Brief Genetic Analysis

Effect of Exercise on Hepatic Gene Expression in an Obese Mouse Model Using cDNA Microarrays

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Abstract

LEE, KYOUNG-YOUNG, SU-JONG KIM, YOUN-SOO CHA, JU-RYUN SO, JOON-SUK PARK, KYUNG-SUN KANG, AND TAE-WON CHON. Effect of exercise on hepatic gene expression in an obese mouse model using cDNA microarrays. *Obesity.* 2006;14:1294–1302.

To understand the molecular mechanisms involved in the effect of exercise training, we examined hepatic transcriptional profiles using cDNA microarrays in exercise-trained and untrained mice with diet-induced obesity. C57BL/6J male mice (n = 10/group) were fed with a normal diet, high-fat diet (HFD), or HFD with exercise training for 12 weeks. The expression level of ~10,000 transcripts in liver tissues from each group was assessed using cDNA microarray analysis. Exercise training improved lipid profiles and hepatic steatosis and decreased body fat mass induced by the HFD. Seventy-three genes were differentially expressed in the HFD- and/or HFD with exercise training-treated groups, compared with the normal diet- and HFD-fed groups, respectively. Interestingly, the expression profiles involved in metabolism, such as elongation of very long chain fatty acids-like 2, lipin, and malic enzyme, were changed by exercise training. In addition, expression of genes altered by exercise training related to defense and stress response, including metallothionein 1 and 2 and heat shock protein, showed interesting findings. Our study showed beneficial effects of exercise training in preventing the development of obesity and metabolic disorders in mice with diet-induced obesity.

Key words: cDNA microarray, C57BL/6J mouse, exercise training, high-fat diet, metabolism

Consumption of high-fat diets (HFDs)[1] for extended periods can lead to the development of obesity and diabetes (1), adversely affecting the health of humans and experimental animals (1,2). To elucidate the effects of exercise on obesity induced by HFD, we studied liver tissues in C57BL/6J mice because the liver plays an integral part in the physiology of exercise. This organ supplies energy substrates to peripheral tissues by the Cori cycle and glycogen catabolism and is important for detoxification.

Exercise training is known to improve the metabolic profile, including reduced hepatic accumulation of total fat and cholesterol in rats fed an HFD (3). Furthermore, exercise regulates the gene expression related to metabolism, defense, and stress responses (3–5). However, the results in most cases have been obtained in a gene-by-gene manner. For this reason, we chose the microarray approach as a global analysis of the genetic basis for the relationship between exercise and obesity. Therefore, we used cDNA microarray, containing ~10,000 mouse transcripts. Some of them were already known to be sensitive to exercise in an obesity model. However, others have been newly identified. We describe important new effects of exercise on the expression of genes related to energy metabolism, defense, and stress responses.

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[1] Nonstandard abbreviations: HFD, high-fat diet; HFD + Ex, HFD with exercise training; SAM, significant analysis of microarray; ND, normal diet; MT, metallothionein; Apcs, serum amyloid P-component; RT, reverse transcription; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; Cpt, Carnitine palmitoyl transferase; HSP, heat shock protein.

Table 1. Weight-related and biochemical parameters in ND, HFD, and HFD + Ex during 12 weeks

