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Endurance training-induced changes in insulin sensitivity and gene expression

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Teran-Garcia, Margarita, Tuomo Rankinen, Robert A. Koza, D. C. Rao, and Claude Bouchard. Endurance training-induced changes in insulin sensitivity and gene expression. *Am J Physiol Endocrinol Metab* 288: E1168–E1178, 2005. First published February 1, 2005; doi:10.1152/ajpendo.00467.2004.—The beneficial effects of regular physical activity on insulin sensitivity (S_I) and glucose tolerance are well documented, with considerable heterogeneity in responsiveness to exercise training (ET). To find novel candidate genes for ET-induced improvement in S_I, we used microarray technology. Total RNA was isolated from vastus lateralis muscle before and after 20 wk of exercise from individuals participating in the HERITAGE Family Study. S_I index was derived from a frequently sampled intravenous glucose tolerance test using MINMOD Millennium software. Sixteen subjects were selected: eight showing no changes in S_I (low responders, LS_IR) and eight displaying marked improvement in S_I (high responders, HS_IR) with ET. The S_I increase was about four times greater in HS_IR compared with LS_IR ($+3.6 \pm 0.5$ vs. -1.2 ± 0.5 $\mu U \cdot ml^{-1} \cdot min^{-1}$, mean ± SE), whereas age, body mass index, percent body fat, and baseline S_I were similar between the groups. Triplicate microarrays were performed, comparing pooled RNA with HS_IR and LS_IR individuals for differences in gene expression before and after ET using in situ-generated microarrays (18, 861 genes). Array data were validated by quantitative RT-PCR. Almost twice as many genes showed at least twofold differences between HS_IR and LS_IR after training compared with pretraining. We identified differentially expressed genes involved in energy metabolism and signaling, novel structural genes, and transcripts of unknown function. Genes of interest upregulated in HS_IR include V-Ski oncogene, four-and-a-half LIM domain 1, and titin. Further study of these novel candidate genes should provide a better understanding of molecular mechanisms involved in the improvement in insulin sensitivity in response to regular exercise.

microarray; MINMOD Millennium; exercise training

THE BENEFICIAL EFFECTS of regular physical activity on insulin sensitivity (S_I) and glucose metabolism are well documented (26, 54). Regular physical activity has been shown to increase insulin mediated glucose transport in peripheral tissues, and this effect is mediated through multiple adaptations in glucose and lipid metabolism (3, 19). Thirty to fifty percent of the in vivo insulin-mediated glucose disposal occurs in skeletal muscle, and decreased insulin-stimulated muscle glucose uptake is one of the mechanisms responsible for the development of insulin resistance (3, 61). In fact, impaired capability of muscle for glucose disposal in response to normal levels of circulating insulin is the earliest detectable abnormality in patients with type 2 diabetes (T2DM) (12, 29, 47).

Exercise improves insulin action (14, 18, 19), and weight loss associated with regular exercise prevents the onset of diabetes (26, 54). Increased expression of the insulin-responsive glucose transport proteins has been observed in response to exercise training and correlated with improved insulin action in skeletal muscle (20, 23, 44). Seven days of exercise have been shown to increase S_I (9, 22). Exercise training has also been associated with increased oxidative capacity of skeletal muscle (7, 39, 48, 59). Even though skeletal muscle in older individuals seems to retain the ability to increase glucose transport into muscle with endurance training (9), recent data indicate that an age-associated decline in mitochondrial function contributes to the insulin resistance observed in the elderly (38, 50). It is not known whether exercise training-associated improvements in glucose uptake are limited primarily by increased expression of glucose transporters or by the improved muscle oxidative potential. Improvements in S_I with exercise training may be related to coordinated changes in activity of proteins involved in insulin signal transduction, fuel partitioning and metabolism, and cytoarchitecture of skeletal muscle.

How gene expression is modulated by acute and chronic exercise is important for the understanding of the mechanisms by which exercise training improves insulin action in skeletal muscle. The effects of exercise training on the expression of several genes involved in insulin signaling [insulin receptor substrate-1 (IRS-1, IRS-2), phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), AMP-activated protein kinase (AMPK)], glucose transport (GLUT1, GLUT4), glycogen metabolism [glycogen synthase (GS), glycogen synthase kinase-3 (GSK-3)], glycolysis [hexokinase II (HKII), phosphofructokinase (PFK)], mitochondrial genes (COX4, ND4), mitochondrial biogenesis [peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1), nuclear respiratory factor-1 (NRF-1), NRF-2, transcription factor A, mitochondrial (TFAM)], and fatty acid oxidation [carnitine palmitoyltransferase I (CPT I), uncoupling protein 3 (UCP3), NADH6] (13, 19, 50, 57, 60) have been investigated. These studies suggest that exercise training entrains a complex program of transcriptional changes in target tissues that has been associated with improved insulin sensitivity and glucose metabolism.

Microarray technology offers a powerful tool to characterize changes in transcript levels on a large scale. The array technology has been helpful in defining a set of insulin-regulated genes in human skeletal muscle of healthy individuals during a

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3-h hyperinsulinemic euglycemic clamp (45). Several studies have provided a catalog of genes differentially expressed in skeletal muscle under diverse clinical conditions, such as in the comparison of healthy nondiabetic subjects, individuals with T2DM (36), obese insulin-sensitive and obese insulin-resistant subjects (58), and old individuals with T2DM having good or poor glycemic control (52). However, none of these studies has focused on gene expression in skeletal muscle of healthy, previously sedentary individuals in response to an endurance exercise training program, taking into account their changes in S_I. There is considerable variability for the changes in S_I in response to exercise training, and several factors may account for this phenomenon. We (6) recently showed that, among 596 participants from the HERITAGE Family Study, only 58% increased their S_I by 10% or more with a standardized 20-wk training program. These changes in S_I were not associated with changes in body weight, waist circumference, or physical fitness. In the current study, we sought to determine what differences in skeletal muscle gene expression patterns were present before and after 20 wk of endurance exercise training in high and low S_I responders by using a subsample from the HERITAGE Family Study subjects.

