Transcriptomic Analysis of the Age-related Response to Endurance Exercise Training

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Abstract

BACKGROUND: Sarcopenia is the age-associated decline in muscle mass and strength. The most effective therapy is promotion of mitochondria function and content by exercising. Interrogation of the transcriptomic response to exercise may give insights into the underlying biology of this process and how this varies between individuals. Such findings would have the potential to improve current recommendations for exercise as well as assist with the development of new therapies for age-related muscle degeneration, improving the quality of life for an ever-increasing elderly population.
METHODS: Skeletal muscle biopsies were taken from six old individuals (71-77 years old) and six young individuals (19-21 years old) after different amounts of exercise; before exercise training (baseline), two hours after a single bout of exercise (2h) and 48 hours after 12 weeks of aerobic exercise training (trained). RNA sequencing was performed on these biopsies and we carried out differential expression analysis on the 2h and trained samples against baseline for both age groups. Our analysis included targeted investigation of biomarkers for changes in mitochondria biogenesis, content and function as well as Gene Ontology (GO) analysis.
RESULTS: We found a common transcriptional response to exercise in old and young individuals, however the young individuals had a larger number of differentially expressed genes (DEGs). The 2h response appeared to be distinct from the trained response, with a limited proportion of DEGs in common. The 2h response was characterised by up-regulation of mitochondria biogenesis and small down-regulation of mitochondria content, while the trained response involved up-regulation of mitochondria content and function. The old-2h response involved changes in pancreatic-related genes and cellular respiration, while the young-2h response mainly comprised repair and damage control processes and cytoskeleton and extracellular-space components. The old-trained response primarily consisted of up-regulation of mitochondria components, functions and processes. In contrast the young-trained response was much more varied with the most significant GO terms including extracellular components, mitochondria and muscle building functions and growth and immune response processes.
CONCLUSIONS: The transcriptional response to prolonged exercise training is distinct from the initial response to exercise and includes an upregulation of mitochondria content and function, indicating this training regime could be helpful in treating sarcopenia. However, the response to training in old individuals lacked the increased muscle building and immune response seen in young people which could limit the potential of this form of exercise in combatting sarcopenia.

Introduction

Motivation and Impact

This study aims to investigate how muscles respond to exercise in different age groups. Understanding the effects of physical activity is not only important for finding the best ways to improve general health, but is also vital for combatting degenerative muscle diseases such as sarcopenia. The word sarcopenia comes from the Greek, meaning *poverty of flesh* and refers to the progressive loss of muscle mass and strength. The condition is characteristic of the aging process, with a 3-10% loss of skeletal muscle mass every 10 years after the age of 25 (Short *et al.*, 2005). The loss of muscle mass is related to decreased strength and muscle quality, leading to incapacitation. The causes include changes in motor neurone numbers, hormonal status, nutrition and inflammatory mediators. (Doherty, 2003). In aging populations, with an increasing retirement age, muscle frailty presents a major societal problem and resulting disability has a significant impact on quality of life and activity in the community (Guralnik, 1996).

Aging and Mitochondria

Age-related decrease in aerobic capacity and muscle mass have been linked to declines in mitochondrial function (Johnson, Robinson and Nair, 2013). Therapies which improve mitochondrial function and content could potentially combat conditions including sarcopenia (Coyle and Holloszy, 1984). Currently, the most effective method of promoting mitochondrial content and respiratory capacity in skeletal muscle is exercise (Coyle and Holloszy, 1984), however the underpinning biological mechanisms of this are not well understood. The prevailing hypothesis is that decreased mitochondrial DNA (mtDNA) copy number and/or accumulation of mtDNA damage leads to the decline in mitochondrial function seen with age. This theory is under dispute as only 13 of the 500 genes which encode mitochondrial proteins are found within mtDNA (Johnson, Robinson and Nair, 2013).

A combination of mitochondrial biogenesis (mitobiogenesis), mitochondrial fusion (mitofusion) and mitochondrial fission (mitofission) regulate mitochondrial quality control and are thought to help avoid the accumulation of protein damage and facilitate maintenance of skeletal muscle health (Green, Galluzzi, and Kroemer, 2011). It is well established that exercise can improve the performance of these quality control processes and it is hoped this study will provide insight as to how exercise affects the transcriptional regulation of these processes, and how this regulation changes with age.

The Original Study

The data used for this investigation originates from published research by Konopka (2014). In this study, seven young males (20±1 years) and six old males (74±3 years) were investigated, with a variety of factors including BMI, diabetes, health and pharmaceutical use controlled for. Measurements of whole muscle size, aerobic capacity and content of mitochondrial proteins in muscle biopsies were taken for each subject. Participants completed a 12-week exercise regime of progressive aerobic training on a cycle ergometer matched in intensity between young and old groups according to heart rate reserve measurements. Following completion of the period of exercise they rested for 48 hours and then measurements were taken as before. At this point

western blots were prepared to show change in concentration of various mitochondrial proteins, with the researchers finding a significant increase of all proteins tested.

The authors recognise the limitations of using small sample sizes and propose a future extension with more participants. The sample size is very close to the minimum of n = 5 required by the British Journal of Pharmacology, which also requires randomization of samples during processing, a procedure which is not carried out in this study (Curtis, 2015). The investigation was further limited by focus on only a small number of proteins involved in mitochondrial proliferation and function as well as by the method used. A proteomics approach using Mass Spectrometry would allow a more extensive evaluation of a greater number of proteins and give more precise quantification compared to western blotting (Aebersold, 2013). Although a comprehensive investigation of proteins was lacking, an in-depth look at changes in gene expression was intended by performing RNA sequencing on muscle biopsies, however these data were not examined at the time. The analysis of this transcriptomics dataset is is the focus of this paper.

Methods

Data Processing and Initial Analysis

RNA sequencing was done on samples from all six old and six of the seven young individuals at three time-points and used for analysis of their transcriptomic response to exercise. The three time-points were; before any exercise (baseline), two hours after a single bout of exercise (2h) and 48 hours after completion of 12 weeks of aerobic exercise training (trained). The data were provided in the form of a matrix of Fragments per Kilobase of transcript per Million mapped reads (FPKM) for 22912 genes for all 36 samples. PCA was carried out using a log2 transformed FPKM matrix for an initial look at the structure of the data. The FPKM values were then extracted into four data frames for four differential analyses; old group baseline vs old group 2h, old group baseline vs old group trained, young group baseline vs young group 2h and young group baseline vs young group trained.

Un-targeted Differential Expression Analysis using NOISeq

Differential expression analysis was conducted in R using the bioconductor package NOISeq 2.22.1 (Tarazona *et. al.*, 2015). This package was chosen as it could analyse data in the form of FPKM values, while other packages such as limma and DESeq2 require raw count data. The noiseqbio function from this package was used which outputs a probability of differential expression and log2 fold-change for each gene. The default filtering option in noiseqbio was used to remove low-count genes and this disgared 45-49% of genes from each analysis. The probability is calculated by using the log2 fold-change between the conditions and the value of the difference between the conditions and comparing these to the noise distribution. Differentially expressed genes (DEG's) were defined as genes with a probability of more than 0.8, meaning the probability of differential expression was at least four times the probability of non-differential expression. After the DEG's for each group were determined, DEG's that were shared between groups were identified, and whether the DEG's were down-regulated or up-regulated was established. To assess the similarities in transcriptomic response between the

different groups, DEG's were extracted and imported into cytoscape 3.6.0 (Shannon *et al.*, 2003) to build a network showing which genes were significant in multiple groups.

Targeted Analysis of Biomarkers for Mitobiogenesis, Content, Function, and Dynamics

Fold-change analyses on biomarkers of mitochondrial biogenesis, content, function, and dynamics were conducted in R using the results of the NOISeq differential expression analysis. To assess age-related response to endurance exercise training the log2 fold-change in the expression of; peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1) as a marker of mitobiogenesis (Ghosh *et al.*, 2011); the metabolic enzymes Citrate synthase (CS) and cytochrome c oxidase, complex IV (COXIV) as markers of mitochondrial content (Vigelsø, Andersen, and Dela, 2014); β-hydroxylacyl Co A dehydrogenase (βHAD) and electron transport protein succinate dehydrogenase (SDH) as markers of mitochondrial metabolism (Heilbronn *et al.*, 2007, Larsen *et al.*, 2012); Mitofusin 1 (MFN1), Mitofusin 2 (MFN2) and optic atrophy protein-1 mitochondrial dynamin like GTPase (OPA1) as markers of mitofusion (Ishihara, 2004; Johnson, Robinson and Nair, 2013; Konopka *et al*., 2014); and Mitochondrial fission 1 protein (FIS1) as a marker of mitofission (Cipolat *et al.*, 2004; Konopka *et al.*, 2014) were investigated.

GO Analysis and Pathway Enrichment

The four result matrices from NOISeq (old/young vs post 2h/trained) were annotated with Gene Ontology (GO) terms and p-values were produced for each term present using R. The gene2go annotation dataset, obtained from the NIH FTP server was merged to each dataset so each gene and associated probability of differential expression were linked to corresponding GO Terms (NCBI, 2018). They were then filtered by GO Category (Component, Function and Process respectively) and contingency tables were created based on the significance of the probabilities of differential expression from the NOISeq matrices (a value of > 0.8 is deemed significant). Fisher's Exact Test was carried out for each term present and a p-value was generated and stored in a table for each dataset, split by GO Category. Tree maps representing significant GO Terms and phylogeny were produced using REVIGO, a web based service for visualising GO data, with default settings (Supek, F et al. 2011). The tree maps use hierarchical terms to group similar GO Terms which is indicated by the colour, with the size proportional to the relative adjusted p-values.