Group	ND	HFD	HFD + Ex
Final body weight (g)	25.20 ± 1.60[c]	41.14 ± 2.38[a]	31.74 ± 1.81[b]
Body weight gain (g/d)	0.13 ± 0.06[b]	0.33 ± 0.10[a]	0.19 ± 0.14[b]
Food intake (g/d)	2.60 ± 0.16[b]	3.35 ± 0.70[a]	2.40 ± 0.24[b]
Abdominal fat (g/100 g body weight)	1.53 ± 0.55[c]	5.86 ± 0.51[a]	4.99 ± 1.08[b]
Back fat (g/100 g body weight)	0.69 ± 0.22[c]	3.77 ± 0.89[a]	2.92 ± 1.27[b]
Liver			
Weight (g)	0.91 ± 0.10[b]	1.24 ± 0.21[a]	0.91 ± 0.02[b]
Total carnitine (nmol/g)	1.36 ± 0.36[b]	1.58 ± 0.41[a]	1.37 ± 0.57[b]
Serum			
HDL-cholesterol (mg/dL)	64.49 ± 7.37[b]	96.92 ± 8.35[a]	90.81 ± 13.63[a]
LDL-cholesterol (mg/dL)	30.99 ± 22.78[b]	66.59 ± 18.28[a]	33.97 ± 20.41[b]
Triglyceride (mg/dL)	142.93 ± 41.59	157.69 ± 36.01	152.39 ± 38.48
Total cholesterol (mg/dL)	130.79 ± 16.90[c]	190.83 ± 18.93[a]	156.74 ± 26.25[b]
Total carnitine (nmol/g)	6.83 ± 0.68[a]	5.12 ± 1.06[b]	5.47 ± 0.58[b]
Leptin (ng/mL)	8.56 ± 5.68[b]	29.72 ± 5.55[a]	13.44 ± 6.09[b]

ND, normal diet; HFD, high-fat diet; HFD + Ex, HFD with exercise training; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are means ± standard error, $n = 10$. Means in a row with superscripts without a common letter differ, $p < 0.05$.

Weight-related and biochemical parameters in C57BL/6J mice are shown in Table 1. After treatment for 12 weeks, the HFD with exercise training (HFD + Ex) group showed decreases in weight-related parameters such as final body weight, liver weight, and relative weights of abdominal fat and back fat, which were increased by the HFD ($p < 0.05$, respectively). The accumulation of serum total cholesterol, low-density lipoprotein-cholesterol, and leptin because of the HFD was lower in the HFD + Ex-treated group ($p < 0.05$, respectively).

Only those genes whose mRNA levels were changed >2.0-fold and considered significantly altered by significant analysis of microarray (SAM) method (6) were designated as differentially expressed. By these criteria, 73 genes were differentially expressed in the HFD- and/or HFD + Ex-treated groups, compared with the normal diet (ND)- and HFD-fed groups, respectively (Tables 2 to 4). Of the 62 gene transcripts altered in the HFD-fed mice, 38 were completely normalized by exercise (Table 2). These included genes related to fatty acid biosynthesis such as elongation of very long chain fatty acids-like 2. This down-regulated gene in the HFD-fed mice was normalized by exercise training. Hepatic genes related to defense or detoxification responses, such as metallothionein (MT) 1 and 2, which are antioxidant factors that protect various tissues including liver (7), had lower levels of expression in the HFD group than in ND group, and their decreased expressions were normalized by exercise training. In contrast, several genes encoding mediators of immune responses or molecular chaperones displayed increased expressions in the HFD-fed mice, but their increased expressions were moderated by exercise training. These genes included the heat shock 70-kDa protein 5 families. The gene-encoding enzymes of electron transport such as P450 (cytochrome) oxidoreductase were lower in the mice fed the HFD than in those fed the ND but were normalized in the mice treated with the HFD + Ex. Solute carrier family 7, a cationic amino acid transporter, which interacts with endothelial nitric oxide synthase (8), was also down-regulated by the HFD but remained normal with exercise training.

Eleven gene transcripts were altered by HFD and exercise training but not by HFD alone (Table 4). HFD did not affect malic enzyme, which is implicated as a lipogenic enzyme, and this gene was down-regulated by HFD and exercise training. Lipin 1, which is a regulator for normal induction of the adipogenic gene (9), was increased by exercise but did not change in the HFD group. Serum amyloid P-component (Apcs), which is one of the acute phase responses (10), was decreased in HFD + Ex-treated group but was not altered in the HFD group.

