TRANSCRIPTION FACTORS IN HUMAN SKELETAL MUSCLE ASSOCIATED WITH SINGLE AND REGULAR STRENGTH EXERCISES

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Skeletal muscle plasticity is the ability to change morphofunctional properties in response to changes in contractile activity. Strength training increases the size of muscle fibers and maximum strength with the activation of protein synthesis. Regulation of these changes at the gene level has not been investigated properly. This study aimed to identify transcription factors associated with changes in the transcriptome of the human skeletal muscle in the context of single and regular strength exercises. We assessed changes in the transcriptomic profile of *m. vastus lateralis* of 10 young men (mean age 23 (20.8 - 25.9) years) before and after 12-week leg extensor muscles strength training course, as well as before, 8 and 24 hours after a single exercise. Transcriptomic profiling involved RNA sequencing, search for binding motifs and the associated transcription factors. Bioinformatic methods of statistics, FastQC, GraphPad Prizm 8, DAVID, R enabled analysis of the data acquired. The strength training course resulted in the enrichment of the functional groups of genes "secreted proteins", "extracellular matrix" and "basal membrane" ($p < 0.05$). Transcriptomic responses and the associated transcription factors differed 8 and 24 hours after a single session as well as after regular training sessions. Transcription factors involved in adjustment to regular and one-time loads participate in myogenesis, angiogenesis, regulation of fiber phenotype, proteostasis and other processes. Thus, regulation of gene expression during adjustment to the resistance training loads is a complex process that involves many transcription factors with different functions. Investigation of the role played by these factors in the context of adjustment to exercising is a potentially rewarding task.

Keywords: gene expression, strength training, muscle plasticity, muscle fibers, hypertrophy

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Compliance with ethical standards: the study was approved by the Ethics Committee of the Lopukhin Federal Research and Clinical Center Of Physical-Chemical Medicine (Minutes № 202/06/01 of June 01, 2021). All participants signed the voluntary informed consent form.

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ТРАНСКРИПЦИОННЫЕ ФАКТОРЫ В СКЕЛЕТНОЙ МЫШЦЕ ЧЕЛОВЕКА, АССОЦИИРОВАННЫЕ С ОДНОКРАТНЫМ И РЕГУЛЯРНЫМИ СИЛОВЫМИ УПРАЖНЕНИЯМИ

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Пластичность скелетной мышцы — способность менять морфофункциональные свойства в ответ на изменение сократительной активности. Силовые тренировки ведут к увеличению размеров мышечных волокон и максимальной силы с активацией синтеза белков. Регуляция этих изменений на генном уровне мало изучена. Целью работы было выявить транскрипционные факторы, ассоциированные с изменением транскриптома скелетной мышцы человека при однократном и регулярных силовых упражнениях. Изменение транскриптомного профиля оценивали в *m. vastus lateralis* 10 молодых мужчин (возраст 23 (20,8–25,9) года) до и после 12-недельной силовой тренировки мышц-разгибателей ног, а также до, через 8 и 24 ч после однократного упражнения. Транскриптомные профили оценивали методом РНК секвенирования, поиска мотивов связывания и ассоциированных транскрипционных факторов. Использовали биоинформатические методы статистики, программы FastQC, GraphPad Prizm 8, DAVID, R. Длительная силовая тренировка привела к обогащению функциональных групп генов «секретируемые белки», «внеклеточный матрикс» и «базальная мембрана» ($p < 0,05$). Транскриптомные ответы и ассоциированные транскрипционные факторы различались через 8 и 24 ч после однократной нагрузки, а также после регулярных тренировок. Транскрипционные факторы, участвующие в адаптации к длительной и однократной нагрузке, участвуют в миогенезе, ангиогенезе, регуляции фенотипа волокон, протеостазе и иных процессах. Таким образом, регуляция экспрессии генов при адаптации к силовым нагрузкам — сложный процесс с участием множества транскрипционных факторов с разными функциями. Изучение роли этих факторов в адаптации скелетной мышцы к упражнениям является перспективной задачей.

