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Effects of high-fat diet and regular aerobic exercise on global gene expression in skeletal muscle of C57BL/6 mice

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ABSTRACT

Exercise training may decrease insulin resistance (IR) and increase glucose tolerance. However, the adaptive responses in skeletal muscle at the molecular and genetic level have not been clearly understood. Here we used oligonucleotide microarray analysis to dissect the effects of high-fat diet (HFD) and regular aerobic exercise on global gene expression in the skeletal muscle of C57BL/6 mice. C57BL/6 male mice (n = 40) were fed with normal chow (n = 20) and HFD (n = 20) for 8 weeks. The animals were then divided into 1 of 4 intervention groups: groups of mice fed with normal chow and HFD accompanied with 6-week treadmill running (60 min/d) at 75% maximum oxygen consumption (NE and HE) and their sedentary control groups (NC and HC). Oligonucleotide microarray was applied to analyze the effect of aerobic exercise and HFD at the transcriptional level, and selected genes were confirmed by real-time polymerase chain reaction. Our data showed that 6 weeks of aerobic exercise improved the plasma lipid profile and reversed the glucose intolerance induced by HFD. A set of 503 genes was differentially expressed in samples of HC mice as compared with those of the NC group. Forty of those genes were identified as involved in the process of aerobic exercise ameliorating IR by comparing the changes in expression profiles between the HE and HC groups. These changes include genes involved in metabolism, defense, and inflammation and genes of unknown function. Aerobic exercise training is able to ameliorate IR of mice maintained with HFD. The biochemical pathways involved in ameliorating IR identified in this study may represent potential targets for the treatment of IR.

1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance (IR), relative insulin deficiency, and hyperglycemia. Insulin resistance is a condition in which regular amounts of insulin are inadequate for normal function in the liver, adipose tissue, and skeletal muscle, resulting in T2DM [1]. Defective insulin action in glucose metabolism causes

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inability to suppress glucose production by the liver and inability to stimulate glucose uptake and metabolism in insulin-sensitive tissues such as the skeletal muscle and adipose tissue. The genetic and molecular basis for the reduction in insulin sensitivity is not fully understood. Skeletal muscle, which makes up about 40% of the body mass in humans and other mammalian species, is the predominant tissue responsible for glucose and lipid disposal under various physiological conditions [2]. In a state of IR, glucose and fatty acid uptake and utilization in skeletal muscle are dramatically decreased. Recently, defects in fatty acid oxidation have been considered the primary cause of muscle IR [3]. Incomplete fatty acid oxidation and mitochondrial overload leading to intramyocellular lipid accumulation may contribute to the development of IR in skeletal muscle [2-4]. Growing evidence also suggests an association between obesity, IR, and chronic, subacute inflammatory state in skeletal muscle [5,6]. Mediators of inflammation and acute-phase response are associated with the risk of subsequent development of IR/T2DM in humans. These mediators can also generate the disease in rodent models [7,8], suggesting that low-grade inflammation precedes and triggers the development of IR/T2DM. Insulin resistance, as a common pathophysiological feature of T2DM, is multifactorial and polygenic. Therefore, it is extremely important to identify the pathogenic and therapeutic relevant target genes.

The concept that regular exercise is essential for maintaining optimal health and preventing metabolic diseases is not new. In the early 20th century, one of the forefathers of diabetes research, Elliot P Joslin, proposed that, along with controlled diet and insulin therapy, regular exercise was a cornerstone of diabetes management [9]. Exercise training could induce a set of adaptive responses including enhanced insulin action on the skeletal muscle glucose transport system, reduced hormonal stimulation of hepatic glucose production, improved blood flow to skeletal muscle, and normalization of an abnormal blood lipid profile [2]. The identification of the responsible genes for the beneficial effects of exercise on skeletal muscle is still incomplete and is worth studying.

