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Perspectives

PERSPECTIVES

Skeletal Muscle Possesses an Epigenetic Memory of Exercise: Role of Nucleus Type-Specific DNA Methylation

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A Perspective on "Nucleus Type-Specific DNA Methylomics Reveals Epigenetic "Memory" of Prior Adaptation in Skeletal Muscle"

The recent paper in *Function* by Wen et al.,[1] adds exciting new knowledge to earlier evidence that human skeletal muscle possesses an epigenetic memory of exercise.[2–4] These works demonstrated retained methylation signatures on the DNA from skeletal muscle tissue following earlier training-induced muscle hypertrophy, that was maintained even during detraining when exercise had completely ceased, and lean mass had returned to pre-exercise levels. For a subset of genes, these observations corresponded with gene expression also being preserved during detraining. Further, other gene sets demonstrated enhanced methylation profiles into later retraining and even larger gene expression after encountering an earlier training period.[3] Using a sophisticated model of exercise in mice, the new study by Wen et al., undertook the same methodological design using a training, detraining, and retraining model together with bisulfite and RNA-sequencing for genome wide DNA methylation and gene expression respectively, with the primary aim to investigate if the muscle demonstrated retained epigenetic signatures after training, even when exercise ceased during detraining. Importantly, authors also undertook an elaborate set of additional advanced "nuclei labelling" experiments in mice to provide an important extension of this field by examining DNA methylation and gene expression in both the myonuclei and interstitial nuclei. This was compared to the earlier human studies in muscle "homogenates" (that assessed DNA from all muscle derived cell nuclei present in a muscle tissue biopsy). This is an important extension to this research area, given that it is unknown as to the predominant contribution towards the retention of epigenetic information from earlier exercise or growth, that is, if epigenetic memory is originating in the muscle fibres themselves or if other cells within the muscle niche are also important retainers of epigenetic modifications. Indeed, Wen et al. identified that promoter hypomethylation slightly outweighed hypermethylation signatures in myonuclei after training, and promoter hypomethylation was also predominant in interstitial nuclei, with these data like those signatures observed in human muscle tissue after the first training period.[3] Indeed, exercise as a "hypomethylating stimuli" has also been supported by studies using different types of exercise (not exclusively resistance exercise), such as sprint interval running exercise [5] and at the candidate gene level after aerobic exercise.[6] Despite this general similarity, the hypomethylated signatures seems to be enriched in divergent gene pathways between exercise types, for example, in growth, actin and ECM related pathways following resistance exercise,[4] compared with MAPK, AMPK, and Insulin signalling after running exercise.[5] At the pathway level in myonuclei in the present study there was enriched hypomethylation of promoter CpGs particularly in Wnt signalling and growth-related pathways, with hypomethylation and upregulation of gene expression in growth-related pathways also observed after resistance exercise in humans.[4] Interestingly, Wen et al., also identified differential regulation of the Wnt pathway in interstitial nuclei, where they demonstrated opposite profiles to myonuclei, that is, hypermethylation of Wnt pathway genes in interstitial compared with hypomethylation in the myonuclei. This demonstrates the importance of such experiments differentiating between nuclei of different muscle derived cell types to determine which pathways are epigenetically regulated and

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contribute to muscle memory after exercise training in the muscle fibre itself.

Importantly, Wen et al., was able to confirm the overarching conclusion from the earlier human studies, that there was a retention of methylation signatures following training into the detraining period, even when the exercise had ceased, suggestive of an epigenetic memory. The authors identified that the retention of hypomethylated signatures were predominant in the interstitial nuclei and the retention of hypermethylation signatures were mainly retained in the myonuclei following training and into the detraining period. In previous human studies, the authors took an approach of investigating the temporal profile of epigenetic changes in the top 500 most significantly differentially methylated CpG sites. In these human sites the predominant profile was retention of hypomethylation which was like the profile observed in interstitial nuclei, but not the myonuclei, in the present study in mice. It is however possible, that in the human studies other CpG sites (still significantly regulated just not as statistically highly ranked as the top 500 sites) may have demonstrated retention of hypermethylation. Furthermore, the new Wen et al., study also identified sites that had retained hypomethylation, these were just fewer in number. These are therefore interesting and subtle differences that require further bioinformatic/comparative analyses between the original human studies and the latest mouse studies. Furthermore, the new and exciting insights from Wen et al., demonstrating differential retention of methylation in myonuclear versus interstitial nuclei is of high interest and requires investigation in humans by separation of different cell types/nuclei from the biopsy tissue.

