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ORIGINAL ARTICLE

Epigenetic changes in leukocytes after 8 weeks of resistance exercise training

Joshua Denham[1,2] · Francine Z. Marques[2,3] · Emma L. Bruns[4] · Brendan J. O'Brien[4] · Fadi J. Charchar[2]

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Abstract
Purpose Regular engagement in resistance exercise training elicits many health benefits including improvement to muscular strength, hypertrophy and insulin sensitivity, though the underpinning molecular mechanisms are poorly understood. The purpose of this study was to determine the influence 8 weeks of resistance exercise training has on leukocyte genome-wide DNA methylation and gene expression in healthy young men.
Methods Eight young (21.1 ± 2.2 years) men completed one repetition maximum (1RM) testing before completing 8 weeks of supervised, thrice-weekly resistance exercise training comprising three sets of 8–12 repetitions with a load equivalent to 80 % of 1RM. Blood samples were collected at rest before and after the 8-week training intervention. Genome-wide DNA methylation and gene expression were assessed on isolated leukocyte DNA and RNA using the 450K BeadChip and HumanHT-12 v4 Expression BeadChip (Illumina), respectively.
Results Resistance exercise training significantly improved upper and lower body strength concurrently with diverse genome-wide DNA methylation and gene expression changes ($p \leq 0.01$). DNA methylation changes occurred at multiple regions throughout the genome in context with genes and CpG islands, and in genes relating to axon guidance, diabetes and immune pathways. There were multiple genes with increased expression that were enriched for RNA processing and developmental proteins. Growth factor genes—*GHRH* and *FGF1*—showed differential methylation and mRNA expression changes after resistance training.
Conclusions Our findings indicate that resistance exercise training improves muscular strength and is associated with reprogramming of the leukocyte DNA methylome and transcriptome.

Keywords Epigenome · Transcriptome · Strength training · mRNA expression · DNA methylation

Abbreviations

ANOVA	Analysis of variance
χ^2	Chi squared
CpG	Cytosine neighbouring a guanine dinucleotide
DAVID	Database for Annotation, Visualization and Integrated Discovery
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
FGF1	Fibroblast growth factor 1
GHRH	Growth hormone-releasing hormone
INS	Insulin
IPAQ	International Physical Activity Questionnaire
MET	Metabolic equivalent of task

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✉ Joshua Denham
jdenham2@une.edu.au

[1] School of Science and Technology, University of New England, Room 259, Botany Building, Armidale, NSW 2351, Australia

[2] Faculty of Science and Technology, Federation University Australia, Mount Helen, VIC, Australia

[3] Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia

[4] Faculty of Health, Federation University Australia, Mount Helen, VIC, Australia

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mRNA	Messenger RNA
NF-kB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
1RM	One repetition maximum
RET	Resistance exercise training
RNA	Ribonucleic acid
TET	Tet methylcytosine dioxygenase

Introduction

Physical inactivity-related chronic diseases, such as obesity, cardiovascular disease and type 2 diabetes, are somewhat preventable and controlled by physical exercise (Booth et al. 2012; Schuler et al. 2013; Pedersen and Saltin 2006). Resistance exercise training (RET) involves muscular contractions against an external force, typically in the form of resistance machines, barbells or body weight. Resistance exercise training improves bone mineral density (Bemben and Bemben 2011; Hinton et al. 2015; Westcott 2012), insulin sensitivity (Dunstan et al. 2002; Gordon et al. 2009; Tresierras and Balady 2009), lipid profile (Kelley and Kelley 2009) and blood pressure (Cornelissen et al. 2011; Cornelissen and Smart 2013) and may lower circulating markers of inflammation (Phillips et al. 2012; Strasser et al. 2012). Increased quality of life, self-efficacy and mood benefits are also gained from RET (Beniamini et al. 1997; Singh et al. 1997; Katula et al. 2008; Westcott 2012). RET is most effective at maintaining muscular strength and mass, which is particularly important to conduct activities of daily living for older adults. Despite the many health benefits conferred by RET, our understanding of the underlying molecular mechanisms governing the health and performance adaptations are not completely understood.