Recent technological advances allow the simultaneous investigation of thousands of genes, and it has been widely applied to the study of global gene expression in IR/T2DM [10] and gene expression alteration induced by physical activity [11,12]. Lifestyle measures including physical activity and caloric restriction have been recognized as the primary strategy for the management of metabolic diseases and the achievement of sustained benefits; however, these are difficult to achieve because of difficulties in maintaining long-term behavioral changes. Consumption of a high-fat diet (HFD) for extended periods of time has been proven to lead to the development of obesity and diabetes in rodents, whereas exercise can ameliorate this effect [11,13]. In the present study, HFD in mice was combined with aerobic exercise to study the health-promoting effects of regular exercise under the condition of an unaltered HFD dietary pattern. Microarray analysis was used to find genes in the biochemical pathways involved in this process and to identify the correlation between gene expression level and the skeletal muscle phenotype.

2. Materials and methods

2.1. Animals

Forty 6-week-old male wild-type C57BL/6 mice were housed in vented cages in a temperature-controlled room (20°C-23°C; 35%-55% humidity) with a 12-hour light/dark cycle and free access to food and water. This research was approved by the Tianjin Medical University Animal Care and Use Committee under the guidelines of the Chinese Academy of Sciences.

Mice were divided into control (n = 20) and HFD-fed (n = 20) groups and fed with normal chow and high-fat diet, respectively, for 8 weeks. After 8 weeks, the control group was randomly divided into normal chow diet control group (NC, n = 10) and normal chow exercise group (NE, n = 10). The HFD mice were randomly divided into HFD control group (HC, n = 10) and HFD exercise group (HE, n = 10). The NE and HE mice were exercised on a motor-driven rodent treadmill for 5 days a week for a total of 6 weeks. The mice initially ran at the intensity of 50% maximum oxygen consumption for 20 min/d during the first week; thereafter, the running intensity and time were increased to 75% maximum oxygen consumption (12 m/min) for 60 min/d [14].

2.2. Oral glucose tolerance test, fasting serum insulin, and metabolic profiles

After the desired length of exercise training (6 weeks), animals were food-restricted for at least 12 hours overnight; and blood samples were taken for fasting serum insulin and lipids assay and administration of oral glucose tolerance test (OGTT). Blood was collected from a cut at the end of the tail immediately before and at 15, 30, 60, 90, and 180 minutes after glucose administration.

2.3. Sample preparation and RNA extraction

Mice were killed 24 hours after the last exercise, and a portion of quadriceps femoris muscle was removed. Six mice of each group were selected randomly for microarray analysis. Total RNA was extracted with TRIZOL reagent (Invitrogen, Gaithersburg, MD) and purified with RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

2.4. DNA microarray

The 32K Mouse Genome Array was constructed by Capital-Bio (Beijing, China). The microarray consists of 5′-amino–modified 70-mer probes representing 32 256 well-characterized mouse genes purchased from Operon Company (http://www.Operon.com). Fluorescent dye (Cy5- and Cy3-dCTP) labeled DNA was produced through the Eberwine linear RNA amplification method and subsequent enzymatic reaction. A hybridization mixture containing 10 μg of the labeled complementary DNA (cDNA) was hybridized to 32K Mouse Genome Array chips, which were then washed, scanned, and analyzed as described elsewhere [15]. For each hybridization, the emission signal was 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.

2.5. Statistical analysis of microarray

Arrays were scanned with a LuxScan 10KA scanner, and obtained images were analyzed with LuxScan 3.0 (Capital-Bio). Genes that have Cy3 and/or Cy5 intensity value higher than 800 were considered as expressed genes. Normalization was performed with a LOWESS program [16]. The Significance Analysis of Microarrays (SAM for Excel Version 3.02, Stanford University, Stanford, CA) method was used to evaluate the significance of differences in gene expression [17]. The fold change represents the median gene expression ratio from each group of 6 independently repeated microarray experiments. A gene was considered to be significantly differentially expressed (overexpressed or underexpressed) if the ratio of normalized intensity was high than 1.5-fold (or lower than 0.67) with a false discovery rate (FDR) less than 5%. The functions of these differentially expressed genes were retrieved using Capital Bio Molecule Annotation System V4.0 (http://bioinfo.capitalbio.com/mas/login.do).

