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Proteomic Analysis in *ob/ob* Mice Before and After Hypoglycemic Polysaccharide Treatments

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In an attempt to discover novel biomarker proteins in type 2 diabetes prognosis, we investigated the influence of hypoglycemic extracellular polysaccharides (EPS) obtained from the macrofungus *Tremella fuciformis* on the differential levels of plasma proteins in *ob/ob* mice using two-dimensional gel electrophoresis (2-DE). The 2-DE analysis demonstrated that 92 spots from about 900 visualized spots were differentially regulated, of which 40 spots were identified as principal diabetes-associated proteins. By comparing control with EPS-fed mice, we found that at least six proteins were significantly altered in *ob/ob* mice, including Apo A-I, IV, C-III, E, retinol-binding protein 4, and transferrin, and their levels were interestingly normalized after EPS treatment. Western blot analysis revealed that the altered levels of the two regulatory molecules highlighted in diabetes and obesity (*e.g.*, resistin and adiponectin) were also normalized in response to EPS. The Mouse Diabetes PCR Array profiles showed that the expression of 84 genes related to the onset, development, and progression of diabetes were significantly downregulated in liver, adipocyte, and muscle of *ob/ob* mice. EPS might act as a potent regulator of gene expression for a wide variety of genes in *ob/ob* mice, particularly in obesity, insulin resistance, and complications from diabetes mellitus.

Keywords: Diabetes, *ob/ob* mice, PCR array, proteome, *Tremella fuciformis*

Recently, the search for appropriate hypoglycemic agents has been done using diverse macrofungi, owing to the various side effects and toxicity raised by current synthetic antidiabetic drugs [11, 36, 37, 65]. Many investigators have studied the hypoglycemic effect of the fruiting body or mycelia from various macrofungi because of their marked antidiabetic activities, whereas the identity of bioactive ingredients responsible for hypoglycemic activity was not clearly elucidated [7, 15, 26, 27, 29, 30].

Tremella fuciformis is a medicinal mushroom used as a folk medicine for a variety of human diseases in several Asian countries. It was reported that intraperitoneal and oral administration of an acidic polysaccharide from fruit bodies of several species of *Tremella* had significant hypoglycemic activity in mice with streptozotocin-induced and genetic diabetes [28]. We also observed a strong hypoglycemic activity of the extracellular polysaccharides (EPS) obtained from mycelial culture of *T. fuciformis* in *ob/ob* mice [3].

In our previous proteomics studies, differential changes of plasma proteins after treatment with streptozotocin (STZ, a diabetogenic chemical) and its reversal by EPS (from the macrofungus *Phellinus baumii*) were observed [31, 32]. However, the mechanism of the reversal of the protein expression profile by EPS was not clearly addressed.

Serum proteome analysis by the combination of 2-DE and mass spectrometry is a powerful method to study the protein expression profile on a large scale and has contributed significantly to the identification of marker proteins and pathways involved in diabetes development [33, 46, 48, 52, 54]. To systematically characterize the reversal of the protein expression in diabetic animals by EPS, we employed such proteomic method, and validated the changes by Western blotting and PCR array profiling methods.

Materials and Methods

Preparation of the EPS

The fungus *Tremella fuciformis* DG-02 was isolated from a mountainous district in Korea. To prepare the EPS sample, a submerged culture of *T. fuciformis* was performed as described previously [4]. Briefly, *T. fuciformis* was cultivated under the following conditions: glucose 20 g/l, tryptone 2 g/l, KH_2PO_4 0.46 g/l, K_2HPO_4 1 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l; temperature, 28°C; aeration rate, 2 vvm; agitation speed,

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200 rpm; initial pH 8.0; and working volume, 3-l. The EPS consisted of mainly mannose (56.1%), xylose (21.7%), and fucose (19.6%) [4].

Animals and Breeding Conditions

All experiments were performed on male C57BL/6J *ob/ob* mice purchased from Japan SLC (Hamamatsu, Japan) and left to acclimatize for 2 weeks before experiment. The breeding room was maintained under a constant 12-h light: 12-h dark cycle with a temperature of 23±2°C and relative humidity of 55±5% throughout the experimental period. They were given free access to standard pellets (Sam Yang, Seoul, Korea) and water. These experiments were approved by the Committee for Laboratory Animal Care and Use, Daegu University. All procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Experimental Design

All the animals were randomly divided into two groups with seven animals in each group: The control group received 0.9% NaCl solution; the diabetic groups were treated with 200 mg/kg EPS from *T. fuciformis* (EPS) daily for 52 days. Fasting blood glucose levels and body weight were measured after fasting the animals for 4 h (starting from 10:00 A.M.). Glucose levels in the tail-vein whole blood samples were determined at 2:00 P.M. using a glucose analyzer (Lifescan, Milpitas, CA, U.S.A.).

Preparation of the Plasma Sample

Blood samples were obtained by resecting the terminal 1–2 mm of the mice' tails; a total of 0.5–0.6 ml of blood was drawn into sodium tubes containing EDTA. Plasma was separated by centrifugation (3,000 ×*g*, 10 min) and then stored at −30°C until analysis. The protein content of plasma sample was determined by the Bradford method [2] using protein assay dye reagent concentrate (Bio-Rad, Hercules, CA, U.S.A.).

Two-Dimensional Gel Electrophoresis

Two-DE images were made in duplicate for three mice in each experimental group (totally 24 gel images for each group) on days 0 and 52. 2-DE images were normalized prior to statistical analysis. IPG IEF of samples was carried out on pH 4–7, 17 cm IPG DryStrips in the PROTEIN IEF cell (Bio-Rad) using the protocol recommended by the manufacturer. The IPG strips were rehydrated passively overnight in strip holders in 350 µl of rehydration solution containing 3 µl of plasma sample. IEF was carried out as follows: 15 min at 250 V, 3 h at 250–10,000 V, 6 h at 10,000 V, and then held at 500 V until ready to run the second dimension. Briefly, 400 µg (~3 µl) of the plasma sample was mixed with 347 µl of rehydration solution containing 7 M urea, 2 M thiourea, 4% CHAPS, 1 mM PMSF, 20 mM DTT, and 2% IPG buffer. After focusing, the gel strips were equilibrated in a solution containing 6 M urea, 2% SDS, 1% DTT, 30% glycerol, and 50 mM Tris-HCl (pH 6.8) for 15 min, followed by further incubation in the same solution, except for replacing DTT with 2.5% iodoacetamide for an additional 15 min. The equilibrated IPG strips were then gently rinsed with electrophoresis buffer. The gel strips were then placed on a 20×20 cm 12% polyacrylamide gel for resolution in the second dimension. The fractionation was performed with the Laemmli SDS-discontinuous system, at a constant voltage of 20 mA per gel for 10 h, after which the separated gels were visualized by silver staining.

Image Acquisition and Data Analysis

Gels were imaged on a UMAX PowerLook 1120 (Maxium Technologies, Inc., Taipei, Taiwan) and the resulting 16-bit images were converted to TIF format prior to export and analysis. Intensity calibration was carried out using an intensity stepwedge before gel image capture. Comparison of the images was performed using a modified version of ImageMaster 2D software V4.95 (Amersham Biosciences, Little Chalfont, Buckinghamshire, England). A reference gel was selected at random from the gels of the control group for each experiment, and detected spots from the other gels in the control data set were matched to those in the selected reference gel. The relative optical density and relative volume were also calculated in order to correct for differences in gel staining. Each spot intensity volume was processed by background subtraction and total spot volume normalization; the resulting spot volume percentage was used for comparison.

