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Isolation stress for 30 days alters hepatic gene expression profiles, especially with reference to lipid metabolism in mice

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Motoyama K, Nakai Y, Miyashita T, Fukui Y, Morita M, Sanmiya K, Sakakibara H, Matsumoto I, Abe K, Yakabe T, Yajima N, Shimoi K. Isolation stress for 30 days alters hepatic gene expression profiles, especially with reference to lipid metabolism in mice. Physiol Genomics 37: 79–87, 2009. First published December 23, 2008; doi:10.1152/physiolgenomics.90358.2008.—To elucidate the physiological responses to a social stressor, we exposed mice to an isolation stress and analyzed their hepatic gene expression profiles using a DNA microarray. Male BALB/c mice were exposed to isolation stress for 30 days, and then hepatic RNA was sampled and subjected to DNA microarray analysis. The isolation stress altered the expression of 420 genes (after considering the false discovery rate). Gene Ontology analysis of these differentially expressed genes indicated that the stress remarkably downregulated the lipid metabolism-related pathway through peroxisome proliferator-activated receptor-α, while the lipid biosynthesis pathway controlled by sterol regulatory element binding factor 1, Golgi vesicle transport, and secretory pathway-related genes were significantly upregulated. These results suggest that isolation for 30 days with a mild and consecutive social stress regulates the systems for lipid metabolism and also causes endoplasmic reticulum stress in mouse liver.

social stress; mouse liver; DNA microarray

HANS SELYE, WHO INTRODUCED original concept of stress, defined stress as nonspecific responses of the body to any demand (41, 42). A stressor is an agent that produces stress at any time. He termed these responses as a general adaptation syndrome that consists of three phases, the alarm reaction, the stage of resistance, and the stage of exhaustion, and is characterized by adrenal hypertrophy, atrophy of the thymus gland, and gastric ulcerations. Physiological and psychological stress increases blood pressure and the plasma levels of catecholamines and glucocorticoid (cortisol or corticosterone) by the activation of the sympathetic-adrenomedullary axis. PHYSIOLOGICAL AND PSYCHOLOGICAL STRESS fibers of the hypothalamic-pituitary-adrenal axis (49).

Recent generations have lived in what is called a “stressful society.” Recent studies have reported that the incidences of diseases related to social and psychological stress are increasing. For example, the morbidity of depression, one of the major mental disorders, has reached 3–10% per year according to a report of World Health Organization (2). Furthermore, it has been reported that daily exposure to social and psychological stress is associated with lifestyle-related diseases such as hyperinsulinemia, hyperglycemia, cardiovascular diseases, and cancer, as well as mental disorders (3, 8, 32, 39). Interestingly, psychosocial stress exacerbated hepatic diseases such as inflammatory and fibrotic changes in alcoholic hepatitis, chronic hepatitis C, and hepatocellular carcinoma (7). Therefore, there is a need for treatments that prevent various disorders resulting from daily exposure to social and psychological stress; and for this an increased understanding of the mechanism underlying response to a social stressor is required. DNA microarray analysis is a useful and powerful tool (50) that allows us to understand which genes might be involved in the stress response.

Recent studies using microarray analysis have identified many novel genes regulated by artificial stressors like immobilization, forced running, or electroshock. Ha et al. (18) reported that hepatic gene expression involved in fatty acid/lipid metabolism and detoxification was elevated in mice exposed to restraint stress for 14 days. Yin et al. (55) identified genes that contribute to the splenocyte apoptosis in mice exposed to 12-h daily restraint stress for 2 days. Sato et al. (40) reported that the expression of duodenal genes related to lipid metabolism, including those encoding mitochondrial HMG-CoA synthase, was induced in rats immobilized for 2 wk. However, the stress models used in these studies are quite excessive situations compared with our daily life. Little is known about tissue-specific gene expression in response to social stressors as reflected in our daily life. Hence, more comparable methods are required to expose test animals to mild and consecutive stress.

Social isolation stress has been shown to accelerate the development and growth of either transplanted or chemically induced tumors (52). Marked amplification of contact sensitivity responses and various modifications of the immune system were observed in the skin of isolated mice (35). We have reported that hypertension of the adrenal glands is induced and that oxidative DNA damage in mouse peripheral blood cells can be detected by single cell gel electrophoresis (36). We also

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reported that urinary excretion of biopyrrins as oxidative metabolites of bilirubin and serum levels of corticosterone increased when the mice were exposed to isolation stress for 7 days (33). These findings suggest that isolation over a long period of time, e.g., 30 days, causes a mild social stress. In the present study, we evaluated changes in the hepatic gene expression profile using a DNA microarray in mice that were exposed to isolation stress for 30 days. The primary reason for targeting our evaluation to the liver is that the liver is one of the essential organs responsible for biological functions such as energy metabolic homeostasis, metabolism and detoxification of endo- and exogenous substances. Another reason is that hepatic gene expression profiles under social stress conditions have not been well defined.