Results

Principal Component Analysis (PCA)

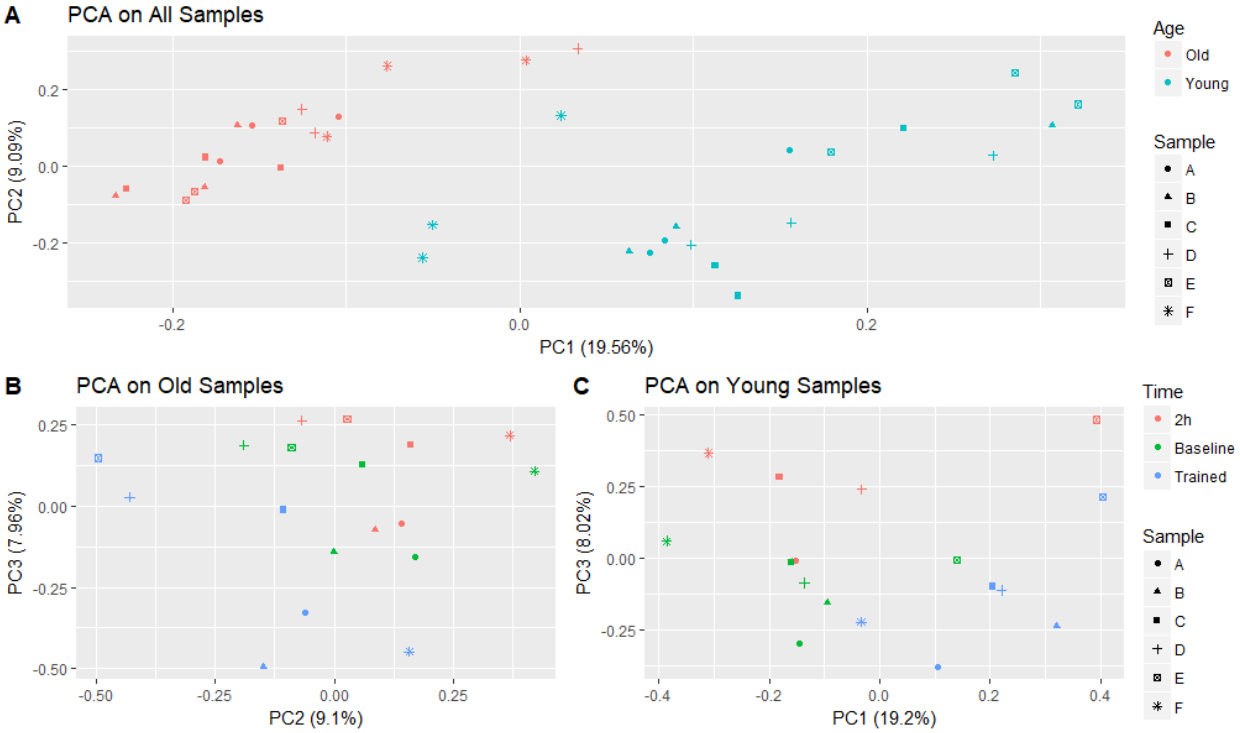

Figure 1: Principal component analysis on (A) all samples, (B) old-group samples and (C) young-group samples.

PCA on all samples shows a clear separation between the gene expression of the old group and the young group by PC1 and PC2, with the young group being more variable than the old group (Figure 1.a). To look at how samples vary at different time-points, PCA was performed on the old group (Figure 1.b) and young group (Figure 1.c) separately. The old group samples group by time best using PC2 and PC3. Although they do not form clearly defined clusters, each individual spreads approximately across PC2 and PC3 from the bottom-left corner to the top-right corner in the order of trained sample, then baseline sample, then 2h sample. Young samples seperate in a similar fashion from the bottom right corner to the top left corner of the PCA plot using PC1 and PC3. Although samples from different individuals behave differently, a general pattern of separation between trained samples and 2h samples, with the baseline samples between them can be seen from PCA. This could indicate the response to initial exercise (2h) is distinct from the response to prolonged exercise training.

Differential Expression Analysis

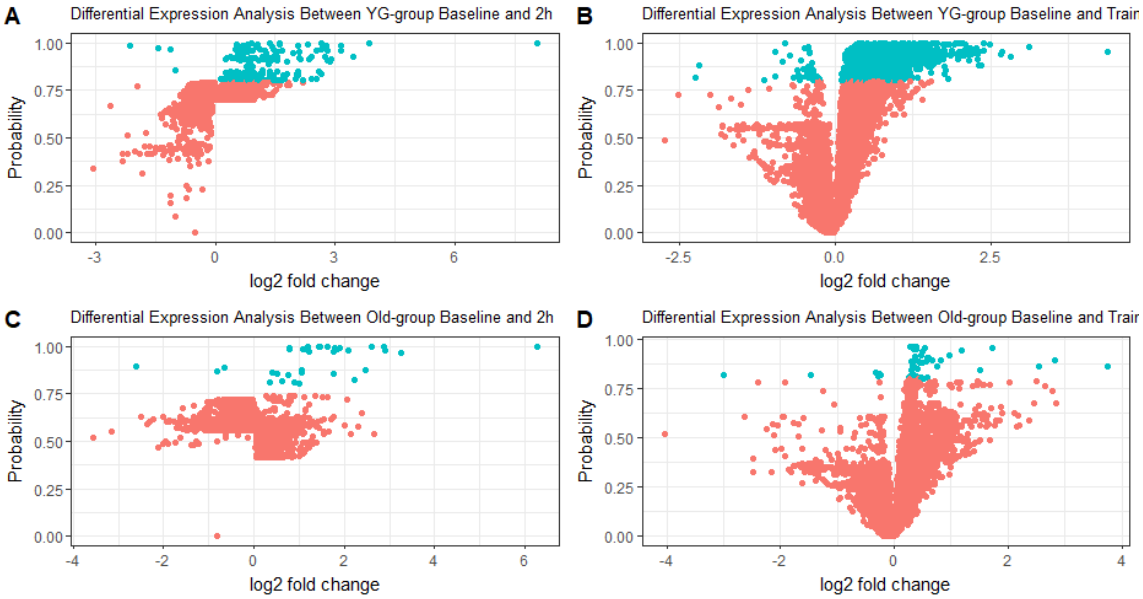

Figure 2: Differential expression of genes between (A) old-group baseline and old-group two hours, (B) old-group baseline and old-group trained, (C) young-group baseline and young-group two hours and (D) young-group baseline and young-group trained. Genes shown in blue have a probability above 0.8.

Figure 2 shows the probability and log2 fold-change of each gene for each differential analysis. There are 35 old 2h DEG's, 55 old trained DEG's, 251 young 2h DEG's and 1661 young trained DEG's. There are more DEG's in the young samples compared to old samples, and the same is true for trained samples compared to 2h samples. In addition there appears to be more genes being significantly up-regulated than down-regulated in all analyses.

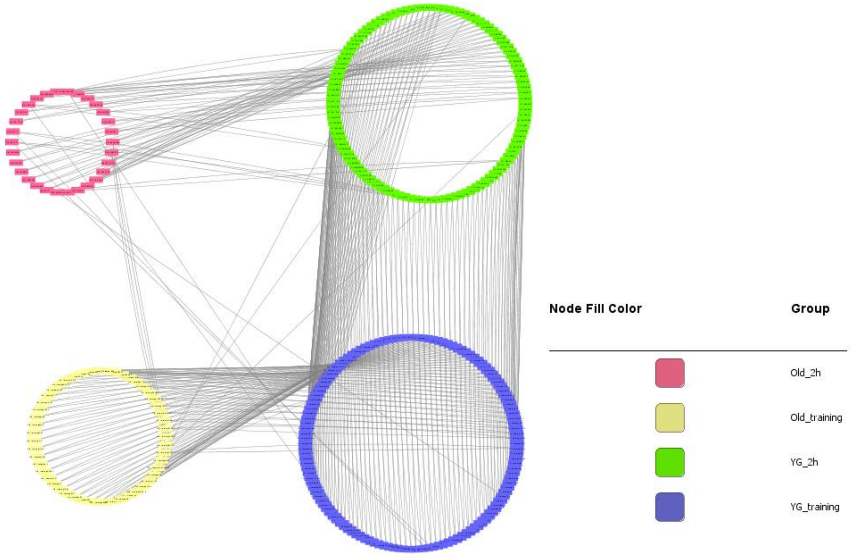

Figure 3: Network of significantly differentially expressed genes. Each node for each group is a gene with a probability of > 0.8 from differential analysis in noiseq. Each edge is a link between a significant gene in one group to the identical gene in another group, showing that gene is significantly differential expressed in both groups.

In Figure 3 the DEG's from all four analyses are depicted in the form of a network produced in cytoscape (Shannon *et al.*, 2003), with common DEG's joined by edges. 34% of young 2h DEG's are also DEG's for young trained, 5.7% of old 2h DEG's are shared with old trained, 83% of old 2h DEG's are DEG's for young 2h, and 89% of old trained DEG's are also young trained DEG's. This indicates the transcriptomic response between age-groups is similar for the same time-point, although the young group shows a bigger response with a larger number of DEG's. There is much less similarity in the response in trained samples compared with 2h samples.

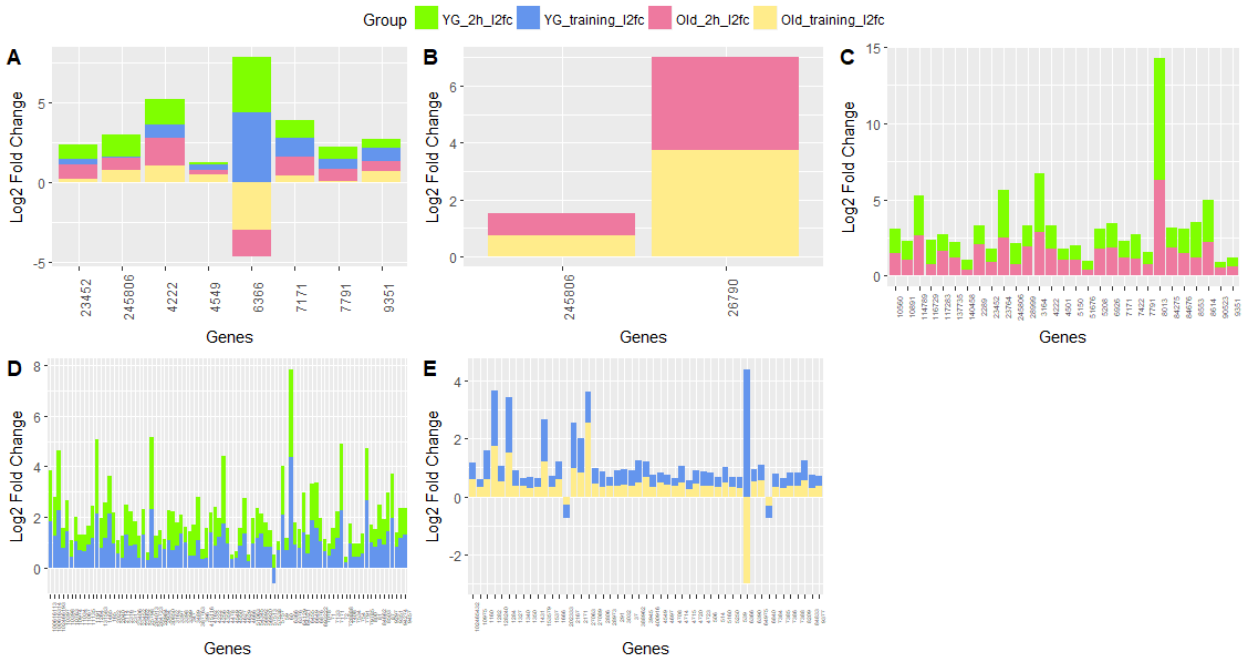

Figure 4: Log2 fold-change of DEGs shared between (A) three groups , (B) old 2h and old training, (C) young 2h and old 2h, (D) young 2h and young training and (E) young training and old training

To take a closer look at the DEG's that are shared between multiple groups, the log2 fold-change of common DEG's are represented in Figure 4. For almost all shared DEG's they are up-regulated in all groups, with a few shared DEG's down-regulated in both. This shows that the direction of change in these genes is the same for all groups where they are significantly differentially expressed. In addition, the magnitude of the change is similar for most of these DEG's. The only DEG's that responds differently in different groups is 6366, which is up-regulated in both young groups and down-regulated in both old groups, and 57538 which is up-regulated in young 2h but down-regulated in young trained. 6366 is the gene ID for CCL21, which functions as a cytokine. 57538 in the gene ID for ALPK3 which is a protein kinase involved in cardiomyocyte differentiation.