RESEARCH DESIGN AND METHODS

Subjects. The HERITAGE Family Study was designed to investigate the role of genetic factors in the cardiovascular and metabolic adaptations to 20 wk of endurance training in white and black subjects (5). All subjects were required to be sedentary and in good health to participate in the HERITAGE Family Study and meet the inclusion criteria (5). None of the HERITAGE subjects was diabetic or taking hypoglycemic or hypertensive medication. Participants from the Quebec Clinical Center were asked to volunteer for a muscle biopsy before and after the training program. Biopsies were obtained from 76 subjects, and 16 volunteers with a mean (±SE) age of 30 ± 5 yr and a BMI of 23.7 ± 1.2 kg/m^2 were selected for the present experiment. The characteristics of these subjects are depicted in Table 1. The selection of these 16 subjects was based on their S_I responses to exercise training: eight subjects (4 males and 4 females) showed no changes in S_I (low responders, LS_IR), and eight subjects (3 males and 5 females) displayed marked improvements in S_I (high responders, HS_IR) with the exercise program. These subjects were matched for sex and baseline S_I. There were no statistical differences in baseline S_I among the whole sample of white individuals from the HERITAGE Family study (4.3 ± 2.9 $\mu U \cdot ml^{-1} \cdot min^{-1}$, mean ± SD, $n = 429$), the subset of those who volunteered for a muscle biopsy (5.1 ± 3.7 $\mu U \cdot ml^{-1} \cdot min^{-1}$, $n = 76$), and the subsample selected for the array studies (4.1 ± 1.3 $\mu U \cdot ml^{-1} \cdot min^{-1}$, $n = 16$). Each institutional review board of the HERITAGE Family Study research consortium approved the study protocol. Written informed consent was obtained from each participant.

Table 1. *Subject characteristics*

	LS_IR ($n = 8$)	HS_IR ($n = 8$)
Age, yr	30.3±5.4	31.2±4.9
BMI, basal, kg/m^2	25.0±1.9	23.4±1.0
%Body fat	24.4±3.6	22.9±4.5
Glucose (baseline), mmol/l	5.0±0.2	5.1±0.2
Insulin (baseline), pmol/l	72.6±12.3	60.1±6.0
S_I, $\mu U \cdot ml^{-1} \cdot min^{-1}$, at:		
Baseline	4.1±0.6	4.0±0.4
After training	3.1±0.4	8.6±1.1*
Response	−1.2±0.5	3.6±0.5†

Values are means ± SE. LS_IR and HS_IR, low and high insulin sensitivity (S_I) responders, respectively; BMI, body mass index. *$P < 0.05$ for difference between after versus baseline. †$P < 0.05$ for differences in response between LS_IR and HS_IR.

Exercise training program. The exercise training program has been described in detail previously (5). Briefly, the exercise intensity of the 20-wk program was customized for each participant on the basis of the heart rate-oxygen uptake relationship measured at baseline. During the first 2 wk, the subjects trained at a heart rate corresponding to 55% of the baseline $\dot{V}O_{2\,max}$ for 30 min per session. Exercise duration was gradually increased to 50 min per session and intensity to the heart rate associated with 75% of the baseline $\dot{V}O_{2\,max}$, and these conditions were then sustained for the last 6 wk of the training program. Training frequency was three times per week, and all training sessions were performed on cycle ergometers under supervision in the laboratory.

Measurement of glucose, insulin, and S_I. Fasting plasma glucose and insulin levels were determined at baseline and after the 20-wk exercise training program. The frequently sampled intravenous glucose tolerance test (FSIVGTT) as described by Walton et al. (55) was administered in the morning after an overnight fast of 12 h. Data were collected at baseline and posttraining 24–36 h after an exercise session as described elsewhere (1). From the FSIVGTT data, S_I was derived using the recently released MINMOD Millennium software (4). S_I is a measure of insulin action and corresponds to the ability of a given increment in plasma insulin to accelerate glucose uptake and suppress glucose production (11).

Experimental study design. Muscle biopsies were taken from the middle of the vastus lateralis muscle by the percutaneous needle biopsy technique (51). Each biopsy was partitioned into two pieces: one was immediately frozen in liquid nitrogen and stored at −80°C until total RNA preparation, and the other was frozen in isopentane cooled by liquid nitrogen and used for histochemistry and enzyme activity assays.

Total RNA preparation. Frozen tissue samples were crushed in liquid nitrogen, and total RNA was extracted using the Tri Reagent (Molecular Research Center, Cincinnati, OH), followed by purification with Qiagen columns from the RNeasy kit (Qiagen, Valencia, CA). The concentration and quality of the RNA were determined spectrophotometrically. Once the integrity of the RNA was confirmed in a nondenaturing agarose gel, RNA pools were prepared. Given the limited amount of material available, it was necessary to use RNA pools for the present study. In addition, using RNA pools provided an advantage by decreasing the variability that tissue heterogeneity might introduce in the gene expression profiles, reducing the "polymorphic noise" present in humans and minimizing the effect of sex differences. Furthermore, we were able to accumulate enough RNA in the pools while still maintaining individual samples for validation of differentially expressed genes by use of quantitative (Q)RT-PCR. Therefore, equal amounts of the individual samples were used to generate four RNA pools: baseline HS_IR, baseline LS_IR, posttraining HS_IR, and posttraining LS_IR. The samples from HS_IR and LS_IR were analyzed, comparing pretraining and posttraining RNA pools.

Array generation. High-density oligonucleotide microarrays were generated by the Pennington Biomedical Research Center Genomics Core Microarray Facility. Briefly, the array was printed using a library of 18,861 human oligonucleotides (Sigma-Genosys, The Woodlands, TX) representing 17,260 unique genes (or transcripts) based on LEADS cluster analyses (Compugen, San Jose, CA). The 60–70mer oligonucleotides were diluted in 50% DMSO and spotted onto poly-L-lysine-coated slides using an OmniGrid Microarrayer equipped with a Stealth SPH32 Micro Spotting Pin Matrix and SMP4 Micro Spotting Pins (Telechem International, Sunnyvale, CA).

RNA amplification and array detection. Microarray analysis was performed in triplicate, comparing pooled RNA from the HS_IR and LS_IR groups to investigate the differences in gene transcript profiles prior to exercise training (pretraining arrays) deposited in the GEO

database as Series GSE1718, with sample ID nos. GSM29507, GSM29523 and GSM29529. We also compared the HS_IR and LS_IR groups after exercise training (posttraining arrays) in a second set of triplicate arrays deposited in the GEO database as Series GSE1718 with sample ID nos. GSM29533, GSM29535, and GSM29536. Dye switching was prepared in one slide of each set to account for incorporation differences between Cy3 and Cy5. RNA was labeled and detected using a tyramide signal amplification (TSA) labeling and detection kit (Perkin-Elmer, Boston, MA). The TSA method (TSA™, NEN Life Science Products, Boston, MA) is highly sensitive and allows for the use of small amounts (2 μg) of total RNA. The TSA method has been shown to provide consistent and reproducible signal amplification across arrays (25). Probe labeling and array hybridization were performed as described in the instruction manual (MICROMAX TSA Labeling and Detection Kit). After hybridization to probes, slides were scanned using a GSI Lumonics ScanArray 5000 (Packard Biochip Technologies, Meriden, CT) and expression data were analyzed with QuantArray V3.0 software (Packard Bioscience, Meriden, CT).