Changes in gene expression discovered by cDNA microarray analysis were further confirmed by analyzing four known genes by reverse transcription (RT)-polymerase chain reaction (PCR). When gene expression profiles ob-

Table 2. Genes that were up- or down-regulated in the liver of C57BL/6J mice fed an HFD compared with mice fed an ND but normalized by exercise (HFD + Ex), compared with mice fed an HFD

Gene bank assession number	Gene name and symbol	Fold change [SAM(δ)]	
		HFD/ND	HFD + Ex/HFD
Metabolism			
BG086604	Methionine adenosyltransferase I, alpha (Matla)	−2.5 (−3.5)	
BG086656	RIKEN cDNA E030003E18 gene (Aldh1a1)	2.9 (4.1)	
BG077894	Serine hydroxymethyl transferase 2 (mitochondrial) (Shmt2)	−2.1 (−2.0)	
BG949059	Elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 2 (Elovl2)	−2.5 (−2.1)	
AA122814	Aldehyde dehydrogenase family 1, subfamily A7 (Aldh1a7)	2.6 (−3.6)	
Defense and stress response			
BG077818	Metallothionein 1 (Mt1)	−6.1 (−4.4)	
AA051654	Metallothionein 1 (Mt1)	−5.0 (−2.9)	
AI427514	Metallothionein 1 (Mt1)	−3.6 (−2.8)	
BG063925	Metallotionein 2 (Mt2)	−5.7 (−6.4)	
BG076621	Heat shock 70kD protein 5 (Hspa5)	2.9 (4.1)	
BG078795	Heat shock 70kD protein 5 (Hspa5)	2.8 (3.6)	
BG087650	Heat shock 70kD protein 5 (Hspa5)	2.8 (3.8)	
BG076460	Glutamate-cysteine ligase, catalytic subunit (Gclc)	2.3 (2.7)	
Signal transduction/ apoptosis/cell cycle			
BG067419	Growth arrest and DNA-damage-inducible 45 gamma (Gadd45g)	−2.2 (−2.4)	
BG072812	Growth hormone receptor (Ghr)	2.2 (2.5)	
BG078806	High mobility group nucleosomal binding domain 2 (Hmgn2)	2.2 (2.8)	
Transcription regulation			
BG081592	Transforming growth factor beta induced transcript 4 (Tgfbli4)	3.8 (4.3)	
AI851042	Activating transcription factor 5 (Atf5)	−2.4 (−2.7)	
BG084173	Transforming growth factor beta induced transcript 4 (Tgfbli4)	2.4 (2.8)	
Transport			
BG070686	P450 (cytochrome) oxidoreductase (Por)	−2.3 (−2.1)	
AA109684	Cytochrome P450, family 4, subfamily a, polypeptide 10 (Cyp4a10)	−2.3 (−3.0)	
AA222205	Calcium binding protein, intestinal (Cai)	2.0 (2.4)	
AA250170	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2 (Slc7a2)	−4.1 (3.1)	
BG078796	Solute carrier family 25 (mitochondrial carrier, peroxisomal membrane protein), member 17 (Slc25a17)	2.4 (−3.1)	
BG087169	Thioredoxin domain containing 7 (Txndc7)	3.0 (4.1)	

Table 2. (continued)

