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Gene Expression Profile in the Liver Tissue of High Fat Diet-Induced Obese Mice

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The purpose of this study was to investigate the gene profiles that were up- or down-regulated in the livers of high-fat diet-induced obese mice and *db*$^-$/*db*$^-$ mice with deficient leptin receptor. C57/BL6 normal mice and *db*$^-$/*db*$^-$ mice, respectively, were divided into two groups and fed a standard or high-fat diet for four weeks. Liver weight was unchanged in the normal mice but the high-fat diet led to a 10% weight increase in the *db*$^-$/*db*$^-$ mice. Adipose tissue mass increased by about 88% in the normal mice that were fed a high-fat diet and by about 17% in the *db*$^-$/*db*$^-$ mice on the high-fat diet. In terms of serum lipids, total cholesterol significantly increased in mice on the high-fat diet. Microarray analysis was carried out using total RNA isolated from the livers of standard or high-fat diet-fed mice of the normal and *db*$^-$/*db*$^-$ strains. The change of gene expression was confirmed by RT-PCR. About 1.6% and 6.8% of total genes, respectively, showed different expression patterns in the normal mice fed the high-fat diet and *db*$^-$/*db*$^-$ mice. As a result of microarray, many genes involved in metabolism and signal pathways were shown to have different expression patterns. Expression of Mgst3 gene increased in the livers of normal and *db*$^-$/*db*$^-$ mice that were fed a high-fat diet. Wnt7b and Ptk9l were down-regulated in the livers of the normal mice and *db*$^-$/*db*$^-$ mice that were fed a high-fat diet. In conclusion, a high-fat diet induced obesity and affected gene expression involved in metabolism and signal pathway.

Key words : High fat diet, Wnt7b, Ptk9l, Liver, Microarray, *db*$^-$/*db*$^-$ mouse

INTRODUCTION

Obesity is a major health concern worldwide. It is estimated that 26% of Americans are overweight[1)], with 5% to 14% of men and 7% to 24% of women considered evidently obese[2)]. Similar or even higher estimates for the prevalence of obesity have been reported in other countries. Obesity contributes to an increased rate of mortality by virtue of its role in the development of cardiovascular disease, type 2 diabetes, pulmonary dysfunction and gallstones.[3,4)] Obesity occurs when energy intake exceeds energy expenditure. It is generally accepted that a high-fat diet promotes obesity under arbitrary feeding conditions.[5)]

In mammals, excessive energy is stored as triglyceride in adipose tissue. Adipose tissue is the major site for regulating energy storage and release by many bioactive molecules called adipocytokine, such as adipsin, angiotensinogen, leptin, tumor necrosis factor α (TNF-α) and adiponectin. Specifically, leptin plays a key role in the regulation of food intake, energy expenditure and adiposity. Leptin, secreted by the adipose tissue, is transferred through the blood and binds leptin receptor (product of the *db* gene) in the hypothalamus. The hypothalamus responds to leptin by secreting neuropeptides that regulate energy metabolism[6)]. If leptin or leptin receptor are deficient, the affected individuals become extremely obese.

A high-fat diet has been shown to impair glucose tolerance and decrease insulin sensitivity in both humans and animals.[7,8)]. A high-fat diet was also reported to decrease insulin suppresibility of hepatic glucose production.[9)] It was reported that a high-fat diet reduces insulin-stimulated skeletal muscle and adipocyte glucose transport.[10,11)] The fatty acid composition of dietary fat may determine its effect on glucose metabolism and insulin action. Saturated fat intake has been shown to be associated with insulin resistance, whereas some of the adverse effects of a high-fat diet on insulin sensitivity can be ameliorated by substitution of n-3 fatty acids.[7,12)].

Many genes are involved in lipid metabolism in the liver.[13)] One of these is FABP (fatty acid binding protein) which is a 14-15 kDa protein and is involved in the absorption, transfer and storage of free fatty acid.[14,15)] Galatz and Veerkamp[16)] reported that a high-fat diet increases FABP in the liver, heart, small intestine and adipose tissue.

In this study, we searched for genes that were differentially expressed in the liver tissue of mice that

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were fed a high-fat diet. To find the candidate genes, we used microarray analysis and found genes whose expression was up or down-regulated significantly.

MATERIALS AND METHODS

Animal Care

Male normal C57BL/6 mice and *db*$^-$/*db*$^-$ mice of the C57BL/6 strain were purchased from Daehan biolink (Seoul, Korea) and Korea Research Institute of Bioscience and Biotechnology (Taejon, Korea), respectively. All mice were acclimatized for at least one week before experimental manipulation. The normal and *db*$^-$/*db*$^-$ mice were divided into two groups. Each group contained 10 mice. One group was fed a standard diet and the other was fed a high-fat diet containing 60% fat calories for four weeks. Composition of the standard diet was AIN-93G, from which the high-fat diet was modified[17] (Table 1). All mice had free access to water and food. The temperature was kept at 22±1℃, and a 12h light-dark cycle was maintained.

Table 1. Composition of standard diet and high fat diet

Components	Standard Diet (%)	High Fat Diet (%)
Casein	20	20
Sucrose	10	10
Corn starch	39.75	14.95
Dyetrose	13.2	5
Lard (80%)		36.5
Soybean oil	7	3.5
Cellulose	5	5
Vitamin mix	1	1
Mineral mix	3.5	3.5
Choline bitartarate	0.25	0.25
L-Cystein	0.3	0.3
BHQ	0.0014	0.0014
Energy (Kcal/100g)	394.8	500.0
Energy from fat (%)	16.0	58.9

Measurement of Serum Lipid Amount

After dissecting the mice, blood was drawn from the heart using a syringe. Levels of total cholesterol (TC) and triglyceride (TG) in serum were detected using TG and TC-detection kit (ASAN pharmaceutical, Seoul, Korea). Free fatty acid (FFA) level was measured by Nescott NEFA kit-U (Azwell Co. Osaka, Japan).