Ключевые слова: экспрессия генов, силовая тренировка, мышечная пластичность, мышечные волокна, гипертрофия

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Plasticity is one of the capabilities of skeletal muscles: they can change their morphofunctional characteristics in response to changes in the level of contractile activity. Investigation of the molecular mechanisms underpinning plasticity is a fundamental task. It also has a practical dimension in the context of optimization of training programs for amateur and professional athletes, as well as for prevention of the negative effect of various diseases on the skeletal muscles. On the one hand, a high-intensity physical load triggers transient increase of the mTORC1-dependent rate of muscle protein synthesis [1–3]; thus, practiced on a regular basis, such loads make muscle fibers and muscle in general grow bigger and stronger. On the other hand, a single session [4–7] and regular resistance training of varying duration [4, 6–11] alter the gene expression profile in the exercised skeletal muscle. The mechanisms regulating these alterations (in particular, transcription factors associated with changes in the transcriptomic profile) have not been sufficiently investigated.

This study aimed to search for transcription factors associated with changes in the transcriptome of human skeletal muscle in the context of single and regular strength exercises. For this purpose, we used RNA sequencing in order to detect changes in the transcriptomic profile of bioptic samples of *m. vastus lateralis*. The samples were taken from 10 young men before and after a 12-week leg extensor (knee joint) training course and 8 and 24 hours after a single exercise session (Fig. 1).

METHODS

Study design

The study involved 10 young men, median age — 23 years (20.8–25.9), median BMI — 22 (20.9–25.1) kg/m^2. The inclusion criteria were: perfect health; lack of acute and chronic diseases; lack of experience of long-term resistance training; lack of traumas and surgeries on the back and lower limbs. The exclusion criteria were: refusal to participate in the training sessions and test procedures; detection of adverse conditions in the context of training sessions or procedures (life- and health-threatening); violation of the recommended diet or practicing vicious habits during the experiment. For 12 weeks, the participants trained leg extensor muscles by doing the seated leg press exercise (both legs). They were surveyed before the experiment; all of the participants reported diverse diets with regular meals containing sufficient amounts of protein, fats and carbohydrates, and adequate volumes of water consumed through the day. The recommendation for them was to continue with their usual diets during the experiment. All participants were non-smokers before and during the study; they did not take bioactive supplements for 3–4 months before the experiment nor while involved therein. None of them was a vegetarian or a vegan. With regular resistance training sessions, it is not the magnitude of the load but doing each exercise to expressed fatigue (failure, inability to continue) that guarantees muscle growths [12]. At the same time, a training program optimal from the viewpoint of strength development includes 25 sets per session and at least 2 sessions a week [13,14]. Therefore, the participants of our study trained 3 times a week, with varying intensity: Monday — (65% MVC, to failure) × 3 sets, Wednesday — (50% MVC, 25 repetitions) × 4 sets, Friday — (75% MVC, to failure) × 4 sets. The participants were allowed 4 minutes of rest between the sets. In addition, each training session started with a warm up (50% MVC, 12 repetitions). All participants reported 8 hours of sleep a day (on average), none of them mentioned any changes in the sleeping patterns during the experiment. We recommended moderation in physical activity and keeping it at the customary level for 24 hours after each training sessions; also, the recommendation was to abstain from alcohol for 24–48 hours thereafter, when the body is restoring.

We took bioptic samples from *m. vastus lateralis* before the training part of the experiment (Fig. 1; B1). Two days later, the participants took an introductory session, and after another 2 days, we determined their MVC, which was a derivative of the maximum load at which the participant could fully extend both legs. We assessed the MVC every 2–3 weeks and after the training part of the experiment (Fig. 1; T2). Separately, we measured the MVC of the leg that was loaded later, during the test training session (TTS); this leg was selected at random in order to mitigate the dominant limb effect (Fig. 1; T1). After 4 days, the participants attended the TTS and did the seated leg press exercise with one leg (Fig. 1): warm-up (50% of the MVC, 12 repetitions) + (65% MVC, to failure) × 4 approaches). Bioptic samples were taken from both legs (donor muscle — *m. vastus lateralis*), loaded and not loaded, 8 and 24 hours after the session. Gene response may change several hours after exertion not only because of the muscle contractions, but also under the influence of systemic factors (circadian oscillations, nutrition, etc.) [15,16]. In our work, seeking to eliminate the