Epigenetics encompasses the study of changes to gene expression independent of genotype alterations (Denham et al. 2014). Epigenetic modifications caused by the internal and external environment act on DNA and histone proteins to rearrange chromatin conformation and ultimately govern gene expression (Bird 2002; Cedar and Bergman 2012). DNA methylation is one of the most commonly studied epigenetic modifications, and aberrant DNA methylation profiles seem to play a role in cancer (Heyn and Esteller 2012), atherosclerosis (Zaina et al. 2014), type 2 diabetes (Volkmar et al. 2012) and psychotic disorders (Grayson and Guidotti 2013). The potential for epigenetic modifications to impact health and disease is extensive, such that aberrant epigenetic modifications can be experienced by future generations through transgenerational epigenetic inheritance (Nilsson and Skinner 2015).

Until now, research has focused primarily on the impact aerobic exercise training has on epigenetic modifications and gene expression. For instance, aerobic exercise training improves cardiovascular health and fitness concurrently with reprogramming genome-wide DNA methylation in skeletal muscle (Lindholm et al. 2014; Rowlands et al. 2014; Nitert et al. 2012), adipose tissue (Ronn et al. 2013), leukocytes (Denham et al. 2015b) and sperm (Denham et al. 2015a). Exercise training may attenuate the risk of chronic diseases through favourable epigenetic modifications (Denham et al. 2014) and these changes may be somewhat heritable and influence the health of future generations. Although DNA methylation is particularly responsive to aerobic exercise training, whether the leukocyte epigenome is reprogrammed by RET is currently unknown.

Considering the health and strength benefits elicited by RET, the purpose of this investigation was to analyse the leukocyte genome-wide DNA methylation and gene expression after 8 weeks of resistance training in healthy young men. We hypothesized that RET would cause DNA methylation and transcriptional changes to genes involved in cellular growth and inflammation leading to improvements in strength.

Methods

Subjects

Eight apparently healthy subjects gave written informed consent and were recruited for this study. Subjects were non-smokers and free from any chronic disease according to a self-administered Physical Activity Readiness Questionnaire (PARQ). The PARQ included questions on family medical history, smoking status, and current and previous medical conditions, including age-related cardiometabolic diseases, cardiac anomalies and disease risk factors (i.e. high blood pressure, uncontrolled blood glucose and hyperlipidaemia). Physical activity levels were assessed by the self-administered International Physical Activity Questionnaire (IPAQ). Physical activity data were cleaned and analysed according to the IPAQ guidelines. The subjects were moderately active as indicated by weekly metabolic equivalent of task (MET)-minutes and estimated energy expenditure data outlined in Table 1. Subjects reported being recreationally active and were not currently engaging in any structured aerobic or resistance exercise training, but were otherwise healthy. Subjects' height, weight and body mass index were recorded. Resting blood pressure was averaged over three measurements recorded after an intermittent 1 min rest using an electronic blood pressure recorder (Microlife BP). This study was approved by the Federation University Australia's Human Research Ethics Committee.

Table 1 Characteristics before and after 8 weeks of resistance exercise training

Characteristics ($n = 8$)	Before RET	After RET	% Change	p value
Age (years)	21.1 ± 2.2			
Height (cm)	177.4 ± 4.7	177.5 ± 4.9	0.03 ± 0.3	0.73
Weight (kg)	73.6 ± 10.4	75.0 ± 8.2	2.2 ± 5.9	0.42
BMI (kg/m^2)	22.5 ± 2.0	23.8 ± 3.0	2.2 ± 5.6	0.07
SBP (mmHg)	127.6 ± 10.7	121.2 ± 9.5	−3.1 ± 4.1	0.11
DBP (mmHg)	74.7 ± 3.9	77.5 ± 18.7	−1.5 ± 8.7	0.70
MAP (mmHg)	90.3 ± 2.7	88.3 ± 9.2	−2.3 ± 4.7	0.21
PP (mmHg)	42.5 ± 3.1	49.4 ± 6.3	−4.3 ± 14.9	0.38
Resting HR	78.5 ± 15.4	74.4 ± 17.8	−9.7 ± 14.6	0.56
Waist girth (cm)	80.9 ± 9.3	79.7 ± 8.5	−1.4 ± 4.5	0.37
Hip girth (cm)	88.8 ± 9.1	85.5 ± 10.7	−3.5 ± 8.8	0.27
Waist/hip ratio	0.91 ± 0.06	0.93 ± 0.07	2.8 ± 9.2	0.43
Waist/height ratio	0.46 ± 0.02	0.45 ± 0.05	−1.4 ± 4.4	0.39
$\sum$ 7 skinfolds (mm)	109.9 ± 54.4	112 ± 49.9	5.22 ± 19.6	0.75
Body fat (%)	13.3 ± 5.1	14.6 ± 6.0	5.0 ± 19.4	0.30
Physical activity				
Physical activity level (MET-min/week)	2802.7 ± 1448.5			
Estimated energy expenditure (Kcal/week)	3512.1 ± 1955.3			
Strength (1RM)				
Squat	71.6 ± 10.6	106.9 ± 13.9	50.6 ± 17.9	**4.1×10^{-5}**
Bench press	52.8 ± 13.0	64.4 ± 11.6	24.3 ± 16.4	**0.001**
Bench pull	55.9 ± 11.5	63.7 ± 11.6	15.0 ± 13.2	**0.01**
Deadlift	67.8 ± 11.3	101.6 ± 14.3	51.4 ± 18.6	**6.5×10^{-5}**
Relative strength (1RM/weight)				
Squat	0.98 ± 0.16	1.44 ± 0.21	47.7 ± 19.4	**1.02×10^{-4}**
Bench press	0.72 ± 0.17	0.86 ± 0.13	22.2 ± 20.9	**0.001**
Bench pull	0.76 ± 0.12	0.85 ± 0.13	12.4 ± 11.5	**0.01**
Deadlift	0.92 ± 0.14	1.36 ± 0.15	48.1 ± 16.2	**4.4×10^{-5}**