Microarray Assay

cDNA chips for microarray were obtained from Gaiagen (Seoul, Korea). Total RNA from the liver was extracted by the acid-phenol method using Trizol (GibcoBRL, Gaithersburg, USA) according to the manufacturer's instructions. From each sample, 20μg total RNA was transcribed into fluorescently labeled cDNA using 5μg of anchored oligo-dT primer, 6ul of 5×reaction buffer, 0.6μl of 50×dNTP, 2μl of reverse transcriptase (GibcoBRL NY, USA) and deoxyuridine- triphosphate (dUTP) labeled with one of two fluorophores, Cy3 or Cy5. Unincorporated deoxyribonucleotide-triphosphates (dNTPs) were removed and the sample was concentrated by speed vac. Microarrays were prehybridized with 15 μl of hybridization solution (poly(dA) 8ul, *E.coli* tRNA 4ug, mouse Cot1 DNA 10 μg, 1% SDS, 5×SSC) for 2 h at 42℃ in a humidified hybridization chamber. For each microarray, Cy5 and Cy3 labeled cDNA from tissue RNA were combined with 15 μl hybridization buffer and heated at 95℃ for 10 min. The combined sample was covered with a coverslip and hybridized for 12-16 h at 42℃. After hybridization, the microarray was washed twice at RT in 0.1×SSC/0.1% SDS solution and 0.1×SSC solution, respectively. The slide was dried by brief centrifugation and scanned according to the manufacturer's instructions with the use of a GSM418 scanner (Affimetrix, CA, USA). The data analysis was performed using ImaGene software (BioDiscovery Co., CA, USA)

Expression of Mgst3, Wnt7b and Ptk9l Genes by RT-PCR

To determine Mgst3, Ptk9l and Wnt7b gene expression in the livers of the normal and *db*$^-$/*db*$^-$ mice that were fed the high-fat diet, total RNA was extracted and RT-PCR was conducted using specific primers. One microgram of total RNA was reverse transcribed for 60 min at 42℃ using RT premix (Bioneer. Daejeon, Korea). Two microliters of the product were used for PCR amplification in a total volume of 20ul containing 1×Tag buffer, 0.25 mM dNTPs, 0.15 mM $MgCl_2$, 1uM of each primer and 1U ExTaq (TAKARA, Shiga, Japan). The primers used were 5'-tcctcttccgacttgattc-3' (left primer) and 5'- ggttctgtgtgtaccagctc-3' (right primer) for Ptk9l, 5'-atcatctgcaacaagattcc-3' (left primer) and 5'-cttacattccagcttcatgc -3' (right primer) for Wnt7b, 5'-GATTTGTGCTTCTCACTGGT 3' (left primer) and 5'-AACTGGAAATCACGGTGAG 3' (right primer) for Mgst3. PCR cycles were performed in GeneAmp PCR system 2700 (Perkin-Elmer Corp, Massachusetts, USA) with the following temperature profile: denaturation at 95℃ for 30 sec, annealing at 58℃ for Mgst3, 55℃ for Ptk9l, 50℃ for Wnt7b for 1 min and extension at 72℃ for 30 sec. The cycle was repeated 25-40 times, followed by a final extension step of 10 min at 72℃. Five microliters of PCR product was electroporesed on a 2% agarose gel and stained with 0.5 μg/ml ethidium bromide to visualize bands by UV transilluminator.

Statistical Analysis

The data were presented as mean ±S.E.M. and were

analyzed statistically by One-Way ANOVA with post hoc Duncan test using SPSS 9.0 software (SPSS inc., Chicago, USA). Differences were considered significant at $p < 0.05$.

RESULTS

Effect of a High-fat Diet on Morphology

A high-fat diet is one of the factors leading to obesity in humans and animals. Body size and weight in normal mice and *db⁻/db⁻* mice fed a high-fat diet was larger than that for mice fed a standard diet. The fur of the mice fed a high-fat diet was fattier than that for the control group, and the amount of fur was less than that for the control group. In the *db⁻/db⁻* mice, their body size was larger than that of the normal mice and the fur of the *db⁻/db⁻* mice fed a high-fat diet tended to fall out compared with the *db⁻/db⁻* mice fed the control diet (Fig. 1).

Effect of a High-fat Diet on Weight and Serum Lipid

To confirm the effects of a high-fat diet on body weight, liver, adipose tissue and serum lipid, normal and *db⁻/db⁻* mice were fed a standard diet or a high-fat diet for four weeks. After four weeks of feeding, body weight had increased 13% in the normal mice on the high-fat diet (Table 2). Epididymal and retro-peritoneal adipose tissue roughly doubled in the normal mice on a high-fat diet. In the *db⁻/db⁻* mice on the high-fat diet, weight increased 11%. Epidydimal and retro-peritoneal adipose tissue increased 17% in the *db⁻/db⁻* mice on the high-fat diet. But liver mass was unchanged in the normal mice on the high-fat diet. In the *db⁻*/b- mice on the high-fat diet, liver mass increased about 10%. Compared with those of the normal mice, the livers of the *db⁻/db⁻* mice turned yellow and contained lipid. TC level in serum increased in the normal mice and the *db⁻/db⁻* mice on the high-fat diet (Table 2). TG and FFA levels increased but were not significantly different in the normal mice and the *db⁻/db⁻* mice on the standard diet.