Previous investigations of gene expression for stress using DNA microarrays have been based on relatively low-resolution analyses with limited assessment for fold-change. Also, Gene Ontology (GO) or Pathway-based analyses have seldom been applied to stress research. Taking this into consideration, we employed a method for extracting the differentially expressed genes (DEGs) more effectively and used gene function enrichment analysis according to GO. In addition, we confirmed our microarray data by real-time quantitative PCR experiments. Finally, we discuss the alteration of gene expression profiles and their relationships to potential risks for several diseases associated with exposure to mild and consecutive social stress.

MATERIALS AND METHODS

Animals and isolation stress. Male BALB/c mice (4 wk old; Japan SLC, Shizuoka, Japan) were housed at five mice per cage. After acclimatization for 10 days, the mice were exposed to isolation stress (one mouse per cage). All cages were placed in a foam polystyrene box to avoid social contact. To enhance the feeling of isolation, the bed volume in each cage for the isolated mice was reduced to ~2 g per cage. Both control and stress groups were composed of five mice each. All mice were housed in an air-conditioned room (room temperature: 23 ± 1°C, humidity: 55 ± 5%) under 12 h dark/12 h light cycles (light on at 8:00), with free access to tap water and MF diet (Oriental Yeast, Tokyo, Japan). This study was conducted according to the guidelines for the care and use of laboratory animals of the University of Shizuoka and was reviewed and approved by the University of Shizuoka Animal Usage Ethics Committee.

Sample preparation. After 30 days of stress, the mice (without fasting) were killed. After anesthesia with ether, the liver was removed. Then the thymus, adrenal glands, and spleen of each mouse were collected and weighed immediately. The liver sample was cut into cubes of ~3 mm, immersed in RNALater (Ambion, Austin, TX) overnight at 4 °C, and then stored at −80°C until RNA isolation. These experiments were executed from 17:00 to 19:00. Three mice were chosen from each group according to the average weight of their adrenal glands and subjected to DNA microarray analysis.

RNA extraction. RNA was extracted from each liver by a method that combined TRIzol RNA extraction (Invitrogen Life Technologies, Carlsbad, CA) with the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the protocol included. Following isolation, RNA quantity, purity, and concentration were determined using a Gene Quant pro spectrophotometer (Amersham Biosciences, Foster City, CA) and agarose gel electrophoresis. The total RNA was used for microarray analysis and real-time quantitative PCR as described below.

Microarray analysis. DNA microarray analysis was performed as described previously (34). In brief, cDNA was synthesized from 2 μg of purified total RNA, and then biotinylated cDNA was transcribed by using T7 RNA polymerase, fragmented, and added to an Affymetrix Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA), which contains probes for >45,000 mouse genes. Following hybridization at 45°C for 16 h, the array was washed and labeled with phycoerythin. Fluorescent signals were scanned using the Affymetrix GeneChip System. All microarray data were submitted to the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo; GEO Series ID GSE12693). Affymetrix GCOS software was used to reduce the array images to the intensity of each probe (CEL files). The CEL files were quantified with the DWF algorithm (6) using the statistical language R (1) and Bioconductor (17). Hierarchical clustering was then performed by the pvclust() function (46) in R. The DEGs were identified using Rank products (5). The annotation file for the Mouse Genome 430 2.0 Array was downloaded from Affymetrix website (March 7, 2007, http://www.affymetrix.com/).

GO analysis. To detect the overrepresented GO categories in each group of DEGs, we used Cytoscape 2.5.2 molecular network visualizing software (43), with BiNGO 2.0 plug-in software (29), according to the manuals available from each on the respective websites (http://www.cytoscape.org/ and http://www.psb.ugent.be/cdb/papers/BiNGO/). We compared these DEGs with a complete annotation table of the mouse genome included within the software (August 29, 2007). Gene symbol was used for the input data format. To extract from these categories statistically overrepresented genes and to correct for multiple testing, a hypergeometric test and the Benjamini and Hochberg false discovery rate (FDR) correction (4) were respectively employed; the default setting was used in both cases.