Enzymatic Digestion of Protein in the Gel

Protein spots were enzymatically digested in the gel in a manner similar to that previously described by Shevchenko *et al.* [50] using modified porcine trypsin. Gel pieces were washed with 50% acetonitrile to remove SDS, salts, and stain. The gel was then dried to remove solvent, rehydrated with trypsin (8–10 ng/µl), and incubated for 8–10 h at 37°C. The proteolytic reaction was terminated by adding 5 µl of 0.5% trifluoroacetic acid. Tryptic peptides were recovered by combining the aqueous phase from repeated extractions of gel pieces with 50% acetonitrile. After concentration, the peptide mixture was redissolved in the buffer and desalted using C_{18}ZipTips (Millipore, Watford, Herts, U.K.), and the peptides were eluted with 1–5 µl of acetonitrile. An aliquot of this solution was mixed with an equal volume of saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, and 1 µl of the mixture was spotted onto a target plate.

Protein Identification

Protein analysis was performed using an Ettan MALDI–TOF (Amersham Biosciences). The peptides were evaporated with a N_2 laser at 337 nm using a delayed extraction mode. They were accelerated with a 20 kV injection pulse for a time-of-flight analysis. Each spectrum was the cumulative average of 300 laser shots. The search program, ProFound, developed by The Rockefeller University (http://129.85.19.192/profound_ bin/ WebProFound.exe), was used for protein identification by peptide mass fingerprinting. Spectra were calibrated with trypsin autodigestion ion peak *m/z* (842.510, 2211.1046) as internal standards. Keratin contamination peaks and trypsin ion peak *m/z* (1045) were excluded from the list if at least two different keratins were identified from the same sample. Peptide masses were matched with the theoretical peptides of all proteins in the NCBI database using the Mascot search program (http://www.matrixscience.com). The following parameters were used for the database search: trypsin as cleaving enzyme; a maximum of one missed cleavage; 0.01% of maximum as peak threshold; iodoacetamide (Cys) as a complete modification; methionine as a partial modification; monoisotopic masses; and a mass tolerance of ±0.1 Da.

Western Blot Analysis

The differentially regulated levels of the eight proteins of interest were further confirmed by Western blot analysis as described below. An aliquot of plasma (70 µg) was diluted in 2× sample buffer [50 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 0.1% bromophenol blue, and

5% β-mercaptoethanol] and heated for 5 min at 95°C before SDS-PAGE (7.5% and 12%). Subsequently, they were transferred to a pure nitrocellulose membrane and incubated overnight with 5% blocking reagent (Amersham Biosciences) in Tris-buffered salt (TBS) containing 0.1% Tween-20 at 4°C. The membrane was rinsed in four changes of TBS with Tween-20 (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20, pH 8.0), incubated twice for 5 min and twice for 10 min in fresh washing buffer, and then incubated for 2 h with blocking solution containing 1:200 dilution of primary antibody [anti-goat apolipoprotein (Apo) A-I, A-IV, C-III, E, anti-rabbit Tf (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) anti-mouse RBP4 (ABNOVA Co., Taipei, Taiwan) anti-mouse resistin (R&D Systems, Minneapolis, MN, U.S.A.) anti-mouse adiponectin (BioVision, Mountain View, CA, U.S.A.)]. After four washes, the membrane was incub 2 h with horseradish peroxidase-conjugated anti-goat IgG, anti-mouse IgG, and anti-rabbit IgG secondary antibody (1:1,000; Santa Cruz Biotechnology) and developed using an enhanced chemiluminescence (ECL) Western blot analysis system kit (Amersham Biosciences). The Western blot was analyzed by scanning with a UMAX PowerLook 1120 (Maxium Technologies, Inc.) and digitalized using an image analysis software (KODAK 1D; Eastman Kodak Company, NY, U.S.A.).

RNA Isolation

Total RNA from the liver, adipose, and muscle tissues of 3 different *ob/ob* mice in each experimental group was isolated with the RNeasy Protect Mini Kit and RNeasy Lipid Tissue Mini Kit (Qiagen, Stanford, CA, U.S.A.). Pelleted cells were resuspended in buffer RLT (liver tissue), QIAzol lysis reagent (adipose and skeletal muscle tissues), and homogenized by passing the lysate 5 times through a 20-gauge needle fitted to a syringe. The samples were then processed according to the manufacturer's instructions. In the final step, the RNA was eluted with 50 μl of RNase-free water by centrifugation for 1 min at 10,000 rpm. The quality of the RNA was analyzed on a 1% agarose gel and the concentration by a Qubit quantitation system (Invitrogen, Eugene, Oregon, U.S.A.).

The RT²Profiler PCR Array System

For PCR array experiments, the Mouse Diabetes RT² PCR Array Profiler (Cat. No. APMM-023B; http://www.superarray.com) was used. The PCR array was a 96-well plate containing primers for a set of 89 related genes, including five housekeeping genes and three controls. The PCR array was performed according to the protocol recommended by the manufacturer (SuperArray Bioscience Co., Frederick, MD, U.S.A.). Briefly, the experimental RNA samples were converted into a PCR template with the RT² First Strand Kit. Then, the template was combined with an instrument-specific and ready-to-use RT² Real-Time SYBR Green PCR Master Mix. Equal aliquots of this mixture (25 μl for 96-well) were added to each well of the same PCR array plate containing the pre-dispensed gene-specific primer sets, and a PCR was performed. Each reaction included 40 ng of total RNA and the proper negative controls (no reverse transcription, no template). RNA from the liver, adipose, and skeletal muscle tissues was analyzed in triplicate, and data were normalized for GAPDH levels by the ΔΔCt method.

Statistical Analysis

All experimental results were compared by one-way analysis of variance (ANOVA) using the Statistical Package of Social Science (SPSS) program, and the data were expressed as means±SE. Group means were considered to be significantly different at $p<0.05$, as determined by the technique of protective least-significant difference (LSD) when ANOVA indicated an overall significant treatment effect ($p<0.05$).

RESULTS

Protein Profiling on 2-DE

To identify differentially expressed proteins, plasma proteins were separated on 2-DE combined with in-gel digestion of proteins and identified by MALDI-TOF-MS of high confidence based on high scores and sequence coverage. As shown in Fig. 1, more than 900 individual spots were detected in the plasma protein map, ranging from 6 to 200 kDa masses between pH 4 and 7. The proteomic analysis demonstrated that 92 spots from a total of about 900 matched spots were differentially expressed, of which 40 spots have been previously identified as diabetes-associated proteins (Table 1, Figs. 1 and 2). EPS treatment induced downregulation of apo A-IV, E, beta-2 glycoprotein I, and retinol-binding protein 4, and upregulation of apo A-I, C-III, and transferrin (Tf), among plasma proteins. When we conducted Western blotting of several target proteins (such as apo A-I, A-IV, C-III, E, RBP4, Tf) to validate the changes of plasma proteins detected in our proteomic analysis, we observed a good agreement between the results of 2-DE and Western blot analysis (Figs. 2 and 3).

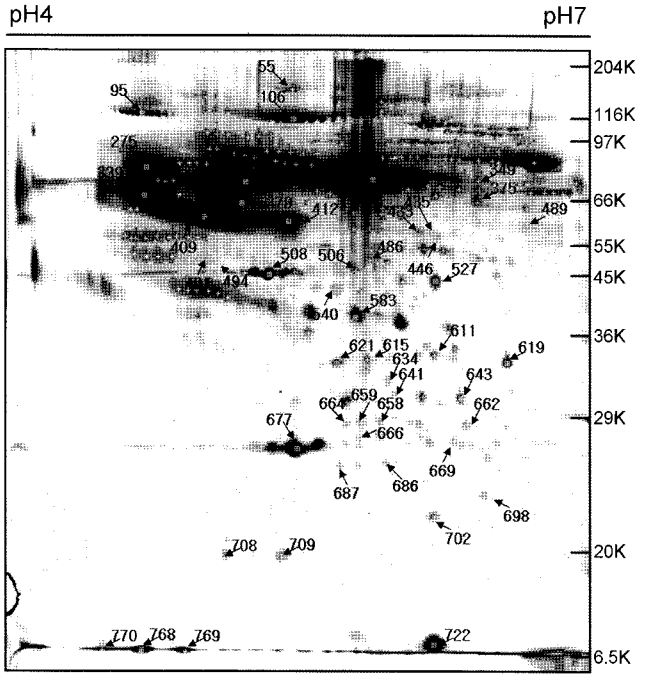

Fig. 1. A representative 2-DE gel image of silver-stained plasma proteins in *ob/ob* mice.
Differentially regulated proteins are marked with arrows together with identified major *ob/ob* mice plasma proteins.