Data are expressed as mean ± SD from paired samples *t* tests

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic BP, *MAP* mean arterial pressure, *PP* pulse pressure, *HR* heart rate, $\sum$ sum of, *1RM* one repetition maximum

Strength testing and RET program

On an occasion separate from the initial assessment, participants' upper and lower body strength was assessed by one repetition maximum (1RM) tests. A brief (5–10 min) aerobic warmup was completed before 1RM testing for each of the squat, bench press, bench pull and deadlift exercises (completed in that order). Before attempting the 1RM for each exercise, participants completed 10, 4 and a single repetition at the estimated 50, 70 and 90 % of 1RM, respectively. The final 1RM tests were conducted 3–4 days after the final RET session.

The initial RET programme included three sets of eight repetitions of the squat, bench press, bench pull and deadlift exercises, at 80 % of 1RM. The training load of each exercise was increased when participants could comfortably complete three sets of 12 repetitions. Participants were instructed to perform the concentric and eccentric phase of each exercise over 1 and 2 s, respectively. A 1.5- to 2-min rest was allocated between sets. Participants trained three times per week for a total of 8 weeks on alternate days to allow 72 h recovery before the next session. Exercise scientists supervised all training sessions. Subjects were asked to adhere to their regular diet throughout the course of the RET intervention.

Blood processing

Subjects donated a resting blood sample before and after 8 weeks of RET. All blood samples were collected from participants in the morning following an overnight fast. The final blood collection was obtained 72–96 h after the final RET session. Briefly, circulating blood was drawn from the antecubital vein into EDTA tubes with participants in a seated position. Immediately after blood collection, DNA and RNA were extracted from whole blood leukocytes

using the Purelink Genomic DNA Mini Kit (Life Technologies, Australia) and miRNeasy Mini Kit (Qiagen, Australia), respectively, according to each manufacturer's guidelines.

Genome-wide DNA methylation analysis

Genome-wide DNA methylation was quantified using an Infinium HumanMethylation450 BeadChip (Illumina, Australia) according to the manufacturer's guidelines, as previously described (Denham et al. 2015a, b). Briefly, DNA quality was assessed by electrophoresis before bisulphite treatment, to convert unmethylated cytosines to uracils. The BeadChip's fluorescence was imaged on the Illumina iScan and data were exported to GenomeStudio Methylation Module where individual CpG site methylation was calculated. All samples quantified on the BeadChip passed the GenomeStudio Methylation Module's quality control procedure and raw β-values were transferred to Partek (Genomic Suite, Singapore). Subset-quantile Within Array Normalization was applied and all β-values were log-transformed into M-values using the logit function, which reduces the heteroscedasticity of β-values. β-values were included in tables and graphs for ease of data interpretation as they correspond to the percentage of DNA methylation.