Array normalization. A uniform scale factor was applied to normalize signal intensities between Cy5 and Cy3. Locally weighted regression and scatter plot smoothing (LOWESS) was applied to account for dye variation between slides and for signal intensity effects. For a given experiment, we assumed that most genes on our slides were present in equal amounts, such that their intensity ratios were equal to 1 (or 0 if data were in log form). However, because there are dye variations across a single slide, as well as between slides, a correction must be applied so that we get an average ratio of 1. LOWESS is one method to do this. LOWESS normalization is based on the concept that any function can be well approximated in a small neighborhood by a low-order polynomial (in our case, we use a linear fit). The LOWESS method fits a curve to a log plot of intensity values and subtracts the fit values from the actual values to get the normalized values. An in-house software program was used to perform the analysis (details of the algorithm can be found at http://bioinfo.pbrc.edu). By use of the LOWESS method, several genes with significant differences in expression across the microarray experiments were identified. The gene expression data were analyzed comprehensively using the software applications of Spotfire (DecisionSite, Somerville, MA) and GeneSpring (Silicon Genetics, Redwood City, CA). The nomenclature adopted for referencing gene names, symbols, and other descriptions is that of the Nomenclature Working Group (http://archive.uwcm.ac.uk/uwcm/mg/docs//mut_nom.html).

QRT-PCR. We selected five genes from the microarray experiments to be validated by QRT-PCR. The genes were selected because they exhibited a minimum of 50% difference ($P < 0.05$) between HS_IR and LS_IR groups either at baseline or in the posttraining experiments. These genes were TTN, pyruvate dehydrogenase kinase-4 (PDK4), V-SKI avian sarcoma viral oncogen homolog (SKI), C-terminal binding protein-1 (CTBP1), and four-and-a-half LIM domains 1 (FHL1). The QRT-PCR studies were performed using TaqMan probe and primer sets to measure the levels of mRNA (Applied Biosystems, Foster City, CA). The four RNA pools from the microarray experiments were also used for the QRT-PCR validation. Total RNA was reverse transcribed using the Reverse Transcription Reagents Kit (Applied Biosystems), according to the manufacturer's instructions, for the two-step RT-PCR methodology. Briefly, cDNA was generated from 150 ng of total RNA of each pool as the template, and a reference sample cDNA was obtained from a commercially available skeletal muscle total RNA source to generate the standard curves (Ambion, Austin, TX). PCR was performed using an ABI PRISM 7900 SDS instrument (Applied Biosystems). The primer-probe sets were either selected from the gene expression system database (Assays-on Demand, Applied Biosystems) or designed using the Primer Express software (Applied Biosystems) and are available upon request. Reactions were carried out in a 384-well plate format with 12 μl of reaction volume. Two concentrations of each cDNA pool were used in triplicate reactions. We also used a 1:50 dilution of the cDNA pools for normalization of the gene signals with 18S rRNA by using the TaqMan PreDeveloped Assay Ribosomal RNA Control Reagents Kit (Applied Biosystems). A standard curve was generated with serial dilutions of the cDNA reference sample and applied to each primer and probe set. The cycle threshold value for each sample was then used to calculate relative expression. The expression level of each gene was normalized to 18S rRNA. Data are reported as arbitrary units. Differential gene expression between the LS_IR and the HS_IR groups as measured by QRT-PCR was analyzed using a one-tailed Student's *t*-test, since we hypothesized that the differences between the groups would follow the same pattern as that measured by the oligonucleotide array analysis.

RESULTS

We report here only the results obtained after normalization by LOWESS and only the expression ratios between HS_IR and LS_IR replicated among the three array experiments ($P < 0.05$). Before exercise training, there were 703 transcripts that met these criteria and 1,166 after exercise training. In addition, we used a cutoff of ±0.5 value from a $\log_2$ scale to describe changes in expression. This corresponds to an increase of 1.4-fold or a decrease of 0.7-fold in the HS_IR-to-LS_IR expression ratio.

Pretraining arrays. At baseline, relatively few transcripts were >1.4-fold more abundant in the HS_IR compared with the LS_IR ($n = 42$). Table 2 lists the HS_IR overexpressed transcripts encoding proteins involved in glycolysis, nitrogen metabolism, signal transduction, transcription regulation, and muscle contraction and development and several other transcripts with unknown or unclassified function. In addition, five transcripts that were less abundant in the HS_IR compared with the LS_IR group (ratio <0.7) are involved in cell growth/maintenance (1) and tRNA processing (1) or have an unknown function (3) (Table 3).

Posttraining arrays. We identified 240 transcripts in the posttraining samples that were at least 1.4-fold more abundant in the HS_IR compared with the LS_IR group. Among these transcripts, 21 were upregulated 2.0-fold or more in the HS_IR group. Upregulated genes are involved in energy metabolism, muscle development and contraction, vesicle transport, signal transduction, cell growth regulation and differentiation, transcription regulation and protein synthesis, extracellular matrix structure and function, and cellular defense response or have unknown functions (Table 4). We also found 121 downregulated transcripts (ratio <0.7; Table 5). The downregulated genes participate in lipid metabolism, oxidative phosphorylation, ribosome biogenesis and transcription regulation, proteolysis and peptidolysis, protein synthesis, and GTPase-mediated signal transduction or have unknown functions.

Integration of pre- and posttraining arrays. A total of 38 genes were differentially expressed at both baseline and posttraining experiments in the two sets of array experiments (Table 6). We developed a list of those genes on the basis of ratios of post/pretraining experiments. Among them, 22 had a known function. Upregulated genes ($n = 8$), with ratios of >1.4 in post/pretraining experiments, are involved in cell adhesion/growth (4), signal transduction (2), and electron (1) and adenine transport (1). Some of the down-regulated genes ($n = 5$) with ratios of <0.7 participate in oxidative phosphor-