Table 1. Identified plasma proteins in *ob/ob* mice.

Spot No.[a]	Protein	Acc. No.	Molecular mass (kDa)		Theoretical pI	No. of matched peptides	Coverage (%)[b]	Z-score[c]
			Experimental	Theoretical				
95	Contraspin	gi:54173	120	46.88	5.0	11	33	2.14
106	Inter alpha-trypsin inhibitor, heavy chain 4	gi:16741341	116	104.78	6.1	10	12	2.40
275	Esterase 1	gi:6679689	66	61.41	5.0	9	19	2.40
297	Albumin1	gi:33859506	66	70.76	5.8	7	15	2.25
339	Contraspin	gi:54173	55	46.88	5.0	11	29	2.38
349	Quiescin Q6 isoform b	gi:12963609	65	64.06	8.7	9	16	2.37
375	Beta-2 glycoprotein I	gi:1938223	60	37.56	9.3	10	31	2.34
379	Serine proteinase inhibitor	gi:18252782	59	52.50	6.1	12	33	2.38
409	Alpha1-antitrypsin precursor	gi:309079	50	46.07	5.3	12	19	2.01
412	Vitamin D-binding protein	gi:193446	50	54.67	5.3	10	24	2.14
433	Alpha-fetoprotein	gi:191765	50	48.81	5.5	5	13	1.99
435	Alpha-fetoprotein	gi:191765	50	48.81	5.5	5	13	1.94
446	Alpha-fetoprotein	gi:191765	49	48.81	5.5	7	16	1.99
486	Alpha-fetoprotein	gi:191765	45	48.81	5.5	7	16	1.93
489	Alpha-fetoprotein	gi:191765	51	48.81	5.5	6	16	1.95
491	Paraoxonase1	gi:7242183	46	39.68	5.1	3	11	1.94
494	Paraoxonase1	gi:7242183	46	39.68	5.1	3	11	1.95
506	Albumin	gi:26986064	45	24.23	5.5	14	32	2.29
508	Apolipoprotein A-IV	gi:191885	45	44.55	5.5	9	28	2.39
527	Transthyretin	gi:7305599	45	15.87	5.8	5	44	1.99
540	Alpha-fetoprotein	gi:191765	44	48.81	5.5	12	18	2.34
583	Alpha2-macroglobulin	gi:2492496, Q61838	40	167.18	6.3	13	6	2.32
611	Albumin	gi:26986064	36	24.23	5.5	5	26	1.97
615	Albumin	gi:26986064	35	24.23	5.5	10	36	1.98
619	Alpha2-macroglobulin	gi:2492496, Q61838	35	167.18	6.3	10	5	2.21

Table 1. Continued.

Spot No.[a]	Protein	Acc. No.	Molecular mass (kDa)		Theoretical pI	No. of matched peptides	Coverage (%)[b]	Z-score[c]
			Experimental	Theoretical				
621	Apolipoprotein E	gi:192005	35	33.21	5.8	19	49	2.36
634	Albumin	gi:26986064	34	24.23	5.5	5	26	1.93
658	MHC class I histocompatibility antigen A149 alpha chain precursor	gi:418849, B60854	30	41.00	6.0	8	35	1.97
662	Anti-DNA Ig light chain IgG	gi:1870378	30	11.75	6.3	5	48	2.32
664	Signal transducer and activator of transcription 1	gi:27502700	29	27.82	5.3	7	35	2.18
669	Phosphatidylinositol 4-kinase type 2 beta isoform 1	gi:13385442	29	53.96	6.4	7	20	1.97
677	Apolipoprotein A-I	gi:6753096	28	30.57	5.6	12	41	2.38
686	Glutathione peroxidase 3	gi:15011841	26	25.43	8.5	5	26	1.95
687	Glutathione peroxidase 3	gi:15011841	26	25.43	8.5	5	19	1.93
698	Retinol binding protein 4	gi:33859612	23	25.53	5.7	7	53	1.99
709	Hypothetical protein LOC66768	gi:21313330	20	27.61	10.4	8	40	1.97
722	Transthyretin	gi:7305599	15	15.87	5.8	6	44	2.30
768	Ig H-chain V-D-J-region	gi:194919	15	12.96	9.5	5	69	1.93
769	Ig H-chain V-D-J-region	gi:194919	15	12.96	9.5	3	47	1.96
770	Apolipoprotein C-III	gi:15421856	15	10.90	4.6	4	49	1.93

[a]Numbers are arbitrarily assigned for depicting Fig. 1.
[b]Coverage: percent of identified sequence to the complete sequence of the known protein.
[c]Z score corresponds to the percentile of the search in the random match population: Z score 1.65=95%, 2.33=99%, 3.09=99.9% confidence.

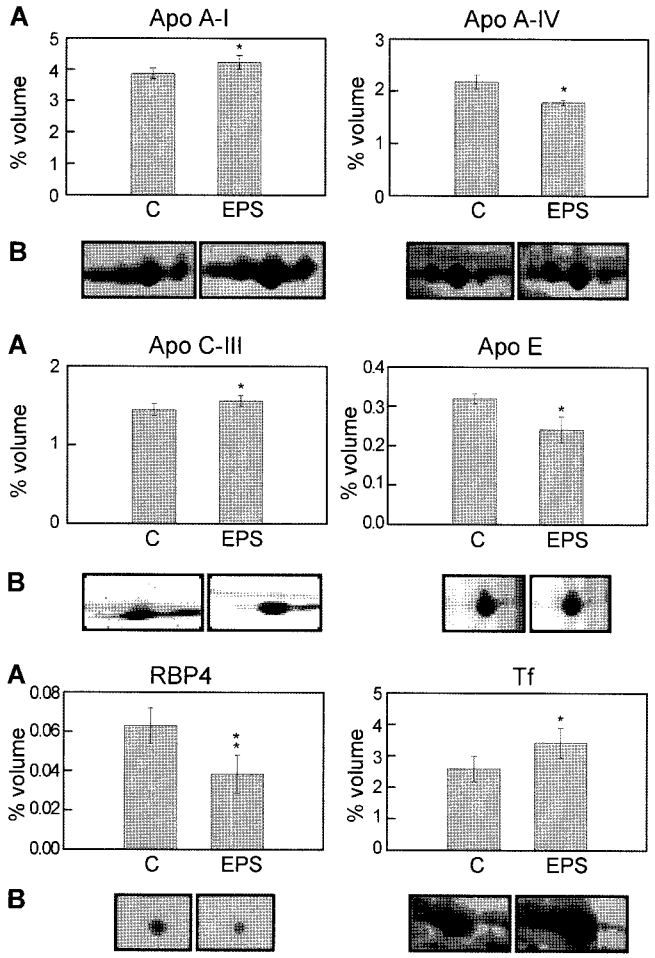

Fig. 2. Alterations of plasma proteins in *ob/ob* mice in response to EPS treatment.
A. Volume density analysis graphs: data (mean ± SEM) are expressed as fold change between EPS-treated and control mice. Significant differences are indicated: *$p<0.05$ control vs. EPS-treated mice; **$p<0.001$ control vs. EPS-treated mice. **B.** 2-DE gel images of selected spots. **C.** Normal control mice group received 0.9% NaCl solution; EPS, diabetic mice group treated with *Tremella fuciformis* 200 mg/kg EPS using an oral administration daily for 52 days.