Real-time quantitative PCR. The RNA sample (300 ng) was added to 20 μl of reaction mixture containing random hexamers, MuLV Reverse Transcriptase, RNase inhibitors, 10 mM PCR Buffer II (Applied Biosystems, Foster City, CA), and 10 mM dNTP mix (Promega, Madison, WI). Synthesis of cDNA was performed at 42°C for 60 min, and the reverse transcription reaction was stopped by heating to 95°C for 7 min followed by chilling on ice. The cDNA was stored at −20°C until further use.

A total of 2 μl of cDNA was added in the 18 μl of PCR mixture containing 10 μl Taq Man Gene Expression Master Mix (Applied Biosystems), 6 μl distilled water DNase Free (Invitrogen, Carlsbad, CA), 1 μl housekeeping gene solution (Gapdh), and 1 μl individual target gene expression reagents for mice: Cyp4a10, Assay ID, Mn01188913_g1; Ppara, Mn00440939_m1; Acox1, Mn00443579_m1; Ethad1, Mn00470099_g1; Pdk4, Mn00443325_m1; Igfbp1, Mn00834347_m1; Srebfl, Mn00550338_m1; Fasn, Mn00662391_m1; and Elovl6, Mn00851223_s1. Real-time quantitative PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems). The samples were amplified by incubation for 2 min at 50°C, then 10 min at 95°C, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. The relative expression level of the target gene product was calculated by the comparative automatic threshold cycles method, using the housekeeping gene, Gapdh, as a calibrator. The relative differences in expression between groups were expressed using cycle time values, and the relative differences between groups were expressed as relative increases, setting the control as 100%.

Statistical analysis for biochemical parameters and real-time quantitative PCR data. Statistical analysis was performed using the Student’s t-test with Pharmaco Analyst II (Hakuhousha, Tokyo, Japan). P values of <0.05 were considered to indicate statistically significant differences.

RESULTS

Effects of isolation stress for 30 days on body and organ weights. The control mice were housed at five per cage, and the five isolated mice were housed one by one. The body and organ weights of the five mice used are summarized in Table 1A. The data from the mice subjected to the DNA microarray analysis are summarized in Table 1B.
Body weight, g  24.6±1.27  24.5±0.86
Thymus, mg/g body weight  1.46±0.13  1.37±0.10
Adrenal gland, mg/g body weight  0.102±0.012  0.178±0.020*
Spleen, mg/g body weight  3.79±0.24  3.41±0.24

A. Mice exposed to isolation stress for 30 days (n = 5)

B. Mice subjected to DNA microarray analysis (n = 3)

There were no significant changes in body weights between control and isolation stress groups. However, adrenal hypertrophy was observed in mice exposed to isolation stress for 30 days (control, 0.105; isolation stress, 0.192 mg/g body wt). The thymus and spleen weights in the mice exposed to isolation stress indicated a lower, though not significant, tendency than those in the control mice.

DNA microarray analysis of gene expression. There are various preprocessing algorithms for Affymetrix type DNA microarray data, and these can result in different subsets of top-ranked genes (23). Furthermore, to detect DEGs under two different conditions, there are many methods for ranking genes. Recently, Kadota et al. (22) proposed some suitable combinations of gene selection method and preprocessing algorithm. Rank product and DFW preprocessing algorithm is one of the best combinations for accurately detecting DEGs (22), and these were applied to our microarray data.

The expression profiles of liver samples were compared between the control mice and the isolation-stressed mice. Hierarchical clustering analysis revealed that the mice in control and stress groups separately formed the apparent cluster (Supplementary Fig. S1). This means that the gene expression profiles between the two groups differ from each other. Considering the FDR, we extracted up- or downregulated genes that theoretically contain fewer than one false positive among true DEGs. The genes whose expression was altered by isolation stress were 202 upregulated (163 annotated and 39 unknown genes) and 218 downregulated (149 annotated and 69 unknown genes). The full lists of the DEGs are shown in Supplementary Tables S1 and S2.

