-abundance genes and thus failed to hybridize with R-amplicons. To confirm upregulated

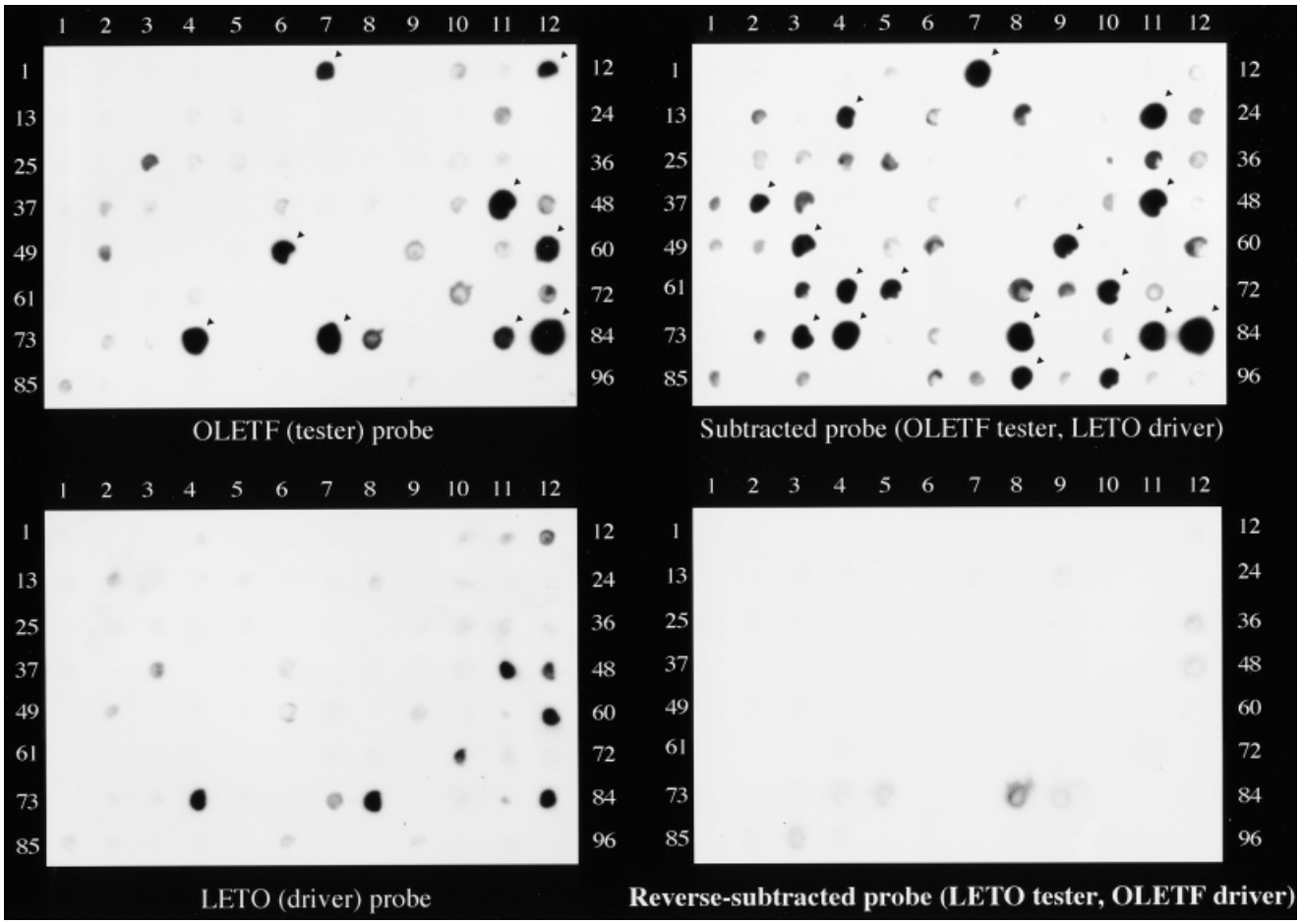


**Fig. 1.** DNA nylon filter array for the screening of upregulated genes. cDNA inserts of 96 randomly selected clones are arrayed onto 4 separate Hybond N+ nylon membranes. Representative amplicons of OLETF rats (tester) and LETO rats (driver), subtracted cDNA, and reverse-subtracted cDNA are radiolabeled with [α-$^{32}$P]dCTP, using the random primer labeling system, and hybridized with the four membranes separately. Clones strongly hybridized with tester and subtracted probes compared with driver and reverse-subtracted probes, respectively, are selected and indicated by arrowheads. By comparison between tester and driver probe, 9 clones are identified (top left). Only cDNA molecules corresponding to highly abundant RNAs, which constitute more than 0.2% of the total cDNA in the R-amplicon, will produce detectable hybridization signals by this method. However, by using forward- and reverse-subtracted probes, an additional 13 clones are identified (top right).