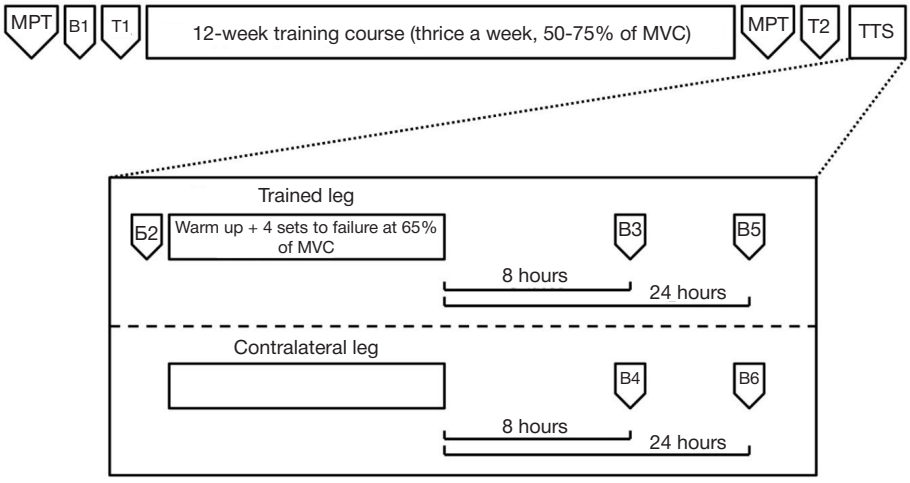

Fig. 1. Physiological experiment diagram. T1, T2 — testing the maximum voluntary contraction (MVC); TTS — test training session (single session with bioptic samples taken from both legs before and after exercising one leg); B1–B6 — sampling from the *m. vastus lateralis*. Biopsies B1, B2, B3, B5 were taken from the leg loaded during TTS. Biopsies B4 and B6 — from the contralateral leg (which was not loaded). Both legs were exercised during twelve-week training course

Table 1. Results of analysis of functional enrichment, genes that changed expression in *m. vastus lateralis* after a training course and a one-time physical load

Comparison	UniProt term	P_{adj}	Number of genes	Genes
After/before training course	Secreted	5,40E-12	39	*COL15A1, SPARC, PCOLCE2, LAMA4, HTRA1, F13A1, C1ORF54, CHRDL1, NID2, FSTL1, THBS4, SERPINA5, CNPY4, CTSK, PENK, S100A13, CCN1, PAMR1, POSTN, CD163, IGFBP3, LAMB1, RNASE1, PLXDC1, ASPN, FNDC5, MFAP5, COL1A1, SFRP4, SMOC2, COL3A1, COL1A2, FNDC1, TCN2, COL5A2, MGP, SAA1, S100A4, MASP1*
	Extracellular matrix	1,70E-06	14	*POSTN, COL15A1, SPARC, LAMA4, LAMB1, NID2, ASPN, THBS4, MFAP5, COL1A1, SMOC2, COL3A1, COL1A2, COL5A2*
	Basement membrane	0.0098	5	*SMOC2, SPARC, LAMA4, LAMB1, NID2*
Loaded/not loaded muscle, 8 hours after exercise	–	n.s.	–	–
Loaded/not loaded muscle, 24 hours after exercise	Cytoskeleton	0.0087	64	*RIPOR2, RIF1, WDR1, CBY1, HSPB1, HNRNPU, NR3C1, TUBA1C, TUBA1B, CSRP3, TUBA1A, SGCD, MPRIP, CEP250, CEP170, DYNLT1, TUBB, ANXA11, CSNK1D, PPP4R3B, ANK3, RANGAP1, MLF1, TUBA4A, ACTA2, KAT2B, KIF9, PALLD, EVL, EZR, PFN1, FKBP4, MACF1, DCTN4, CEP85L, PXN, UACA, AURKA, FGD4, TTC21B, FLNB, CEP192, FLNC, CCT5, MAP2K6, CEP350, RAB3IP, SYNJ2, PARVB, ARHGAP26, ARHGAP24, SEPTIN7, RAB10, DIAPH1, KITLG, TTLL4, ACTC1, APPBP2, KATNBL1, JMY, SPIRE1, PKN2, PTPN4, CALM2*

influence of systemic factors on gene expression after a one-time load, we evaluated the differences in the transcriptomic profile in samples taken from both the loaded and the not loaded (control) muscle of the contralateral limb.