One other important difference between the human and mouse studies was the exercise performed. PoWeR in mice is a model of weighted wheel running and is associated with an increase in size of the plantaris with a shift towards an oxidative phenotype.[7] While fast/slow phenotype was not investigated in the human study the participants trained for hypertrophy using a progressive resistance exercise of a higher load (four sets of 8–10 repetitions at 80% of one repetition maximum /1RM), and was therefore perhaps more likely to demonstrate a more predominant glycolytic phenotype in the quadriceps. Subsequently, this may affect whether the same gene pathways and individual sites would be identified between the human and mouse studies. Despite this, an initial crude, but new comparison of data between the mouse and human study, suggests 26% and 28% of the annotated genes differentially methylated in mice after PoWER training in myonuclei and interstitial nuclei respectively, also demonstrated differential methylation in the same genes after resistance exercise in the muscle tissue of humans. Suggesting that there may be common exercise induced differentially methylated genes following resistance type training, irrespective of the intensity of the loading regime, across species.

It is important to also highlight that this latest paper in *Function*, is still one of a small number of studies to use bisulifte sequencing in skeletal muscle following resistance type training, which can also provide insights to DNA methylation profiles in ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) compared with microarray technology. Indeed, recent papers using the similar methods also identified that resistance exercise differentially methylated rDNA [8] and can hypomethylate the aged mitochondrial methylome.[9] However, one limitation of the methodology used includes the use of low-coverage reduced representation bisulfite sequencing (RRBS), rather than traditional RRBS or whole genome bisulfite sequencing (WGBS), which may have been a confounding factor between the lack of agreement between methylation and gene expression data and perhaps why more differentially methylated regions (DMR) were not identified. However, WGBS is currently prohibitively expensive and requires a larger input of DNA, which is not always possible, especially in small human biopsy samples or when separating DNA from different populations of cells or nuclei.

Finally, as Wen et al. correctly suggested, one way of determining an epigenetic memory was if there was retained epigenetic profiles into detraining from the initial training period, which is what the authors confirmed. However, Wen et al., were unable to directly compare epigenetic profiles between later retraining compared with earlier training due to various practical and financial implications of running these analyses. This was unfortunate as one of the other main outcomes of the Seaborne et al., study in humans identified another epigenetic memory signature where methylation was decreased after training and then further enhanced in the same direction during later retraining. For example, Seaborne et al., identified the cluster of genes (UBR5, RPL35a, HEG1, PLA2G16, and SETD3) to have this profile of hypomethylated (gene turned on) after training, a return to baseline methylation/gene expression during detraining, and then enhanced hypomethylation together with even greater gene expression following retraining.[3] Therefore, this epigenetic memory profile remained untested in the present study and would be important follow up in future studies using this experimental protocol, especially to identify if this memory profile occurs in the myonuclei and/or interstitial nuclei.

From these studies, one of the most pertinent future experiments for this research field, would be to separate out alterations of the methylome and transcriptome in the important regenerative “stem” cell in skeletal muscle, the satellite cell. Then compare this with data derived from the myonuclei in fibres before and after the fusion of satellite cells that occurs due to training, detraining, and retraining. This is because myonuclei are terminally fused into the myofibres (terminally differentiated). Therefore, for longer term epigenetic memory in muscle it may be the satellite cells (that can be activated and divide), that can pass these retained epigenetic modifications to future nuclear progeny. After all, it is the activated satellite cells that are fused/incorporated into the fibre as myonuclei during the repair or growth of muscle in later life, originally proposed in.[10] In the present study, in the interest of looking at the “resident” myonuclei, which were the myonuclei in the muscle fibres at the onset of training, the authors first labelled the myoncuclei at the beginning of the training. However, by the end of the training some satellite cells had fused into the fibre and those satellite cell derived myonuclei would therefore not be labelled and would be included in the interstitial nuclei fraction upon analysis (and not the myonuclear fraction therefore “contaminating” the interstitial fraction). So instead, the authors had another group where myonuclei were labelled at the end of the training to help avoid this contamination. While this can be commended to get a “pure” interstitial fraction, it then begs the question as to which cell type/nuclei within the interstitial fraction was driving the observation of retained hypomethylation profiles following detraining. Was it the nuclei from satellite cells or other non-muscle cell types such as fibroblasts and minority cell types such as endothelial cells, immune cells, neural/glial cells, and fibroadipogenic progenitors (FAPs)? Further, it raises an additional question of whether the satellite cells, that had fused into the fibres during training, contributed a greater or

lesser extent to the epigenetic profiles compared to the already present myonuclei, as the fused satellite cells during training could not be distinguished from the already present myonuclei. Overall, there is no doubt that this study published in *Function* brings the field closer to a better understanding of the mechanisms governing epigenetic muscle memory, and importantly provides insights into the next generation of research required on this exciting topic.

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Conflict of Interest Statement

None declared.

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