Gene bank assession number	Gene name and symbol	Fold change [SAM(δ)] HFD/ND	Fold change [SAM(δ)] HFD + Ex/HFD
Cellular adhesion, cytoskeleton and trafficking			
BG077976	Actin related protein 2/3 complex, subunit 4 (Arpc4)	−2.6 (−3.2)	
BG064608	Calreticulin (Calr)	2.2 (2.8)	
Unclassified			
BG066690	RIKEN cDNA 0610033H09 gene (0610033H09Rik)	−2.9 (−3.2)	
BG071239	ESTs	−2.2 (−2.8)	
BG066267	ESTs	−2.4 (−2.7)	
BG071318	ESTs	−2.3 (−2.4)	
BG070310	ESTs	−2.0 (−2.1)	
BG082494	Translocase of outer mitochondrial membrane 22 homolog (yeast) (Tomm22)	−2.8 (−3.5)	
AA419665	Similar to argininosuccinate synthase (EC 6.3.4.5) - mouse	−2.8 (−3.5)	
BG083639	Mannose-P-dolichol utilization defect 1 (Mpdu1)	2.8 (3.8)	
BG081451	ESTs	−2.3 (−3.0)	
BG086605	Thioredoxin interacting protein (Txnip)	−2.1 (−2.3)	
AI840949	Similar to argininosuccinate synthase (EC 6.3.4.5) - mouse	−2.6 (−3.4)	

HFD, high-fat diet; ND, normal diet; HFD + Ex, HFD with exercise training. The fold change shown represents the median gene expression ratio from each of 4 independently repeated microarray experiments, comparing the HFD with the ND-fed group and HFD + Ex with the HFD-fed group. The Significance Analysis of Microarrays (SAM) method was used to evaluate the significance of differences in gene expression. The criterion for inclusion of a gene in this table was that the observed fold change was >2 and [SAM(δ)] > 0.66.

tained by microarray analysis and RT-PCR were compared, their patterns came out to be changed in similar directions and degrees (Figure 1). Expression levels of peroxisome proliferator-activated receptor (PPAR) α, PPARγ, carnitine palmitoyl transferase (Cpt)1 liver, and Cpt2, which were not spotted on the microarray or were rejected according to technical criteria of the microarray, were also examined using RT-PCR. The expression of these genes was increased in the HFD-fed mice, and these increases were augmented by exercise training (Figure 1).

Of the 16 metabolism-related genes affected by HFD treatment, the expressions of five genes were maintained at normal level with exercise training. Of them, elongation of very long chain fatty acids-like 2, which is involved in fatty acid biosynthesis, was decreased by the HFD but remained normal when exercise training was added. Unfortunately, due to limited transcripts, we could not detect several known metabolism-related genes in the obesity model. These genes included PPARs and Cpts. PPARα is a pivotal regulator of lipid metabolism, stimulating β-oxidation of polyunsaturated fatty acids in peroxisomes and of saturated fatty acids in mitochondria (11). Also, PPARγ has been identified as a potential regulator of skeletal muscle fatty acid metabolism (12). The gene expression of PPARs increased >1.0-fold in HFD-fed mice, and their increases were augmented by exercise training. Cpts, which are PPAR-regulated enzymes, also showed similar expression patterns. Therefore, it might be assumed that the increased expression of Cpts by exercise may be effective in controlling obesity. Another study of exercise training and antilipogenic effects demonstrated changes in malic enzyme gene expression. Malic enzyme, which is a lipogenic enzyme, was decreased by exercise training. There is a possibility that these changes may be related to the augmentation of weight-related changes induced by the HFD and biochemical effects of exercise training.

One of the most interesting findings from the microarray data was that gene-related stress responses between MT and heat shock protein (HSP) expressions were contrary to each other. We suggest that this finding may be a result of the

Table 3. Genes that were up- or down-regulated in the liver of C57BL/6J mice fed an HFD compared with mice fed an ND, and mice not normalized by exercise (HFD + Ex) compared with mice fed an HFD