All bioptic samples were taken after 30 minutes of rest in the supine position, from the middle third of *m. vastus lateralis*, under local anesthesia (2 ml of 2% lidocaine), using a 6 mm suction-modified Bergström needle [17]. The site of each subsequent sampling was 4 cm proximal to the previous one. The tissue samples were quickly cleaned of blood and connective tissue, frozen in liquid nitrogen and stored at –80 °C.

Transcriptomic analysis

Muscle tissue samples (~20 mg) were homogenized in a TissueLyser II homogenizer (Qiagen; Germany), two cycles of 1 minute each, at the frequency of 30 Hz; RNA was isolated with an RNeasy mini kit (Qiagen; Germany). We used a Qubit 3.0 fluorimeter (Thermo Scientific; USA) to measure concentration of the RNA, and a Bioanalyzer 2100 capillary electrophoresis device (Agilent; USA) to establish its integrity. RNA was cleaned of DNA contamination with the help of a Turbo DNA-free Kit (Thermo Scientific, USA). Double-stranded cDNA was synthesized in a Mint-2 kit (Eurogen; RF). The technology used to purify the resulting PCR product was SPRI, the process involved AMPure XP beads (Beckman-Coulter; USA); double-stranded cDNA was split in a ME220 focused-ultrasonicator (Covaris; USA) into double-stranded DNA fragments of 250 pn, strips of eight 50 µl tubes (Peak Incident Power 75W, Duty Factor 20%, Cycles per Burst 1000, Treatment Time 75 s). The resulting double-stranded cDNA fragments were also purified using the SPRI technology and AMPure XP beads (Beckman-Coulter; USA).

Universal DNA Library Prep Set (MGI-Tech; PRC) was used to prepare libraries for 10 ng fragments of the acquired double-stranded cDNA. The protocol included repair and phosphorylation of ends of the fragments, ligation of asymmetric adapters and 47 cycles of amplification of the ligation products for quantitative library development. RNA were sequenced in a DNBseq-G400 analyzer (MGI; PRC) as per the manufacturer's instructions, using reagents from the DNBSEQ-G400RS High-throughput Sequencing Set (PE100), read length — 100 nucleotides, depth — 50 million pairs of reads per sample.

Bioinformatic processing of the RNA sequencing data

We used the FastQC v0.11.9 software (Babraham Institute; UK) to control quality of the sequencing data. Low-quality reads and adapter sequences were removed from the analysis using the Trimomatic tool v0.39 (USADELLAB; USA), standard parameters. We mapped the paired reads to the reference human genome, version GRCh38.p13 (gencode v37), using STAR v2.7.4a (Cold Spring Harbor Laboratory, USA) with standard parameters. The number of unique reads aligned to known exons of each gene was determined using the featureCounts function of the Rsubread package (R programming language, Lucent Technologies, USA), with genome annotation gencode v37.

We used the DESeq2 package of the R programming language to find differentially expressed genes (DEGs) in the compared groups. The DEG registration threshold was Padj < 0.1 (BH-adjusted *p*-value). To analyze the functional enrichment of DEGs, we used DAVID tools (Frederick National Laboratory for Cancer Research; USA) and UniProt resources.

Searching for transcription factors potentially regulating gene expression in response to strength exercises, as well as the corresponding binding motifs, we analyzed promoter sites of DEGs (open chromatin sites around the transcription initiation start that were determined for skeletal muscle and reported earlier [18]). The search for motifs (and the associated transcription factors) was performed by the GeneXplain platform and the TRANSFAC v2022.1 positional weight matrix database, as described earlier [18]. The maximum enrichment (FEadj, adjusted odds ratio of site frequency with a confidence interval of 99%) was determined for each positional weight matrix (PWM) relative to a random set of 5000 promoters. The adjusted enrichment value (FEadj) > 1.5 for binding sites with transcription factors (binomial test) and FDR < 0.05 were selected as significance criteria.

Statistical processing

We used the GraphPad Prizm 8 program (GraphPad Software, Dotmatics; USA) and Wilcoxon test ($p < 0.05$) to assess changes in the MVC post training.