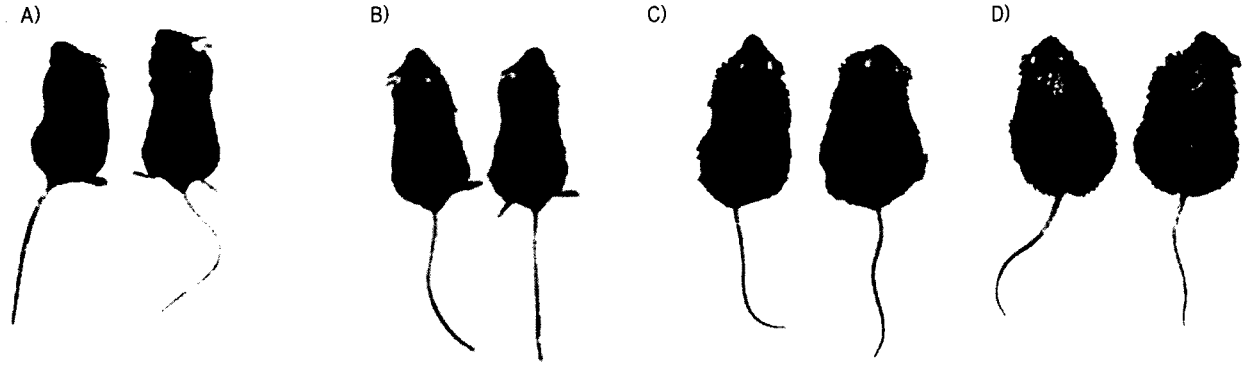

Fig. 1 Photograph of normal and *db⁻/db⁻* mice that were fed different diet for 4 week. A) and B) were normal mouse. C) and D) were *db⁻/db⁻* mouse. A) and C) were fed standard diet and B) and D) were fed high fat diet.

Table 2. Body weight, liver and adipose tissue of normal and *db⁻/db⁻* mice fed standard diet or high fat diet. Serum lipid levels of normal and *db⁻/db⁻* mice fed standard diet or high fat diet.

	Normal mice Standard diet	Normal mice High fat diet	*db⁻/db⁻* mice Standard diet	*db⁻/db⁻* mice High fat diet
Weight (g)				
Body weight	26.21 ± 0.29[a]	29.60 ± 0.53[b]	40.78 ± 0.77[c]	45.20 ± 0.39[d]
Liver	1.27 ± 0.05[a]	1.24 ± 0.02[a]	1.97 ± 0.06[b]	2.17 ± 0.07[c]
Retro-peritoneal adipose tissue	0.22 ± 0.02[a]	0.42 ± 0.02[b]	0.63 ± 0.04[c]	0.74 ± 0.03[d]
Epididymal adipose Tissue	0.74 ± 0.05[a]	1.39 ± 0.06[b]	2.06 ± 0.08[c]	2.42 ± 0.06[d]
Serum lipid				
Cholesterol (mg/dL)	176.96 ± 8.46[a]	252.38 ± 16.12[b]	215.48 ± 15.20[ab]	344.64 ± 19.58[c]
Triglyceride (mg/dL)	51.15 ± 4.03[a]	70.76 ± 7.36[a]	100.51 ± 7.56[a]	171.08 ± 34.52[b]
Free fatty acid (ug/dL)	1579.09 ± 123.86[a]	1801.06 ± 129.79[a]	2119.74 ± 137.44[ab]	2524.47 ± 167.68[b]

Gene Expression Profile of the Liver in Normal and *db⁻/db⁻* Mice on the High-fat Diet

A high-fat diet caused obesity and changed the morphology in the normal and *db⁻/db⁻* mice. It also led to an increase in adipose tissue and fat in the liver. To identify genes whose expression is modulated by a high-fat diet, we compared gene expression profiles for the livers of normal and *db⁻/db⁻* mice on the high-fat diet versus those for mice on the standard diet.

The total number of cDNA clones spotted on the microarray was 10,379. That result showed that many genes were affected by leptin signal. For example, expression of lipase, which is involved in lipid catabolism, was not affected in the normal mice on the high-fat diet, but it increased 2.7 fold in the *db⁻/db⁻* mice. Genes that were up- or down-regulated more than three-fold in the livers of normal mice on the high-fat diet are shown in Table 3. Table 4 and table 5 shows genes for which expression was four-fold above or below in *db⁻/db⁻* mice on the standard or high-fat diets compared with normal mice on the standard diet.