To suggest a molecular mechanism for the improvement of glucose profile by EPS, the plasma levels of adiponectin and resistin that were not detected by 2-DE were further studied by Western blot analysis (Fig. 4). Resistin was downregulated but adiponectin was upregulated by EPS treatment.

Analysis of the Mouse Diabetes RT^2 PCR Array

To investigate the differential gene expression patterns in the liver, adipose tissue, and muscle of *ob/ob* mice in response to EPS treatment, the Mouse Diabetes PCR Array was performed in triplicate. As shown in Fig. 5 and Table 2, the expression level of many genes related to the onset, development, and progression of diabetes was significantly downregulated by EPS. The responsive genes could be divided into six functional categories: (1) receptors, transporters,

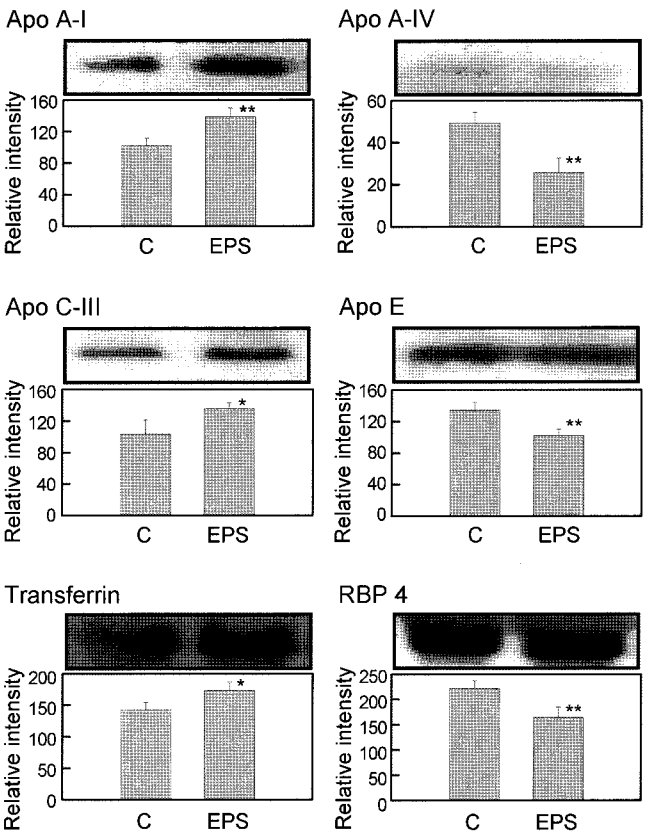

Fig. 3. Detection of transporter proteins of interest by Western blotting.
Band density was digitized with software and the mean±SE of three independent experiments are shown. *$p<0.05$ control vs. EPS; **$p<0.001$ control vs. EPS-treated mice. Notations for each group are the same as in Fig. 2.

and channels; (2) nuclear receptors; (3) metabolic enzymes; (4) secreted factors; (5) signal transduction proteins; and (6) transcription factors. On the whole, the number of genes downregulated was higher than those upregulated after EPS treatment in all three tissues, particularly in the liver and muscle (Fig. 5). Of the 89 genes tested, the expressions of 34 genes were decreased and that of 2 genes increased after EPS treatment in all three tissues (data not shown). In Table 2, genes showing extremely altered expression by EPS are listed. The expressions of Cd28, Foxc2, p38 MAPK, Nfkb1, Retn, and tumor necrosis factor (TNF) were significantly downregulated in all tissues, in response to EPS treatment. In contrast, there were no coherent changes in the expression of Mapk8 (JNK1) and peroxisome-proliferator-activated receptor alpha (PPARa).

Discussion

On the Significance of Lipid Metabolism Proteins

One of the main features of insulin resistance includes dyslipidemia, which is characterized by high triglyceride,

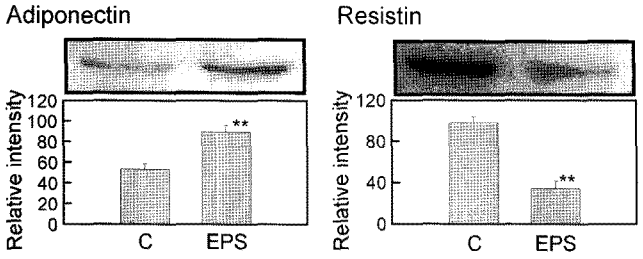

Fig. 4. Detection of two proteins of interest (adiponectin and resistin) by Western blotting.
Band density was digitized with software and the mean±SE of three independent experiments are shown. *$p<0.05$ control vs. EPS-treated mice; **$p<0.001$ control vs. EPS-treated mice. Notations for each group are the same as in Fig. 2.

low high-density lipoprotein (HDL), and small and dense low-density lipoprotein (LDL) in the blood [13]. In our previous study, we found that EPS from *T. fuciformis* significantly increased lipid metabolism in *ob/ob* mice [3]. Thus, we first examined the changes of the protein related to the lipid metabolism such as apolipoproteins (Apo) A, C, and E, because exchangeable Apo plays critical roles in lipid transport and lipoprotein metabolism in diabetic subjects.

In animal models of diabetes and in cell cultures treated with high concentrations of glucose, apo A-I expression is reduced [25]. Since the major lipoprotein constituents of HDL are apo A-I and A-II, identifying the mechanisms that modulate apo A-I gene expression will contribute to the new development of therapeutic agents that increase plasma apo A-I and HDL concentrations.

The activation of adenosine monophosphate-activated protein kinase (AMPK) leads to an increase in glucose uptake in skeletal muscle and a decrease in gluconeogenesis in the liver [6]. Through this mechanism, apo A-I stimulates glucose utilization and improves insulin resistance in peripheral tissues. In this regard, an increase in apo A-I after EPS treatment in this study has a dual protective effect in type 2 diabetes mellitus (T2DM), both by lowering the prevalence of diabetes-associated cardiovascular diseases and improving insulin resistance *via* activation of AMPK. In this respect, apo A-I seems to be an ideal target for the prevention and therapy of T2DM and the metabolic syndrome. However, it remains unclear at present whether HDL and/or apo A-I directly protect against diabetes, and if so, what the underlying molecular mechanism is.

Blood levels of apo A-IV generally correlate with triglyceride levels and are increased in diabetic patients. Few studies have attempted to elucidate the regulation of apo A-IV at the promoter level [17]. Apo C-III is the most abundant apolipoprotein in very low-density lipoprotein (VLDL) particles and is correlated closely with concentrations of serum total and VLDL triglycerides [20]. This protein modulates metabolism of triglyceride-rich lipoproteins (TRLs) at two subsequent steps. Firstly, apo C-III is an inhibitor of