2.6. Real-time reverse transcriptase polymerase chain reaction

To confirm the results obtained by microarray, acetyl-CoA acyltransferase 2 (ACAA2), cytochrome c oxidase subunit VIIa (COX7A), fatty acid synthase (FASN), and transformation-related protein 53 (Trp53) were selected for real-time reverse transcriptase polymerase chain reaction (RT-PCR) analyses. Total RNA of quadriceps femoris muscle of each sample was subjected to reverse transcription using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) for cDNA synthesis. Synthesized cDNA was used for PCR with specific primers at optimized cycles. The following sets of primers were used: acetyl–coenzyme A acyltransferase 2 (ACAA2) forward, 5′-TCGAGGTGGAAAGTACGCAGTG-3′, reverse, 5′-TAAGGCCTCCGATGGTTG-GT- 3′; COX7A forward, 5′-GCTGAGGACGCAAAATGAGG-3′, reverse, 5′- AAGCAG-TATAA-GCAGTAGGCAGTG-3′; FASN forward, 5′-CATGCCGTGGTGCTG-GAGATT-3′, reverse, 5′-AAGGCGTTAGGGTTGACATTGAT-3′; Trp53 forward, 5′-TCAGCTCCCGGAA-CATCTCG- 3′, reverse, 5′-GCCGACCTATCCTTACCATCATCAC- 3′. Real-time PCR was performed using Bio-Rad iQ5 real-time PCR system (Bio-Rad Laboratories, Hercules, CA) and FastStart Universal SYBR Green Master (Roche Applied Science, Indianapolis, IN) according to the manufacturers' instructions. Relative expression level of target gene was determined as $2^{-\Delta\Delta C_T}$.

2.7. Statistical analysis

The data regarding body weight and plasma composition analyses were analyzed using 2-way analysis of variance with SPSS (Chicago, IL) software and expressed as mean ± SEM.

3. Results and discussion

Excess weight gain and increased accumulation of fat mass in skeletal muscle that result from overeating and a sedentary life style are considered to be the main risk factors for the development of T2DM. In our study, 2 × 2 (diet × exercise) factorial analysis of variance was used to test the effects of diet, aerobic exercise, and their interactions on body weight and plasma parameters. Final body weight and biochemical parameters in C57BL/6 mice are shown in Tables 1 and 2. Exercise training improved plasma lipid profiles and decreased body weight induced by HFD. Curve for glucose levels during OGTT showed that glucose intolerance induced by an HFD was also reversed by 6 weeks of aerobic exercise (Fig. 1).

Only the genes with messenger RNA (mRNA) level changes that were higher than 1.5-fold were considered as significantly expressed genes. By these criteria, a set of 503 genes was shown to have the differential expression profiles between the HC and the NC groups (Supplemental Data 1). To identify functional differences, we conducted a simple cluster analysis using Capital Bio Molecule Annotation System V4.0 based on Kegg Pathway to cluster genes into functional groups (Supplemental Data 2). When comparing the expression profiles of the HE and HC groups, 117 gene transcripts were found to be differentially expressed in the muscles of the HE group as opposed to the HC group (Supplemental Data 3).

Comparisons of the differentially expressed genes, induced by aerobic exercise under conditions of normal chow and HFD (NE/NC and HE/HC), showed a distinctly different program of change and produced an overlapping set of only 29 genes, which were commonly differentially expressed in both diet patterns (Fig. 2). When these differentially expressed genes were clustered into functional groups, the 88 genes observed in the HFD group were mainly related to energy metabolism, mitochondrial proteins, stress, and defense. The 278 genes regulated in the normal diet group were related to muscle structure, muscle development, energy metabolism, and mitochondrial proteins. The outcome demonstrated a marked difference in the effect of physical activity on muscle gene expression in different diet patterns (data were not shown).