RESULTS

Twelve weeks of strength training increased the maximum voluntary contraction by 1.19 times ($p = 0.002$), which is

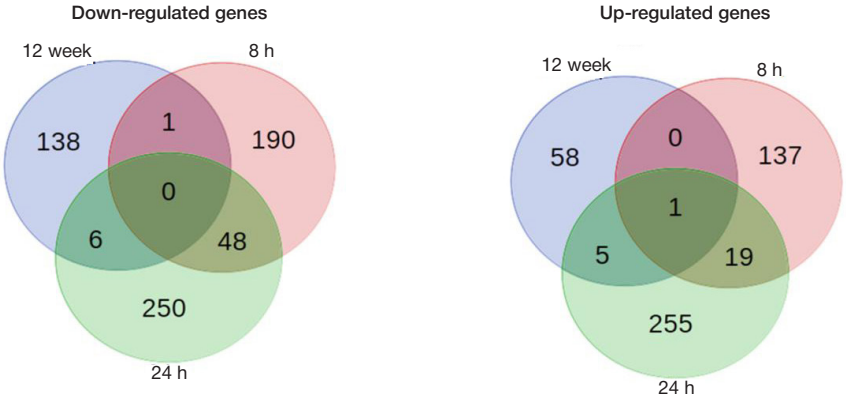

Fig. 2. Number of genes that changed expression in *m. vastus lateralis* after a 12-week training program and 8 and 24 hours after a single load. Venn diagrams show the number of mRNAs unique and common to different experimental conditions for genes that increased and decreased expression

comparable to the results of studies involving training with a similar design [19, 20]. The figure confirms effectiveness of the training program we selected.

The effect of regular strength training on changes in the basal transcriptome

Training changed expression of 209 genes, with the content of 145 mRNAs increasing and 64 mRNAs decreasing (comparison B2-B1; Figure 1). Functional enrichment analysis revealed significant enrichment for the functional terms "extracellular matrix", "secreted proteins", and "basement membrane". Among these genes, there were various collagens, calmodulin-like proteins and adhesion molecules (Table 1). This result, despite the minor character of alterations, is consistent with the findings of meta-analyses that reviewed transcriptome changes in response to regular strength training [21, 22]. On the one hand, activation of expression of protein genes of extracellular matrix is probably one of the mechanisms involved in the adjustment of the trained skeletal muscle to regular physical exertion. On the other hand, our and other studies report a relatively weak effect of prolonged strength training on the skeletal muscle transcriptome, even when training sessions are regular for 15 years or more [23]. This may be due to the fact that strength exercises, first of all, activate translation and not transcription.

Transcriptome change in response to a single session

Eight and twenty-four hours after a single session, the content of 396 and 584 mRNAs changed, respectively; more than half of them increased expression: 239 mRNAs and 304 mRNAs, respectively. There was little overlap between the sets of genes that changed expression 8 and 24 hours after a single session (Fig. 2). The analysis of enrichment revealed no functional categories 8 hours after the load. Nevertheless, we detected activation of expression of a number of genes known from previous studies as markers of early response to contractile activity (including during aerobic exercise): *ATF3*, *DDIT3*, *JUND*, *MAFF*, *NR4A3*, *VDR*, *PRKAG2*, *PPARGC1A*, etc. [22,24–26]. Genes that changed expression 24 hours after a single session were associated with the term "cytoskeleton" (Table 1). More than half of them increased expression and were represented by genes of motor proteins (alpha- and beta-tubulin, actins *ACTA2* and *ACTC1*, components of the kinesin-dynein complex KIF9 and *DYNLT1*), chaperones (*CRYAB1*, *HSPB1*), etc. It should be noted that some contractile activity response expression markers (*ATF3*, *DDIT3*, *VDR*, *PRKAG2*) remained activated during the post-exercise recovery period for up to 24 hours, which suggests their important role in regulating the response to physical exertion. Interestingly, there is even less overlap between gene response to a single exertion and regular training sessions (Fig. 2).

After a 12-week strength training program that involved both legs, we have registered a change in the transcriptomic profile of *m. vastus lateralis* that is comparable to that described earlier in similar studies. Using a test model, which was the exercise done with one leg, and comparing gene expression in the loaded and not loaded *m. vastus lateralis*, we, for the first time ever, managed to describe the transcriptomic response (on the 8th and 24th hours of recovery) in human skeletal muscle that is specific to strength exercises, i.e., this response was independent of circadian and systemic influences. There was only a slight overlap between the sets of genes that changed expression between different experimental conditions, which can be explained by the specific mechanisms of gene expression regulation peculiar to the said conditions.