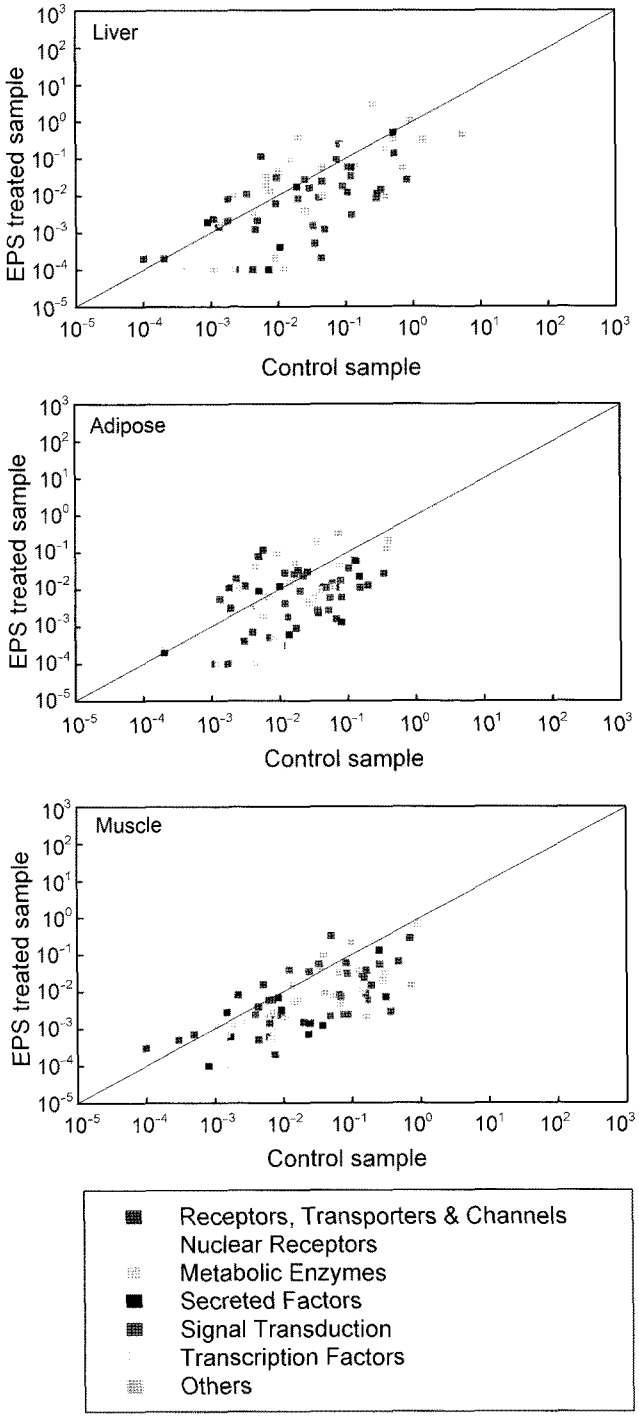

Fig. 5. Analyses of mRNA expression of diabetes-specific genes in *ob/ob* mice by the RT^{2}Profiler PCR Array System. Notations for each group are the same as in Fig. 2.

lipoprotein lipase activity, and secondly, it interferes with apolipoprotein E-mediated receptor binding, delaying removal of remnant particles. To date, little attention has been paid to the distribution of apo C-III between VLDL subspecies. Likewise, only a few studies have reported apo C-III levels in a diabetic population [20]. These reports emphasize the

Table 2. Significant alterations of genes related to diabetes by the RT^{2}Profiler PCR Array System.

Symbol	Description	GenBank Access. No.	Fold changes of regulation					
			Liver		Adipose		Muscle	
			Treated/ Control	*p* Value	Treated/ Control	*p* Value	Treated/ Control	*p* Value
Cd28	CD28 antigen	NM_007642	-16.15	0.4068	-19.52	0.4572	-28.18	0.4161
Foxc2	Forkhead box C2	NM_013519	-28.71	0.2862	-40.55	0.4326	-14.44	0.4534
Mapk8 (JNK1)	Mitogen-activated protein kinase 8	NM_016700	3.20	0.3826	-2.28	0.4518	3.10	0.2707
Mapk14 (p38 MAPK)	Mitogen-activated protein kinase 14	NM_011951	-3.60	0.4884	-4.58	0.4090	-4.76	0.4473
Nfkb1	Nuclear factor of kappa light chain gene enhancer in B-cells 1, p105	NM_008689	-4.64	0.4587	-5.47	0.4232	-6.31	0.4557
PPARa	Peroxisome-proliferator-activated receptor alpha	NM_011144	2.38	0.4225	-5.90	0.4564	-3.96	0.3665
Retn	Resistin	NM_022984	-5.46	0.0633	-22.65	0.3653	-17.96	0.4926
Tnf	Tumor necrosis factor	NM_013693	-28.61	0.4562	-15.80	0.4456	-31.16	0.4660

need to study the distribution and composition of VLDL subclasses separately to capture the atherogenicity of TRL subpopulations in T2DM. In the present study, the expression of apo A-IV was decreased, whereas that of apo C-III was unexpectedly increased in EPS-treated mice, which is probably attributable to the enhanced lipid metabolism.

Apo E is a glycoprotein that circulates as a surface component of plasma lipoproteins and is expressed in numerous mammalian cell types [63]. Reports over many years have demonstrated that apo E is involved in maintaining important aspects of organismal and cellular homeostasis. For example, disordered apo E function/expression has a well-established role in the pathophysiology of important human diseases such as atherosclerosis and dementia [5, 37]. Apo E is involved in HDL metabolism, as demonstrated by decreased plasma clearance of HDL-cholesteryl ester in apo E knock-out mice [18]. The associations between plasma apo A-I and E concentrations and phospholipids transfer protein (PLTP) activity suggest that these apolipoproteins are important regulators of PLTP activity *in vivo* [55]. The increase in PLTP activity in T2DM is partly related to the changes in these apolipoproteins. The decreased level of apo E after EPS treatment in this work implies a positive role in HDL metabolism.

Adiponectin is a peptide predominantly synthesized in the adipose tissue that plays an important role in carbohydrate and lipid metabolism and vascular biology [41, 58, 61]. Plasma adiponectin levels are reduced in individuals with abdominal obesity, metabolic syndrome, and/or T2DM [41]. Thus, adiponectin concentration has been found negatively correlated with abdominal obesity and insulin resistance in humans and has been shown to predict the development of T2DM. Adiponectin is also related to lipid metabolism, principally high levels of HDL cholesterol and lower levels of triglycerides. The mechanism for the association between plasma adiponectin and HDL cholesterol is still unknown and has not been previously investigated.

Furthermore, adiponectin stimulates glucose metabolism by promoting the phosphorylation and activation of adenosine monophosphate activation of adenosine monophosphate-activated protein kinase (AMPK), a stress-responsive kinase, in skeletal muscle, liver, and adipocytes [44]. It was suggested that down regulation of the adiponectin-AMPK regulatory pathway may participate in endothelial cell dysfunction and vessel rarefaction that are observed in obese states and T2DM. Taken together, increased adiponectin level in EPS-treated mice supported a hypothesis that EPS played a critical role in AMPK activation, thereby promoting insulin sensitivity in *ob/ob* mice.

On the Significance of Retinol-Binding Protein 4 and Transferrin

More recently, it has been demonstrated that elevated serum RBP4 levels are associated with many metabolic syndromes such as obesity and T2DM [10, 14, 60, 62]. For example, increased levels of serum RBP4 led to impaired glucose uptake into skeletal muscle and increased glucose production by the liver, whereas lowered serum RBP4 levels greatly enhanced insulin sensitivity [60]. It has been reported that adipocyte GLUT4 protein and serum RBP4 levels were inversely correlated. Although the molecular evidence for the relationship between these two molecules is not clarified yet, it might involve sensing of glucose by adipocytes [14]. Moreover, RBP4 is an adipocyte-secreted molecule that is elevated in the serum before the development of overt diabetes and predicts insulin resistance and associated cardiovascular risk factors in subjects with varied clinical presentations [14]. In this study, the plasma level of RBP4 was significantly decreased in EPS-treated *ob/ob* mice, suggesting the possibility of EPS as a possible antidiabetic agent for lowering the serum RBP4 levels.