Targeted Analysis of Markers for Mitobiogenesis, Content, Function & Dynamics

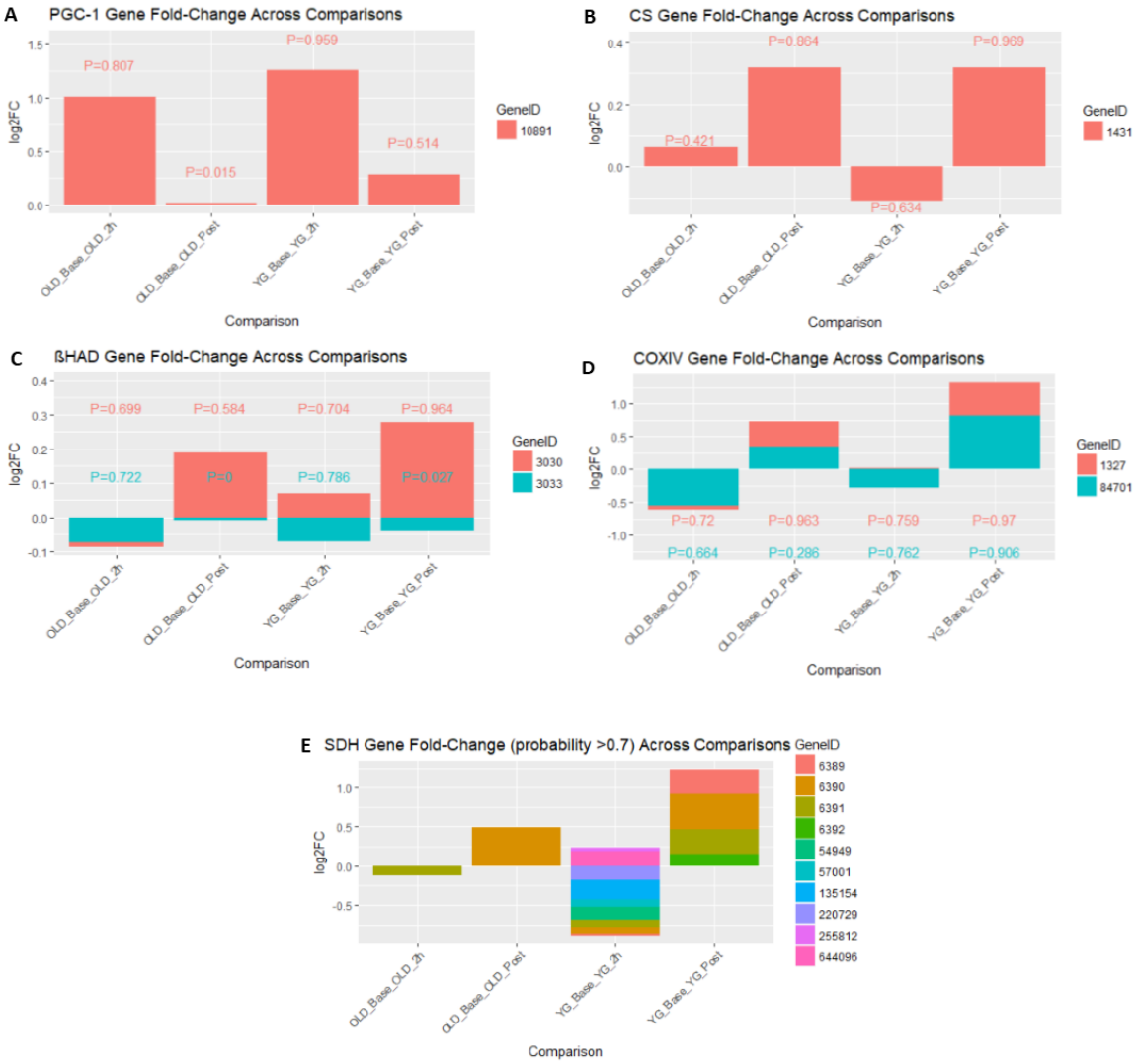

Figure 5: Figure 5: log2 fold-change in the expression of (A) PGC-1, (B) CS, (C) βHAD, (D) COXIV, and (E) SDH across each group compared to baseline. Where P is the probability of differential expression given by NOISeq

Figure 5 shows the log2 fold-change in the selected mitochondrial biomarkers of mitobiogenesis, content and function across all four analyses. Figure 5.A shows a significant increase (Probability > 0.8) in PGC-1 expression in both the young and old 2h groups and to a similar degree (log2FC 1.251, 1.005 respectively). PGC-1 is the primary regulator of mitobiogenesis (Ghosh et al. 2011) and as such is responsible for the generation of new mitochondria (Yan, Lira and Greene, 2013).

Figure 5.B shows a significant increase (Probability > 0.95) in CS expression in both the young and old trained groups and to the same degree (log2FC 0.319, 0.320 respectively). Mitochondrial metabolic enzyme, CS, is a valid biomarker for the density of mitochondria within skeletal muscle (Vigelsø, Andersen, and Dela 2014), and has been shown to correlate with

mitochondrial content (Larsen et al. 2012). Figure 5.D shows: a weak signal of down regulation in COXIV 84701 in both the young and old 2h groups (Probabilities: 0.664, 0.762, log2FC: -0.284, -0.550, respectively); a significant increase (Probability > 0.95) in COXIV 1327 expression in both young and old trained groups, with the young trained group showing a larger relative increase (log2FC 0.510, 0.380 respectively); and a significant increase (Probability > 0.9, log2FC 0.812) in COXIV 84701 expression in the young trained group. Levels of electron transport protein COXIV are commonly used as a marker of mitochondrial content (Picard et al. 2011), and correlate with mitochondrial oxidative phosphorylation capacity (Larsen et al. 2012).

Figure 5.C shows: a significant increase (Probability > 0.95, log2FC 0.277) in βHAD 3030 expression in the young trained group; and a very weak signal of up-regulation (Probability > 0.5, log2FC 0.188) in βHAD 3030 expression in the old trained group. βHAD encodes an enzyme which catalyses beta oxidation of fatty acids to acetyl Co-A within mitochondria (Flack et al. 2016), the expression in which has been shown to correlate with mitochondrial lipid metabolism (Heilbronn et al. 2007).

Figure 5.E shows: a weak signal of down regulation (Probability > 0.7, log2FC <-0.15) in expression of SDH genes 54949, 220729 and 135154 and of up regulation (Probability > 0.7, log2FC 0.183) in SDH 644096 in the young 2h group; a significant increase (Probability > 0.9) in SDH 6390 expression after in both young and old trained groups (log2FC 0.497, 0.446 respectively); and a significant increase (Probability > 0.9) in expression of SDH genes 6389 and 6391 in the young trained group (log2FC 0.318, 0.319 respectively). SDH is a mitochondrial electron transport protein shown to correlate with mitochondrial respiratory activity (Larsen et al. 2012).

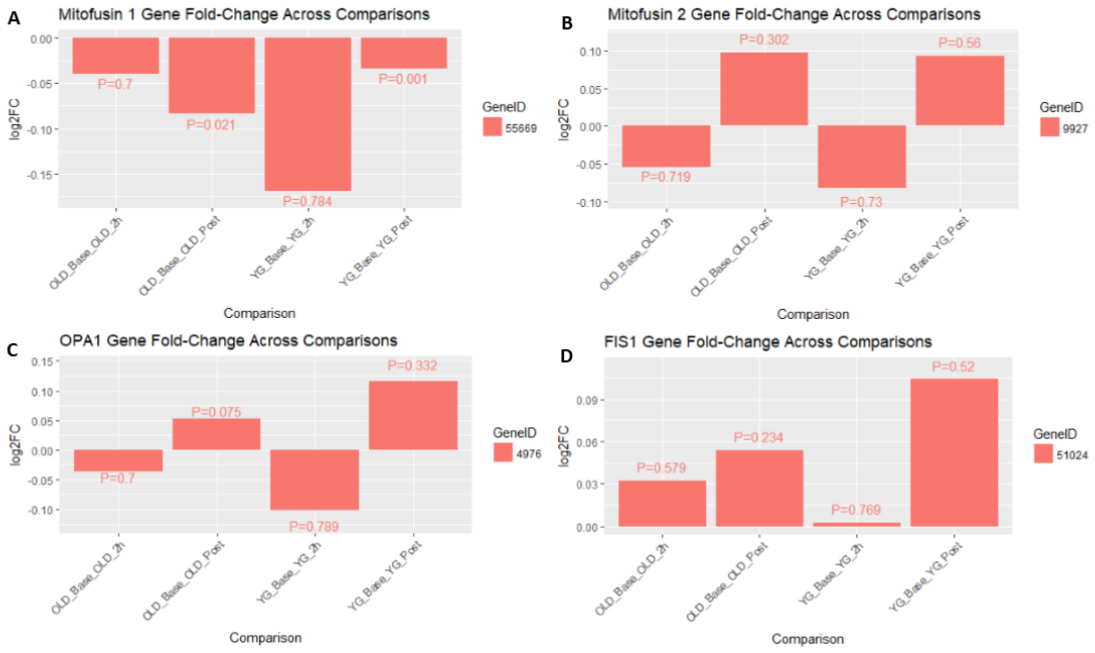

Figure 6: Log2 fold-change in the expression of (A) Mitofusin 1, (B) Mitofusin 2, (C) OPA1, and (D) FIS1 across each group compared to baseline. Where P is the probability of differential expression given by NOISeq

Figure 6 shows the log2 fold-change in the selected biomarkers of mitochondrial dynamics across all four analyses. Figure 6.A indicates a weak signal of down regulation (Probability > 0.7, log2FC -0.169) in MFN1 expression in the young 2h group. MFN1 is essential to the process of mitochondrial fusion (Ishihara 2004). Figure 6.B shows no apparent change across any comparison in the expression of MFN2, an important regulator of mitofusion (Johnson, Robinson and Nair, 2013). Figure 6.C suggests a weak signal of down-regulation (Probability > 0.7, logFC -0.102) in OPA1 expression in the young 2h group. OPA1 regulates fusion of inner mitochondrial membranes (Liesa and Shirihai 2013). Levels of OPA1 expression correlate with the extent of mitochondrial fusion (Cipolat et al. 2004). Figure 6.D shows no apparent change across any of our comparisons in the expression of FIS1 expression, which is involved in the process of mitfission (Cipolat et al. 2004).

GO Analysis and Pathway Enrichment

Presented here are graphs showing significant GO Terms for all groups and treemaps showing the significantly represented Biological Process Terms for old and young trained response (Figures 7-8 respectively). The response for the old group following a 2 hour session of exercise highlighted only three GO Terms, against a wider array from the young group largely consisting of transcriptional and translational activities. Comparatively the response after the training regime is more varied, in particular in the young-group. Further illustrations of all significant GO Terms can be found in the Appendix.