Genome-wide gene expression analysis

The HumanHT-12 v4 Expression BeadChip (Illumina) was used to determine the abundance of 99.9 % of refseq genes with over 47,000 probes. Of these, 20,000 transcript analysed on the Expression BeadChip are the same of those analysed for CpG methylation on the 450K BeadChip. Briefly, the RNA sample integrity was assessed on an Agilent BioAnalyzer before samples were amplified and hybridized to the Expression BeadChip. Fluorescent signals were scanned on the Illumina iScan and after subtracting the Chip background signal, expression data were obtained using the GenomeStudio Gene Expression Module (Illumina). Genome-wide gene and DNA methylation were analysed using the statistical software, Partek (Genomic Suite, version 6.6), and pathway analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (da Huang et al. 2009).

Statistical analysis

Participant phenotypes were assessed for normal distribution using Kolmogorov–Smirnov and Shapiro–Wilks tests. Parametric data are expressed as mean ± SD or otherwise detailed. Non-parametric data was log-transformed before further analyses. Two-tailed paired samples *t* tests were used to identify statistically significant changes ($p < 0.05$) to phenotypes and strength after 8 weeks of resistance training. DNA methylation changes caused by RET were determined by a two-way ANOVA, χ^2 tests and Wilcoxon signed-rank tests as previously described (Denham et al. 2015a, b). Expression BeadChip data was assessed using a paired *t* test on log-transformed data. Array data was analysed using Partek (Genomic Suite, version 6.6). To prevent type 1 error and considering our modest sample size, the statistical significance for genome-wide analyses was set at $p < 0.01$.

Results

Exercise compliance

All eight subjects successfully completed the 8-week RET program. All subjects completed a minimum of 20 out of a possible 24 scheduled training sessions and the average exercise compliance was 93.2 %.

Phenotypes

All participants successfully completed the RET intervention and significantly improved their upper and lower body strength (Table 1). Aside from a trend for BMI to increase ($p = 0.07$), no other phenotypes were influenced by the 8 weeks of RET (Table 1).

Genome-wide DNA methylation

57384 CpG sites were altered (0.1–27.2 %) after RET ($p < 0.01$). Of these, 28,397 showed increased methylation and 28,987 were demethylated after RET ($p < 0.01$, Fig. 1a, b, respectively). When assessed in relation to the closest gene region and CpG islands, CpG methylation was altered after RET ($p < 0.01$, Fig. 1c, d, respectively). While CpG sites in the promoter region, 5′ untranslated region and intronic regions were demethylated (Infinium II), exonic and intronic CpGs were further methylated (Infinium I) after RET ($p < 0.0001$, Fig. 1e). Infinium II north and south shores were demethylated, and Infinium I open sea CpGs were further methylated after RET ($p < 0.0001$, Fig. 1f). The genes with the largest DNA methylation changes (5 %) are listed in Supplementary Table 1 ($p < 0.01$). DAVID pathway analysis was conducted on differentially methylated CpGs (at least a 2 % change) and revealed that genes were enriched for numerous signalling pathways including cell adhesion molecules, cancer, type I and II diabetes, and axon guidance (Fig. 2a). Resistance exercise also altered CpG sites in a number of growth factors associated with anabolic signalling ($p < 0.01$, Fig. 2b).