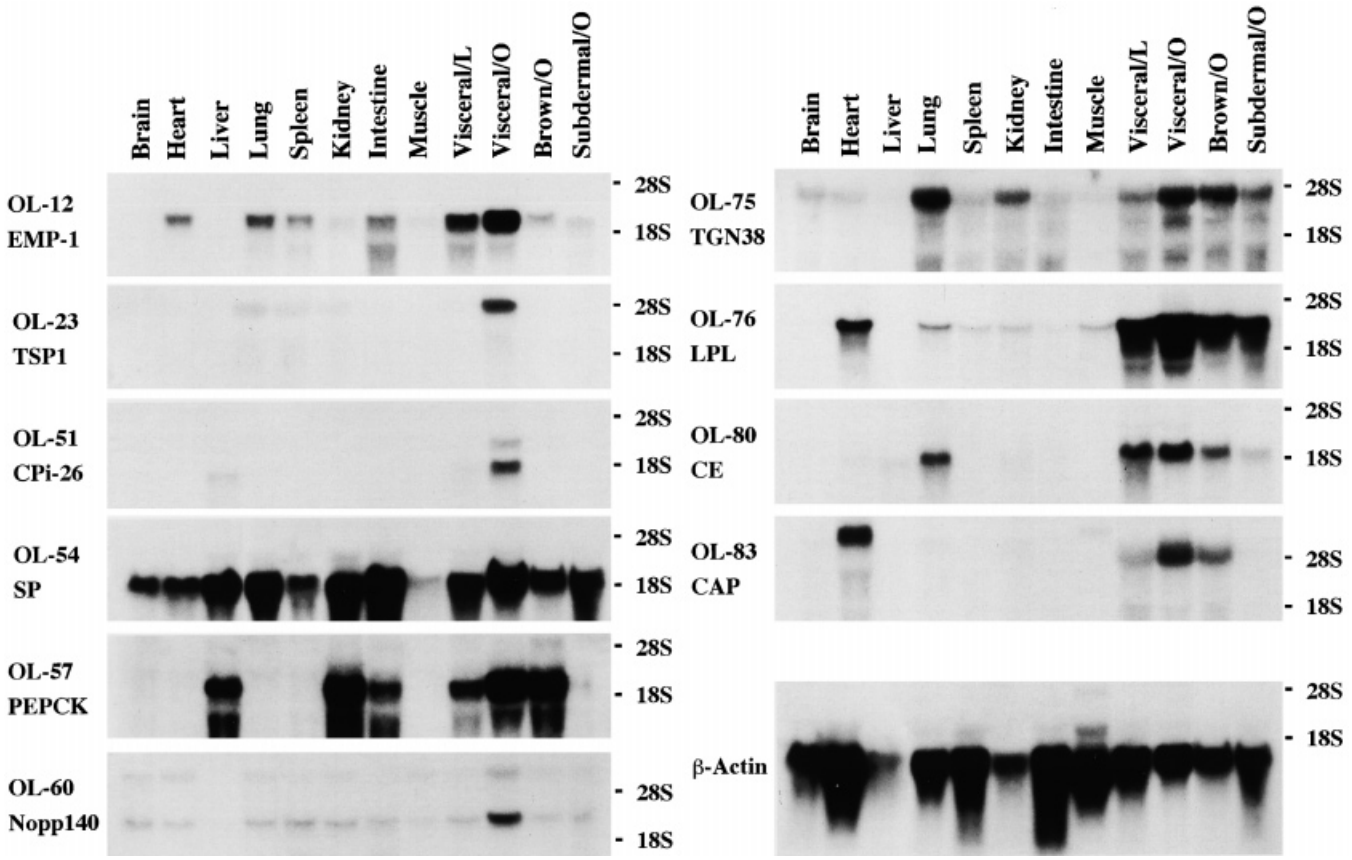


**Fig. 2.** Known genes upregulated in visceral adipose tissue of OLETF rats and their tissue distribution. Two micrograms of mRNAs extracted from 11 tissues of OLETF rats and visceral adipose tissue of LETO rats are subjected to Northern blot analysis. The membranes are hybridized with α-$^{32}$P-radiolabeled cDNA probes of 10 known genes and β-actin. Abbreviations: Visceral/L, visceral adipose tissue of LETO rats; Visceral/O, visceral adipose tissue of OLETF rats; Brown/O, brown adipose tissue of OLETF rats; Subdermal/O, sudermal adipose tissue of OLETF rats; EMP-1, epithelial membrane protein 1; TSP1, thrombospondin 1; CPi-26, contrapsin-like protease inhibitor; SP, selenoprotein P; PEPCK, phospho*enol*pyruvate carboxykinase; Nopp140, nucleolar phosphoprotein 140kD; TGN38, *trans*-Golgi network integral membrane protein; LPL, lipoprotein lipase; CE, cholesterol esterase; CAP, c-Cbl-associated protein.

expression of low-abundance genes, nylon filters were hybridized with the $^{32}$P-labeled forward-subtracted and reverse-subtracted probes. Clones representing mRNAs that are differentially expressed in adipose tissue of OLETF rats will hybridize only with $^{32}$P-labeled forward-subtracted probe. By this method, an additional 13 clones were identified (Fig. 1, right panels). mRNAs derived from adipose tissues of LETO and OLETF rats were subjected to Northern blot analyses, using 22 candidate cDNA clones as radiolabeled probe. By Northern blot analysis, the differential expression of 10 known and 3 unknown genes was confirmed (**Figs. 2** and **3**). The differential expression of nine other genes was not confirmed, because mRNA signals were not detected by Northern blot analysis.