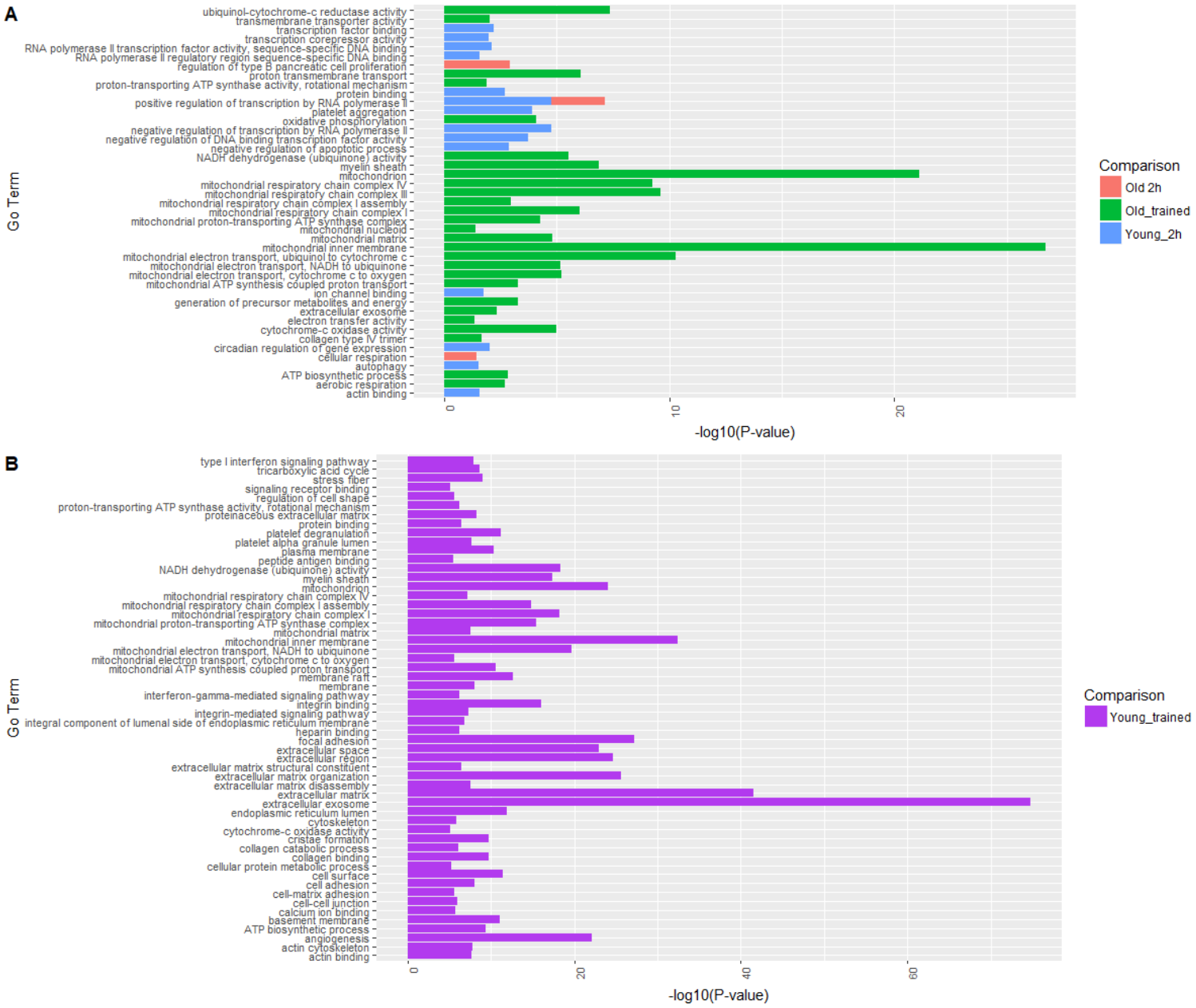

Figure 7: -log10 adjusted p-value for (A) all significant GO Terms for up and down regulated genes for the old 2h, old-trained and young 2h groups and (B) all GO Terms with -log10 adjusted p-value > 5 for up and down regulated genes for the young-trained group. N.B. scales differ between A and B.

Figure 7 shows the young response was significantly more varied and pronounced over all GO Terms for both after 2 hours of exercise and for after the training regime. The post exercise range of GO Terms demonstrates much more diversity and significance for both old- and young-groups.

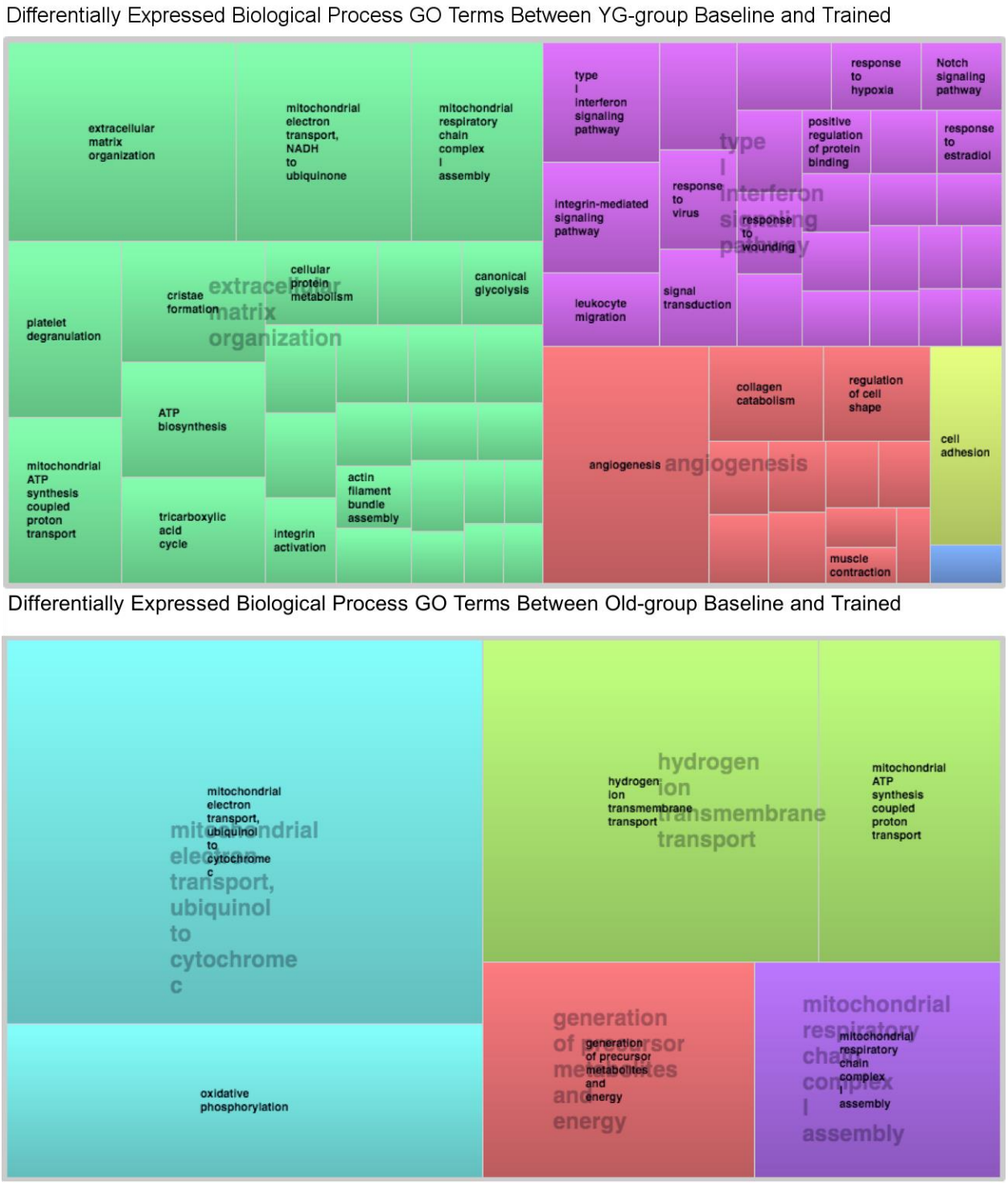

Figure 8: Treemaps showing all significantly differentially expressed GO Terms from the Biological Process Category. Size of the quadrangles represents adjusted p-value relative to others present.

Figure 8 demonstrates the Biological Process GO Terms are more differentiated in the young group than old. There is evidence of mitochondrial processes in both cohorts but the young group shows additional immunological responses, this could relate to skeletal muscle acting as a secretory organ, releasing peptides and cytokines (or “myokines”) to the wider body, aiding the immune system (Pedersen, 2013).

There are also aspects for muscle growth present such as angiogenesis, which is known to be up-regulated after exercise, via genes such as vascular endothelial growth factor (VEGF). This occurs in order to supply the muscle more effectively with oxygen (Bloor, 2005).

Discussion

How does the Transcriptional Response to Exercise Vary between the 2h and Trained Groups?

Comparisons of the results from differential expression analysis demonstrated minimal similarities between the 2h response and the trained response for both age groups. This leads to the conclusion that the response to a single bout of exercise is distinct from the response to prolonged exercise training, which agrees with multiple other studies investigating the response to exercise before and after training (Hinkley *et al*., 2017; Schmutz *et al.*, 2006).

Our targeted analysis of the 2h groups shows evidence of a significant up-regulation (Probability > 0.8) in markers of mitobiogenesis and weak evidence of down-regulation (Probability > 0.6) in markers of mitochondrial content. Other studies have found mitobiogenesis to increase following bouts of acute exercise (Menshikova et al., 2006). The transient increase in PGC-1 (log2FC > 1) which suggest increased mitobiogenesis, has also been demonstrated by similar studies 2h following acute exercise (Pilegaard, Saltin, and Neufer, 2003). The decrease in COXIV 84701 in the young and old 2h groups (Probabilities: 0.664, 0.762, log2FC: -0.284, -0.550, respectively), contradicts other studies which have found COXIV expression and other biomarkers of mitochondrial content to increase following acute exercise (Cobley et al. 2012), which casts doubt as to whether there is an actual decrease in mitochondrial content.

In contrast, our targeted analysis of the trained groups shows evidence of a significant up-regulation (Probability > 0.8) in markers of mitochondrial content and mitochondrial function (Probability >0.9), but no apparent change in mitobiogenesis. The increase in COXIV 1327 expression seen in both young and old trained groups (Probability > 0.95, log2FC 0.510, 0.380 respectively) and COXIV 84701 expression seen in the young trained group (Probability > 0.9, log2FC 0.812), is in agreement with other studies which found COX IV expression (Yan, Lira, and Greene 2013) and markers of mitochondrial content to increase following a training regime (Menshikova et al., 2006). The increase in expression of SDH 6390 (Probability > 0.9, log2FC >0.445) in both trained groups, and SDH 6389 and 6391 in the young trained group (Probability > 0.9, log2FC 0.318, 0.319 respectively), has previously been demonstrated to occur following training along with increased mitochondrial respiration (Psilander 2014). The higher level of βHAD 3030 expression (Probability > 0.95, log2FC 0.277) in the young trained group, was also demonstrated following training by Flack et al. 2016, and suggests an increase in mitochondrial lipid metabolism following training (Heilbronn et al. 2007). No significant changes in markers of mitobiogenesis were seen in the trained groups, which is in agreement with other studies that show an apparent attenuation of PGC-1 up-regulation in individuals subject to a training regime (Hinkley et al. 2017).