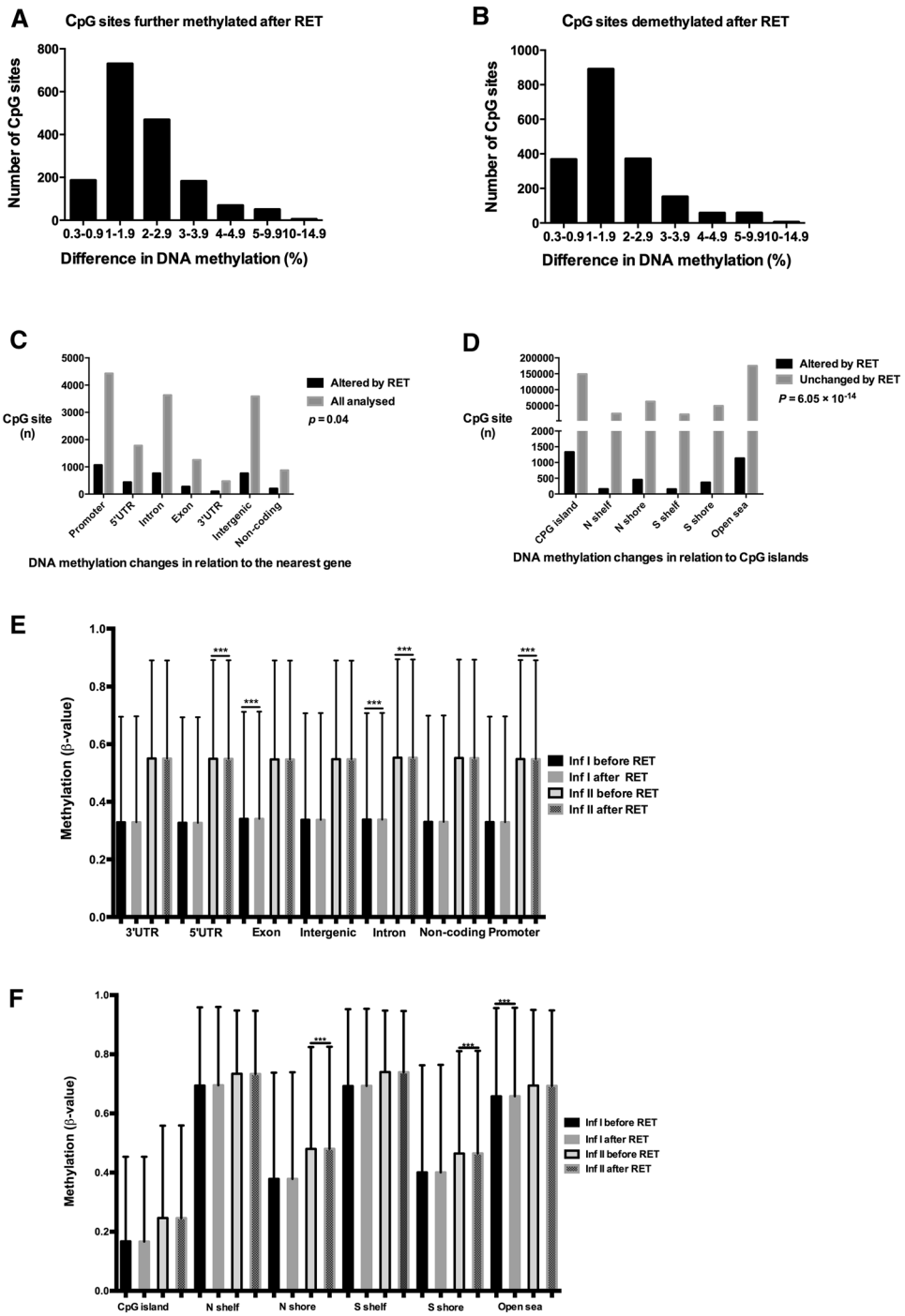

Fig. 1 Genome-wide DNA methylation changes caused by resistance training. The magnitude of change and number of CpG sites further methylated and demethylated by RET are displayed (**a**, **b**, respectively). RET induced genome-wide DNA methylation changes in context with genomic regions (**c**) and CpG islands (**d**). Data are from χ^2 tests. The genome-wide DNA methylation changes that occurred in relation to genomic locations (**e**) and CpG islands (**f**) for Infinium I and II assays are outlined. Data are from Wilcoxon signed-rank tests and are expressed as mean ± SE. *RET* resistance exercise training, *3′UTR* three prime untranslated region, *5′UTR* five prime untranslated region, *Inf* infinium, *TSS200* 200 bases upstream of the transcription start site; 1500 bases upstream of the transcription start site; ***$p < 0.0001$

Genome-wide gene expression

There were 495 differentially expressed transcripts after 8 weeks of RET ($p < 0.01$). Of these, 280 were up-regulated and 215 were down-regulated compared to pre-RET levels ($p < 0.01$). The up- and down-regulated genes with the largest changes (fold change >1.15) after RET are listed in Supplementary Table 2. The functional annotation analysis for up- and down-regulated transcripts after RET ($p < 0.01$) are displayed in Fig. 3a, b, respectively. While genes with increased expression after RET were associated with alternative splicing and RNA processing, genes with decreased expression related to differentiation, lysosome, spermatogenesis and cytoplasmic regulation (Fig. 3a, b).