### Homology search of isolated cDNA clones

A GenBank homology search revealed that 10 clones matched with full-length known genes. They included the genes of epithelial membrane protein 1 (OL-12), thrombospondin 1 (OL-23), contrapsin-like protease inhibitor (OL-51), selenoprotein P (OL-54), phospho*enol*pyruvate carboxykinase (OL-57), nucleolar phosphoprotein of 140 kDa, Nopp140 (OL-60), *trans*-Golgi network integral membrane protein TGN38 (OL-75), lipoprotein lipase (OL-76 and OL-84), cholesterol esterase (OL-80), and c-Cbl-associated protein (CAP; OL-83). Densitometric analyses revealed their 1.8- to 4.2-fold upregulated expression in adipose tissue of OLETF rats. Notably, the transcripts of thrombospondin 1 and contrapsin-like protease inhibitor were barely detectable in LETO rats and they were abundantly expressed in visceral adipose tissue of OLETF rats (**Table 1** and Fig. 2).

In addition to the 10 known genes, the differential expression of 3 novel genes was confirmed by Northern blot analyses. All unknown genes, OL-16, -38, and -64, were dominantly expressed in visceral adipose tissue of OLETF rats (Table 1 and Fig. 3). A homology search was performed through the EST database of rat, mouse, and human at the NCBI, and the clustered EST clones were retrieved from the UniGene database at http://www.ncbi.nlm.nih.gov/UniGene/.

OL-16 completely matched to rat UniGene Rn.40141; however, it did not show significant homology to any EST clones of human and mouse. Only two EST clones clustered in Rn.40141, indicating that OL-16 was not a ubiquitously expressed gene. Furthermore, a BLASTX search did not reveal any significant homology with other known