Table 2. *Genes upregulated pretraining*

Gene	Unigene	GenBank Identifier	Gene Ontology	Fold Difference	*P* Value
H. sapiens fc fragment of iga, receptor for (FCAR)	Hs.193122	NM_002000	Immune response	1.8	0.050
H. sapiens kelch-like 1 protein (KLHL1)	Hs.106808	AF252283	Developmental processes	1.7	0.042
H. sapiens cDNA: FLJ21940 FIS, clone hep04512	Hs.104916	AK025593	Developmental processes	1.6	0.025
H. sapiens sh3-domain grb2-like 3 (SH3GL3)	Hs.80315	NM_003027	Central nervous system development	1.6	0.044
H. sapiens clone pp591 unknown	Hs.118666	AF218022	Amino acid & derivative metabolism	1.5	0.034
H. sapiens kraken-like (BK126B4.1)	Hs.301947	NM_014509	Nitrogen metabolism	1.5	0.036
H. sapiens unnamed herv-h protein (LOC51581)	Hs.274490	NM_015875	Cell motility	1.5	0.052
H. sapiens aquaporin 2 (collecting duct) (AQP2)	Hs.37025	NM_000486	Water transport	1.5	0.047
H. sapiens aldo-keto reductase family 7, member a2 (aflatoxin aldehyde reductase) (AKR7A2)	Hs.6980	NM_003689	Aldehyde metabolism	1.4	0.048
H. sapiens inositol(myo)-1(or-4)-monophosphatase 2 (IMPA2),	Hs.5753	NM_014214	Signal transduction	1.4	0.048
H. sapiens sorting nexin 10 (SNX10)	Hs.106260	NM_013322	Endocytosis	1.4	0.006
H. sapiens lengsin (LGS)	Hs.149585	NM_016571	Cell adhesion	1.4	0.018
H. sapiens cDNA: FLJ22662 fis, clone hsi08080	Hs.178470	AK026315	Retrograde (Golgi to ER) transport	1.4	0.020
H. sapiens complexin 2 (CPLX2)	Hs.321567	NM_006650	Neurotransmitter release	1.4	0.037
H. sapiens cDNA FLJ13448 fis, clone place1002993	Hs.288945	AK023510	Nitrogen metabolism	1.4	0.050
H. sapiens cDNA: FLJ23360 fis, clone hep15172	Hs.161279	AK027013	Cell death	1.4	0.053
H. sapiens ris (LOC51285)	Hs.27018	NM_016563	Synaptic transmission	1.4	0.006
H. sapiens HSPC265, partial cds	Hs.284207	AF161383	Muscle contraction	1.4	0.009
H. sapiens tumor necrosis factor receptor superfamily, member 9 (TNFRSF9)	Hs.73895	NM_001561	Signal transduction	1.4	0.001
Human clone h3		U03672	Regulation of transcription	1.4	0.047
H. sapiens diacylglycerol kinase, delta (130kd) (DGKD)	Hs.115907	NM_003648	Signal transduction	1.4	0.023
H. sapiens cDNA FLJ14356 fis, clone y79aa1002103, similar to zinc finger protein zfp-36	Hs.253193	AK024418	Transcription regulation	1.4	0.008
H. sapiens cgi-127 protein (LOC51646)	Hs.184542	NM_016061	Leading strand elongation	1.4	0.050
H. sapiens cDNA FLJ12576 fis, clone nt2rm4001032	Hs.193261	AK022638	Muscle development	1.4	0.007

This is a partial list of genes with known function; the complete list of genes can be accessed in the GEO database series GSE1718. Fold difference represents the average ratio of HS_IR to LS_IR in 3 microarray experiments.

ylation [2], transcription regulation [1], and protein biosynthesis [1].

QRT-PCR validation. The genes selected for validation exhibited a minimum of 50% difference ($P < 0.05$) in expression between HS_IR and LS_IR groups at either baseline or posttraining. SKI and FHL1 are genes involved in cell growth and/or maintenance, TTN is a structural constituent of muscle and plays a role in sarcomere elasticity and the regulation of muscle contraction, PDK4 has protein kinase activity and participates in glucose metabolism, and CTBP1 is a functional dehydrogenase. The differences in expression levels among the four groups are depicted in Fig. 1 for PDK4, FHL1, SKI, and TTN.

PDK4 levels were 1.8-fold greater in the HS_IR group, compared with the LS_IR group before exercise training by QRT-PCR; this is concordant with the difference observed between the two groups in the pretraining arrays (Fig. 1). There were no differences in PDK4 levels between the HS_IR and LS_IR groups with either the array data or QRT-PCR in the posttraining arrays.

The differences between the HS_IR and LS_IR groups in the posttraining arrays for the TTN gene were also validated by QRT-PCR. We found a 2.6-fold difference in TTN transcript levels between the HS_IR and LS_IR groups after exercise training (Fig. 1). This difference is smaller than the average 4.7-fold difference observed in the posttraining array experiments but within the 2.6 to 6.9-fold range observed across the triplicate experiments.

The levels of FHL1, SKI, and CTBP1 in the sedentary state were not different between the HS_IR and LS_IR groups by QRT-PCR, as we had found in the pretraining arrays (Fig. 1). However, after exercise training, the QRT-PCR results revealed significantly higher FHL1 and SKI levels in the HS_IR compared with the LS_IR group, as observed in the posttraining arrays. The 1.7-fold difference in CTBP1 observed in the posttraining arrays in the HS_IR vs. LS_IR group could not been replicated by the QRT-PCR. Instead, we observed only a 20% difference in CTBP1 levels favoring the HS_IR group after exercise training.

Table 3. *Genes downregulated pretraining*

Gene	Unigene	GenBank Identifier	Gene Ontology	Fold Difference	*P* Value
H. sapiens fbj murine osteosarcoma viral oncogene homolog b (FOSB), mRNA	Hs.75678	NM_006732	Cell cycle control	0.7	0.04
H. sapiens pre-b lymphocyte gene 3 (VPREB3), mRNA	Hs.136713	NM_013378	Humoral defense mechanism	0.7	0.01
H. sapiens retinoic acid- and interferon-inducible protein (58kd) (RI58), mRNA	Hs.27610	NM_012420	Immune response	0.6	0.04
H. sapiens creatine kinase, brain (CKB) mRNA	Hs.173724	NM_001823	Transferase activity, phosphorus-containing groups	0.6	0.03

This is a partial list of genes with known function; the complete list of genes can be accessed in the GEO database series GSE1718.