Growth hormone-releasing hormone (*GHRH*) and fibroblast growth factor 1 (*FGF1*), growth factors important

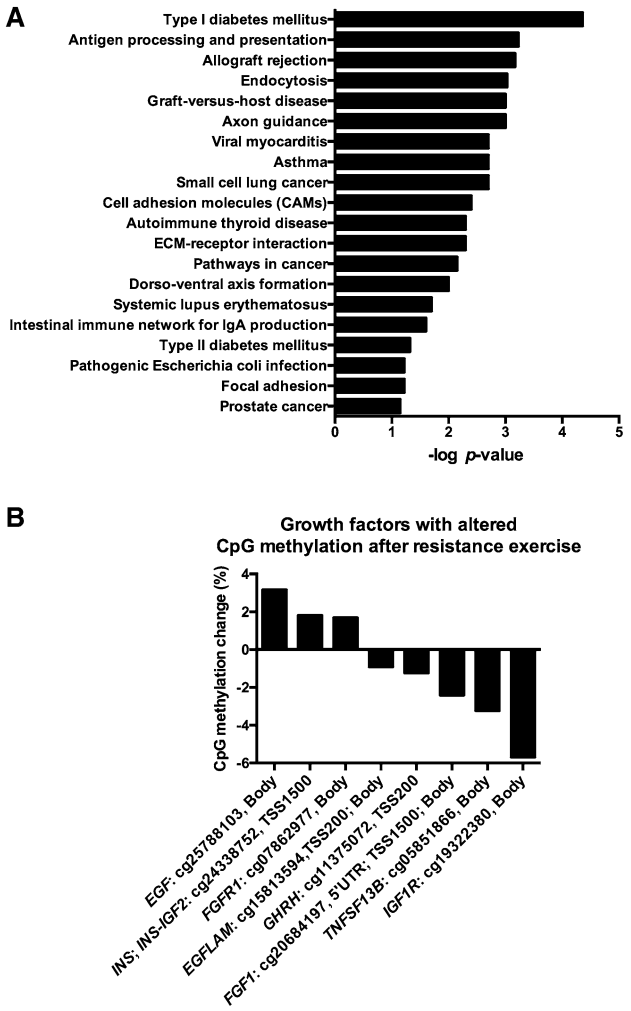

Fig. 2 Pathway analysis on differentially methylated CpG sites caused by resistance training. **a** Pathway analysis on genes with methylation changes (>2 %) caused by resistance exercise training. Negative log-transformed enrichment *p* values for KEGG pathways are displayed. Pathway analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7. **b** Growth factors with altered CpG methylation caused by resistance exercise training. *RET* resistance exercise training, *EGF* epidermal growth factor, *INS* insulin, *IGF2* insulin-like growth factor 2, *FGFR1* fibroblast growth factor 1 receptor 1, *EGFLAM* EGF-like, fibronectin type III and laminin G domains, *GHRH* growth hormone-releasing hormone, *FGF1* fibroblast growth factor 1, *TNFSF13B* tumour necrosis factor (ligand) superfamily, member 13b, *IGF1R* insulin-like growth factor 1 receptor, *5′UTR* five prime untranslated region, *TSS200* 200 bases upstream of the transcription start site, 1500 bases upstream of the transcription start site

for anabolic signalling, exhibited differential mRNA expression and demethylated CpG sites (cg11375072, −1.2 % and cg20684197, −2.4 %, respectively) after RET, indicating that these genes had undergone epigenetic reprogramming.

Discussion

This study determined the leukocyte DNA methylation and transcriptomic responses to 8 weeks of resistance exercise training in healthy young men. We are the first to show that resistance exercise training significantly increases muscular strength and concurrently reprograms the leukocyte methylome to impact genome-wide gene expression. DNA methylation changes occurred throughout the genome in relation to CpG islands and genomic regions, and in particular genes important for anabolic signalling and numerous other biological pathways. We also provide the first evidence of leukocyte transcriptomic regulation induced by 8 weeks of RET in healthy young men and demonstrate that particular mRNA expression is reprogrammed via DNA methylation.