Gene bank assession number	Gene name and symbol	Fold change [SAM(δ)]	
		HFD/ND	HFD + Ex/HFD
Metabolism			
BG080926	Hydroxysteroid dehydrogenase-1, delta<5>-3-beta (Hsd3b1)	2.8 (3.6)	2.4 (2.7)
AI841574	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (Hmgcs1)	4.6 (3.3)	2.8 (2.1)
BG079850	Glutamate oxaloacetate transaminase 1, soluble (Got1)	−3.5 (−4.1)	−2.3 (−2.7)
BG071805	Cystathionase (cystathionine gamma-lyase) (Cth)	−3.1 (−4.4)	−2.6 (−3.1)
BG081903	Hydroxysteroid dehydrogenase-1, delta<5>-3-beta (Hsd3b1)	3.2 (4.1)	2.6 (2.3)
AA028760	Hydroxysteroid dehydrogenase-1, delta<5>-3-beta (Hsd3b1)	2.8 (3.2)	2.5 (2.3)
BG082495	Argininosuccinate lyase (Asl)	−3.9 (−4.6)	−2.0 (−2.0)
AA060494	Glutamate oxaloacetate transaminase 1, soluble (Got1)	−7.4 (−7.0)	−3.6 (−4.3)
AA122925	Carbonic anhydrase 2 (Car2)	5.5 (3.5)	3.6 (1.9)
AA274685	Hydroxysteroid dehydrogenase-4, delta<5>-3-beta (Hsd3b4)	3.8 (4.4)	2.4 (2.6)
AU022809	Deleted in polyposis 1 (Dp1)	−2.1 (−2.1)	−2.2 (−1.3)
Defense and stress response			
AA274682	Glutathione S-transferase, alpha 3 (Gsta3)	2.8 (3.7)	2.6 (2.2)
Signal transduction/ apoptosis/cell cycle			
AA034857	RNA binding motif protein 3 (Rbm3)	−2.9 (−3.2)	−2.1 (−2.2)
W83086	Insulin-like growth factor binding protein 1 (Igfbp1)	−4.2 (−5.6)	−2.8 (−2.6)
AA250120	Gila cell derived neurotrophic factor family receptor alpha 1 (Gfra1)	−2.5 (−2.8)	−2.0 (−2.2)
Transcription regulation			
BG082588	Ets homologous factor (Ehf)	−3.6 (−5.1)	−2.1 (−2.1)
Transport			
AA271121	Aminoadipate-semialdehyde synthase (Aass)	−3.0 (−4.5)	−2.5 (−2.7)
BG064959	Hypothetical LOC403343 (Apoa4)	−4.6 (−5.2)	−4.3 (−4.1)
AI893661	Cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14)	−3.2 (−4.0)	−2.3 (−2.3)
AA098524	Cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14)	−3.7 (−4.8)	−2.6 (−3.0)
AA060595	Cytochrome P450, family 4, subfamily a, polypeptide 14 (Cyp4a14)	−3.7 (−4.4)	−2.5 (−2.5)

Table 3. (continued)

Gene bank assession number	Gene name and symbol	Fold change [SAM(δ)] HFD/ND	Fold change [SAM(δ)] HFD + Ex/HFD
Cellular adhesion, cytoskeleton and trafficking			
BG065049	Procollagen, type II, alpha 1 (Col2a1)	−3.8 (−4.5)	−3.5 (−3.8)
Unclassified			
AA097421	Progesterone receptor membrane component 2 (Pgrmc2)	−4.4 (−4.6)	−4.0 (−4.5)
AA276254	Transcribed locus	3.4 (3.8)	2.7 (2.3)

HFD, high fat diet; ND, normal diet; HFD + Ex, HFD with exercise training. The fold change shown represents the median gene expression ratio from each of 4 independently repeated microarray experiments, comparing the HFD with the ND group and the HFD + Ex with the HFD group. The Significance Analysis of Microarrays (SAM) method was used to evaluate the significance of differences in gene expression. The criterion for inclusion of a gene in this table was that the observed fold change was >2 and [SAM(δ)] > 0.66.

interaction between antioxidant and repair systems. First line antioxidant systems such as superoxide dismutase, catalase, glutathione peroxidase, and Mt, as seen in the present study, were stimulated during training (13,14). Moderate regular exercise such as swimming in the present experiment induces so much first line protection against reactive oxygen species that a secondary repair system like HSP can be partly shut down (15). In addition to HSP and Mt, Apcs, which is an acute-phase response protein like C-reactive protein, was reduced by exercise training in obese mice fed HFD. A few studies have shown that Apcs response increases after various insults such as infection and injury in liver (10). However, none of these studies reported on the effects of exercise training or acute exercise. Therefore, to our knowledge, this is the first finding that exercise training may increase Apcs in fatty liver induced by HFD. According to these results, exercise training might attenuate the inflammatory response.