To understand the underlying mechanisms by which aerobic exercise affects insulin sensitivity and plasma lipid profiles, we tried to identify the pathways that were improved by exercise training that had been previously dysregulated because of HFD. As described above, a set of 503 genes had a significantly altered gene expression levels between the HC and NC groups; and those genes were considered to be closely related to the pathogenesis of IR and obesity. Of the 503 genes

Table 1 – Univariate analyses of variance on body weight and plasma parameters

	Significance		
	Diet	Exercise	Diet + exercise
Body weight	.020	.001	<.001
Insulin	<.001	.020	.025
FFAs	.010	<.001	.015
TC	<.001	.010	.011
TG	<.001	.013	.016
HDL	.010	.005	.253

FFA indicates free fatty acid; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein.

Table 2 – Body weight and plasma analyses after 14 weeks of intervention

	NC	NE	HC	HE
Body weight (g)	28.70 ± 1.1	27.95 ± 1.24	36.42 ± 1.68†	30.02 ± 0.99§
Insulin (ng/mL)	0.22 ± 0.04	0.26 ± 0.04	0.62 ± 0.26†	0.30 ± 0.04‡
FFAs (mmol/L)	1.12 ± 0.10	0.90 ± 0.06	1.45 ± 0.12†	1.07 ± 0.13§
TC (mmol/L)	2.38 ± 0.18	2.06 ± 0.27*	3.68 ± 0.24†	2.96 ± 0.26§
TG (mmol/L)	0.66 ± 0.05	0.59 ± 0.09*	0.92 ± 0.14†	0.65 ± 0.17‡
HDL (mmol/L)	2.20 ± 0.20	3.10 ± 0.14†	2.38 ± 0.16	3.42 ± 0.18§

Values are expressed as mean ± SE; n = 10 for each of group.
* $P < .05$ vs NC.
† $P < .01$ vs NC.
‡ $P < .05$ vs HC.
§ $P < .01$ vs HC.

altered in HFD-fed mice, only 4 were found to be partially or completely normalized by 6 weeks of aerobic exercise. This determination was made by comparing the changes of expression profiles between the HE and the HC groups (HE/HC) with an FDR at 5%, whereas 40 genes were identified when FDR was adjusted to 20% (Table 3). Gene Ontology analysis was performed to identify the signaling pathways and biological processes in which these genes may have been involved (Table 3). To confirm the results obtained via microarray, ACAA2, COX7A, FASN, and Trp53 were selected to perform real-time RT-PCR analyses. The fold change of the RT-PCR results correlated to the fold change reported by the microarray (Fig. 3).

Fatty acids in animals come from 2 different sources: exogenously derived (dietary) and de novo endogenously synthesized ones. The latter biosynthesis is catalyzed by FASN [18]. In the present study, expression of FASN in skeletal muscle was decreased in the HFD group, indicating a decreased activity of FASN under the conditions of excessive fat consumption; however, this decrease was completely reversed by 6 weeks of exercise training. 5′-Adenosine monophosphate (AMP)–activated protein kinase subunit beta-2, one regulatory subunit of 5′-AMP-activated protein kinase that is another key player in regulating energy metabolism [19], was decreased

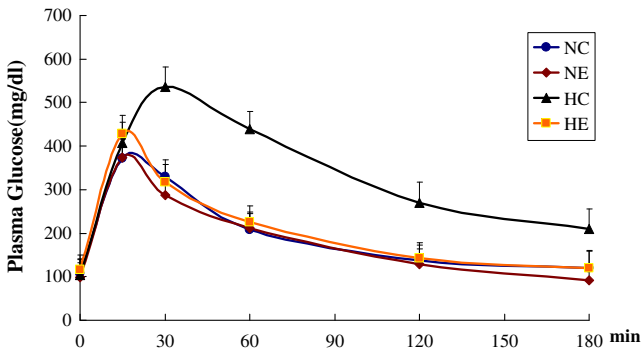

Fig. 1 – Effects of exercise (NE), high-fat diet (HC), and their combination (HE) on glucose response during an OGTT after 14 weeks intervention (n = 10). Mean values ± SEM for the concentration of blood glucose was shown.