Table 3. Genes that were up or down regulated more than 3-fold in liver of normal mice by high fat diet compared with standard diet

UniGene	Title	Expression ratio
Gene profile which were expressed lower of 3 fold		
Mm.154390	FK506 binding protein 5 (51 kDa)	0.14
Mm.3785	protein phosphatase 2, regulatory subunit B	0.18
Mm.2904	zinc finger protein 216	0.20
Mm.8858	RAR-related orphan receptor alpha	0.21
Mm.137	small inducible cytokine A6	0.21
Mm.12919	insulin-like growth factor 2, binding protein 1	0.24
Mm.41887	chondroitin 4-sulfotransferase	0.27
Mm.40672	potassium voltage-gated channel, shaker-related subfamily, member 3	0.28
Mm.3333	Apolipoprotein B editing complex 1	0.29
Mm.140785	stearoyl-Coenzyme A desaturase 1	0.29
Mm.22421	telomerase binding protein, p23	0.32
Mm.6161	phosphate cytidylyltransferase 1, choline, alpha isoform	0.33
Gene profile which were expressed over of 3 fold		
Mm.6799	four and a half LIM domains 2	35.03
Mm.4429	ubiquitin-conjugating enzyme E2E 1, UBC4/5 homolog (yeast)	4.77
Mm.154804	carbonic anhydrase 7	4.66
Mm.17640	proteasome (prosome, macropain) 26S subunit, non-ATPase, 10	4.18
Mm.34102	ornithine decarboxylase, structural	4.01
Mm.1302	SFFV proviral integration 1	3.89
Mm.34408	glycoprotein 49 B	3.75
Mm.8575	SRY-box containing gene 13	3.71
Mm.182255	CD97 antigen	3.58
Mm.42083	Phospholipase C, beta 1	3.51
Mm.31570	potassium channel, subfamily K, member 2	3.49
Mm.25516	Heterogeneous nuclear ribonucleoprotein methyltransferase-like 1	3.49
Mm.1428	GATA binding protein 4	3.40
Mm.29106	protease, serine, 15	3.25
Mm.6522	chemokine orphan receptor 1	3.12
Mm.18535	myelin basic protein expression factor 2, repressor	3.10

Table 4. Genes that were up or down-regulated more than 4 fold in liver of *db/db* mice fed standard diet compared with normal mice fed standard diet

UniGene	Title	Expression ratio
Gene profile which were expressed lower of 4 fold		
Mm.18300	solute carrier family 3, member 1	0.010
Mm.12914	ubiquitin specific protease 2	0.051
Mm.5820	Musculin	0.053
Mm.30271	chorionic somatomammotropin hormone 1	0.072
Mm.903	B-cell translocation gene 2, anti-proliferative	0.073
Mm.14768	reduced expression 3	0.073
Mm.14926	ATPase, Cu++ transporting, alpha polypeptide	0.075
Mm.1287	Microtubule-associated protein tau	0.084
Mm.102752	core 1 UDP-galactose:N-acetylgalactosamine-alpha-R beta 1,3-galactosyltransferase	0.085
Mm.87312	glia maturation factor, beta	0.095
Mm.154045	tumor-associated calcium signal transducer 2	0.105
Mm.107441	zinc finger protein 26	0.108
Mm.2020	cysteine-rich protein 2	0.116
Mm.19101	DEAD box polypeptide 5	0.12
Mm.7106	bone morphogenic protein receptor, type II	0.122
Mm.36520	Geranylgeranyl diphosphate synthase 1	0.123
Mm.4235	kit ligand	0.123
Mm.29581	hairy/enhancer-of-split related with YRPW motif 1	0.129
Mm.42034	zinc finger protein, subfamily 1A, 4	0.137
Mm.781	gene trap ROSA 26 antisense, Philippe Soriano	0.145
Mm.10303	nucleolar protein 5	0.149
Mm.388	adenosine deaminase	0.151
Mm.26865	Mitochondrial ribosomal protein S6	0.153
Mm.2313	calcium modulating ligand	0.156
Mm.46856	PRKC, apoptosis, WT1, regulator	0.162
Mm.22684	solute carrier family 35 (CMP-sialic acid transporter), member 1	0.164
Mm.10818	syntaxin 7	0.165
Mm.27481	A kinase (PRKA) anchor protein (gravin) 12	0.166
Mm.42024	origin recognition complex, subunit 1 homolog (S. cerevisiae)	0.166
Mm.26378	Tescalcin	0.169
Mm.182067	inositol polyphosphate-5-phosphatase, 72 kDa	0.17
Mm.2642	poly A binding protein, cytoplasmic 1	0.172
Mm.830	Proteasome (prosome, macropain) 28 subunit, alpha	0.176
Mm.25808	guanosine monophosphate reductase	0.179
Mm.2411	Ras-GTPase-activating protein (GAP<120>) SH3-domain binding protein 2	0.183
Mm.2028	nuclear receptor coactivator 1	0.189
Mm.22701	growth arrest specific 1	0.19
Mm.3868	YY1 transcription factor	0.194
Mm.18856	mitogen-activated protein kinase 6	0.2
Mm.2374	tachykinin 2	0.2
Mm.426	Glutathione S-transferase, pi 2	0.205
Mm.194	FMS-like tyrosine kinase 3	0.208
Mm.2251	plexin A2	0.209
Mm.14802	H19 fetal liver mRNA	0.214
Mm.396	ubiquitin specific protease 9, X chromosome	0.216
Mm.19131	Complement component 3	0.217
Mm.29170	f-box and WD-40 domain protein 5	0.218
Mm.218851	Eukaryotic translation initiation factor 2, subunit 3, structural gene X-linked	0.22
Mm.28959	Serologically defined colon cancer antigen 1	0.22
Mm.141187	trans-golgi network protein 2	0.225