In conclusion, the present study demonstrated that exercise training regulates the expression of many genes related to metabolism, stress and defense, transport, etc. in obese mice. The unbiased approach toward characterization of gene expression in the present study indicates that previously unappreciated transcripts play significant roles in the development, inhibition, and maintenance of the obesity phenotype in vivo. In addition, these transcriptional regulations by exercise training have beneficial effects in this animal model of obesity.

Research Methods and Procedures

Animals, Diet, and Exercise

This study was conducted in conformity with the policies and procedures of the Institutional Animal Care and Use Committee of Jeonbuk National University (Jeonbuk, Korea). Four-week-old C57BL/6J male mice were obtained from the Jeonbuk National University animal laboratories and housed individually in stainless steel wire mesh cages in a room kept at 23 ± 1 °C with a 12-hour light/dark cycle (light period, 8 AM to 8 PM). After familiarization with the facility for 1 week, mice were randomly assigned to one of three groups for 12 weeks (n = 10, respectively): ND, HFD, or HFD + Ex. In the HFD, fat supplied 35% of total energy, compared with 4% fat in the ND [ND: cornstarch, 315 g/kg diet; sucrose, 350 g/kg diet; maltodextrin, 35 g/kg diet; lard, 20 g/kg diet; soybean oil, 25 g/kg diet; casein, 200 g/kg diet; HFD: cornstarch, 0 g/kg diet; sucrose, 68.8 g/kg diet; maltodextrin, 125 g/kg diet; lard, 245 g/kg diet; soybean oil, 25 g/kg diet; casein, 200 g/kg diet (16)]. The diets were based on modified recommendations of the American Institute of Nutrition (16). Mice had free access to both food and distilled water, which were provided fresh every day. Food intake and body weights were recorded every day and every other day, respectively. The HFD + Ex mice were made to swim in an acrylic plastic pool (90 × 45 × 45 cm) filled with water (maintained at 34 °C) to a depth of 38 cm (17). The exercise group completed 12 weeks of swimming exercise in the swimming pool, where they swam for 1 h/d at a 4l/min flow rate, and swimming exercise was carried out every day at the same time of the day. For familiarization, the mice in the exercise group swam at zero flow rate during the 1st week.

Sample Collection and Analytical Procedure

Feed was removed 12 hours before sacrificing. Blood samples were collected from each mouse by orbital venipuncture and incubated on ice water for 1 hour. Serum was

Table 4. Genes that were not altered in the liver of C57BL/6J mice fed an HFD compared with mice fed an ND, and were up- or down-regulated by HFD + Ex compared with mice fed an HFD

Gene bank assession number	Gene name and symbol	Fold change [SAM(δ)]	
		HFD/ND	HFD + Ex/HFD
Metabolism			
BG064680	Malic enzyme, supernatant (Mod1)		−2.9 (−3.3)
AI846934	Lipin 1 (Lpin1)		2.2 (2.4)
BG064859	Fumarate hydratase 1 (Fh1)		−2.0 (−2.1)
BG079455	Adenine phosphoribosyl transferase (Aprt)		−2.2 (−1.8)
Defense and stress response			
AA268587	Serum amyloid P-component A (Apcs)		−2.5 (−2.8)
Transcription regulation			
BG065507	5′-3′ exoribonuclease 2 (Xrn2)		−2.6 (−1.4)
Transport			
BG078850	Serum proteinase inhibitor, clade A, member 6 (Serpina6)		2.1 (2.0)
Unclassified			
BG078849	ESTs		2.9 (2.4)
AW124439	ESTs		2.9 (3.2)
BG081140	ESTs		−2.1 (−1.3)
AA116548	Trinucleotide repeat containing 6 a (Tnrc6)		−2.6 (−1.8)