Analysis of transcription factors associated with changes in gene expression

Figure 3 presents the results of search for transcription factors associated with changes in gene expression under the studied experimental conditions. Adjustment to regular strength training sessions triggered changes in basement gene expression in *m. vastus lateralis* that were associated with diverse families of transcription regulators; the most enriched of them were the poorly studied factors with zinc finger domains. In addition, we identified a number of factors that quite expectedly altered their activity after regular strength training sessions. Thus, activation of gene expression was associated with factors directly related to the contractile activity; for example, NFATC component of the Ca^{2+}–dependent calcineurin-NFAT signaling pathway [27]. NFATC1 is known to control muscle growth [28–30] and the ratio of muscle fiber types in mice, as well as suppress the activity of MyoD-dependent promoters [31].

DISCUSSION

Regular training sessions lead to activation of the expression of extracellular matrix genes, including genes encoding angiogenesis regulatory proteins. Among the transcription factors we found, potential regulators of angiogenesis are ERG and SOX18. ERG is known to regulate angiogenesis by controlling the expression of E-cadherin and the Wnt/β-catenin signaling pathway [27]. SOX18 is expressed mainly in endothelial cells; it regulates angiogenesis by activating their migration and proliferation, while the pattern of SOX18

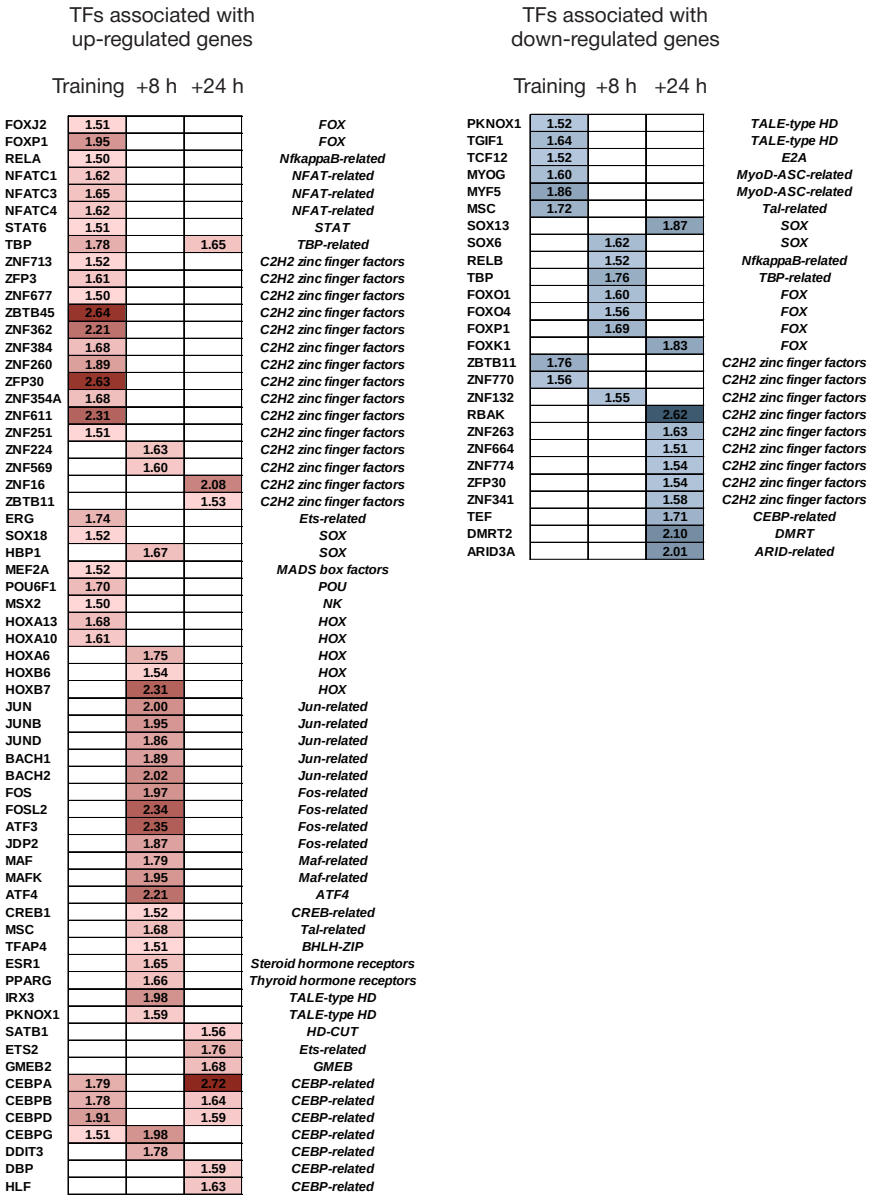

Fig. 3. Transcription factors (TFs) associated with genes that increased and decreased expression after regular strength training sessions (Training) and 8 hours and 24 hours after a single physical load. Shades of color and numbers indicate the amount of enrichment of the binding motif with the transcription factor in individual promoters of genes that have changed expression, relative to 5000 random genes that have not changed expression (see METHODS).

expression in endothelial cells coincides with VEGFA and its receptor [32]. We have expectedly found MEF2A, regulator of myogenesis, and MSX2 among the factors associated with gene expression growth. Unexpectedly, a drop in expression of some genes was associated with myogenic E-box-binding factors (MYOG, MYF5, MSC) controlling the differentiation of myoblasts at different stages. It can be assumed that increased activity of some myogenic factors and suppression of others is associated with a change in the phenotype of the muscle after training. Such strength training programs are known to predominantly increase the size of type II muscle fibers and have only a weak effect on the type I fibers [1].

It is difficult to assess the functions of other transcription factors associated with changes in the transcription profile in the context of regular strength training sessions. For example, FOXP1 was previously described as a transcription repressor, its overexpression causing atrophy and loss of muscle mass in mice [33]. In addition, FOXP1 inhibits the activity of MyoD [34]. RELA and STAT6 are known as regulators of inflammation, but they also play a role in the regulation of myogenesis and atrophy [35, 36].