Table 4. *Genes upregulated posttraining*

Gene	Unigene	GenBank Identifier	Gene Ontology	Fold Difference	*P* Value
H. sapiens for cardiac titin, clone ziss (TTN)		X98114	Structural constituent of muscle	4.7	0.04
H. sapiens; cDNA dkfzp761m222 (from clone dkfzp761m222)	Hs.273186	AL137511	Electron transport	3.9	0.03
H. sapiens corin (PRSC)	Hs.62794	NM_006587	Proteolysis and peptidolysis	2.3	0.01
H. sapiens ras-gtpase activating protein sh3 domain-binding protein 2 (KIAA0660)	Hs.6727	NM_012297	RAS protein signal transduction	2.2	0.02
H. sapiens for KIAA1157 protein, partial cds	Hs.21894	AB032983	Protein dephosphorylation	2.2	0.01
H. sapiens cyclin-dependent kinase inhibitor lb (p27, KIP1) (CDKN1B)	Hs.238990	NM_004064	Cell cycle arrest	2.2	0.005
H. sapiens forssman glycolipid synthetase precursor RNA, complete cds		AF163572	Glycolipid biosynthesis	2.2	0.03
H. sapiens vesicle transport-related protein (KIAA0917)	Hs.27023	NM_016106	Intracellular protein traffic	2.2	0.02
H. sapiens cug triplet repeat, RNA-binding protein 2 (CUGBP2)	Hs.211610	NM_006561	RNA processing	2.1	0.02
H. sapiens neuronal protein (NP25)	Hs.169330	NM_013259	Central nervous system development	2.0	0.02
H. sapiens biglycan (BGN)	Hs.821	NM_001711	Histogenesis and organogenesis	2.0	0.03
H. sapiens nuclear factor of kappa light polypeptide gene (NFKBILI)	Hs.2764	NM_005007	Transcription factor	1.9	0.01
H. sapiens for unknown liver orphan	Hs.118463	X56789	Leading strand elongation	1.9	0.01
H. sapiens delta-5 desaturase; delta-5 fatty acid desaturase (D5D)	Hs.132898	NM_013402	Fatty acid desaturation	1.9	0.04
H. sapiens nitrilase 1 (NITI)	Hs.146406	NM_005600	Nitrilase	1.9	0.04
H. sapiens disrupted in schizophrenia 1 protein (DISC1) complete cds	Hs.26985	AF222980	Cell growth and/or maintenance	1.9	0.02
H. sapiens progesterone binding protein (HPR6.6)	Hs.90061	NM_006667	Cation transport	1.8	0.04
H. sapiens quinolinate phosphoribosyltransferase (QPRT)	Hs.8935	NM_014298	Cell cycle	1.8	0.01
H. sapiens von Willebrand factor (VWF)	Hs.110802	NM_000552	Cell adhesion	1.8	0.02
H. sapiens neu1 protein (LOC51162)	Hs.91481	NM_016215	Signal transduction	1.8	0.03
H. sapiens G protein-coupled receptor salpr (LOC51289)	Hs.170146	NM_016568	Chemotaxis	1.8	0.04
H. sapiens C-terminal binding protein 1 (CTBP1)	Hs.239737	NM_001328	Negative control of cell proliferation	1.8	0.03
H. sapiens zic family member 3 (odd-paired *Drosophila* H.log, heterotaxy 1) (ZIC3)	Hs.111227	NM_003413	Transcription regulation	1.7	0.04
H. sapiens tissue inhibitor of metalloproteinase 3 (TIMP3)	Hs.245188	NM_000362	Developmental processes	1.7	0.01
H. sapiens cDNA FLJ12323 fis, clone mammal 002094	Hs.214979	AK022385	Cell growth and/or maintenance	1.7	0.02
H. sapiens tubulin, beta, 4 (TUBB4)	Hs.159154	NM_006086	Microtubule-based process	1.7	0.03
H. sapiens cDNA FLJ12688 fis, clone nt2rm4002534	Hs.13526	AK022750	Iron homeostasis	1.7	0.02
H. sapiens udp-galbetaglcnac beta 1,4-galactosyltransferase, polypeptide 2 (B4GALT2)	Hs.206713	NM_003780	Lactose biosynthesis	1.7	0.04
H. sapiens four and a half LIM domains 1 (FHL1)	Hs.239069	NM_001449	Histogenesis and organogenesis	1.7	0.01

This is a partial list of genes with known function; the complete list of genes can be accessed in the GEO database series GSE1718.

Therefore, differences in gene expression between HS_IR and LS_IR groups, either in the pre- or posttraining states, were confirmed by QRT-PCR in four of the five genes identified by microarray analysis as differentially expressed.

DISCUSSION

The objective of this study was to identify genes that are differentially expressed in the skeletal muscle of individuals who markedly improved or failed to improve S_I after 20 wk of endurance training. The uniqueness of the present microarray study resides in the selection of previously sedentary individuals with similar baseline S_I but with a fourfold difference in S_I response after 20 wk of an endurance exercise program. The differences in gene expression observed between the HS_IR and LS_IR groups cannot be attributed to the influences of sex, age, BMI, or body fat, since high and low responders were carefully matched for them (Table 1). It has been recommended for the analysis of skeletal muscle gene expression in microarray experiments to use pools of individual samples to decrease the influence of interindividual variability (2, 46). This strategy has been used successfully in other studies aimed at defining patterns of gene expression (2, 24, 46, 56). We pooled the individual samples of eight individuals in each of the high- and low-responder groups before and after the exercise program.

The genes differentially expressed between HS_IR and LS_IR groups in the posttraining arrays likely include a distinctive set of genes involved in the divergent S_I response to the training program. Moreover, because there were no differences in mean S_I at baseline, the genes differentially expressed in the pretraining arrays between HS_IR and LS_IR groups are likely to represent another set of genes that contribute to S_I response to the exercise training program.

Several microarray studies have provided a catalog of differentially expressed skeletal muscle genes under diverse clinical conditions associated with insulin resistance such as TD2M, obesity, and aging (36, 50, 52, 58). A direct comparison of these reports with our study is, however, difficult, since the selection of the individuals in the HS_IR and LS_IR groups was based on S_I responses to an exercise training program. Our study contrasted the differences in gene expression between LS_IR and HS_IR groups and not the effects of exercise training as such on gene expression patterns. Nonetheless, we intentionally searched our data for results on genes that, according to the literature, are modified by regular exercise or insulin-