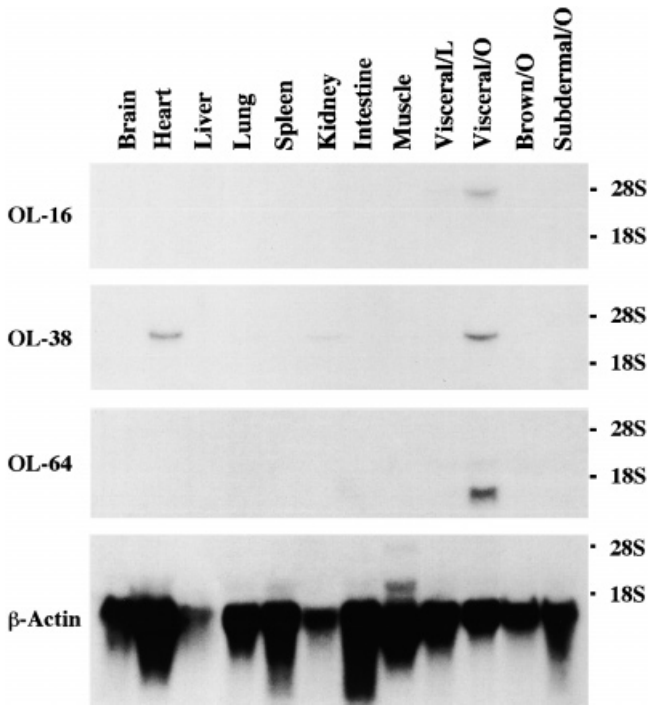


**Fig. 3.** Novel genes upregulated in visceral adipose tissue of OLETF rats and their tissue distribution. Two micrograms of mRNAs extracted from 11 tissues of OLETF rats and visceral adipose tissue of LETO rats are subjected to 2.2 M formaldehyde-1% agarose gel electrophoresis and capillary transferred to the Hybond N+ nylon membranes. As size marker, 20 μg of total RNA, is also loaded and 28S and 18S are indicated. Northern blot of β-actin is also shown. The Northern signals of OL-16, -38, and -64 in visceral adipose tissue of LETO are barely detectable. Notably, OL-16 and OL-64 are visceral adipose tissue-specific genes and not expressed in any other tissues including brown and subdermal adipose tissues.

protein sequences. Because Rn.40141 stretched over only 581 bp and was accompanied by a poly(A) signal, the obtained cDNA fragment of OL-16 corresponded to a 3′-untranslated region (Table 1).

OL-38 showed a nucleotide sequence identical to that of rat UniGene Rn.41961. UniGene Rn.41961 contained only one 340-bp EST clone and a poly(A) signal was observed. The BLASTX search also failed to reveal known protein domain structures or motifs (Table 1).

OL-64 revealed strong similarity with clustered EST clones of mouse UniGene Mm.20286 and human UniGene Hs.99476. The matched rat EST clones were not found. Alignment of the deduced partial coding sequences indicated that they shared a conserved amino acid sequence (**Fig. 4A**). A BLASTX search of OL-64 showed that the deduced partial amino acid of OL-64 revealed ~40% homology with $\alpha_1$-antitrypsin of human (1313184B, g2392173), mouse (P22599, P07758), and rat (P17475). The sequence alignment of OL-64 and rat $\alpha_1$-antitrypsin is shown in Fig. 4B. The significant homology with $\alpha_1$-antitrypsin suggested that the gene product of OL-64 is a novel member of the serine proteinase inhibitor (SERPIN) family.

### Tissue distribution of differentially expressed known and unknown genes

To demonstrate tissue distribution of the isolated genes, multiple tissue Northern blot analyses were performed. An equal amount of mRNA (2 μg) was loaded into each lane, and the nylon membranes were subjected to Northern blot analysis. The relative expression ratio of β-actin mRNA to total amount of mRNA in various tissues was similar to that described in a previous report (19), ensuring the quality of isolated mRNAs. The expression pattern of the known and novel genes in various adipose tissues indicated that they were divided into three groups. They were as follows: *1*) the genes that exist in all adipose tissues: selenoprotein P, phospho*enol*pyruvate carboxykinase (PEPCK), Nopp140, TGN38, lipoprotein lipase (LPL), and cholesterol esterase (CE); *2*) the genes distributed in visceral and brown adipose tissues: CAP, OL-7, and OL-94; and *3*) the genes specifically expressed in accumulated visceral adipose tissue of OLETF rats: thrombospondin 1,

TABLE 1. Differentially expressed known and novel genes in visceral adipose tissue of OLETF rats