Resistance exercise training elicits numerous health benefits and is particularly effective in improving insulin sensitivity, lean body mass, muscle hypertrophy and strength (Braith and Stewart 2006). The underpinning molecular mechanisms responsible for these favourable effects are only beginning to be explored. Epigenetic modifications programme gene expression regulated by the internal and external environment. Evidence that exercise training modulates the epigenome of multiple tissues is accumulating, but aerobic exercise has been the primary focus of previous research (Denham et al. 2014). For instance, 6 months of moderate intensity aerobic exercise training significantly reorganizes the skeletal myocyte (Nitert et al. 2012) and adipocyte (Ronn et al. 2013) DNA methylome. Three months of isokinetic knee extension exercise altered genome-wide DNA methylation and gene expression in the skeletal muscle of sedentary volunteers (Lindholm et al. 2014). In an integrated analysis of the skeletal muscle methylome, transcriptome and proteome, resistance exercise training caused unique DNA methylation, gene and microRNA expression changes compared to aerobic exercise in obese middle-aged Polynesian individuals with type 2 diabetes, emphasizing the mode-specific effect exercise has on epigenetic reprogramming and corresponding regulation of gene and protein expression (Rowlands et al. 2014). We previously found that shorter (4-week) high-intensity interval training intervention significantly modulated the leukocyte methylome and improved cardiorespiratory fitness in healthy young men (Denham et al. 2015b). Consistent with previous reports in skeletal muscle (Rowlands et al. 2014), the amount of CpG sites differentially methylated and the magnitude of methylation changes observed in the present study were not as marked compared to those revealed in leukocytes after aerobic exercise training (Denham et al. 2015b). Our data, however, supports the premise that resistance exercise training can influence the

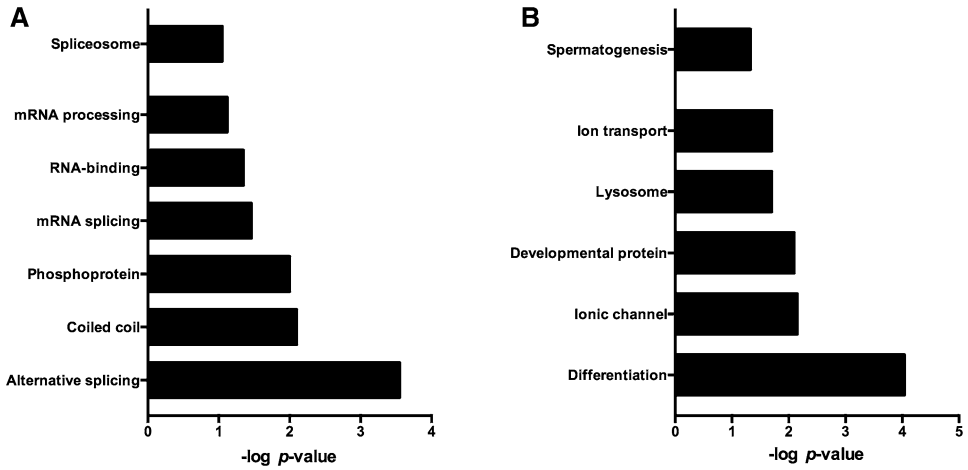

Fig. 3 Functional annotation analysis on differentially expressed genes after resistance training. Functional annotation analysis on up- and down-regulated genes (**a**, **b**, respectively). Negative log-transformed enrichment *p* values for KEGG pathways are displayed. Functional annotation analysis was conducted using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7

leukocyte methylome, though not as significantly as aerobic exercise training.

Genes with differentially methylated CpG sites after resistance exercise training in the present study were involved in numerous biological pathways. Interestingly, genes in pathways such as those involved in type 1 and 2 diabetes, calcium signalling and axon guidance were differentially methylated after resistance training. Given the hypertrophy and strength-specific adaptations conferred by resistance training and the marked increase in muscular strength observed in subjects involved in our study, it is possible that these pathways may be important regulators. We also found many overlapping pathways effected by resistance training that were modulated by high-intensity aerobic training (focal adhesion, extracellular matrix receptor interaction, pathways in cancer, dilated cardiomyopathy and calcium signalling, amongst others), which is not unexpected given some favourable health benefits conferred by resistance training are comparable to those induced by aerobic training (e.g. improved insulin sensitivity, glucose and blood pressure control, reduced low-grade inflammation). Alternative genes involved in RNA splicing and other processes were up-regulated in the functional annotation analysis, but down-regulated genes were involved in cellular differentiation, lysosomal activity, development and cytoplasmic restructuring. Therefore, resistance training seems to cause alterations to the DNA methylome and transcriptome in unique pathways and functional categories and to some of which overlap with those regulated by aerobic exercise.