Table 5. *Genes downregulated posttraining*

Gene	Unigene	GenBank Identifier	Gene Ontology	Fold Difference	*P* Value
H. sapiens mRNA; cDNA dkfzp434b1231 (from clone dkfzp434b1231); partial cds	Hs.35945	AL137493	Cell adhesion	0.3	0.02
H. sapiens glucocorticoid receptor af-1 specific elongation factor mRNA, partial cds	Hs.181165	AF174496	Protein synthesis elongation	0.3	0.01
H. sapiens cDNA FLJ13533 fis, clone place1006371	Hs.143671	AK023595	Transcription regulation	0.4	0.003
H. sapiens collapsin response mediator protein 1 (CRMP1) mRNA	Hs.155392	NM_001313	Neurogenesis	0.4	0.004
H. sapiens ribosomal protein s29 (RPS29) mRNA	Hs.539	NM_001032	Protein biosynthesis	0.4	0.001
H. sapiens death-associated protein kinase-related 2 (DRAK2) mRNA	Hs.120996	NM_004226	Protein phosphorylation	0.5	0.02
H. sapiens ribosomal protein 16 (RPL6), mRNA.	Hs.174131	NM_000970	Protein biosynthesis	0.5	0.003
H. sapiens ribosomal protein 19 (RPL9), mRNA.	Hs.157850	NM_000661	Ribosome biogenesis	0.5	0.03
H. sapiens ras-related associated with diabetes (RRAD) mRNA	Hs.1027	NM_004165	Small GTPase mediated signal transduction	0.5	0.04
H. sapiens collagen, type xii, alpha 1 (COL12A1), mRNA	Hs.101302	NM_004370	Cell adhesion	0.5	0.02
H. sapiens claudin 2 (CLDN2), mRNA	Hs.16098	NM_020384	Pathogenesis	0.5	0.02
H. sapiens mRNA for MEGF7, partial cds	Hs.4930	AB011540	Lipid metabolism	0.5	0.005
H. sapiens dynein light chain-a (LOC51143), mRNA	Hs.266483	NM_016141	Cell growth and/or maintenance	0.5	0.05
H. sapiens ribosomal protein 110a (RPL10a), mRNA	Hs.252574	NM_007104	Protein biosynthesis	0.5	0.005
H. sapiens microfibril-associated glycoprotein-2 (MAGP2), mRNA	Hs.300946	NM_003480	Posterior midgut invagination	0.5	0.03
H. sapiens natural killer-tumor recognition sequence (NKTR), mRNA	Hs.241493	NM_005385	Immune response	0.5	0.006
H. sapiens atp synthase, h+ transporting, mitochondrial f0 complex, subunit e (ATP5I)	Hs.85539	NM_007100	Proteolysis and peptidolysis	0.5	0.02
H. sapiens cathepsin 12 (CTSL2) mRNA	Hs.87417	NM_001333	Proteolysis and peptidolysis	0.6	0.05
H. sapiens hn1 protein (LOC51155), mRNA	Hs.109706	NM_016185	Mesoderm development	0.6	0.02
H. sapiens cdc2-related protein kinase 7 (CRKRS), mRNA	Hs.123073	NM_016507	Developmental processes	0.6	0.03
H. sapiens cd5 antigen-like (scavenger receptor cysteine rich family) (CD5L) mRNA	Hs.52002	NM_005894	Cellular defense response	0.6	0.03
H. sapiens mitochondrial proteolipid 68mp H.log (PLPM) mRNA	Hs.109052	NM_004894	Complex III (ubiquinone to cytochrome *c*)	0.6	0.02
Human hepg2 3′ region mboi cDNA, clone hmd1c1 1m3		D17129	RNA splicing	0.6	0.0001
H. sapiens kaptin (actin-binding protein) (KPTN), mRNA	Hs.25441	NM_007059	Actin filament organization	0.6	0.05
H. sapiens abcg5 (ABCG5) mRNA, complete cds	Hs.132992	AF320293	Transport	0.6	0.05
H. sapiens cDNA: FLJ20896 fis, clone adka03527	Hs.26756	AK024549	Signal transduction	0.6	0.03
H. sapiens karyopherin alpha 4 (importin alpha 3) (KPNA4) mRNA	Hs.302499	NM_002268	NLS-bearing substrate-nucleus import	0.6	0.01
H. sapiens ran binding protein 3 (RANBP3), transcript variant ranbp3-a, mRNA	Hs.176657	NM_003624	Protein targeting	0.6	0.004
H. sapiens ribosomal protein 124 (RPL24) mRNA	Hs.184582	NM_000986	RNA processing	0.6	0.006

This is a partial list of genes with known function; the complete list of genes can be accessed in the GEO database series GSE1718.

resistant states. Interestingly, there were no remarkable effects before or after exercise training on HS_IR/LS_IR expression ratios for genes involved in pathways of insulin signaling (IRS-1, IRS-2, PI3K, MAPK, AMPK), glucose transport (GLUT1, GLUT4), glycogen metabolism (GS, GSK-3), glycolysis (HKII, PFK), mitochondrial function (COX4, ND4), mitochondrial biogenesis (PGC-1, NRF-1, NRF-2, TFAM), and fatty acid oxidation (CPT I, UCP3, NADH6). These findings are undoubtedly due to the uniqueness of the experimental design on which the current study is based.

Differences in S_I responses are expected to involve the transcriptional reprogramming of sets of skeletal muscle genes. Differences in gene expression, particularly in clinical studies, are represented by modest changes in groups of related genes such as those associated with key metabolic pathways (13, 21, 49). Such small differences are often difficult to identify in microarray studies but can be readily evaluated using QRT-PCR methodologies.

Genes upregulated before exercise training. Given the matching criteria between the HS_IR and LS_IR groups, we did not expect to find dramatic differences in gene expression before the exercise training intervention. Nevertheless, we verified whether there were differences in gene expression that could predispose the HS_IR groups to increase S_I by regular exercise more than the LS_IR group. None of the differences was greater than 2.0-fold, and the differentially expressed transcripts encode proteins involved in glycolysis, nitrogen metabolism, signal transduction, transcription regulation, muscle contraction and development, and several new sequences with unknown or unclassified functions.

Among the novel candidate genes upregulated in the HS_IR before exercise training we identified were sorting nexin 10

Table 6. *List of genes that were consistently up- or downregulated in the pre- and posttraining experiments*