Plasma transferrin itself may be involved in the pathogenesis of insulin resistance because it has an antagonistic effect on insulin action [57]. A recent study showed that transferrin is a major determinant of the lipolytic activity of human serum in adipocytes [47]. An increase in adipose tissue lipolysis due to higher transferrin levels could increase availability of free fatty acids to the liver and skeletal muscle, which could in turn lead to insulin resistance. Accordingly, elevated serum transferrin by EPS treatment suggests the stimulatory role of this molecule in lipolysis, which probably occurs through a prooxidant mechanism mediated by iron.

On the Significance of Cytokines

Adipocytes produce numerous hormones and cytokines such as TNF-α, plasminogen activator inhibitor type 1 (PAI-1), leptin, adiponectin, and resistin. These factors play an important role locally (within adipose tissue) but may also have important effects as circulating bioactive factors [38]. The ability of adipokines such as resistin to directly modulate endothelial function and incite endothelial activation may represent an important link between insulin resistance and cardiovascular disease [35, 56]. However, the role of resistin in insulin sensitivity and obesity is still controversial. For example, some authors suggested that increased serum resistin levels were associated with obesity, insulin resistance in T2DM, and inflammation, whereas others failed to observe such correlations. Plasma resistin levels were highly positively correlated with triglycerides, waist circumference, waist/hip ratio, systolic blood pressure, and apo A-I/apo B ratio, whereas they were inversely correlated with high-density lipoprotein and apo A-I levels [42]. Resistin was also identified by screening the genes that were induced during the differentiation of the adipocytes exposed to PPARγ ligands. Taken together, it is likely that serum resistin level is associated with insulin sensitivity, suggesting that resistin plays an important role

in the development of insulin resistance in T2DM patients [35, 56]. The markedly reduced levels of resistin after EPS treatment in the present study is another evidence for the preventive role of EPS in diabetic mice.

Functional Analysis by PCR Array System

Global gene expression, or transcriptional profiling, has been used to identify molecular markers for various pathological states, and can be used to generate novel hypotheses [8, 23, 24, 39]. DNA microarrays have been used to identify candidate genes for disease diagnosis and to characterize gene expression patterns associated with potential disease treatments including diabetes [12, 21, 24, 39, 59]. However, the genetic changes involved in the pathologenesis of diabetes have not been fully identified so far [67]. To date, there is still a lack of knowledge about the changes in gene expression in diabetic subjects before and after treatments with antidiabetic drugs or other candidate materials.

Nadler *et al.* [40] have employed DNA microarrays to identify differences in gene expression between the adipose tissue from lean, obese, and that from obese-diabetic mice. By using different strains of mice, they were able to identify those genes whose expression changed regardless of the strain background and are therefore most likely to be relevant to obesity and diabetes. Of the over 11,000 genes examined, over 200 showed consistent changes with obesity. Another DNA microarray approach provided several candidate genes for muscle insulin resistance, complications associated with poor glycemic control, and effects of insulin treatment in T2DM [53]. The expression of five genes (*e.g.*, MHC-I, GLUT4, IGFBP-5, SOD2, and UCP-3) significantly changed in T2DM patients compared with the control subjects.

RT^2 Profile PCR Arrays are the most reliable and sensitive gene expression profiling technology for analyzing a panel of genes in signal transduction pathways, biological processes, or disease-related gene networks. More recently, the PCR arrays have been used for research on cancer, diabetes, immunology, toxicology, biomarker discovery and validation, and phenotypic analysis of cells and transgenic animals [1, 16, 43, 45, 64, 66]. The Mouse Diabetes RT^2 *Profile* PCR Array profiled the expression of 84 genes related to the onset, development, and progression of diabetes. They include genes that contribute to obesity, insulin resistance, the early onset of diabetes, and complications from diabetes mellitus. It is interesting to mention that the number of genes downregulated was much higher than those upregulated, in response to EPS treatment in all three tissues of *ob/ob* mice, particularly in the liver and muscle. Therefore, this result is strong evidence for the hypoglycemic role of EPS in *ob/ob* mice at the gene level, and is the first report showing that EPS directly signals rat tissues to regulate the expression of many important diabetes-related genes.

Among 84 diabetes genes, 7 genes (JNK1, Tnf, Nfkb1, p38 MAPK, PPARα, Retn, and Cd28) would significantly contribute to amelioration of the diabetic condition in *ob/ob* mice by EPS treatment. JNK (Jun N-terminal kinase) deficiency is protective in different mouse models of obesity-induced glucose intolerance because it increases the sensitivity of target tissues to insulin [9, 19, 51]. Interestingly, our results indicate that JNK in adipose tissues strongly decreased, whereas the level in muscle tissues and liver increased in *ob/ob* mice by EPS treatment. Elevated production of tumor necrosis factor (TNF)-α by adipose tissue decreases sensitivity to insulin and has been detected in several experimental obesity models and obese humans [9]. Free fatty acids (FFAs) are also implicated in the etiology of obesity-induced insulin resistance, although the molecular pathways involved in their action remain unclear [51].

In skeletal muscle, TNF-α induces insulin resistance *via* the p38 MAPK pathway [49]. The reason for the elevation of TNF-α in T2DM is still unclear. Some have ascribed it to increased adiposity associated with T2DM and the consequence of fat cell-dependent TNF-α production [22, 34, 36]. Igarashi *et al.* [22] suggested p38 MAPK as a possible target in vascular cells, which can be activated by high glucose levels and diabetes. In addition, these findings support those of Kotlyarov *et al.* [34] who showed that lipopolysaccharide (LPS)-induced TNF-α production was dependent on posttranscriptional modifications regulated by MAPKAP kinase 2, a kinase directly downstream of p38 kinase, and not to changes in mRNA expression and stability. Insulin-dependent activation of PI3K is reduced in T2DM. It is likely that decreased p38 MAPK levels by EPS will have biologic and possible pathologic importance in the preservation of diabetic vascular complications. It is probable that some of these changes by EPS treatment are related to the changes in circulating substrates such as glucose, fatty acids, and amino acids.