GO analysis of top-ranked up- or downregulated genes. Using BiNAGO, the DEGs by isolation stress were classified into functional categories according to GO. The output graphs drawn by BiNAGO are also shown in Supplementary Figs. S2 and S3. The significantly enriched categories of the gene set that were up- or downregulated by the exposure to isolation stress are summarized in Figs. 1 and 2, respectively. The hierarchical structure of GO means that the more specific categories appear deeper in the hierarchy. Therefore, the most important categories appear at the lower end of the graph (e.g., represented as square nodes in Supplementary Figs. S2 and S3). Due to the hierarchical character of GO, the same gene can be associated with multiple GO terms. Therefore, the Venn and Euler diagrams in Fig. 3, A and B, show the association of genes with multiple GO terms and the resulting complex interdependencies of categories sharing DEGs that were up- or downregulated by the exposure of isolation stress for 30 days.

As shown in Fig. 1, the most specific (i.e., appearing in the deepest hierarchy) overrepresented categories in the upregulated genes were “ER to Golgi vesicle-mediated transport,” “protein transport,” “lipid biosynthetic process,” “cholesterol metabolic process,” “carboxylic acid biosynthetic process,” and “ER overload response.” Using BiNAGO, we identified 30 genes in 17 GO terms enriched in the gene set upregulated by isolation stress. These genes and GO terms are summarized by Venn and Euler diagrams with 28 genes in 8 GO terms (Fig. 3A). Figure 3A shows that these GO terms fell into three clusters of the lipid metabolic process, intracellular transport, and endoplasmic reticulum (ER) overload. The genes involved in the lipid metabolic process fell into a single cluster of interconnected categories relating to the lipid biosynthetic process, cholesterol metabolic process, and carboxylic acid metabolic process. This cluster included some key elements in the lipid metabolic process such as Fasn, Srebf1, Elovl6, Ldlr, and Ppapl. Moreover, the genes involved in intracellular transport fell into a single cluster including ER to Golgi transport as a core category.

Similarly, the most specific overrepresented categories in the downregulated genes were “glucose metabolic process,” “pyruvate metabolic process,” “fatty acid β-oxidation,” “acyl-CoA metabolic process,” “long-chain fatty acid transport,” “hemopoietic or lymphoid organ development,” “cell differentiation,” “negative regulation of protein kinase activity,” “activation of MAPKKK activity,” and “activation of MAPKK activity” (Fig. 2). We identified 50 genes in 26 GO terms enriched in the gene set downregulated by isolation stress. These genes and GO terms are also summarized by Venn and Euler diagrams with 34 genes in 9 GO terms (Fig. 3B). As shown in Fig. 3B, these GO terms fell into three partially overlapping clusters of glucose metabolic process, lipid metabolic process, and cell differentiation. Especially, Ppara is a core element that commonly appeared in the categories “glucose metabolic process,” “lipid metabolic process,” “fatty acid β-oxidation,” and “fatty acid transport.”

As mentioned above, a common category in upregulated and downregulated genes was lipid metabolism in mice exposed to isolation stress for 30 days. Isolation stress remarkably downregulated the lipid metabolism-related pathway through Ppara, while lipid biosynthesis pathway regulated by Srebf1 was upregulated. Thus, our results indicate that exposure to isolation stress for 30 days affects lipid metabolism, leading to energy accumulation.

Confirmation of the gene expression by real-time quantitative PCR analysis. Our microarray results indicated that genes involved in major energy-accumulating biochemical pathways were upregulated following exposure to isolation stress for 30 days. Therefore, to confirm the DNA microarray results, real-time quantitative PCR was applied to nine selected genes involved in the lipid metabolic process and the glucose metabolic process based on the results of microarray analysis. We
selected genes involved in the lipid biosynthetic process (Fasn, Elovl6, and Srebf1), fatty acid metabolism (Cyp4a10 and Ppara), fatty acid β-oxidation (Acox1 and Ehhadh), the pyruvate metabolic process (Pdk4), and the glucose metabolic process (Igfbp1). Among these genes, Igfbp1 indicated the most dramatic change in the microarray analysis (Supplementary Table S2).

The expression levels of Srebf1, Fasn, and Elovl6 were increased significantly (Fig. 4A). These results confirm the results from microarray experiments. Similarly, the expression of Igfbp1, Ppara, Cyp4a10, Ehhadh, Acox1, and Pdk4 significantly decreased, indicating the same changes compared with that of microarray analysis (Fig. 4B).