Leukocytes are implicated in the inflammatory repair process of damaged skeletal muscle (Tidball and Villalta 2010; Tidball 2005), and as such the genome-wide methylation and transcriptomic changes observed in the present study may be reflective of adaptations gained from resistance training that improve muscle repair, hypertrophy and strength. Macrophages infiltrate damaged muscle caused by exercise-related muscle contractions and activate a pro-inflammatory state through the NF-kB pathway to facilitate skeletal muscle repair (Pillon et al. 2013). We found growth factors known for anabolic effect on muscle differentially methylated after resistance exercise training (Fig. 2b). In particular, insulin (INS), insulin growth factor 1 receptor (IGF1R) and growth hormone-releasing hormone (GHRH) are potent anabolic proteins that showed altered methylation after resistance training. Consistent with the reprogramming of growth factors after resistance training, we found that *GHRH* and *FGF1* were demethylated concurrently with mRNA expression changes. While GHRH is secreted by the hypothalamus to increase growth hormone concentrations, FGF1 increases satellite cell numbers by encouraging proliferation. Although the biological significance of the differential expression and methylation of these growth factors in leukocytes is unclear, these data suggest that resistance training reorganizes the leukocyte DNA methylation and changes gene expression in growth factor genes, with a role in muscle repair and proliferation.

Although the transcriptional responses to resistance training in skeletal muscle are well documented (Liu et al. 2010; Raue et al. 2012; Hulmi et al. 2009; Stepto et al. 2009), the impact of resistance training on leukocyte genomic changes is a relatively unexplored area that deserves attention. Habitual resistance training was associated with lowered leukocyte toll-like receptor 4 expressions of older adult postmenopausal women (McFarlin et al. 2004). More recently, the influence of a single 30-min bout of resistance exercise training on the peripheral blood mononuclear cell (PBMC) transcriptome was reported (Carlson et al. 2011). Similar to the effect of aerobic exercise training (Radom-Aizik et al. 2008; Connolly et al. 2004; Buttner et al. 2007; Thompson et al. 2010), a bout of resistance training had a marked influence on the peripheral blood mononuclear cell transcriptome, such that 167 genes were differentially expressed 2 h after the cessation of

exercise in genes relating to immune diseases and cell signalling pathways (Carlson et al. 2011). Whereas the gene expression changes elicited by a single bout of resistance training reflect cellular stress and signalling events that provide insight into growth and recovery, our novel data present the expression profile caused by cumulative repeated exposure to resistance training and is reflective of strength and hypertrophy adaptation that seems to be somewhat governed by epigenetic reprogramming.

The DNA methylation reprogramming found in our study could be due to modulated DNA methyltransferase (DNMT) enzyme activity. Whereas DNMT1 is responsible for maintaining DNA methylation patterns during DNA replication (Schermelleh et al. 2007), DNMT3A and DNMT3B are crucial for embryonic development and facilitate de novo methylation modifications (Okano et al. 1999). We did not quantify DNMT3A/B protein expression or enzyme activity due to financial constraints. We did, however, find decreased *DNMT3B* mRNA expression after resistance training, which could partly explain the DNA methylation changes observed in our subjects. Alternatively, the Tet methylcytosine dioxygenase proteins (TET-1, -2, -3) convert 5-methylcytosine to 5-hydroxymethylcytosine through an oxidative process (Tahiliani et al. 2009). 5-hydroxymethylcytosine is not recognized by DNMT1 during replication (Inoue and Zhang 2011) and this could lead to passive demethylation during replication occurring over the 8-week study intervention. 5-Hydroxymethylcytosine can also be further oxidized to form 5-formylcytosine and 5-carboxylcytosine and removed by the base excision repair pathway (He et al. 2011). The molecular mechanisms regulating resistance training-induced DNA methylation changes will no doubt require future study.

There are some limitations associated with our study. Firstly, we were unable to determine if some of the DNA methylation changes could have been due to subset shifts or differential leukocyte counts, because these were not performed. Diet also influences DNA methylation (Anderson et al. 2012) and was not recorded in this study. Therefore, we were unable to account for any dietary influences on the observed changes to leukocyte DNA methylation and gene expression. The modest sample size included in our study limited our statistical power and no CpG sites or mRNA expression survived false discovery rate correction (q value). However, we believe our preliminary data are grounds for future studies to explore epigenetic modifications in context with resistance training with more robust numbers of participants.

Conclusions

In summary, we have provided evidence that 8 weeks of resistance training modulates the leukocyte DNA methylome and transcriptome in genes relating to growth factors and numerous biological pathways that may underpin the favourable health adaptations in chronic training. The time course and signalling mechanisms governing these epigenetic changes and what effects these have on other phenotypes remain topics for future studies.

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Compliance with ethical standards

Conflict of interest None declared.

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