Fig. 2 – The overlapping networks of the differently expressed genes between NE/NC and HE/HC. Comparison of the differentially expressed genes induced by aerobic exercise under conditions of normal chow and HFD showed a distinctly different program of change and produced an overlapping set of only 29 genes, which were commonly expressed differentially in both diet patterns.

by HFD but subtly increased with exercise training (Table 3), indicating the elevated expression of 5′-AMP–activated protein kinase in response to aerobic exercise.

Sixteen of the differentially expressed genes altered by HFD are highly related to oxidative phosphorylation (OXPHOS, Supplemental Data 2). Cytochrome *c* oxidase, polypeptide 1 of subunit 7A (COX7A1) is a nuclear encoded OXPHOS gene encoding polypeptide 1 of subunit 7A, a subunit of cytochrome *c* oxidase (COX), which plays an essential role in catalyzing the electron transfer from reduced cytochrome *c* to oxygen. The COX7A1 was significantly downregulated in response to HFD feeding, which was in line with the research of Mootha et al [20] that COX7A1 is downregulated in muscle of diabetics. Our results showed for the first time that decreased expression of COX7A1 induced by HFD feeding could be totally normalized by 6 weeks of aerobic exercise, indicating an improved mitochondrial OXPHOS capacity in the mice fed HFD associated with exercise training treatment (Table 3). Dehydrogenase/reductase member 7C, as a NAD+- or NADP+-dependent oxidoreductase belonging to the short-chain dehydrogenase/reductase family, was also downregulated by the HFD but remained normal with exercise training (Table 3).

The chronic inflammatory response appears to underlie obesity-induced metabolic deterioration including IR and T2DM [6,7]. In skeletal muscle, there was a change in expression of mRNAs coding for molecules associated with immune response and inflammation in HFD-fed animals, including genes related to cytokines-cytokine receptor interactions and leukocyte transendothelial migration (Kegg pathways analyses, Supplemental Data 2). Furthermore, a set of genes encoding immunoglobulin light- or heavy-chain repertoire proteins, including IGKV6-32, IGKV6-17, IGKV6-14, IGKV8-27, IGKV8-28, and IGHG2B, displayed the concordantly increased expression profiles in HC, indicating an increased immune response in HFD-fed mice. However, the increased expression of these genes was moderated by exercise training (Table 3).

Our data also showed that the expression of Trp53, also termed *tumor suppressor protein p53*, was increased in the HFD-

Table 3 – Genes that were up- or downregulated in HC were partially or completely normalized by 6 weeks of aerobic exercise by comparing the changes of expression profiles between HE and HC with an FDR at 20%