<table>
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<th>GO-ID</th>
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*GO term with no p-value means not significant.  †For shadowed p-value representation, see Fig. 1.
In this study, we evaluated the hepatic gene expression of mice exposed to isolation stress for 30 days using DNA microarray with probes representing >45,000 genes. After 30 days of isolation, adrenal hypertrophy, which is a characteristic change in response to physical and psychological stress loading (33), was observed (Table 1). Thus, this indicates that the mice isolated for 30 days received mild and consecutive social stress. After consideration of the FDR, we extracted 202 upregulated genes and 218 downregulated genes. The DEGs were classified according to their functional categories by GO analysis (Figs. 1; 2; and 3, A and B), and real-time quantitative PCR analysis was performed to confirm the alteration of gene expression of Srebf1, Fasn, Elovl6, Igfbp1, Ppara, Cyp4a10, Ehhadh, Acox1 and Pdk4 (Fig. 4, A and B).

Microarray analysis identified that a common category in upregulated and downregulated genes was for lipid metabolism in mice exposed to isolation stress for 30 days. The expression of Pdk4 decreased in the isolated mice. Pdk4 is a key regulatory enzyme involved in switching the energy source from glucose to fatty acids in response to physiological conditions, resulting in the inhibition of the production of acetyl-CoA from pyruvate in mitochondria (45). Therefore, the downregulation of Pdk4 contributes to the increase in lipogenesis by increasing the production of acetyl-CoA in the mitochondria. Transcription of the Pdk4 gene is activated by the administration of a Ppara ligand (54). Moreover, our data suggested that isolation stress increased expression of Srebf1 in the liver. This gene is a lipogenic transcription factor and is known to be responsible for the upregulation of genes involved in the fatty acid biosyn-
thesis pathway (19), such as Fasn and Elovl6. The upregulation of both of these genes was confirmed by the microarray and real-time quantitative PCR analyses. The Elovl6 gene encodes an elongase that catalyzes the conversion of palmitate to stearate and is involved in insulin sensitivity (31). Matsuzaka et al. (31) reported that Elovl6-deficient mice, as well as the wild-type mice, became obese and developed hepatosteatosis when fed a high-fat diet. However, they showed marked protection from hyperinsulinemia, hyperglycemia, and hyperleptinemia. Elovl6 was thus a new determinant for development of insulin resistance, diabetes mellitus, and cardiovascular illness risk, even in the presence of persistent obesity.

The expression of the group of genes encoding Ppara, Cyp4a10, Acox1, and Ehhadh, which control fatty acid degradation, decreased. Ppara is one of three structurally related transcriptional factors, which belong to the steroid hormone receptor superfamily of ligand activated intracellular receptors (14, 20), and mediates transcriptional modulation of genes encoding enzymes of peroxisomal and mitochondrial fatty acid β-oxidation as well as genes encoding fatty acid transport proteins in rodent liver (12, 26). Ppara knockout mice show low activity of fatty acid β-oxidation and low level of ketone body compared with wild-type mice in the stationary state (47). The Cyp4a10 gene is known to have a peroxisome proliferator response element in its promoter (15, 16, 51). Cyp4a10, which is an enzyme of ω-hydroxylation pathway, was reported to be upregulated and shown to be highly capable of peroxidizing lipids (27). Acox1 and Ehhadh are the key enzymes in the β-oxidation pathway of peroxisomal fatty acid (44). Consequently, it is suggested that isolation stress raises the possibility of decreased ketone production in the circulating blood. These results indicate that mild and consecutive stress changes the expression of genes related to lipid metabolism in the mouse liver and enhances energy storage by both enhancement of the fatty acid synthesis pathway and reduction of the fatty acid degradation pathway. In the present study, no significant changes were observed in body weights between control and isolation stress groups. Fat weight and food consumption were not measured in this study. Body weights or fat weight may be increased by more than 30 days. The relationship between body weight, food consumption, fat weight, and isolation stress is now under investigation.

On the other hand, Ha et al. (18) reported that chronic restraint stress elevated expression of genes involved in fatty acid/lipid metabolism, including Fasn and Cyp4a10. In contrast with our results, their results suggested that the gene expression related to lipid metabolism was stimulated by the stress and was expected to elevate the production of ketone bodies. They used mice exposed to a restrained stress, in which mice were kept in a polypropylene conical tube for 8 h per day for 14 consecutive days. Such stress is thought to be quite acute for mice compared with that used in our study. We previously reported that significant increase of serum levels of corticosterone and urinary levels of biopyrrins was observed in mice exposed to isolation stress for 7 days and that these levels decreased after 30 days (33). Therefore, isolation stress for <7 days may show similar results as Ha’s experiment (18). Stress responses probably indicate variable patterns according to the individual stress type and/or their levels.