Gene	Unigene	GenBank Identifier	Gene Ontology	Pretraining HS_IR/LS_IR Arrays		Posttraining HS_IR/LS_IR Arrays		Post/PreTraining H/LS_IR Arrays Ratio
				Fold difference	*P* value	Fold difference	*P* value	
H. sapiens mRNA; cDNA DKFZp686K23233 (from clone DKFZp686K23233)	Hs.273186	AL137511	Electron transport	0.9	0.03	3.9	0.03	4.2
H. sapiens clone flb4630		AF113688	Transcription factor activity	0.7	0.03	1.7	0.02	2.4
H. sapiens von Willebrand factor (VWF), mRNA	Hs.110802	NM_000552	Cell adhesion	0.8	0.02	1.8	0.02	2.3
H. sapiens voltage-dependent anion channel 3 (VDAC3), mRNA	Hs.7381	NM_005662	Adenine transport	0.8	0.03	1.5	0.01	1.8
H. sapiens toll-like receptor 4 (TLR4), mRNA	Hs.159239	NM_003266	Signal transduction	0.8	0.05	1.4	0.05	1.7
H. sapiens colony stimulating factor 2 receptor, beta, low-affinity (csf2rb), mRNA	Hs.285401	NM_000395	Signal transduction	0.8	0.02	1.3	0.03	1.6
H. sapiens 3-hydroxyanthranilate 3,4-dioxygenase (HAAO), mRNA	Hs.108441	NM_012205	Cell adhesion	0.8	0.01	1.3	0.03	1.6
H. sapiens KIAA1018 protein (KIAA1018), mRNA	Hs.5400	NM_014967	Cell growth and/or maintenance	0.9	0.05	1.4	0.05	1.5
H. sapiens LIM domain-containing preferred translocation partner (LPP), mRNA	Hs.180398	NM_005578	Cell adhesion	0.9	0.002	1.3	0.01	1.4
H. sapiens mRNA for KIAA1160 protein, partial cds	Hs.33122	AB032986	Ubiquinone metabolism	0.9	0.01	1.3	0.04	1.3
H. sapiens mitogen-activated protein kinase kinase kinase 11 (MAP3K11), mRNA	Hs.89449	NM_002419	Protein kinase	0.9	0.03	1.2	0.04	1.3
H. sapiens complement clq a chain precursor, (LOC51089), mRNA	Hs.9641	NM_015991	Cell adhesion	0.9	0.03	0.9	0.04	1.0
H. sapiens interleukin 11 (IL11B), mRNA		NM_000881	Mesoderm determination	1.3	0.04	1.3	0.01	1.0
H. sapiens mRNA for KIAA0226 protein, partial cds	Hs.141296	D86979	Cytoskeleton organization and biogenesis	0.9	0.05	0.8	0.05	0.9
H. sapiens cDNA: FLJ23529 fis, clone lng06042	Hs.246306	AK027182	Cell motility	0.9	0.01	0.8	0.05	0.9
H. sapiens complexin 2 (CPLX2), mRNA	Hs.321567	NM_006650	Neurotransmitter release	1.4	0.04	1.2	0.03	0.9
H. sapiens cytochrome c oxidase subunit via H.log mRNA, complete cds		AF020589	Energy pathways	0.9	0.004	0.7	0.05	0.8
H. sapiens map 19p13.3; 18.87 cr from lb1264 repeat region, complete sequence	Hs.124161	AF064851	Behavior	0.8	0.05	0.6	0.01	0.7
H. sapiens hypothetical protein FLJ11155 (FLJ11155), mRNA	Hs.176227	NM_018342	Apyrase	1.2	0.01	0.8	0.05	0.7
H. sapiens POU domain, class 4, transcription factor 2 (POU4F2), mRNA	Hs.266	NM_004575	Transcription regulation	0.9	0.01	0.6	0.0003	0.6
H. sapiens NADH dehydrogenase (ubiquinone) 1B subcomplex, 7 (ndufb7), mRNA	Hs.661	NM_004146	Complex I (NADH to ubiquinone	1.1	0.04	0.7	0.02	0.6
H. sapiens mitochondrial proteolipid 68mp H.log (PLPM), mRNA	Hs.109052	NM_004894	Complex III (ubiquinone to cytochrome *c*)	1.1	0.03	0.6	0.02	0.5
H. sapiens ribosomal protein s21 (RPS21), mRNA	Hs.1948	NM_001024	Protein biosynthesis	1.1	0.01	0.2	0.05	0.1

This is a partial list of genes with known function; the complete list of genes can be accessed in the GEO database series GSE1718.

(SNX10), inositol (myo)-1(or -4)-monophosphatase 2 (IMPA2), diacylglycerol kinase-δ (DGKD), and PDK4. SNX10 contains a phosphoinositide-binding domain and belongs to a family of proteins involved in intracellular trafficking. IMPA2 shares similar enzyme activity with enzymes of the inositol phosphate second-messenger signaling pathway. DGKD is a cytoplasmic enzyme that phosphorylates diacylglycerol to produce phosphatidic acid, which, in turn, acts as second messenger in signaling cascades; therefore, DGKD is thought to play an important role in cellular signal transduction. Whether these genes play a role in the regular exercise-induced S_I response will require additional studies.

We further validated the increased PDK4 expression in the HS_IR group before exercise training. Skeletal muscle PDK4 expression increases in situations where carbohydrate availability and insulin levels are decreased and free fatty acids are increased, such as in fasting, high-fat/low-carbohydrate diets, and acute exercise (37, 40, 41). In skeletal muscle, PDK4 is induced during metabolic states in which there is a perceived deficit in whole body glucose availability (i.e., insulin resistance). A shift in substrate utilization in exercising muscle is driven by the increased delivery and oxidation of fatty acids, eliciting a progressive rise in acetyl-CoA that allosterically inhibits pyruvate dehydrogenase (PDH) activity (43). The induction of PDK4 suggests that PDK4-mediated inhibition of PDH in muscle represents a mechanism for conserving carbohydrate substrates by gradually limiting the entry of glycolytic products into the mitochondria for oxidation and thereby con-

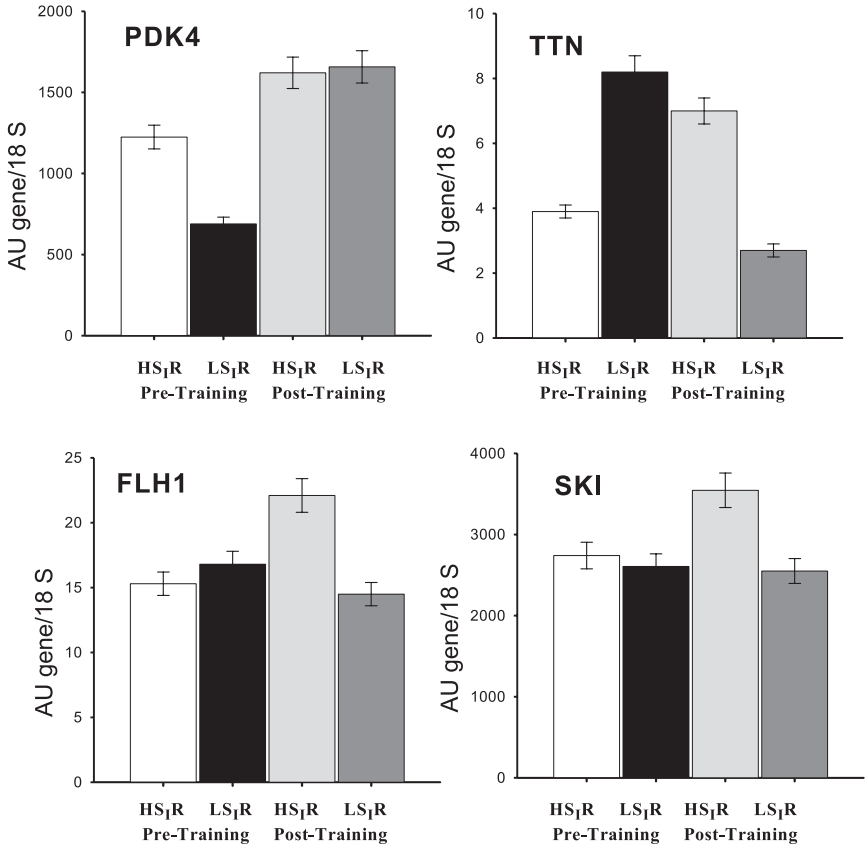

Fig. 1. Quantitative (Q)RT-PCR validation of genes for the insulin sensitivity response to exercise training identified in microarray experiments. Data represent samples analyzed in triplicates using 2-step QRT-PCR methodology. PDK4, pyruvate dehydrogenase kinase 4; TTN, titin; SKI, V-Ski avian sarcoma viral oncogene homolog; FHL1, four-and-a-half LIM domain 1. Levels normalized over the corresponding 18S rRNA value.