HFD, high fat diet; ND, normal diet; HFD + Ex, HFD with exercise training. The fold change shown represents the median gene expression ratio from each of 4 independently repeated microarray experiments, comparing the HFD with the ND group and the HFD + Ex with the HFD group. The Significance Analysis of Microarrays (SAM) method was used to evaluate the significance of differences in gene expression. The criterion for inclusion of a gene in this table was that the observed fold change was >2 and [SAM(δ)] > 0.66.

prepared by the centrifugation of blood at 1000*g* for 15 minutes at 4 °C and stored at −80 °C until analysis. Immediately after the mice were killed by exsanguination, livers were perfused in situ with ice-cold saline, collected, and weighed. Three portions (~1 grams each) from each liver were frozen immediately in liquid N_2 and stored at −80 °C until the preparation of RNA and lipid. Lipid values were measured using standardized procedures, as previously described (18). Serum leptin concentration was analyzed with a radioimmunoassay kit (Linco Research Inc., St. Louis, MO). Total carnitine in serum and liver was assayed using a modified version of the radioisotopic method.

Microarray Analysis

The mouse 10K cDNA microarray used in the present study consisted of 10,336 spots, as previously described (18). Total RNA was isolated from livers using Trizol (Invitrogen, Carlsbad, CA). Fluorescence-labeled cDNA probes were prepared from 20 μg of total RNA using an amino-allyl cDNA labeling kit (Ambion, Austin, TX). Equal amounts of the RNA from six mice of each group were mixed, and each sample was equally divided; one-half was used to generate Cy3-labeled cDNA, and the other one-half was used to generate Cy5-labeled cDNA for dye swapping. The Cy5 and Cy3 probes were mixed, and hybridization was carried out at 55 °C for 16 hours. The two fluorescent images (Cy3 and Cy5) were scanned separately by a GMS 418 Array Scanner (Affymetrix, Santa Clara, CA), and the image data were analyzed using ImaGene 4.2 (Biodiscovery, El Segundo, CA) and MAAS (Gaiagene, Seoul, Korea) software. For each hybridization, the emission signal data were normalized by multiplying the Cy3 signal values by the ratio of the means of the Cy3 and Cy5 signal intensities for all spots on the array. To eliminate the unreliable data, the previously described criteria were adopted (18).

Semiquantitative RT-PCR

To confirm the cDNA microarray analysis, RT-PCR was performed for nine genes. The first strand cDNA was generated from 2 μg of total RNA by RT with 1 μL of poly dT primer (12 to 18 mer) and 50 U of SuperScript II RNase H^- Reverse Transcriptase in a 20-μL reaction mixture (Life Technologies Inc., Gaithersburg, MD). The following sets of primers were used: Mt1 forward, 5′-CACCAGATCTCGGAATGGAC-3′, reverse, 5′-CGGTAGAAAA-