Gene Ontology enrichment analysis demonstrate a stark difference in the wider scale genetic response to acute exercise against a training regime. A far wider range of cellular functions and

process were promoted after training, with the immediate activities carried out following acute exercise being transcriptional and translational centred in the young-group and only three Process Terms present in the old group, including cellular respiration, transcription and cell proliferation.

How does the Transcriptional Response to Exercise Vary between Age Groups?
The majority of genes found to be differentially expressed in old individuals were also shown to be differentially expressed in young individuals at the equivalent time-point, however the young individuals demonstrated additional responses with many more DEG's than the old-group. A possible explanation is that the response to exercise in old individuals is a suppressed version of a young person's response, with a focus on mitochondrial genes, as revealed by the GO analysis. This conclusion correlates with the findings from the original study that found the increase in mitochondria proteins in response to exercise to be age-independent.

While targeted analysis of the 2h groups showed age independent increases in mitobiogenesis, the young 2h group shows weak evidence of down regulation in markers of mitofusion: MFN1 and OPA1 (Probability > 0.7, log2FC -0.169, -0.102) which is not seen the old 2h group. Mitofusion is essential to the maintenance of mitochondrial function (Westermann 2012) and declines with age (Johnson, Robinson and Nair, 2013), as does OPA1 expression (Tezze et al. 2017), so this result is counterintuitive. Combined with the probability of this change in both markers being below our probability threshold of 0.8, further investigation is necessary to clarify this finding.

In both the young and old trained groups there was significant up-regulation (Probability > 0.9, log2FC >0.3) in SDH a marker of of mitochondrial function, but in the young group an additional marker of mitochondrial function βHAD was up-regulated (Probability > 0.95, log2FC 0.277). It has been well established that mitochondrial function declines with age (Johnson, Robinson and Nair, 2013), so to see more pronounced evidence of improved mitochondrial function as a result of training in the young group, is as expected. This supports the notion that training can improve mitochondrial health, but the effects may be more pronounced in younger individuals.

Analysis of GO Terms reveals largely up-regulated genes, as expected from the DEG's largely showing increased transcription. There was a much more streamlined response to the exercise regime from the old-group, focussed largely on upkeep of mitochondria and provision of energy metabolites. The increase in mitochondrial gene expression could demonstrate that the training could improve their life outcomes, due to an increase in mitochondrial activity (Johnson, Robinson and Nair, 2013). The young-group presented a far more varied response, including extracellular functions, including immunological processes not seen in the older subjects. The loss of immune response in the old group may potentially highlight a possible link with the high levels of comorbidity related with sarcopenia. Muscle cells not contributing by secreting molecules which help prevent or manage disease may accelerate onset or allow increases in severity (Pedersen, 2013). General upkeep of the cells and angiogenesis provide an insight into muscle development, cell proliferation and health of the tissue (Bloor, 2005).

Limitations of Mitochondrial Biomarkers

The relationship between RNA transcription levels and translation of the associated gene product is not well understood, and highly variable (Johnson, Robinson and Nair, 2013). Therefore, through analysis of RNA expression alone, an absolute phenotypic response or protein level change cannot be determined. However, by choosing to focus on experimentally validated biomarkers, useful insights into changes in biological variables were discovered. Additionally, the absolute expression changes seen in our data cannot be compared across studies unless a standardized analytical method is used (Vigelsø, Andersen, and Dela 2014).

Gene Ontology Limitations

Extended work can be carried out on this dataset surrounding GO Terms. Network analysis tracing back from the ontology to the genes contributing to significant p-values would be useful to understand how the larger effect is being produced. The treemaps produced show relative p-values rather than quantifying the level of significance which is useful for giving a wide view of what is being affected compared to other terms, however it does obscure the actual size of the effect. Also the lack of hierarchy and removal of terms which are deemed to be too similar leads to a loss of information and reduces how holistic a characterisation is conveyed.

Future Direction

Possible approaches to developing this study further include performing gene coexpression analysis and building a predictive model to see if the initial response to exercise (2h) can predict the response to prolonged aerobic exercise training. This could not be achieved in the current study due to the limited number of samples, therefore future studies should aim to collect a larger number of samples. In addition, the collection of more data types would be valuable to get a broader view of the response to exercise. Ideally this would include proteomics data as well as phenotypic data such as muscle size and aerobic capacity. Samples taken at another time-point after the discontinuation of training to assess if changes in transcription revert back to baseline would also be interesting to investigate. As well as additional data types and time-points, different types of exercise such resistance training and strength training would be useful to research as possible countermeasures to sarcopenia, with the potential to explore the effects on comorbid conditions. Finally, pathway analysis would be another useful avenue to explore, which would require improved KEGG annotation.

References

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Appendix

Data and code

Results data as well as code for analysis and plot generation is available at https://github.com/annegarrett/Group_Project.

Additional Enrichment Analysis

Please note that the size of the quadrangles shows the relative adjusted p-values. The colour is simply to differentiate the different zones.

Supplementary Figure 1. Summary Statistics for GO Enrichment (number of significant GO Terms per set)

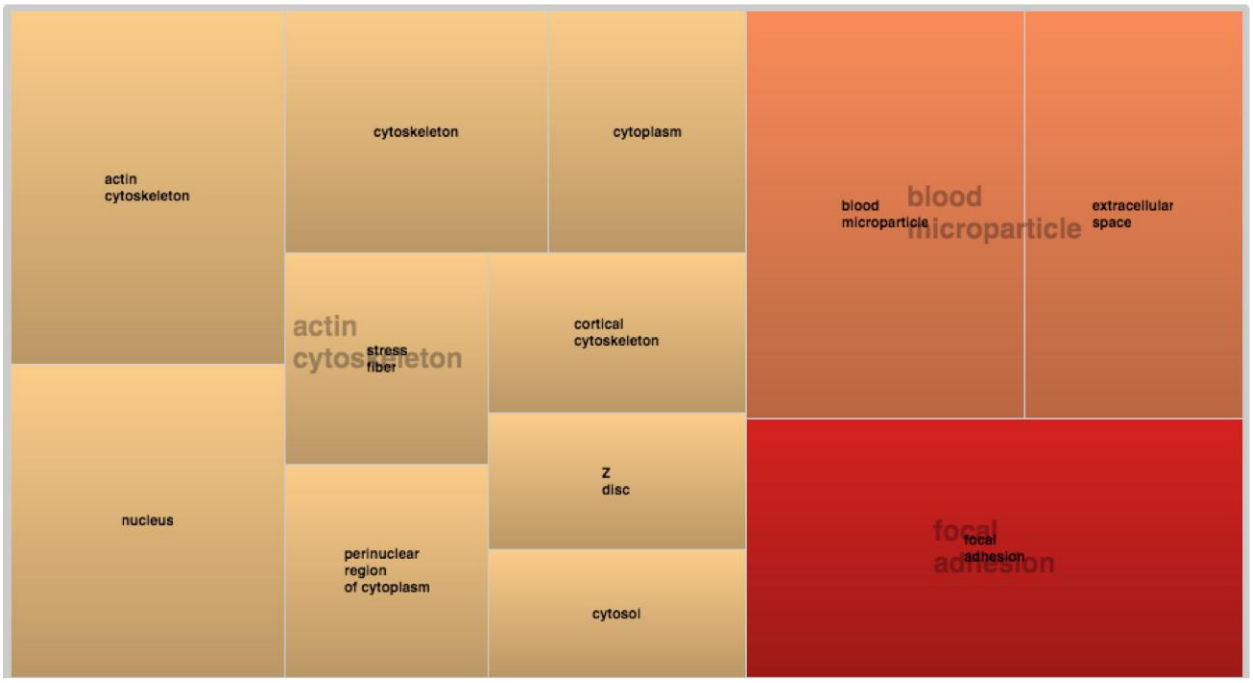
	Number of significant GO Terms		
Category	Up & Down Regulated	Up Regulated	Down Regulated
Baseline vs 2 hours			
Old			
Component	0	0	0
Function	0	0	0
Process	3	0	0
Young			
Component	13	6	0
Function	7	1	0
Process	7	6	0
Baseline vs Trained			
Old			
Component	11	10	0
Function	6	4	0
Process	10	9	0
Young			
Component	82	51	5
Function	38	27	3
Process	136	71	0

Supplementary Figure 2 (A-G) - Component Treemaps

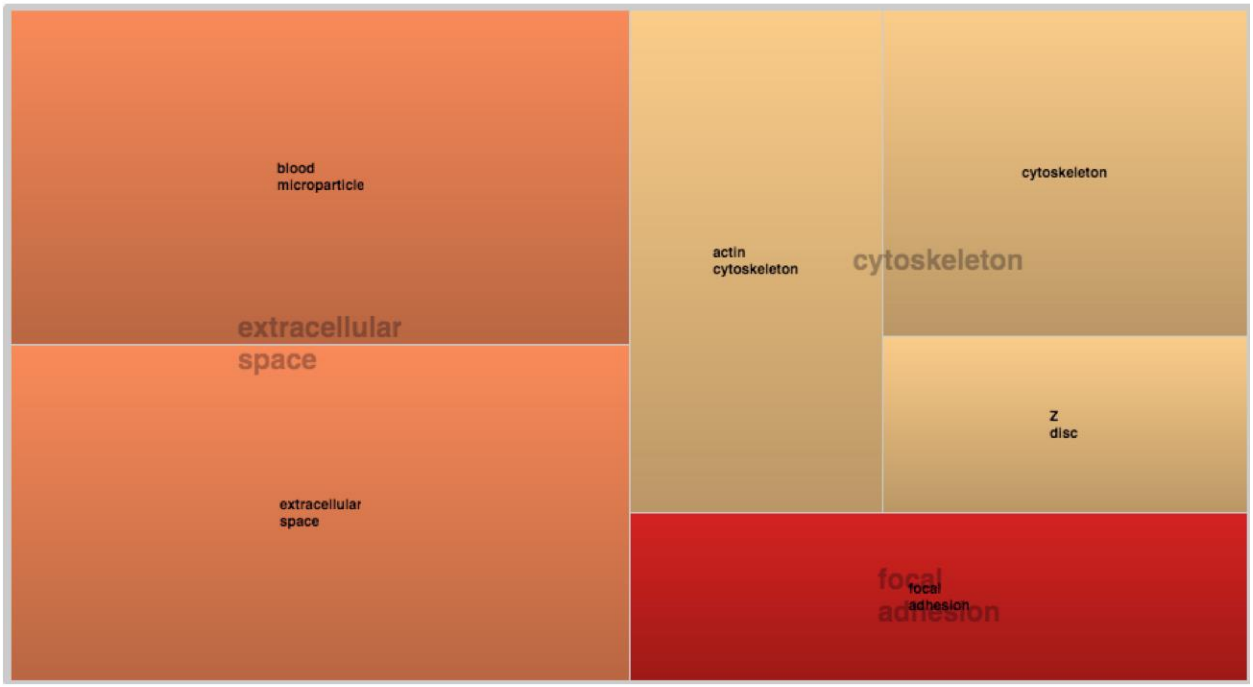
Component GO Terms - Up and Down Regulated Genes - Between Old-group Baseline and Trained