Table 4. continue

UniGene	Title	Expression ratio
Mm.28278	caveolin, caveolae protein, 22 kDa	0.226
Mm.4876	Reticulocalbin	0.227
Mm.140785	stearoyl-Coenzyme A desaturase 1	0.23
Mm.20115	D-amino acid oxidase	0.232
Mm.382	discs, large homolog 1 (Drosophila)	0.232
Mm.4451	hairy and enhancer of split 1, (Drosophila)	0.232
Mm.6856	pituitary tumor-transforming 1	0.232
Mm.1574	Wiskott-Aldrich syndrome-like (human)	0.233
Mm.1548	Glycoprotein galactosyltransferase alpha 1, 3	0.237
Mm.10681	osteoblast specific factor 2 (fasciclin I-like)	0.237
Mm.12898	Tnf receptor-associated factor 1	0.238
Mm.38306	Macrophage erythroblast attacher	0.24
Mm.4415	kinesin heavy chain member 2	0.243
Mm.16767	Heterogeneous nuclear ribonucleoprotein A2/B1	0.244
Mm.12921	Baculoviral IAP repeat-containing 6	0.245
Mm.18561	cysteine and histidine rich 1	0.246
Mm.2419	cadherin 4	0.247
Mm.2878	SRY-box containing gene 18	0.247
Mm.141443	lactate dehydrogenase 1, A chain	0.248
Mm.87142	forkhead box J2	0.249
Gene profile which were expressed over of 4 fold		
Mm.142740	Metallothionein 2	15.766
Mm.9537	lipocalin 2	7.764
Mm.18628	CD36 antigen	7.22
Mm.34663	NTF2-related export protein 1	5.532
Mm.14781	Cytochrome P450, 2a4	5.348
Mm.7459	Cytochrome P450, 4a14	5.222
Mm.144028	U2 small nuclear ribonucleoprotein auxiliary factor (U2AF),35 kDa	4.926
Mm.14894	plasma glutamate carboxypeptidase	4.821
Mm.7459	Cytochrome P450, 4a14	4.744
Mm.21761	interferon alpha responsive gene, 15 kDa	4.735
Mm.21882	CD84 antigen	4.733
Mm.142814	actin related protein 2/3 complex, subunit 4	4.694
Mm.2423	Procollagen, type II, alpha 1	4.511
Mm.2974	fructose bisphosphatase 2	4.502
Mm.4777	Orosomucoid 1	4.469
Mm.2769	MARCKS-like protein	4.417
Mm.3906	mitogen activated protein kinase kinase 7	4.247
Mm.173304	syntaxin 1B2	4.223
Mm.195898	Phosphatidylethanolamine binding protein	4.108

k-means clustering of the expression levels of these genes in the livers of normal and *db*$^-$/*db*$^-$ mice on the standard or high-fat diets identified 19 distinct groupings of genes with significant expression level (Fig. 2). Group A, B, C, D, M, N and P showed genes in which expression increased in *db*$^-$/*db*$^-$ mice on the standard diet but in other mice, there was no change or a slight decrease. Group K, Q and S decreased in *db*$^-$/*db*$^-$ mice on the standard diet but were unchanged or increased in the other mice. Group L and O increased in the *db*$^-$/*db*$^-$ mice on the high-fat diet compared with the *db*$^-$/*db*$^-$ mice that were fed a standard diet, but group G and H showed the opposite pattern. Group F, R only increased in the normal mice on the high-fat diet. Group I and J showed highly decreased gene expression in *db*$^-$/*db*$^-$ mice.

Table 5. Genes that were up or down-regulated more than 4 fold in liver of *db*$^-$/*db*$^-$ mice fed high fat diet compared with normal mice fed standard diet

UniGene	Title	Expression ratio
Gene profile which were expressed lower of 4 fold		
Mm.5032	peripherin 2	0.050
Mm.140785	stearoyl-Coenzyme A desaturase 1	0.083
Mm.916	H2A histone family, member Z	0.110
Mm.180333	mutY homolog (E. coli)	0.114
Mm.8155	TG interacting factor	0.132
Mm.4092	wingless-related MMTV integration site 7B	0.134
Mm.426	glutathione S-transferase, pi 2	0.155
Mm.7563	retinol dehydrogenase type 5	0.182
Mm.30266	hemoglobin, beta adult major chain	0.190
Mm.426	glutathione S-transferase, pi 2	0.211
Mm.38869	protein inhibitor of activated STAT 1	0.213
Mm.29910	ribosomal protein S6 kinase polypeptide 1	0.224
Mm.20365	Glycoprotein Ib, beta polypeptide	0.229
Mm.39093	short stature homeobox 2	0.236
Mm.10681	osteoblast specific factor 2	0.237
Mm.4451	hairy and enhancer of split 1, (Drosophila)	0.239
Mm.27801	solute carrier family 30 (zinc transporter), member 4	0.242
Mm.28223	inhibitor of DNA binding 4	0.248
Mm.194	FMS-like tyrosine kinase 3	0.249
Mm.22662	keratin complex 1, acidic, gene 10	0.251
Gene profile which were expressed over of 4 fold		
Mm.142740	Metallothionein 2	21.730
Mm.18628	CD36 antigen	8.477
Mm.2020	cysteine-rich protein 2	7.771
Mm.4533	Apolipoprotein A-IV	5.144
Mm.9537	lipocalin 2	4.656
Mm.1786	adenine phosphoribosyl transferase	4.590
Mm.17912	Bcl2-interacting killer-like	4.515
Mm.12051	Suppression of tumorigenicity 7	4.495
Mm.142814	actin related protein 2/3 complex, subunit 4	4.476
Mm.38017	single Ig IL-1 receptor related protein	4.440
Mm.30728	cyclic AMP phosphoprotein, 19 kDa	4.264
Mm.34319	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	4.205
Mm.141187	trans-golgi network protein 2	4.042
Mm.3863	P450 (cytochrome) oxidoreductase	4.010