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References

1. Bowers, R. R., J. W. Kim, T. C. Otto, and M. D. Lane. 2006. Stable stem cell commitment to the adipocyte lineage by

inhibition of DNA methylation: Role of the BMP-4 gene. *Proc. Natl. Acad. Sci. U.S.A.* **103:** 13022–13027.
2. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
3. Cho, E. J., H. J. Hwang, S. W. Kim, J. Y. Oh, Y. M. Baek, J. W. Choi, and J. W. Yun. 2007. Hypoglycemic effects of exopolysaccharides produced by mycelial cultures of two different mushrooms *Tremella fuciformis* and *Phellinus baumii* in *ob/ob* mice. *Appl. Microbiol. Biotechnol.* **75:** 1257–1265.
4. Cho, E. J., J. Y. Oh, H. Y. Chang, and J. W. Yun. 2006. Production of exopolysaccharides by submerged mycelial culture of a mushroom *Tremella fuciformis*. *J. Biotechnol.* **127:** 129–140.
5. Curtiss, L. K. and W. A. Boisvert. 2000. Apolipoprotein E and atherosclerosis. *Curr. Opin. Lipidol.* **11:** 243–251.
6. Drew, B. G., N. H. Fidge, G. Gallon-Beaumier, B. E. Kemp, and B. A. Kingwell. 2004. High-density lipoprotein and apolipoprotein AI increase endothelial NO synthase activity by protein association and multisite phosphorylation. *Proc. Natl. Acad. Sci. U.S.A.* **101:** 6999–7004.
7. Ewart, R. B., S. Kornfeld, and D. M. Kipnis. 1975. Effect of lectins on hormone release from isolated rat islets of Langerhans. *Diabetes* **24:** 705–714.
8. Fathman, C. G., L. Soares, S. M. Chan, and P. J. Utz. 2005. An array of possibilities for the study of autoimmunity. *Nature* **435:** 605–611.
9. Gao, X., S. Belmadani, A. Picchi, X. Xu, B. J. Potter, N. Tewari-Singh, S. Capobianco, W. M. Chilian, and C. Zhang. 2007. Tumor necrosis factor-alpha induces endothelial dysfunction in Lepr(db) mice. *Circulation* **115:** 245–254.
10. Gavi, S., L. M. Stuart, P. Kelly, M. M. Melendez, D. C. Mynarcik, M. C. Gelato, and M. A. McNurlan. 2007. Retinol-binding protein 4 is associated with insulin resistance and body fat distribution in nonobese subjects without type 2 diabetes. *J. Clin. Endocrinol. Metab.* **92:** 1886–1890.
11. Gochukwu, N. H., and N. E. Abady. 2003. Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sci.* **73:** 1925–1938.
12. Golub, T. R., D. K. Slonim, P. Tamayo, C. Huard, M. Gaasenbeek, J. P. Mesirov, *et al.* 1999. Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. *Science* **286:** 531–537.
13. Gordon, D. J. and B. M. Rifkind. 1989. High-density lipoprotein – the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
14. Graham, T. E., Q. Yang, M. Bluher, A. Hammarstedt, T. P. Ciaraldi, R. R. Henry, *et al.* 2006. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N. Engl. J. Med.* **354:** 2552–2563.
15. Gray, A. M. and P. R. Flatt. 1998. Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom). *J. Endocrinol.* **157:** 259–266.
16. Gude, N., J. Muraski, M. Rubio, J. Kajstura, E. Schaefer, P. Anversa, and M. A. Sussman. 2006. Akt promotes increased cardiomyocyte cycling and expansion of the cardiac progenitor cell population. *Circ. Res.* **99:** 381–388.
17. Hanniman, E. A., G. Lambert, Y. Inoue, F. J. Gonzalez, and C. J. Sinal. 2006. Apolipoprotein A-IV is regulated by nutritional and metabolic stress: Involvement of glucocorticoids, HNF-4 alpha, and PGC-1 alpha. *J. Lipid Res.* **47:** 2503–2514.
18. Hime, N. J., K. J. Drew, C. Hahn, P. J. Barter, and K. A. Rye. 2004. Apolipoprotein E enhances hepatic lipase-mediated hydrolysis of reconstituted high-density lipoprotein phospholipid and triacylglycerol in an isoform-dependent manner. *Biochemistry* **43:** 12306–12314.
19. Hirosumi, J., G. Tuncman, L. Chang, C. Z. Gorgun, K. T. Uysal, K. Maeda, M. Karin, and G. S. Hotamisligil. 2002. A central role for JNK in obesity and insulin resistance. *Nature* **420:** 333–336.
20. Hiukkam, A., J. Fruchart-Najib, E. Leinonen, H. Hilden, J. C. Fruchart, and M. R. Taskinen. 2005. Alterations of lipids and apolipoprotein CIII in very low density lipoprotein subspecies in type 2 diabetes. *Diabetologia* **48:** 1207–1215.
21. Hu, J. S., M. Durst, R. Kerb, V. Truong, J. T. Ma, E. Khurgin, D. Balaban, T. R. Gingeras, and B. B. Hoffman. 2000. Analysis of drug pharmacology towards predicting drug behavior by expression profiling using high-density oligonucleotide arrays. *Ann. N. Y. Acad. Sci.* **91:** 9–15.
22. Igarashi, M., H. Wakasaki, N. Takahara, H. Ishii, Z. Y. Jiang, T. Yamauchi, *et al.* 1999. Glucose or diabetes activates p38 mitogen-activated protein kinase *via* different pathways. *J. Clin. Invest.* **103:** 185–195.
23. Iida, S., Y. Sato, A. Nakaya, Y. Shinohara, Y. Hayashi, A. Sawada, *et al.* 2006. Genome wide expression analysis of white blood cells and liver of pre-diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats using a cDNA microarray. *Biol. Pharm. Bull.* **29:** 2451–2459.
24. Jakupciak, J. P., G. D. Dakubo, S. Maragh, and R. L. Parr. 2006. Analysis of potential cancer biomarkers in mitochondrial DNA. *Curr. Opin. Mol. Ther.* **8:** 500–506.
25. Kawashiri, M. A., C. Maugeais, and D. J. Rader. 2000. High-density lipoprotein metabolism: Molecular targets for new therapies for atherosclerosis. *Curr. Atheroscler. Rep.* **2:** 363–372.
26. Kiho, T., A. Yamane, J. Hui, S. Usui, and S. Ukai. 1996. Polysaccharides in fungi. XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. *Biol. Pharm. Bull.* **19:** 294–296.
27. Kiho, T., H. Morimoto, M. Sakushima, S. Usui, and S. Ukai. 1995. Polysaccharides in fungi. XXXV. Anti diabetic activity of an acidic polysaccharide from the fruiting bodies of *Tremella aurantia*. *Biol. Pharm. Bull.* **18:** 1627–1629.
28. Kiho, T., Y. Tsujimura, M. Sakushima, S. Usui, and S. Ukai. 1994. Polysaccharides in fungi. XXXIII. Hypoglycemic activity of an acidic polysaccharide (AC) from *Tremella fuciformis*. *Yakugaku Zasshi* **114:** 308–315.
29. Kim, D. H., B. K. Yang, S. C. Jeong, J. B. Park, S. P. Cho, S. Das, J. W. Yun, and C. H. Song. 2001. Production of a hypoglycemic, extracellular polysaccharide from the submerged culture of the mushroom, *Phellinus linteus*. *Biotechnol. Lett.* **23:** 513–517.
30. Kim, D. H., B. K. Yang, S. C. Jeong, N. J. Hur, S. Das, J. W. Yun, J. W. Choi, Y. S. Lee, and C. H. Song. 2001. A preliminary study on the hypoglycemic effect of the exo-polymers produced by five different medicinal mushrooms. *J. Microbiol. Biotechnol.* **11:** 167–171.

