THE MOLECULAR ARCHITECTURE OF TRAINABILITY EXPLAINED BY GENETICS

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Abstract

Introduction

There is a substantial interindividual variability in responses to exercise training, which is influenced by both environmental and genetic factors. However, the influence these genes is relatively unknown. Therefore, we have investigated the association between robust genetic variants, using the Exercise Polygenic Score (EPS), on mitochondrial and physiological response to four weeks of High-Intensity Interval Training (HIIT) in the Gene SMART (Skeletal Muscle Response to Training) study. Thus, **the overarching aim of this thesis is to evaluate the association of robust genetic variants using the Exercise Polygenic Score (EPS) in mitochondrial and physiological response to exercise phenotypes to four weeks of High Intensity Interval Training (HIIT).**

Methods

116 adults from the Gene SMART cohort study completed four weeks of HIIT to measure physiological and mitochondrial responses. Maximal oxygen uptake (VO_{2max}), lactate threshold (LT), and peak power output (W_{peak}) were assessed before and after the exercise training. Muscle biopsies were collected before and after 4-weeks of HIIT to assess mitochondrial markers: citrate synthase (CS), cytochrome c oxidase (COX), succinate dehydrogenase (SDH), mitochondrial copy number (mtCN) and mitochondrial health index (MHI). DNA isolated from blood samples was genotyped using the Genome-Wide Genotyping Array, and genotype data was then used to derive exercise polygenic scores for each participant using exercise-related SNPs identified in **Chapter 3.**

Results:

We found significant increases in mitochondrial markers CS and COX after the HIIT intervention ($p < 0.05$), but no changes in mitochondrial health index (MHI). We also found that changes in LT were found to be positively correlated with changes in both CS ($r = 0.2$, p

= 0.014,) and COX (r = 0.19, p = 0.019). Also, significant correlations were found between changes in VO_{2max} and changes in two mitochondrial markers, CS (r = 0.24, p = 0.0025) and SDH (r = 0.20, p = 0.011). Finally, a significant correlation was found between changes in W_{peak} and changes in CS (r= 0.24, p = 0.0024). We did not observe significant associations between MHI changes and changes in physiological measurements.

We found no associations between EPSs and physiological and mitochondrial markers either before or after four weeks of HIIT. However, we found a significant association between baseline mtCN and the ***(PPARGC1A)*** rs8192678 SNP (p= 0.012). We further showed several associations between SNPs and mitochondria factors i) baseline mtCN and rs8192678 (p= 0.021), ii) 4-week change in mtCN and **(*BIRC*)** rs6090327 (p <0.001), iii) 4-week change in CS and **(*AGT*)** rs699 (p=0.0381), iv) 4-week change in SDH and rs609037 (p= 0.030) and **(*DAAM1*)** rs12891759 (p= 0.035) and v) baseline MHI and ***(PPARA)*** rs4253778 (p= 0.027), ***(RGS18)*** rs10921078 (p= 0.029), and ***(ACTN3)*** rs1815739 (p= 0.027). We found no significant differences between SNP genotypes and least square means of VO_{2max}, LT, and W_{peak}.

Summary

The experimental design of this study enables a better understanding of the roles of genes contributing to the complexity of exercise responses in humans. Future research should also integrate physiological molecular and omics (epigenomics, transcriptomics, metabolomics) to elucidate the mechanisms of exercise training in humans.

Student Declaration

I, Javier Alejandro Alvarez Romero, declare that the PhD entitled "The molecular architecture of trainability explained by genetics" is not more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

I have conducted my research in alignment with the Australian code for Responsible Conduct of Research and Victoria University´s Higher Degree by Research Policy and procedures. This research was approved by the Human Ethics Committee at Victoria University **(HRE13-223 and HRE 21-122).**

Signature: **Date: 17/02/2023**

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List of Publications

The following work has been accepted for publication at peer-reviewed journals in support of this thesis:

1. **Alvarez Romero J,** Voisin S, Eynon N, Hiam D. Mapping Robust Genetic Variants associated with Exercise Responses**.** Int J Sports Med 2020; 41: 1–15.

2. **Alvarez Romero J,** Jones P, Hiam D, Jacques M, Landen S, Garnham A, Abraham G, & Eynon N. Physiological and mitochondrial phenotypes in response to high intensity interval training in male and female skeletal muscle: The Gene SMART study. Medicine & Science in Sports & Exercise 2022. Under Review

During my candidature, the following work was published or submitted in a peer-reviewed journal and is outside the scope of this thesis:

1. Patrice R Jones**,** Sarah Voisin, Brendan J Nolan**,** Shanie Landen**,** Macsue Jacques**,** Beau Newell**,** Sav Zwickl**,** Teddy Cook, Alex Wong**,** Ariel Ginger**,** Andrew Palmer**,** Andrew Garnham**, Javier Alvarez-Romero,** Namitha Mohandas**,** Kirsten Seale**,** Ada Cheung**,** Nir Eynon. (2022). Uncovering the effects of gender affirming hormone therapy on skeletal muscle and epigenetics: protocol for a prospective matched cohort study in transgender individuals (the GAME study) *BMJ Open* 2022;**12:**e060869. doi: 10.1136/bmjopen-2022-060869.
2. **Alvarez-Romero, J.**, Seale, K., Laguette, MJ., Voisin, S., Fuku, N., Hiam, D., Jacques, M., Eri, M., Feller, JA., Tirosh, O., Collins, M., September, AV., Eynon, N. (2021). Collagen genetic variants within the COL*5A1* gene are associated with ligament injuries in Australian, South African, and Japanese Populations. *European Journal of Sport Science*
3. Jacques M, Landen S**, Alvarez-Romero J**, Yan X, Garnham A, Hiam D, Siegwald M, Mercier E, Voisin S, Eynon N (2021). Individual physiological and

mitochondrial responses during 12 weeks of intensified exercise. *Physiological Reports.*

4. Jacques, M., Kuang, J., Bishop, D. J., Yan, X., **Alvarez-Romero, J.,** Munson, F., Garnham, A., Papadimitriou, I., Voisin, S., & Eynon, N. (2020). Mitochondrial respiration variability and simulations in human skeletal muscle: The Gene SMART study. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, *34*(2), 2978–2986.