Gene Expression Pattern Involved in Metabolism

Carbohydrate metabolism. Genes involved in carbohydrate metabolism are metabolic genes composed in glycolysis/gluconeogenesis, TCA, glycogen synthesis and the carbohydrate transport pathway. Genes involved in carbohydrate catabolism showed a repressive pattern

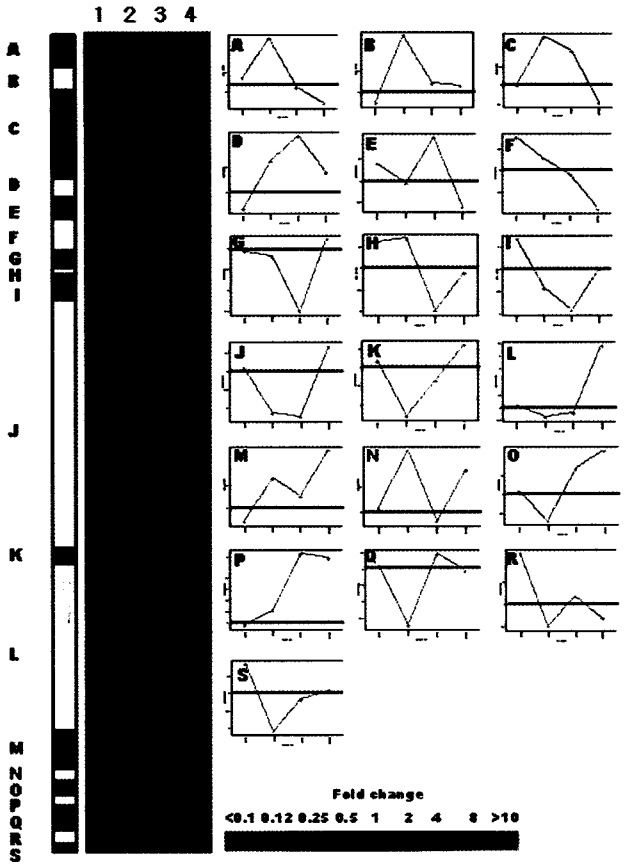

Fig. 2 *k*-means cluster analysis of genes changing in abundance in liver of normal and *db*$^-$/*db*$^-$ mice fed standard or high fat diet.

Left, 1172 genes were clustered 19 groups according their expression profile. The clusters were labeled A-S and boundaries between clusters were indicated by the alternating red and yellow colorbar. Upper numbers were hybridization pair. 1: normal mice fed high fat diet compared with standard diet, 2: *db*$^-$/*db*$^-$ mice with normal mice fed stadndard diet , 3: *db*$^-$/*db*$^-$ mice fed high fat diet compared with normal mice standard diet, 4: *db*$^-$/*db*$^-$ mice fed high fat diet compared with standard diet.

Right, the normalized mean expression level was shown for each cluster of genes in graphical form. X axis was indicated 1, 2, 3, 4 shown in left cluster and Y axis was expression ratio. Thick line was zero point.

owing to a high-fat diet or *db* deficiency (Fig. 3A). For example, pyruvate kinase and glucose phosphate isomerase, part of glycolysis, showed a decrease pattern in normal and *db*$^-$/*db*$^-$ mice on the high-fat diet. But pyruvate decarboxylase, which converts pyruvate to acetyl-CoA, showed an increase pattern in normal mice on the high-fat diet and in *db*$^-$/*db*$^-$ mice on the standard diet. In *db*$^-$/*db*$^-$ mice, citrate synthase showed an increase pattern but succinate-CoA ligase showed a repressive pattern. Citrate synthase and succinate-CoA ligase are important enzymes in the TCA cycle. So, the TCA cycle may be inhibited and citrate may accumulate in the mitochondria. Glycogen synthetase showed a repressive pattern but Glycogen synthetase kinase increased. The results showed that glycogen synthesis was inhibited and extra carbohydrates were inverted in lipid synthesis.

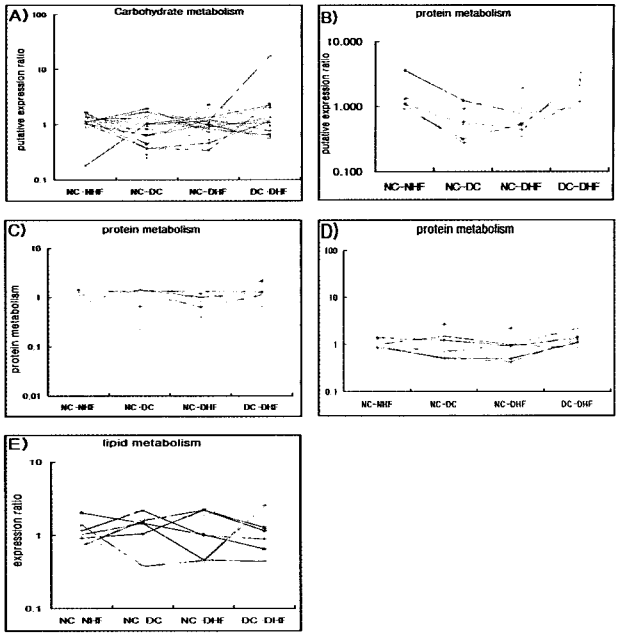

Fig. 3 Genes expression profile which were involved in each of metabolism.

left of each graph were shown gene expression pattern.
NC-NHF: normal mice fed high fat diet compared with standard diet, NC-DC: *db*$^-$/*db*$^-$ mice with normal mice fed stadndard diet , NC-DHF: *db*$^-$/*db*$^-$ mice fed high fat diet compared with normal mice standard diet, DC-DHF: *db*$^-$/*db*$^-$ mice fed high fat diet compared with standard diet.
A) carbohydrate metabolism, B) protein metabolism, C) fatty acid metabolism, D) cholesterol metabolism, E) lipid metabolism.