As mentioned above, the gene expression of Srebf1 was significantly increased in the liver of mice exposed to isolation stress for 30 days. Sterol regulatory element binding factors (SREBFs) are lipogenic transcriptional factors bound to the ER membrane in addition to being related to the fatty acid synthesis pathway. This gene family is associated with increased expression of genes responsible for cholesterol or triglyceride biosynthesis and also uptake and/or intracellular accumulation of cholesterol (19). Recent studies demonstrated that SREBFs contribute to ER stress (9, 24, 37, 53). Malhotra et al. (30) reported that protein folding and generation of reactive oxygen species (ROS) as a byproduct of protein oxidation in the ER are closely linked events and suggested that persistent oxidative stress and protein mis-folding initiate apoptotic cascades and play predominant roles in the pathogenesis of multiple human diseases including diabetes, atherosclerosis, and neurodegenerative diseases. Our previous study indicated that ROS were generated in the isolated mice (33, 36). The results obtained in this study show that the function of Golgi vesicle transport and
the function of ER overload response were notably induced in the mice exposed to isolation stress for 30 days. Our results support the hypothesis that social stress may induce ER stress.

Based on the results that genes related to protein transport were notably increased by isolation stress as well as the changes of gene expression-related lipid metabolism and discussion mentioned above, it is possible that mild and consecutive social stress decreases the plasma level of free fatty acid and ketone bodies and increases triglyceride. On the other hand, the $Igfbp1$ gene was downregulated to the largest extent in the mouse liver after exposure to isolation stress for 30 days in the DNA microarray analysis. Recently, $Igfbp1$ was reported to be a primary target gene of peroxisome proliferator activated receptors (PPARs) (11). The present study found that the $Ppara$-mediated signal pathway was downregulated in the liver under the social stress (Fig. 5), consequently gene expression of $Igfbp1$ decreased. $Igfbp1$ is one of six homologous proteins that specifically bind and modulate the mitogenic and metabolic actions of insulin-like growth factor-I (21). Some studies have reported that $Igfbp1$ is secreted into blood (28, 48).

Therefore, $Igfbp1$ is also a useful candidate for a predictive biomarker for evaluation of exposure to mild and consecutive social stress. Further investigations are required to ascertain whether biochemical parameters such as free fatty acid, ketone bodies, triglyceride, and $Igfbp1$ will be predictive biomarkers for evaluation of social stress.

Recently, Kuo et al. (25) investigated the relationship between stress and obesity using stressed mice on a high-fat, high-sugar diet and reported that stress exaggerates diet-induced obesity through a peripheral mechanism in the abdominal white adipose tissue that is mediated by neuropeptide Y. $Ppara$-deficient mice showed abnormalities in triglyceride and cholesterol metabolism and became obese with age (10). This study demonstrates that isolation stress for 30 days decreased gene expressions of $Ppara$ and its target such as $Cyp4a10$, $Acox1$, and $Ehhadh$. Our results also support the hypothesis that social stress may induce and/or exacerbate obesity. However, in this study body weight did not increase after 30 days of stress. Perhaps a longer period of exposure to stress is needed for body weight gain. Therefore, we will repeat the
experiments with and without high-fat diet to evaluate whether mild and consecutive stress will induce the obesity by same model used in this study. It has been reported that isolation stress induces aggressive behavior in mice (13). Recently, Pibiri et al. (38) reported that enhanced fear responses and impaired fear extinction in mice exposed to isolation stress for 3–4 wk were involved in a decrease of 5α-reductase type I gene in the medial prefrontal cortex, hippocampus, and basolateral amygdala and that contextual freezing time was increased. These results suggest that isolation stress decreases the locomotion activity of mice. The reduction of locomotion activity may result in the alteration of lipid metabolism. However, the freezing time in their study was evaluated in the fear conditioning apparatus after isolation stress, but not in the cage where mice were housed. Therefore, behavioral analysis of mice including food intake and sleep in the cage is needed for further study.

In conclusion, we present a list of 420 liver-expressed genes whose expression was altered in response to isolation stress for 30 days; these consisted of 202 upregulated genes and 218 downregulated genes. A notable feature of the gene expression profile was the involvement of the lipid metabolism pathway including enhancement of fatty acid synthesis and decrease in fatty acid degradation and ER stress. These results indicate that mild and consecutive stress probably induces and/or exacerbates various lifestyle-related diseases such as obesity and cardiovascular disease.

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GRANTS

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