Gene ID	Gene symbol	Gene ontology	Description	Fold change			
				HC/NC fold change	HC/NC *q* value (%)	HE/HC fold change	HE/HC *q* value (%)
M300010457	H2-Ea	1. Antigen processing and presentation 2. Type 1 diabetes mellitus	Histocompatibility 2, class II antigen E alpha	8.24	0	0.58	4. 79
M300013417	Tmem58	1. Integral to membrane	Transmembrane protein 58	3.71	0.79	0.41	2. 41
M400005854	IGKV6-32	None	Immunoglobulin Kappa light chain V gene segment	3.66	2.79	0.23	0.12
M300010445	Fxyd1	1. Ion transport	FXYD domain-containing ion transport regulator 1	3.10	1.32	0.47	0
M400000025	Ccl3	1. ATPase activity 2. Chemokine activity 3. Toll-like receptor signaling	Chemokine (C-C motif) ligand 3	2.77	0	0.57	3.11
M200007299	Slc41a3	1. Cation transport	Solute carrier family 41, member 3	2.58	0.29	0.63	0.59
M200009507	0610006I08Rik	None	None	2.54	2.30	0.43	1.62
M300020782	Bex1	1. Nervous system development	Brain expressed gene 1	2.54	1.32	0.32	4.90
M400009428	IGKV8-27	None	Immunoglobulin Kappa light chain V gene segment	2.43	0	0.67	3.62
M400010640	IGKV6-17	None	Immunoglobulin Kappa light chain V gene segment	2.43	0	0.60	1.17
M400012178	Igj	None	Immunoglobulin joining chain	2.34	0	0.62	2.02
M200009516	Hist1h4h	1. Nucleosome assembly	Histone cluster 1, H4h	2.27	1.32	0.62	0.72
M300010133	Cerk	1. Transferase activity 2. Ceramide kinase activity 3. Protein kinase activity	Ceramide kinase	2.26	4.60	0.38	0.12
M400002441	IGKV6-14	None	Immunoglobulin Kappa light chain V gene segment	2.18	0.44	0.66	0
M400010435	IGKV8-28	None	Immunoglobulin Kappa light chain V gene segment	2.15	0.53	0.50	3.91
M200003310	IGHG2B	None	Immunoglobulin heavy chain C gene segment	2.13	0	0.54	4.32
M200002313	Fosl1	1. Transcriptional factor activity 2 Wnt signaling	Fos-like antigen 1	2.12	0.75	0.50	1.73
M300003876	Epb4.1l3	1. Actin binding	Erythrocyte protein band 4.1-like 3	2.10	0.79	0.52	0.77
M400011714	Krt8	1. Development 2. TNF-mediated signaling	Keratin 8	2.02	0.44	0.64	2.31
M200005435	Racgap1	1. GTPase activator activity 2. Cell cycle	Rac GTPase-activating protein 1	1.89	1.49	0.66	4.17
M300011445	Ncapd2	1. Binding	Non-SMC condensin I complex, subunit D2	1.69	0.53	0.66	0.42
M200000084	Trp53	1. MAPK signaling 2. Wnt signaling 3. Apoptosis	Transformation related protein 53	1.56	1.49	0.62	0.12
M400007082	Hist4h4	1. Nucleosome assembly	Histone cluster 4, H4	1.53	1.49	0.50	3.56
M400011504	Cenpm	None	Centromere protein M	1.51	1.64	0.66	0
M400004085	Immt	None	Inner membrane protein, mitochondrial	0.68	1.15	1.52	3.50
M400010990	Cox7a1	1. Cytochrome c oxidase activity	Cytochrome c oxidase, subunit VIIa 1	0.66	4.02	1.84	3.98
M300003128	Ghr	1. Growth hormone receptor activity 2. Jak-STAT signaling	Growth hormone receptor	0.66	3.57	1.54	4.5
M200005595	Rnmt	1. mRNA (guanine-N7-)-methyltransferase activity	RNA (guanine-7-) methyltransferase	0.65	0.75	1.60	0.59
M400001234	Zfp97	1. Zinc ion binding	Zinc finger protein 97	0.65	0.59	1.51	3.50
M400012530	Prkab2	1. AMP-activated protein kinase activity	5′-AMP-activated protein kinase subunit beta-2	0.62	4.02	2.69	0.79
M400001953	EG210853	1. Zinc ion binding	Predicted gene, EG210853	0.61	0.69	1.54	0
M400003291	Q32P07_MOUSE	None	None	0.59	0.55	1.57	0.49
M400011399	Myoz1	1. Protein binding	Myozenin 1	0.53	0.56	3.12	4.71

Table 3 (*continued*)