Component GO Terms - Up Regulated Genes - Between Old-group Baseline and Trained

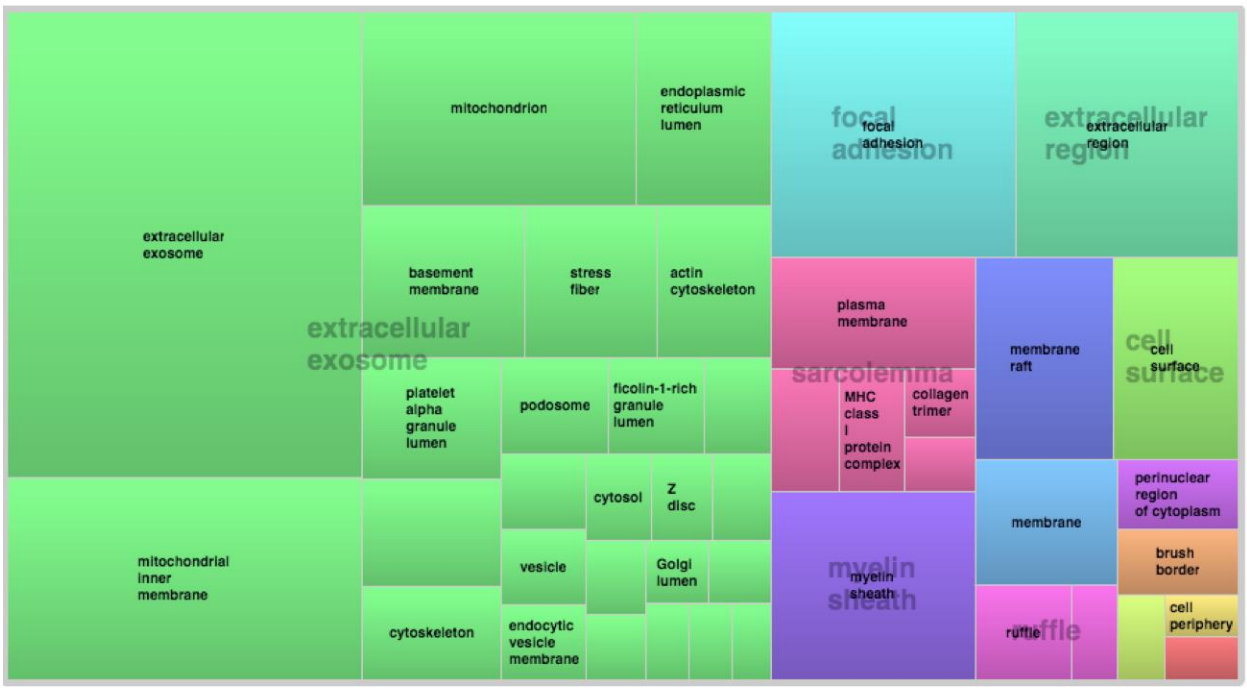
Component GO Terms - Up and Down Regulated Genes - Between YG-group Baseline and 2 hours



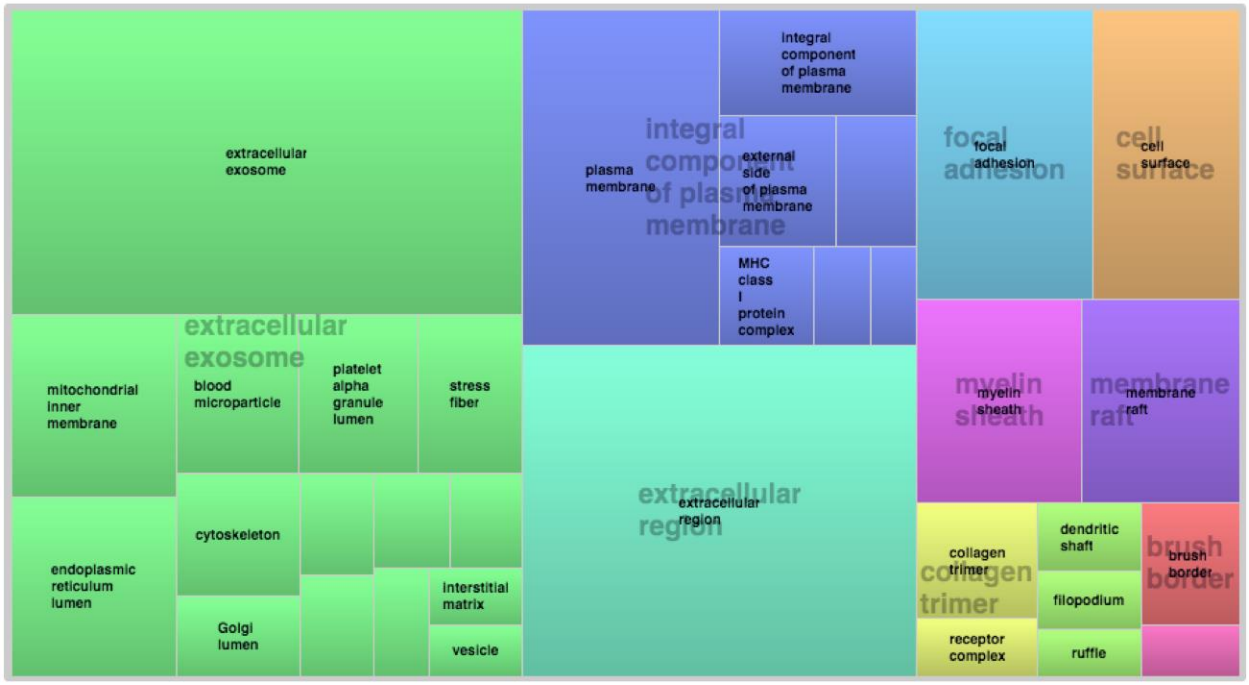
Component GO Terms - Up Regulated Genes - Between YG-group Baseline and 2 hours



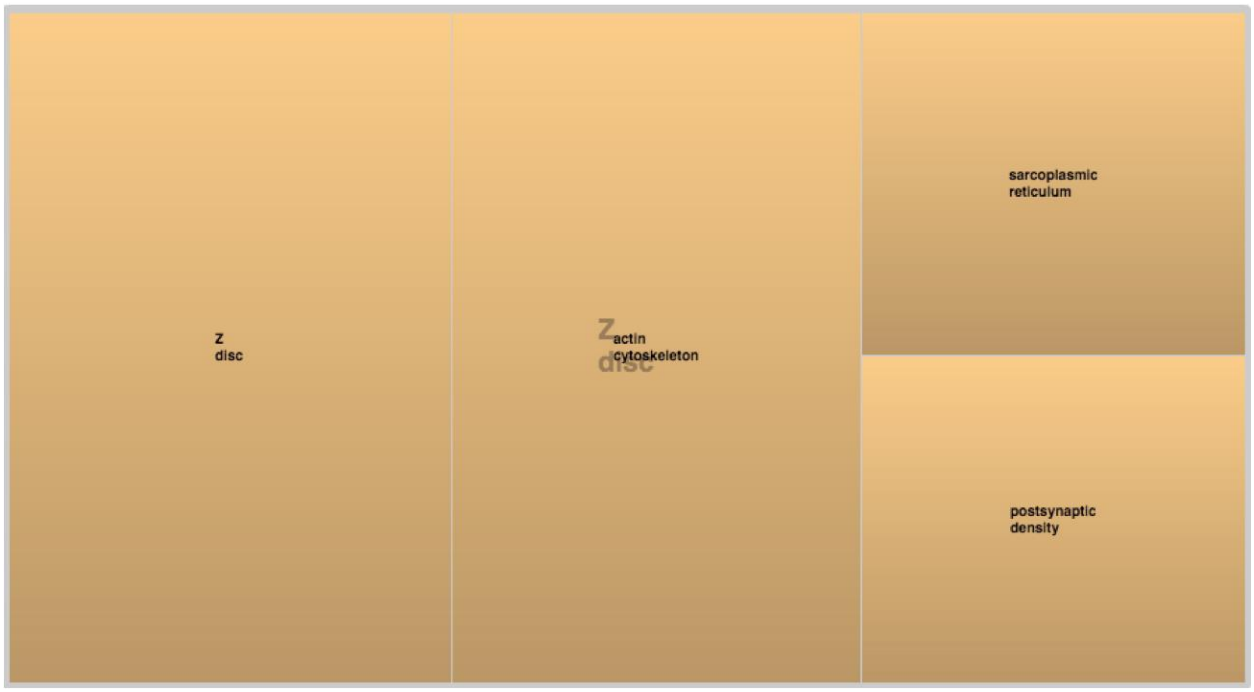
Component GO Terms - Up and Down Regulated Genes - Between YG-group Baseline and Trained



Component GO Terms - Up Regulated Genes - Between YG-group Baseline and Trained

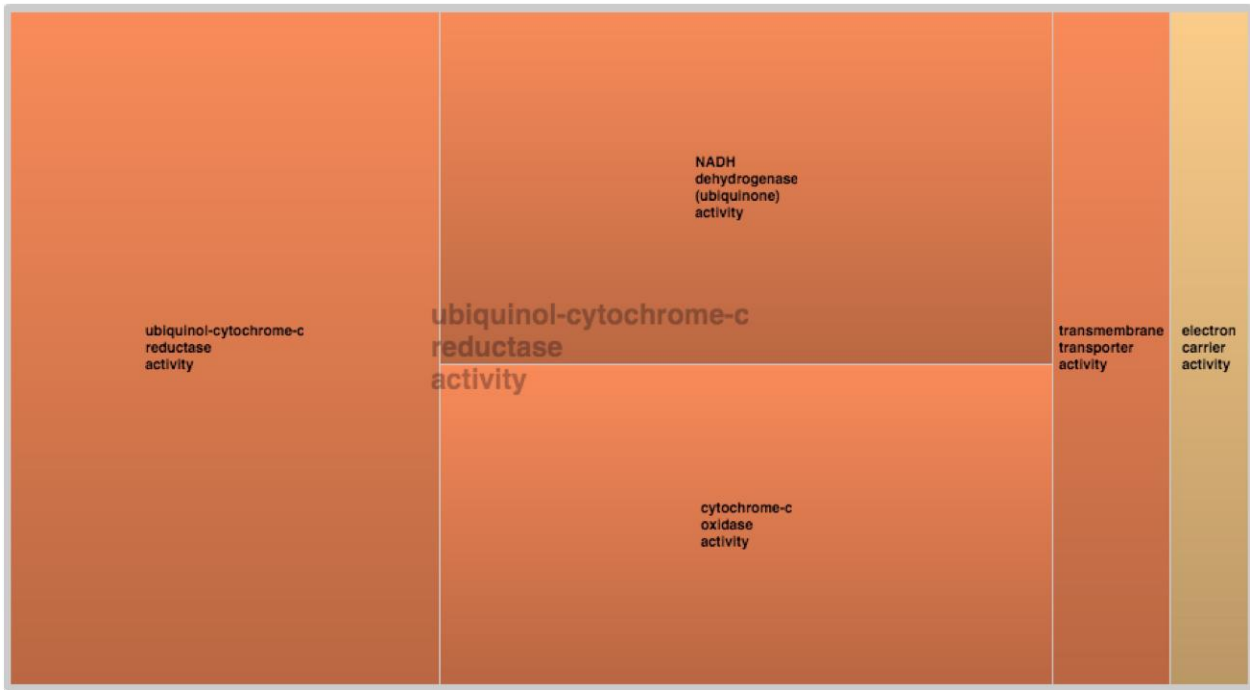


Component GO Terms - Down Regulated Genes - Between YG-group Baseline and Trained