| Clone | m RNA Size (kb) | Northern Blot Up-regulation (-fold)[a] | Accession Number | Gene Name |
|---|---|---|---|---|
| OL-12 | 2.8 | 1.8 | X98403 | Mouse mRNA for epithelial membrane protein 1 |
| OL-23 | 6.0 | OL-Spec | M62470 | Mouse thrombospondin gene |
| OL-51 | 2.2 and 4.0 | OL-Spec | D00753 | Rat mRNA for contrapsin-like protease inhibitor (CPi-26) |
| OL-54 | 2.4 | 2.0 | D25221 | Rat mRNA for selenoprotein P |
| OL-57 | 2.6 | 3.4 | K03248 | Rat phospho*enol*pyruvate carboxykinase (GPT) gene |
| OL-60 | 3.6 and 8.0 | 4.2 | M94228 | Rat nucleolar phosphoprotein of 140 kDa, Nopp140 mRNA |
| OL-75 | 5.0 | 3.2 | X53565 | Rat mRNA for *trans*-Golgi network integral membrane protein TGN38 |
| OL-76, -84 | 3.7 | 2.0 | L03294 | Rat lipoprotein lipase mRNA |
| OL-80 | 2.3 | 1.8 | L46791 | Rat cholesterol esterase (Es-HVEL) mRNA |
| OL-83 | 7.0 and 8.0 | 3.2 | U70668 | Mouse c-Cbl-associated protein CAP mRNA |
| OL-16 | 6.0 | OL-Spec | Rn.40141[b] | Unknown |
| OL-38 | 5.0 | OL-Spec | Rn.41961[b] | Unknown |
| OL-64 | 1.8 | OL-Spec | Mm.20286[b] Hs.99476[b] | Unknown |

Abbreviations: OL-Spec: mRNA signal is not observed in visceral adipose tissue of LETO rats.
[a] mRNA expression is visceral adipose tissue of OLETF rats is compared with that of LETO rats by densitometric analysis.
[b] Sequence is available at http://www.ncbi.nlm.nih.gov/UniGene/.