Gene ID	Gene symbol	Gene ontology	Description	Fold change			
				HC/NC fold change	HC/NC *q* value (%)	HE/HC fold change	HE/HC *q* value (%)
M400007615	NP_001034298.1	None	None	0.53	0.55	1.55	3.52
M200001441	Fasn	1. Fatty acid biosynthesis 2. Insulin signaling pathway	Fatty acid synthase	0.44	4.02	2.03	3.02
M200007639	Galns	1. Hydrolase activity	Galactosamine (N-acetyl)-6-sulfate sulfatase	0.41	1.01	3.12	4.06
M200002893	Myom2	1. Structural constituent of cytoskeleton	Myomesin 2	0.40	3.16	1.73	4.13
M200000728	Ttk	1. Protein serine/threonine kinase activity	Ttk protein kinase	0.39	1.15	2.90	1.19
M400006572	NP_001030031.1	None	None	0.33	2.52	1.84	3.47
M400001526	Dhrs7c	1. Oxidoreductase activity	Dehydrogenase/reductase (SDR family) member 7C	0.16	1.64	2.36	0.68

fed mice (Table 3). Minamino et al [21] reported that deficiency of p53 decreases inflammation and improves insulin sensitivity in obese agouti mice on regular diet or obese wide-type mice on HFD compared with wide-type lean mice. These results were consistent with our gene array data. Conversely, in the research of Minamino et al, transgenic overexpression of p53 in adipose tissue caused an inflammatory response that led to IR [21], making it likely to be an early event and therefore a potential primary cause of T2DM, whereas the increased expression of p53 in mice maintained with HFD was normalized by 6 weeks of aerobic exercise (Table 3) in our experiment, suggesting a potential mechanism underlying physical activity ameliorating IR and T2DM.

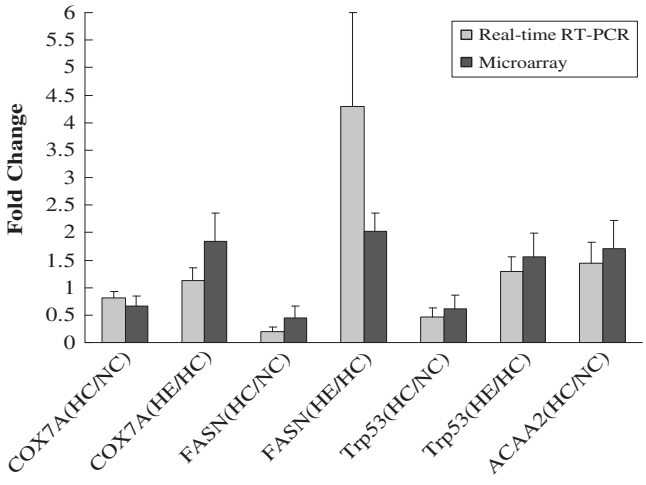

Fig. 3 – Comparison of microarray data to real-time PCR (n = 6 each). ACAA2 had a higher level of expression in HC group (HC/NC). COX7A, FASN, and Trp53 were found to be down-regulated by HFD feeding (HC/NC), whereas an upward tendency was induced by exercise training (HE/HC). The results showed that the fold change of real-time PCR correlated to the fold change reported by the microarray.

In summary, the major finding of the present study is that aerobic exercise ameliorating HFD-induced IR is accompanied with changes in skeletal muscle gene expressions of specific pathways related to metabolism, defense, inflammation, etc. In addition, we recognize that not all changes in mRNA expression are directly connected to the experiment conducted. Conversely, because of the severe criteria chosen for defining the differences in gene expression, the differences we detected in this experiment are merely part of the overall program of change. Special attention should be devoted to the genes we detected with no description or unknown Gene Ontology functions (Table 3), which may also highly related to the physiology and pathology of IR/T2DM and the process of exercise combating T2DM.

Supplementary materials related to this article can be found online at doi:10.1016/j.metabol.2011.06.017.

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Conflict of Interest

The authors declare that there is no duality of interest associated with this manuscript. The authors also declare that they have no competing interests as defined by *Metabolism* or other interests that might be perceived to influence the results and discussion reported in this paper.

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