Supplementary Figure 3 (A-G) - Function Treemaps

Function GO Terms - Up and Down Regulated - Between Old-group Baseline and Trained

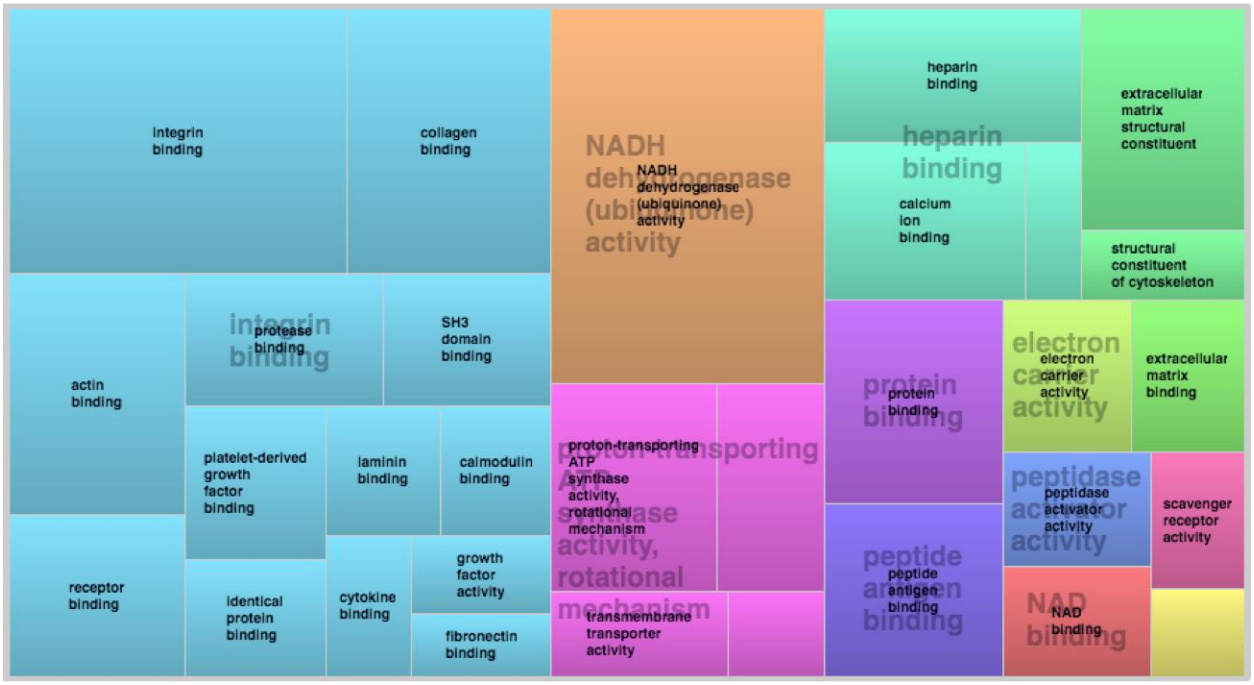


Function GO Terms - Up Regulated - Between Old-group Baseline and Trained

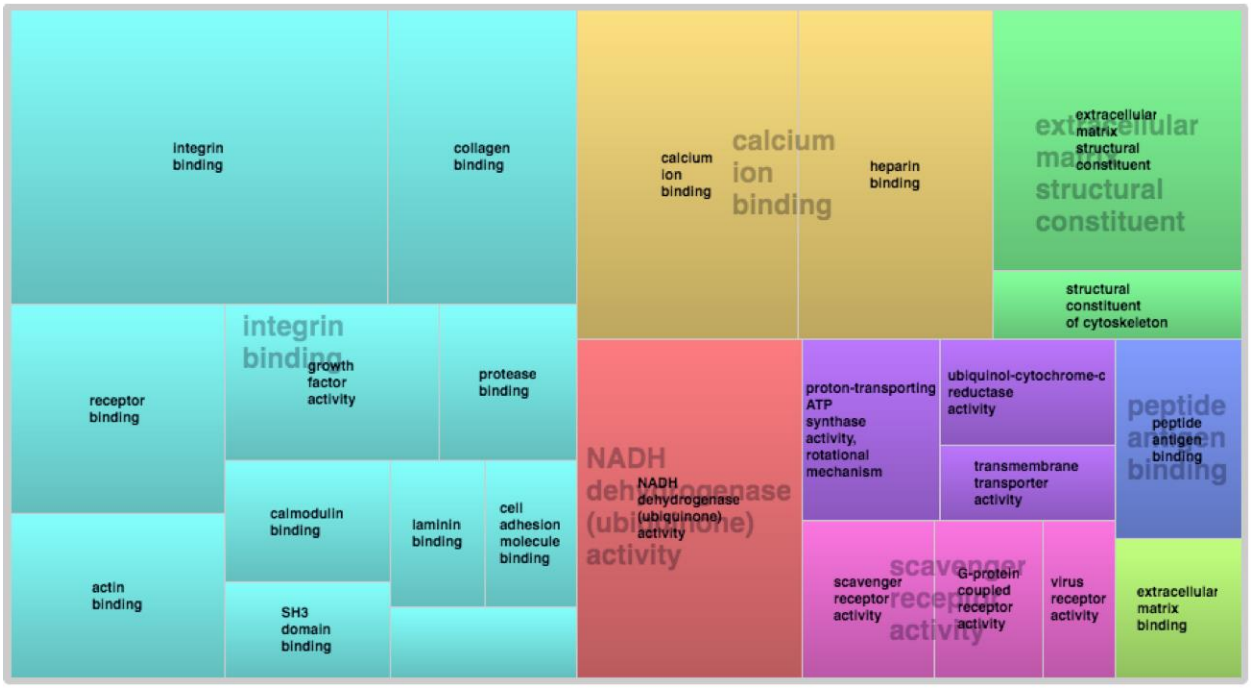
Function GO Terms - Up & Down Regulated - Between Young-group Baseline and 2 hours

Function GO Terms - Up Regulated - Between Young-group Baseline and 2 hours

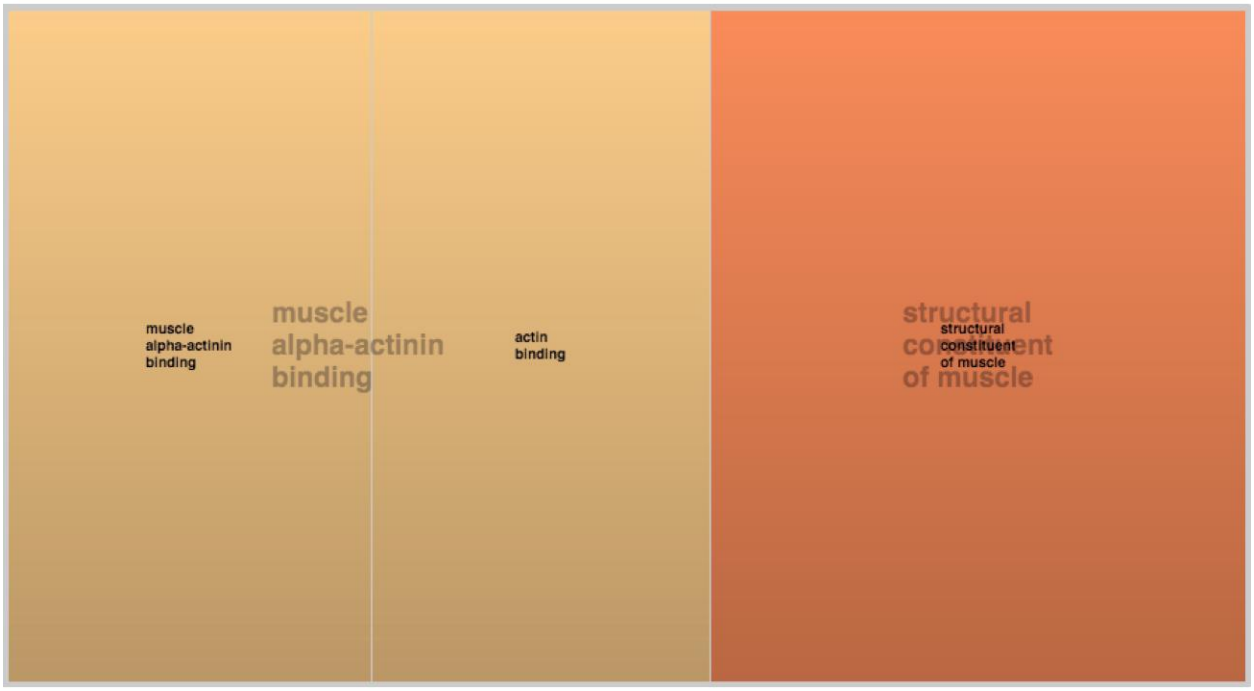
Function GO Terms - Up & Down Regulated - Between Young-group Baseline and Trained