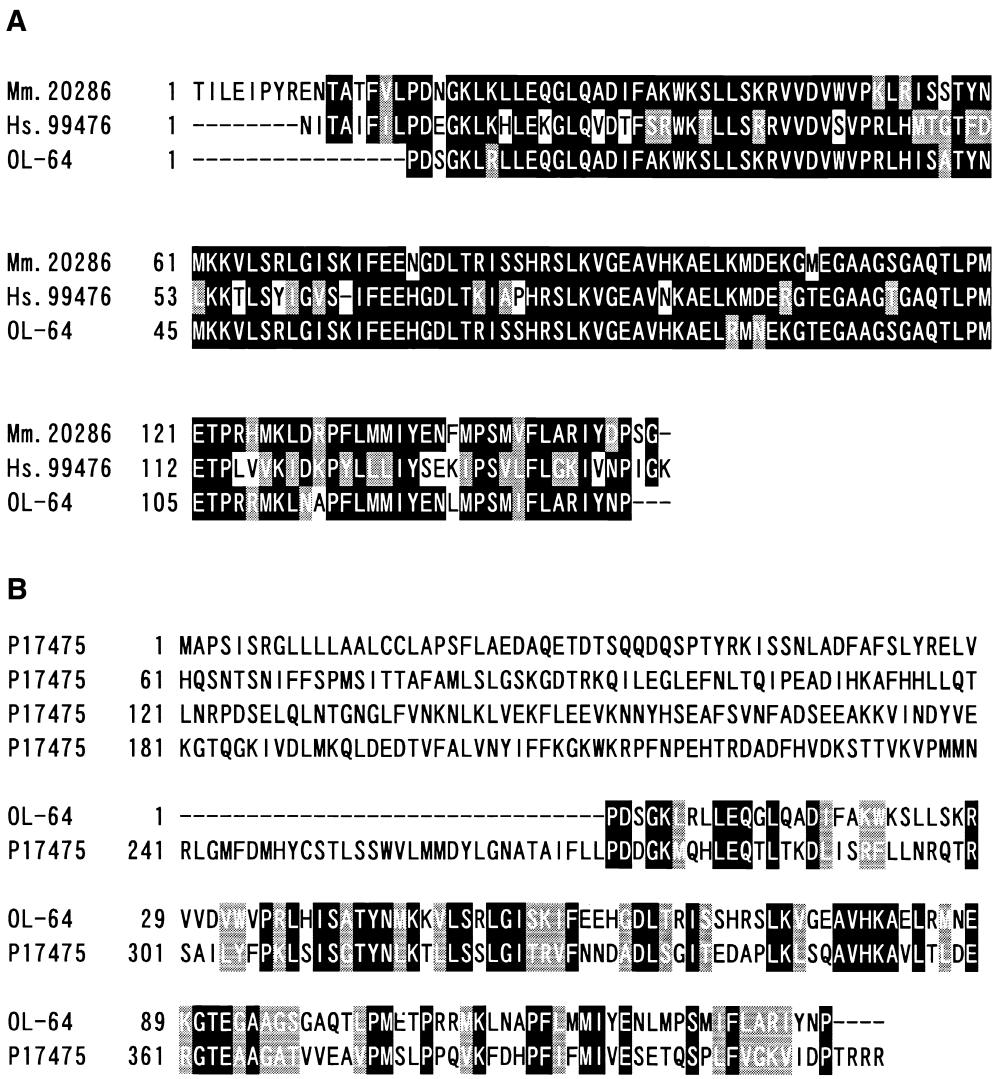


**Fig. 4.** Multiple alignment of amino acid sequences. (A) The CLUSTALW multiple alignment program, using the deduced amino acid sequence of OL-64, mouse UniGene Mm.20286, and human UniGene Hs.99476, indicates that they are orthologous genes. (B) CLUSTALW multiple alignment of the deduced amino acid sequences of OL-64 and $\alpha_1$-antitrypsin of rat (P17475). The gene product of OL-64 is a novel member of the serine proteinase inhibitor (SERPIN) gene family. Black boxes indicate identical amino acids and gray boxes indicate similar amino acids.

contrapsin-like protease inhibitor-related protein, OL-16, OL-38, and OL-64 (Figs. 2 and 3). Notably, Northern blot analyses of various nonadipose tissues indicated that two novel genes, OL-16 and OL-64, were visceral adipose tissue-specific genes and exclusively expressed in the accumulated adipose tissue of OLETF rats (Fig. 3).

## DISCUSSION

The successful isolation of the genes expressed in visceral adipose tissue of obese OLETF rats indicated that the expression profile of actively transcribed mRNA species in accumulated abdominal fat is unique compared with other adipose tissues. Although attempts were made to isolate the white adipose tissue-specific genes by randomly sequencing the nonbiased library (5), PCR-based subtractive screening using the visceral adipose tissue of both obese and nonobese rats enabled us to efficiently isolate such genes. This finding indicated that the investigation of visceral adipose tissue in obesity would open a new area of exploration in adipocyte biology. The isolated genes in this investigation fall into three groups: *1*) known genes with characterized functions (some of these were already reported to be upregulated in accumulated visceral adipose tissue), *2*) known genes with unexplored roles in adipose tissue, and *3*) novel and totally uncharacterized genes.

In the first category, enzymes related to lipid and glucose metabolism, that is, lipoprotein lipase (LPL), phospho*enol*pyruvate carboxykinase (PEPCK), cholesterol esterase (CE), and CAP, are included. It is well known that higher triglyceride uptake and turnover are observed in visceral adipose tissue compared with subdermal adipose

tissue (20). The increased expression of lipoprotein lipase mRNA during the development of obesity in ventromedial hypothalamus-lesioned rats was reported (19) and lipoprotein lipase is responsible for the delivery of fatty acid to the visceral adipose tissue (21). Another key enzyme responsible for lipid metabolism in adipose tissue is PEPCK. In liver, PEPCK is a rate-limiting gluconeogenic enzyme, and it is also involved in glyceroneogenesis (22). In adipose tissue, PEPCK provides glycerol 3-phosphate needed for fatty acid reesterification, that is, glyceroneogenesis, and upregulated PEPCK reflects the active turnover cycle of lipolysis and fatty acid reesterification in visceral adipose tissue of OLETF rats (23). The product of the c-*cbl* protooncogene is tyrosine phophorylated in response to insulin in 3T3-L1 adipocytes and not in 3T3-L1 fibroblasts. c-Cbl-associated protein (CAP) is isolated by a yeast two-hybrid cDNA library prepared from fully differentiated 3T3-L1 adipocytes by screening with full-length c-Cbl as a bait protein (24). CAP contains a unique structure with three adjacent Src homology 3 (SH3) domains and associates with insulin receptor. Because insulin stimulation results in the dissociation of CAP from the insulin receptor, CAP seems to participate in the insulin signaling events in adipocytes (24). Upregulated expression of CAP may reflect the augmented insulin signaling in the accumulated visceral adipose tissue of OLETF rats.