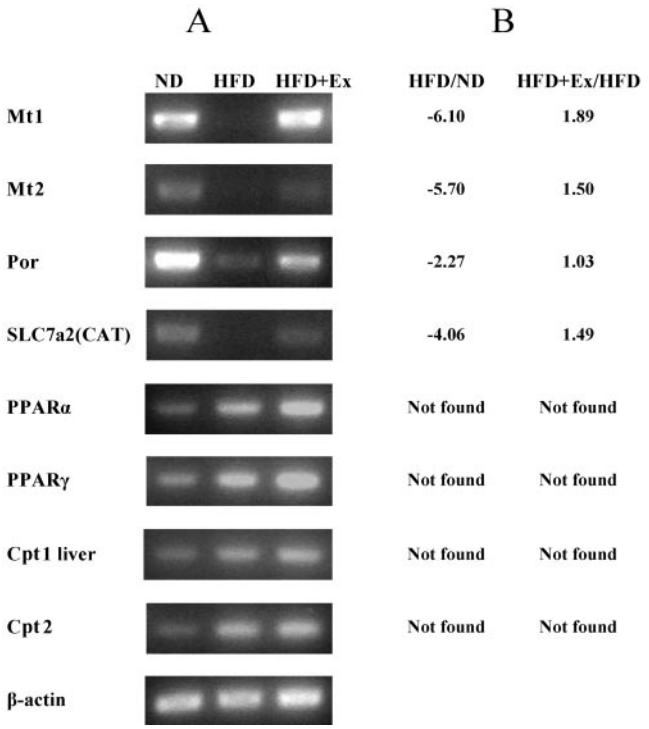

Figure 1: Confirmation by RT-PCR of microarray results using RNA from ND, HFD, and HFD + Ex. (A) Mt1, Mt2, Por [P450 (cytochrome) oxidoreductase], solute carrier family 7, cationic amino acid transporter, PPARα, Cpt1 liver, Cpt2, and β-actin were amplified, and products were separated in a 1.5% agarose gel and stained with ethidium bromide. RT-PCR was carried out under linear amplification conditions. β-Actin was used as the control for equivalent RNA template among the three groups in the PCR reactions. (B) The fold changes of microarray shown represent the median gene expression ratio from four independently repeated microarray experiments.

CGGGGGTTTA-3′; Mt2 forward, 5′-GGAGAACGAGT-CAGGGTTGT-3′, reverse, 5′-CCGATCTCTCGTCGATC-TTC-3′; solute carrier family 7, cationic amino acid transporter forward, 5′-GAATAGGCCTCCGAATCACA-3′, reverse, 5′-AAAATCCTTCAACGTGCTCA-3′; P450 (cytochrome) oxidoreductase forward, 5′-ATTCCAAGGGCCAG-GTTATT-3′, reverse, 5′-AACTTCCTTCTGCCGACCTC-3′; Cpt1 liver forward, 5′-AGAATCTCATTGGCCACCAG-3′, reverse, 5′-CAGGGTCTCACTCTCCTTGC-3′; Cpt2 forward, 5′-CACAACATCCTGTCCACCAG-3′, reverse, 5′-CA-TTGCAGCCTATCCAGTCA-3′; PPARα forward, 5′-GTG-GCTGCTATAATTTGCTGTG-3′, reverse, 5′-GAAGGTG-TCATCTGGATGGGT-3′; PPARγ forward, 5′-CAAGAC-TACCCTTTAAGTGAA-3′, reverse, 5′-CTACTTTGATC-GCACTTTGGT-3′; and β-actin forward, 5′-TTGCTGA-CAGGATGCAGAAG-3′, reverse, 5′-TGATCCACATCT-GCTGGAAG-3′. PCR amplification was measured using a Hybaid DNA thermal cycler. The thermal profiles consisted of 94 °C for 2 minutes for initial denaturing, followed by optimized cycle numbers of 94 °C for 45 seconds, annealing at 55 °C to 65 °C for 45 seconds, and 72 °C for 30 seconds (19). β-Actin was used as an internal quantitative control.

Statistical Analysis

Data are expressed as means ± standard error. Data were analyzed by one-way ANOVA with Tukey's post hoc test using the SPSS/PC statistical program (version 10.0 for Windows; SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $p < 0.05$. In the microarray analysis, we calculated the median gene expression ratios from four independently performed microarray experiments. We used the SAM method for multiclass response data to test whether differences in gene expression were significant (6). We carried out the genome-wide significance level at the SAM (δ) = 0.66 and adopted a cut-off of a 2.0-fold change as meaningful based on our experience. Genes presenting significant differences in expression were classified into different functional categories based on gene ontology with modifications (20).

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