Function GO Terms - Up Regulated - Between Young-group Baseline and Trained

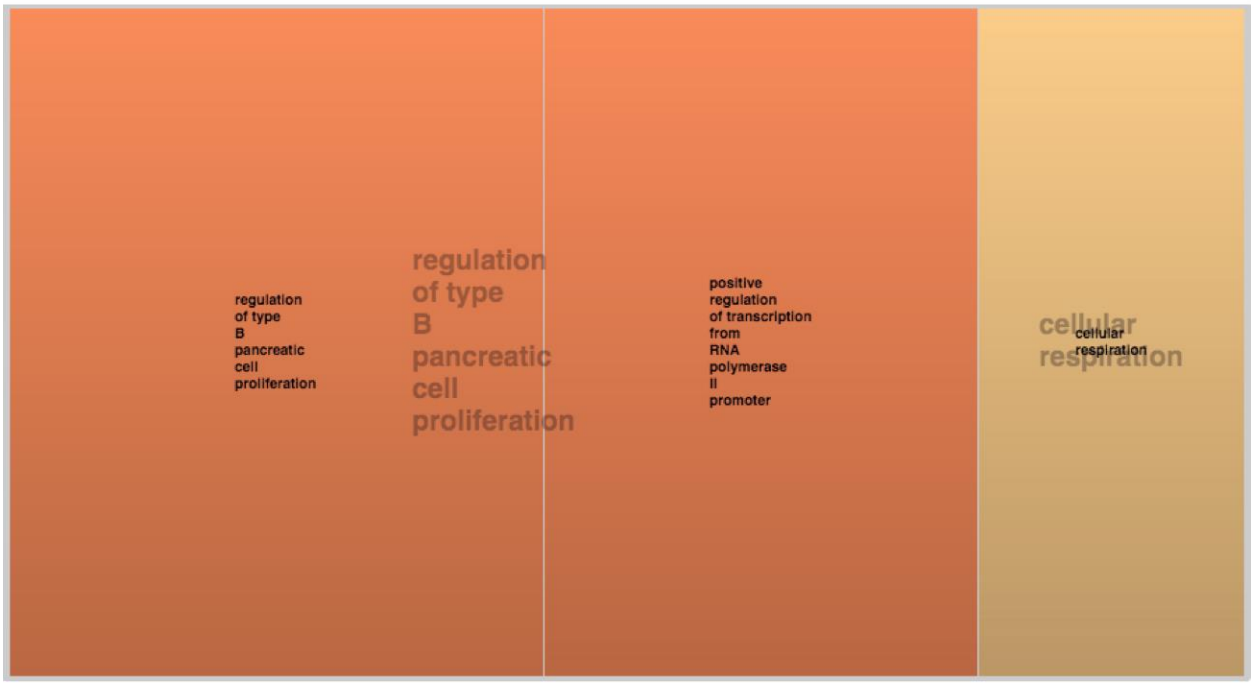


Function GO Terms - Down Regulated - Between Young-group Baseline and Trained

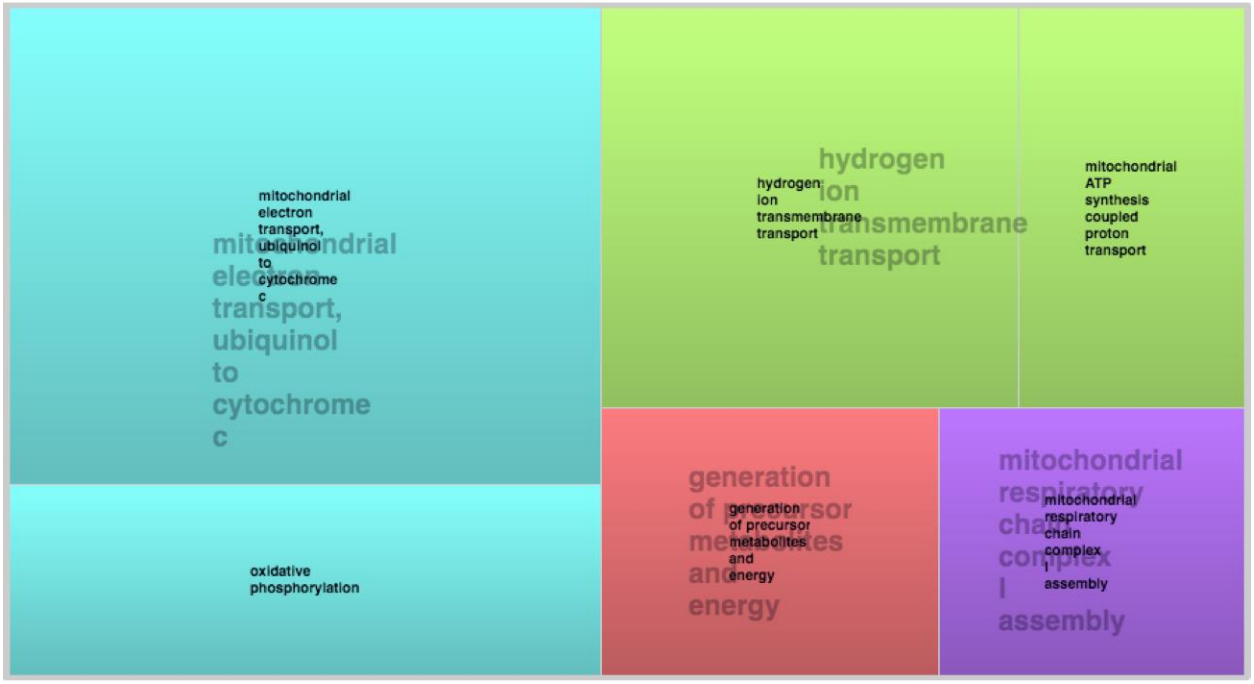


Supplementary Figure 3 (A-G) - Component Treemaps

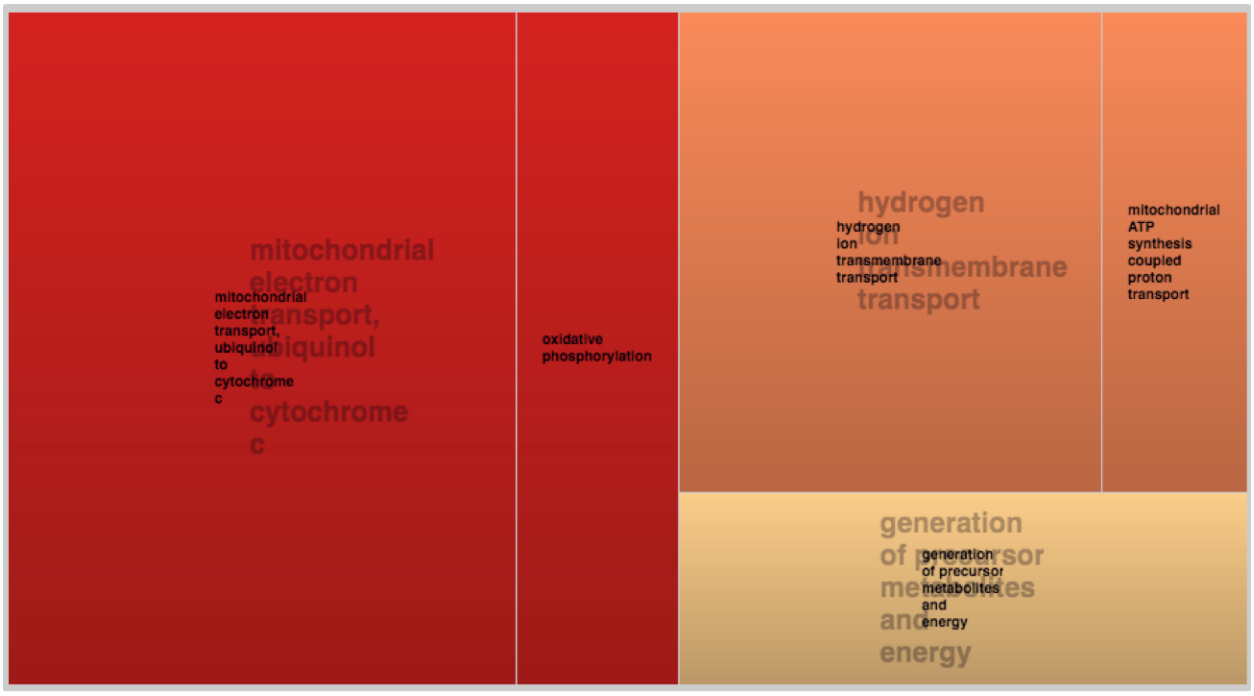
Process GO Terms - Up & Down Regulated - Between Old-group Baseline and 2 hours



Process GO Terms - Up and Down Regulated - Between Old-group Baseline and Trained



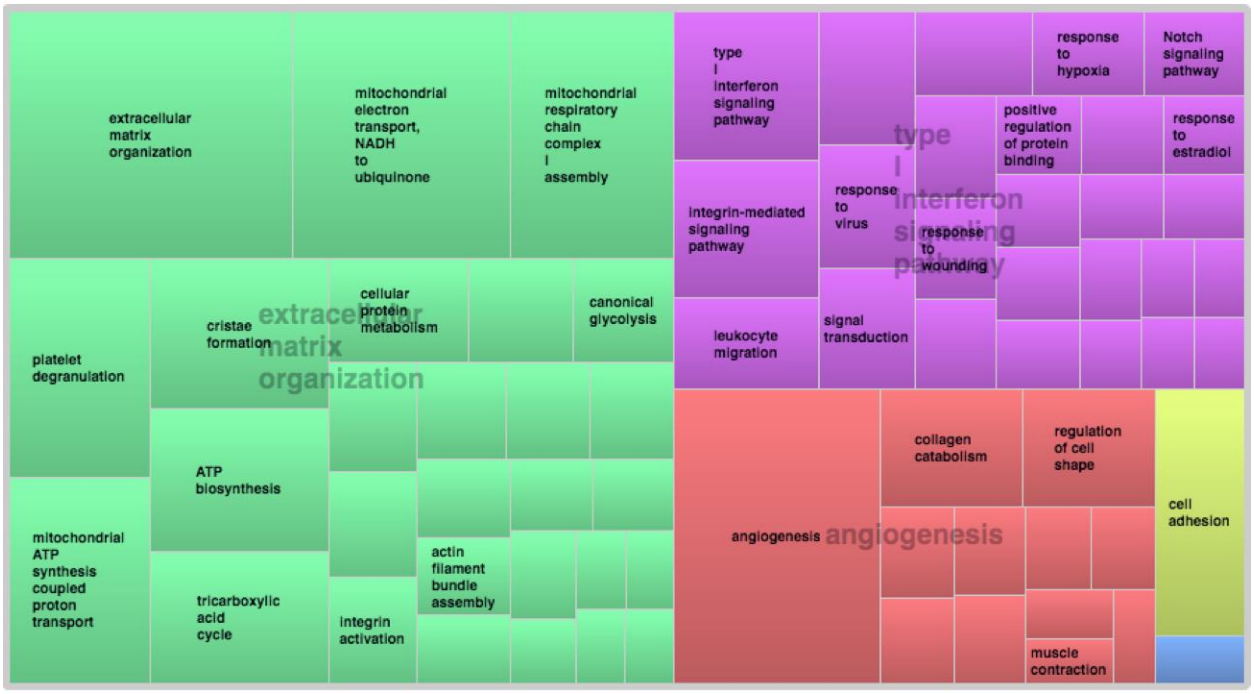
Process GO Terms - Up Regulated - Between Old-group Baseline and Trained



Process GO Terms - Up & Down Regulated - Between YG-group Baseline and 2 hours

Process GO Terms - Up Regulated - Between YG-group Baseline and 2 hours

Process GO Terms - Up & Down Regulated - Between YG-group Baseline and Trained



Process GO Terms - Up Regulated - Between YG-group Baseline and Trained