In the second group, Nopp140, TGN38, selenoprotein P, thrombospondin 1, contrapsin-like protease inhibitor, and epithelial membrane protein 1 are included. Among these gene products, Nopp140 and TGN38 are intracellular proteins. Nopp140 is a nucleolar phophoprotein of 140 kDa, which was originally isolated as a nuclear localization signal (NLS)-binding protein (25). Nopp140 was suggested to function as a chaperone for import into and/or export from the nucleolus. TGN38 is also widely expressed in various tissues; it is an integral membrane protein predominantly localized to the *trans*-Golgi network (TGN) and constitutively trafficks between the TGN and the cell surface. TGN38 reaches the cell surface and again it is internalized and delivered back to the TGN (26, 27). The significance of these intracellular traffic molecules, that is, Nopp140 and TGN38, in adipose tissue remains unexplored. In addition to these intracellular proteins, the secreted proteins were isolated, including selenoprotein P, thrombospondin 1, and contrapsin-like protease inhibitor. It has been reported that 19.6% of genes expressed in human adipose tissue result in secreted proteins, and this evidence suggested that adipose tissue might play a central role in the development of multiple risk factor syndrome and subsequent atherosclerosis by secreting various bioactive substances (5). Selenoprotein P is an extracellular protein that contains multiple selenocysteines. It binds heparin and associates with endothelial cells in the liver, kidney, and brain (28). The function of selenoprotein P is currently unknown; however, indirect evidence suggested that selenoprotein P is a free radical scavenger (29). Thrombospondin 1 is multifunctional secreted extracellular matrix glycoprotein and it has been recognized that thrombospondin 1 reveals inhibitory activities against angiogenesis and may be related to the pathogenesis of atherosclerosis (30, 31). Contrapsin-like protease inhibitor also revealed visceral adipose tissue-specific gene expression, although it was originally cloned from liver (30). Contrapsin-like protease inhibitor was found to inhibit trypsin activity but not chymotrypsin or elastase activity, and three related genes (CPi-21, -23, and -26) were isolated by homology screening (32). The final known gene with uncharacterized function is a membrane-bound protein, that is, epithelial membrane protein 1 (EMP-1) (33). EMP-1 is a putative four-transmembrane protein of 160 amino acids with 40% amino acid homology with 22-kDa peripheral myelin protein (PMP22) and belongs to PMP22/EMP/MP20 gene family (34). PMP22 is mainly expressed in myelinating Schwann cells in the peripheral nervous system (35); however, EMP-1 is predominantly found in gastrointestinal tract, skin, and lung (34). Although EMP-1 is speculated to play a role in proliferation and differentiation of the epithelial cells, its role in adipose tissue is totally unknown.

The third group includes three novel and uncharacterized genes, which are differentially expressed in the visceral adipose tissue of OLETF rats. Among the novel genes, OL-64 is exclusively expressed in the visceral adipose tissue of OLETF rats and it seems to belong to the serine proteinase inhibitor (SERPIN) gene family. It is interesting to note that PAI-1 is also a member of the SERPIN family, and enhanced expression of the PAI-1 gene in visceral fat is closely related to elevated PAI-1 plasma levels. Furthermore, it has been postulated that it plays a role in the development of vascular disease in visceral obesity (11). In this regard, the presence of the protein product of OL-64 in the plasma and its biological activities need to be investigated.

In conclusion, the data obtained in this investigation suggest that the visceral adipose tissue of obese OLETF rats has a unique gene expression profile and that the differentially expressed genes may be associated with visceral adipocyte hypertrophy. Further expression studies in various fat tissues, using other strains of obese rats and mice, may be necessary to elucidate whether their transcriptional activity depends on the maturation and hypertrophy of adipocytes in visceral fat. The secreted proteins including thrombospondin 1, contrapsin-like protease inhibitor, and OL-64 reveal specific expression in visceral adipose tissue of obese OLETF rats, and they may participate in the pathogenesis of both abdominal obesity and its vascular complications. Because visceral obesity in rats may not necessarily be representative of human visceral obesity, the role and significance of these gene products in pathobiology of human obesity need to be addressed in future studies.

This work was supported by the Uehara Memorial Foundation, the Naito Foundation, and the ONO Medical Foundation; by a Grant-in-Aid for Encouragement of Young Scientists (Ministry of Education, Science and Culture, Japan; 10770199) to J. Wada; and by a Grant-in-Aid for Scientific Research (B) (Ministry of Education, Science and Culture, Japan; 11470218) and by the Uehara Memorial Foundation to H. Makino. H. Zhang

was supported by an International Society of Nephrology/Kirin Fellowship Award.

*Manuscript received 21 March 2000 and in revised form 30 May 2000.*

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