Protein metabolism. Many genes involved in protein biosynthesis and proteolysis showed a repressive or non-different expression pattern (Fig. 3B). Specifically, peptiases increased in the *db*$^-$/*db*$^-$ mice compared with the control mice. In the normal mice, gene expression involved in proteolysis increased slightly on the high-fat diet.

Fatty acid metabolism. Fatty acid metabolism was divided into degradation and biosynthesis. In the normal and *db*$^-$/*db*$^-$ mice on the high-fat diet, the genes involved in fatty acid degradation increased slightly whereas the genes involved in fatty acid biosynthesis decreased slightly (Fig. 3C). Specifically, fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1) decreased significantly.

Cholesterol metabolism. Genes whose products compose cholesterol metabolism, except 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Fig 3D), decreased in the liver owing to the high-fat diet and *db* deficiency. But sterol carrier protein 2, which transports cholesterol out of the hepatocyte, increased. These increased in *db*$^-$/*db*$^-$ mice on the high-fat diet versus those that were fed the standard diet.

Lipid metabolism. Apolipoproeins that perform lipid transport decreased in the normal mice on the high-fat diet but increased in the *db*$^-$/*db*$^-$ mice that were fed a standard or high-fat diet (Fig. 3E). Hepatic, hormone

sensitive and lysosomal lipase showed a repressive pattern. Phospholipid transfer protein and phospholipid scramblase 2 decreased more than two-fold in *db*$^-$/*db*$^-$ mice but not in normal mice.

Gene expression Pattern Involved in Signal Pathway

We performed gene expression that was part of the cell signal pathway in the livers of normal and *db*$^-$/*db*$^-$ mice (Fig. 4). These were divided into six groups. Genes involved in group A were highly repressed in *db*$^-$/*db*$^-$ mice but no different than that for the mice on the high-fat diet. Group B genes were those for which expression decreased in normal mice on the high-fat diet but were unchanged or increased slightly in *db*$^-$/*db*$^-$ mice fed the standard and high-fat diets. Group C genes slightly increased in normal mice on the high-fat diet but were unchanged in *db*$^-$/*db*$^-$ mice. Group D and Group E genes slightly decreased or increased, respectively, only in *db*$^-$/*db*$^-$ mice on the standard diet. Group F genes showed a repressive pattern in normal mice on the high-fat diet but were unchanged in *db*$^-$/*db*$^-$ mice. Specifically, genes involved in calcium and Wnt signal pathway showed a repressive pattern in normal or *db*$^-$/*db*$^-$ mice on the high-fat diet, but genes in the transforming growth factor (TGF) signal pathway showed an increase pattern.

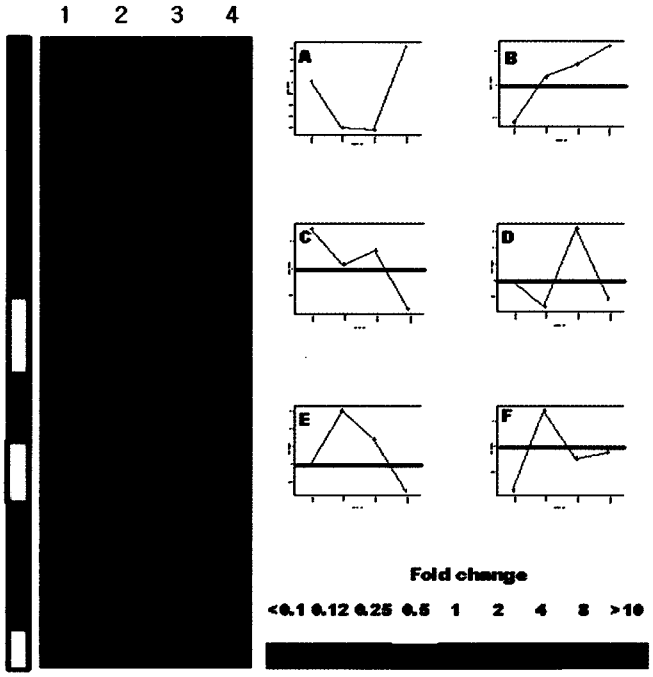

Fig. 4 *k*-means cluster analysis of genes changing in liver of normal and *db*$^-$/*db*$^-$ mice fed standard or high fat diet, which were involved in signal pathway.

left, genes which were clustered 6 groups according their expression profile. Upper numbers were hybridization pair.
1: NC-NHF, 2: NC-DC, 3: NC-NHF, 4: DC-DHF.
Right, the normalized mean expression level was shown for each cluster of genes in graphical form. X axis was indicated 1, 2, 3, 4 shown in left cluster and Y axis was expression ratio. Thick line was zero point.