Eight hours after a single session, the regulation of gene expression was primarily associated with factors of the bZIP class (families of early response factors JUN, FOS, MAF, etc.). Some of them (ATF4, AP-1 factors (FOS, JUN), DDIT3, CEBP) are known to be are activated against violation of proteostasis and EPR stress [37, 38]. Activation of these factors is quite expected, since high-intensity strength exercises cause pronounced metabolic and mechanical stress, however, these factors were not found to activate at later stages of recovery (24 hours) after a single session. On the contrary, dropping gene expression at the 8th hour of recovery was associated with factors of the FOXO family, which regulate the activity of the ubiquitin-proteasome system in the muscle [39–41].

Twenty-four hours after the exercise, the change in gene expression was associated with a small number of transcription factors: growth — mainly with the factors of the CEBP family, suppression — factors containing zinc finger domains, KRAB domain containing RBAK repressor in particular.

Thus, we have sufficient uniqueness of the sets of genes that changed expression in response to a 12-week

strength training course and a single training session, as well as the transcription factors associated with them. Apparently, the reason behind these findings is the availability of many signaling pathways regulating activation of various sets of transcription factors and their target genes in the basal state after a course of regular aerobic training and at different stages of recovery after a single training session. There are published papers that describe the role in regulation of myogenesis played by some transcription factors that we have predicted in our work, which indirectly confirms correctness of the bioinformatic analysis methods we use. The role of other transcription factors in the regulation of myogenesis is not so obvious. Investigation of the role played by these factors in the context of adjustment of a skeletal muscle to high-intensity exercises is a potentially rewarding task.

CONCLUSIONS

We have shown pronounced changes in the transcriptome of skeletal muscle in response to a single exercise session and a 12-week strength training course building up contractile capacities of the trained muscles. These changes are quite consistent with the results of other works that involved similar training routines. Notably, transcriptomic responses and the associated transcription factors differed markedly both 8 hours and 24 hours both after a single training session and after a 12-week regular exercising course. Our results indicate complexity of regulation of gene expression during adjustment to resistance loads, with the apparent reason therefore being the large number of processes involved in the regulation growth of muscle mass.

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