31. Kim, S. W., H. J. Hwang, E. J. Cho, J. Y. Oh, Y. M. Baek, J. W. Choi, and J. W. Yun. 2006. Time-dependent plasma protein changes in streptozotocin-induced diabetic rats before and after fungal polysaccharide treatments. *J. Proteome Res.* **5:** 2966–2976.
32. Kim, S. W., H. J. Hwang, H. M. Kim, M. C. Lee, M. S. Lee, J. W. Choi, and J. W. Yun. 2006. Effect of fungal polysaccharides on the modulation of plasma proteins in streptozotocin-induced diabetic rats. *Proteomics* **6:** 5291–5302.
33. Korc, M. 2003. Diabetes mellitus in the era of proteomics. *Mol. Cell Proteomics* **2:** 399–404.
34. Kotlyarov, A., A. Neininger, C. Schubert, R. Eckert, C. Birchmeier, H. D. Volk, and M. Gaestel. 1999. MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis. *Nat. Cell Biol.* **1:** 94–97.
35. Lee, J. H., J. W. Jr. Bullen, V. L. Stoyneva, and C. S. Mantzoros. 2005. Circulating resistin in lean, obese, and insulin-resistant mouse models: Lack of association with insulinemia and glycemia. *Am. J. Physiol. Endocrinol. Metab.* **288:** E625–E632.
36. Li, G., E. J. Barrett, M. O. Barrett, W. Cao, and Z. Liu. 2007. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells *via* a p38 mitogen-activated protein kinase-dependent pathway. *Endocrinology* **148:** 3356–3363.
37. Mazzone, T. 1996. Apolipoprotein E secretion by macrophages: Its potential physiological functions. *Curr. Opin. Lipidol.* **7:** 303–307.
38. McTernan, P. G., C. L. McTernan, R. Chetty, K. Jenner, F. M. Fisher, M. N. Lauer, J. Crocker, A. H. Barnett, and S. Kumar. 2002. Increased resistin gene and protein expression in human abdominal adipose tissue. *J. Clin. Endocrinol. Metab.* **87:** 2407–2410.
39. Murtaza, I., G. Marra, R. Schlapbach, A. Patrignani, M. Kunzli, U. Wagner, J. Sabates, and A. Dutt. 2006. A preliminary investigation demonstrating the effect of quercetin on the expression of genes related to cell-cycle arrest, apoptosis and xenobiotic metabolism in human CO115 colon-adenocarcinoma cells using DNA microarray. *Biotechnol. Appl. Biochem.* **45:** 29–36.
40. Nadler, S. T., J. P. Stoehr, K. L. Schueler, G. Tanimoto, B. S. Yandell, and A. D. Attie. 2000. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *Proc. Natl. Acad. Sci. U.S.A.* **97:** 11371–11376.
41. Neumeier, M., A. Sigruener, E. Eggenhofer, J. Weigert, T. S. Weiss, A. Schaeffler, H. J. Schlitt, C. Aslanidis, and P. Piso. 2007. High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. *Biochem. Biophys. Res. Commun.* **352:** 543–548.
42. Norata, G. D., M. Ongari, K. Garlaschelli, S. Raselli, L. Grigore, and A. L. Catapano. 2007. Plasma resistin levels correlate with determinants of the metabolic syndrome. *Eur. J. Endocrinol.* **156:** 279–284.
43. Olivero, O. A., A. M. Tejera, J. J. Fernandez, B. J. Taylor, S. Das, R. L. Divi, and M. C. Poirier. 2005. Zidovudine induces S-phase arrest and cell cycle gene expression changes in human cells. *Mutagenesis* **20:** 139–146.
44. Ouchi, N., R. Shibata, and K. Walsh. 2006. Cardioprotection by adiponectin. *Trends Cardiovasc. Med.* **16:** 141–146.
45. Rangel-Moreno, J., L. Hartson, C. Navarro, M. Gaxiola, M. Selman, and T. D. Randall. 2006. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. *J. Clin. Invest.* **116:** 3183–3194.
46. Ritorto, M. S. and J. Borlak. 2008. A simple and reliable protocol for mouse serum proteome profiling studies by use of two-dimensional electrophoresis and MALDI TOF/TOF mass spectrometry. *Proteome Sci.* **6:** 25.
47. Rumberger, J. M., T. Jr. Peters, C. Burrington, and A. Green. 2004. Transferrin and iron contribute to the lipolytic effect of serum in isolated adipocytes. *Diabetes* **53:** 2535–2541.
48. Sharma, A., S. Chavali, A. Mahajan, R. Tabassum, R. Banerjee, N. Tandon, and D. Bharadwaj. 2005. Genetic association, post-translational modification, and protein–protein interactions in type 2 diabetes mellitus. *Mol. Cell Proteomics* **4:** 1029–1037.
49. Sherry, C. L., J. C. O'Connor, J. M. Kramer, and G. G. Freund. 2007. Augmented lipopolysaccharide-induced TNF-alpha production by peritoneal macrophages in type 2 diabetic mice is dependent on elevated glucose and requires p38 MAPK. *J. Immunol.* **178:** 663–670.
50. Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal. Chem.* **68:** 850–858.
51. Solinas, G., W. Naugler, F. Galimi, M. S. Lee, and M. Karin. 2006. Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc. Natl. Acad. Sci. U.S.A.* **103:** 16454–16459.
52. Sparre, T., R. Bergholdt, J. Nerup, and F. Pociot. 2003. Application of genomics and proteomics in type 1 diabetes pathogenesis research. *Expert Rev. Mol. Diagn.* **3:** 743–757.
53. Sreekumar, R., P. Halvatsiotis, J. C. Schimke, and K. S. Nair. 2002. Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. *Diabetes* **51:** 1913–1920.
54. Sundsten, T., M. Eberhardson, M. Göransson, and P. Bergsten. 2006. The use of proteomics in identifying differentially expressed serum proteins in humans with type 2 diabetes. *Proteome Sci.* **4:** 22.
55. Tan, K. C., S. W. Shiu, Y. Wong, W. K. Wong, and S. Tam. 2006. Plasma apolipoprotein E concentration is an important determinant of phospholipid transfer protein activity in type 2 diabetes mellitus. *Diabetes Metab. Res. Rev.* **22:** 307–312.
56. Tokuyama, Y., H. Osawa, T. Ishizuka, H. Onuma, K. Matsui, T. Egashira, H. Makino, and A. Kanatsuka. 2007. Serum resistin level is associated with insulin sensitivity in Japanese patients with type 2 diabetes mellitus. *Metabolism* **56:** 693–698.
57. Vargas, L., M. E. Kawada, S. Bazaes, P. A. Karplus, and C. H. Faerman. 1998. Insulin antagonism: A novel role for human serum transferrin. *Horm. Metab. Res.* **30:** 113–117.
58. Verges, B., J. M. Petit, L. Duvillard, G. Dautin, E. Florentin, F. Galland, and P. Gambert. 2006. Adiponectin is an important determinant of apoA-I catabolism. *Arterioscler. Thromb. Vasc. Biol.* **26:** 1364–1369.
59. Welsh, J. B., P. P. Zarrinkar, L. M. Sapinoso, S. G. Kern, C. A. Behling, B. J. Monk, D. J. Lockhart, R. A. Burger, and G. M. Hampton. 2001. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. *Proc. Natl. Acad. Sci. U.S.A.* **98:** 1176–1181.
60. Wolf, G. 2007. Serum retinol-binding protein: A link between obesity, insulin resistance, and type 2 diabetes. *Nutr. Rev.* **65:** 251–256.

61. Yamauchi, T., J. Kamon, H. Waki, Y. Imai, N. Shimozawa, K. Hioki, *et al.* 2003. Globular adiponectin protected *ob/ob* mice from diabetes and ApoE-deficient mice from atherosclerosis. *J. Biol. Chem.* **278:** 2461–2468.
62. Yang, Q., T. E. Graham, N. Mody, F. Preitner, O. D. Peroni, J. M. Zabolotny, K. Kotani, L. Quadro, and B. B. Kahn. 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* **436:** 356–362.
63. Yue, L., N. Rasouli, G. Ranganathan, P. A. Kern, and T. Mazzone. 2004. Divergent effects of peroxisome proliferator-activated receptor gamma agonists and tumor necrosis factor alpha on adipocyte ApoE expression. *J. Biol. Chem.* **279:** 47626–47632.
64. Zhang, C. Y., Y. X. Feng, Y. Yu, W. J. Sun, J. Bai, F. Chen, and S. B. Fu. 2006. The molecular mechanism of resistance to methotrexate in mouse methotrexate-resistant cells by cancer drug resistance and metabolism SuperArray. *Basic Clin. Pharmacol. Toxicol.* **99:** 141–145.
65. Zhang, H. N., and Z. B. Lin. 2004. Hypoglycemic effect of *Ganoderma lucidum* polysaccharides. *Acta Pharmacol. Sin.* **25:** 191–195.
66. Zhan, Q., S. Signoretti, D. Whitaker-Menezes, T. M. Friedman, R. Korngold, and G. F. Murphy. 2007. Cytokeratin15-positive basal epithelial cells targeted in graft-versus-host disease express a constitutive antiapoptotic phenotype. *J. Invest. Dermatol.* **127:** 106–115.
67. Zisman, A., O. D. Peroni, E. D. Abel, M. D. Michael, F. Mauvais-Jarvis, B. B. Lowell, *et al.* 2000. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat. Med.* **6:** 924–928.