Mgst3, Ptk9l and Wnt 7b Genes Expression in the Livers of Mice Fed a High-fat Diet

In the results of microarray analysis, some of genes for which expression was modulated by the high-fat diet were selected. These were Mgst3 (microsomal glutathione S-transferase 3), Ptk9l (protein tyrosine kinase 9-like, A6 related protein) and Wnt 7b (wingless-related MMTV integration site 7B). Ptk9l and Wnt7b were involved in calcium and the Wnt signal pathway, respectively. Gene expression showed a repressive pattern in the results of microarray assay. Ptk9l and Wnt7b expressions decreased by about 60% in normal mice and by 45%, 43% in *db*$^-$/*db*$^-$ mice on the high-fat diet. The expression patterns for normal and *db*$^-$/*db*$^-$ mice on the high-fat diet are identified by RT-PCR (Fig. 5). As a result of RT-PCR, Ptk9l and Wnt7b decreased by more than half in normal mice on the high-fat diet. But, in *db*$^-$/*db*$^-$ mice, Ptk9l expression slightly decreased compared with that of the control mice and Wnt7b was not identified. Otherwise, Mgst3 showed increased expression pattern owing to obesity increase.

DISCUSSION

The purpose of this study was to identify genes that are differentially regulated in the liver owing to a high-fat diet. We used normal and *db*$^-$/*db*$^-$ mice of the C57BL/6 strain and harvested their livers and adipose tissue after four weeks on standard or high-fat diets. In normal and *db*$^-$/*db*$^-$ mice that were fed a high-fat diet, adipose tissue increased significantly compared with those on the standard diet[18] and induced obesity. Levels of lipids in serum also increased in mice on a high-fat

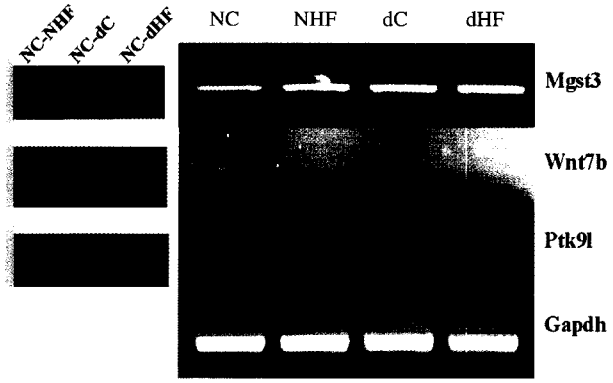

Fig. 5 Expression of Mgst3, Wnt7b and Ptk9l genes in liver by different nutrient and *db* deficient mouse.

The results were obtained by RT-PCR using specific primer of each gene. Left was results of Microarray assay and right was expression different of same genes.
NC: C57/BL6 mouse fed control diet, NHF : C57/BL6 fed high fat diet, dC: *db*$^-$/*db*$^-$ mouse fed control diet, dHF: *db*$^-$/*db*$^-$ mouse fed high fat diet. Graph was used as internal control

diet.[19]

To identify gene expression in the livers of obese mice, we performed microarray analysis and searched gene expression profiles by high-fat diet in normal and *db*/*db* mice. Genes in glycogen synthesis were repressed in normal and *db*/*db* mice that were fed a high-fat diet and the carbohydrate oxidation rate was lower.[20] Fatty acid synthesis showed the same pattern.[21,22] Genes whose function is protein lysis increased owing to high fat and *db* deficiency. This result may mean that a high-fat diet and *db* gene deficiency activate protein lysis.[23-25] In lipid metabolism and cholesterol metabolism, apolipoprotein A and 3-hydroxy-3-methylglutaryl-Coenzyme A synthase increased in normal and *db*/*db* mice owing to high fat.[26,27]

We studied Ptk9l and Wnt7b gene expression in the livers of mice that were fed standard and high-fat diets. Each of the genes are involved in the calcium signal and wnt signal pathways. Ptk9l gene product is one of a family of protein tyrosine kinases (PTKs).[28] PTKs are involved in the calcium signal pathway, and Ptk9l product was phosphorylated and activated by protein kinase Cζ(PKCζ).[28,29] But its expression with a high-fat diet was not identified. From our results, Ptk9l gene expression decreased in mice that were fed the high-fat diet compared with that for mice on the standard diet. Expression of calmodulin and PKCs related to PTK were also repressed by a high-fat diet. Reduction of calmodulin, PKCs and PTKs expression may be caused by decreased Ca^{2+} concentration in serum. A high-fat diet inhibits Ca^{2+} absorption in the intestine.[30]

Wnt7b gene expression decreased significantly in normal mice that were fed the high-fat diet and in *db*/*db* mice and we obtained the same result by RT-PCR using specific primer. Wnt7b is ligand binding with frizzled receptor and activates the Wnt signal pathway. The Wnt signal pathway is related to glycogen synthesis in the liver. Glycogen synthase kinase 3β was identified for its ability to phosphorylate and inhibition glycogen syntase[31,32] and Wnt signaling causes inhibition of GSK3β.[33] From the results of microarray, Wnt ligand and receptor showed a repressive expression pattern and GSK3β expression increased owing to the high-fat diet. A high-fat diet causes a reduction of glycogen synthesis in liver.[34] Libal-Weksler *et al* [35] reported that glycogene synthase expression was significantly lower in rats that were fed a high-fat diet and Eldar-Finkelman *et al*[36] reported that glycogen synthase kinase-3 activity increased in diabetes- and obesity-prone C57BL/6J mice. So Wnt7b expression was affected by a high-fat diet and reduction of Wnt7b expression resulted in inhibition of glycogen synthesis in the liver.

The results indicate that many genes were affected by a high-fat diet in normal and *db*/*db* mice and expressions of Mgst3, PtK9l and Wnt 7b increased or repressed in the livers of mice fed a high-fat diet.

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