serving tricarboxylic acids for gluconeogenesis. This function may be particularly important in muscle fibers that rely heavily on glycolytic metabolism, such as vastus lateralis. We speculate that a persistent elevation in PDK4 expression may ensure that glucose entering the cell is preferentially used for muscle glycogen resynthesis, reflecting the high metabolic priority given to the replenishment of energy reserves in the HS_IR group, even in the sedentary state. This could represent one of the biological markers of the HS_IR group's predisposition to benefit more from a training program in terms of S_I increment than the LS_IR group. Interestingly, there was no difference in PDK4 expression levels between the HS_IR and LS_IR groups after exercise training. Further studies will be required to more thoroughly examine whether the expression of PDK4 or other PDK isoforms like PDK2, PDH phosphorylation, and/or activity contribute to the changes in S_I observed in certain individuals with exercise training.

Genes upregulated after exercise training. More transcripts were upregulated in the HS_IR group after exercise training than in the sedentary state. These upregulated transcripts relate to genes involved in energetic and signal functions, muscle development and contraction, cell growth regulation and differentiation, and sequences with unknown functions (Table 4). Some of the upregulated genes were validated by QRT-PCR methodologies, including SKI, TTN, and FHL1.

SKI is a nuclear proto-oncoprotein that can induce both oncogenic transformation and terminal muscle differentiation (OMIM no. 164780). SKI could function as either a positive or negative regulator of transcription, depending on its tissue expression and/or the promoter context of the genes that use SKI as a transcription factor. In transgenic mice, Ski overexpression induces muscle differentiation in embryo fibroblasts and causes postnatal hypertrophy of type II muscle fibers. The Ski-null mice show a marked decrease in skeletal muscle mass in addition to other abnormalities (for review see ref. 31). The human SKI gene has been associated with congenital fiber type-disproportion type myopathy (CFTD), hypotonia, and craniofacial dysmorphism (1p36 deletion syndrome) (35). Severe hyperinsulinemia and insulin resistance have been described in patients with CFTD (OMIM no. 255310). It remains to be clarified whether the increased expression of the SKI gene that we observed in the HS_IR group is causally related to the improved S_I in muscle.

FHL1, also known as skeletal muscle LIM-1 (SLIM1) protein, is mainly expressed in skeletal muscle; FHL proteins are new members of the LIM-only protein family. FHL1 plays an important role in muscle development (28, 34), and its expression is more abundant in oxidative fibers (30). FHL1 is also thought to function as a scaffold for protein assembly in the actin-based cytoskeleton; it is located in an integrin-dependent manner to the nucleus, focal adhesions, and stress fibers. It has been suggested that one of the FHL1 LIM domains interacts with α-actinin, a component of the Z-discs, whereas interactions of the other LIM domains with actin filaments and spectrin have been demonstrated for FHL3 (8). FHL1 was one of the genes identified by Roth et al. (46) as being upregulated in response to a 4-mo strength training program. It was recently reported that FHL1 might play a role during the early stages of skeletal muscle differentiation, specifically in the $\alpha_5\beta_1$-integrin-mediated signaling pathway (32). In addition, FHL pro-

teins have been implicated in one of the downstream mechanisms by which the extracellular signal-regulated kinase 2 (ERK2) signaling pathway affects the regulation of differentiated growth (42). Interestingly, FHL2 (a cardiac muscle FHL protein) binds TTN, and one of its domains may couple the metabolic enzymes creatine kinase, adenylate kinase, and PFK to the sarcomeric structures (27). The binding partners for the homologous FHL1 and FHL3 proteins have not been defined.

TTN is a giant protein, the largest known polypeptide (3 MDa) and contributes to the maintenance of sarcomere organization and myofibrillar elasticity (for review see ref. 53). TTN may also participate in myofibrillar cell signaling. Tissue-specific expression of various TTN isoforms results in differential myocyte elasticity. Mutations located in the Z-disc binding region of TTN have been identified in tibial myopathies (17). Disruption of TTN has been reported to cause impaired sarcomerogenesis and results in thin, poorly contractile muscle cells. More than half of the TTN molecule is attached to the thick filament, where it appears to control the exact assembly of myosin and other filament components. From the end of the thick filament and the Z-line, TTN forms elastic connections. The Z-line domain of TTN interacts with telethonin/T-cap, which in turn interacts with the muscle growth factor myostatin and the muscle LIM protein (16). Near the middle of the thick filament, TTN has a kinase domain, but its functions and substrate(s) are not fully understood. Both ends of the molecule have potential phosphorylation sites that may be involved in protein signaling. For example, the COOH terminus of a TTN molecule is integrated in the myosin lattice and contains a Ser/Thr kinase domain whose absence leads to sarcomeric disassembly (15). The main functions of the TTN family seem to be to interconnect myosin and actin filaments axially and provide passive elasticity. TTN also makes it possible for equal forces to be distributed by myosin in both halves of the sarcomere (53).

Because FHL1 and TTN were upregulated after exercise training in the HS_IR group, it is tempting to speculate on the potential role of these genes in the improved S_I. The mechanisms by which exercise training improves S_I in healthy skeletal muscle involve enhanced insulin action and signaling, glucose transport, and overall metabolic capability (10). The adaptive response to exercise training requires the sensing of biomechanical signals involving the interface between the contractile cytoskeleton (myofibrils) and the sarcolemma at specialized cell-cell junctions (intercalated discs) and cell-substrate adhesion complexes (costameres). New evidence suggests that complexes associated with the TTN protein sense myocyte stretch, and TTN has recently been proposed as an ideal biomechanical sensor (33). If one of the complexes associated with TTN is FHL1, which in turn could interact with metabolic enzymes, it could explain how increased TTN and FHL1 contribute to an improved S_I response with exercise training. Thus far, there is little direct evidence to support this hypothesis, and experimental data will be required to validate this concept.

In conclusion, we have identified a unique profile of genes that are differentially expressed between individuals with high S_I response to exercise training vs. others carefully matched who showed absolutely no improvement. Among the genes identified with this approach we found that, before exercise training, PDK4 is overexpressed by 80% in the high S_I responders to regular exercise. In those who expressed the highest S_I improvement, SKI, FHL1, and TTN were overexpressed by 50–470% compared with those whose S_I remained stable. The proteins encoded by these novel genes could play a role through either cell-matrix interactions or enhanced signaling pathways involved in the S_I response. Moreover, our studies have identified other transcripts of unknown functions that could participate in the regulation of S_I response to regular exercise. The data presented here offer new candidate genes to account for human variation in the ability to improve S_I in response to regular exercise.

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