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Conferences Presentations

- Exercise slows the ageing process in skeletal muscle. June 2019, **VU conference**.

- The molecular architecture of trainability explained by genetics. 2021 **IHeS Seminar.**

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DETAILS OF INCLUDED PAPERS: THESIS WITH PUBLICATION

Please list details of each scholarly publication and/or manuscript included in the thesis submission. Copies of published scholarly publications and/or manuscripts submitted and/or final draft manuscripts should also be included in the thesis submission.

This table must be incorporated in the thesis before the Table of Contents.

Chapter No.	Publication Title	Publication Status • Published • Accepted for publication • In revised and resubmit stage • Under review • Manuscript ready for submission	Publication Details • Citation, if published • Title, Journal, Date of acceptance letter and Corresponding editor's email address • Title, Journal, Date of submission
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List of Abbreviations

1RM	1 repetition maximum
4WP	four weeks post (training)
ACE	angiotensin converting enzyme
ACSL1	acyl-coa synthetase long chain 1
ACTG1	actin gamma 1
ACTN3	alpha-actin-3
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BMI	body mass index
CCL2	chemokine c-c motif ligand 2
CCR2	chemokine c-c motif ligand 2 receptor
CMJ	counter movement jumps
COX	cytochrome c oxidase
CON	control period
CRISPR	clustered regularly interspaced short palindromic repeats
CTNF	ciliary neurotrophic factor
CS	citrate synthase
DAAM1	dishevelled-associated activator of morphogenesis 1
DNA	deoxyribonucleic acid
ENDCON	end of control period
EPS	exercise polygenic score
ERRα	estrogen-related receptor alpha
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GWAS	genome-wide association study

GXT	graded exercise test
HR	heart rate
HIIT	high intensity interval training
IL-15RA	interleukin 15 receptor subunit alpha
IL-6	interleukin 6
LEPR	leptin receptor
LT	lactate threshold
MCAD	medium chain acyl-coa dehydrogenase
MFF	mitochondrial fission factor
MFN1	mitofusin 1
MFN2	mitofusin 2
MHI	mitochondrial health index
mtDNA-cn	mitocondrial DNA copy number
MVC	maximal voluntary contraction
NADH	nicotinamide adenine dinucleotide + hydrogen
NAMPT	nicotinamide phosphoribosyltransferase
NHMRC	National Health and Medical Research Council of Australia
NR3C1	glucocorticoid receptor subfamily 3 group c
NRF2	nuclear factor erythroid 2
PARK2	parkin rbr e3 ubiquitin protein ligase
PDK4	pyruvate dehydrogenase lipoamide kinase isozyme 4
PGC1α	peroxisome proliferator activated receptor-γ coactivator alpha
PPAR	peroxisome proliferator activated receptor
PPARD	peroxisome proliferator activated receptor delta
PPARG	peroxisome proliferator activated receptor gamma
PPARGC1A	peroxisome proliferator activated receptor gamma coactivator 1alpha
PPARGC1B	peroxisome proliferator activated receptor gamma coactivator 1beta

PTK2	protein tyrosine kinase 2
QTL	quantitative trait loci
SMART	skeletal muscle response to training
SDH	succinate dehydrogenase
SNP	single nucleotide polymorphism
RNA	ribonucleic acid
TFPα	tri functional protein alpha
VLCAD	very long acyl-coa dehydrogenase
VO2max	velocity of maximal oxygen uptake
Wpeak	working peak rate

Chapter 1: Introduction

Exercise training produces multiple adaptations on whole-body human phenotypes; however, these adaptations differ between individuals at both the physiological and molecular level (also called trainability) [1-3]. The molecular mechanisms underpinning the variance in exercise adaptations are largely unknown. Thus, to better understand trainability it is crucial to decipher the influencing components involved. Uncovering the drivers of trainability at physiological and molecular level will advance our understanding of adaptations to exercise training. Current evidence shows that the magnitude of exercise adaptations depends on the speed, force, duration and intensity of exercise interventions and related muscle contractions [4]. High intensity interval training (HIIT) has two general categories [5], one category is referred to as “aerobic HIIT” (the selected method for this thesis), and the other is ”body weight HIIT” or “resistance HIIT”. HIIT can be diverse in duration, and studies has shown that as little as a few bouts of HIIT are sufficient to elicit physiological response [6]. Our chosen 4 weeks HIIT protocol was based on a balance between responses and feasibility. Our results show significant increase in physiological responses after 4 weeks. Previous research has suggested that HIIT induce greater responses than continuous moderate - intensity exercise and need less time spent [7] in markers of cardiovascular health, metabolic capacity and aerobic performance [8-12] Moreover, a growing body of literature suggests that biological determinants (i.e., genetics or baseline fitness) play a crucial role in regulating exercise adaptations [13]. Previous studies suggested that trainability is mediated by our genes [14-16]. However, it is also reported that regardless of the genetic component involved in trainability, no single gene has been shown to be absolutely responsible for a physiological phenotype due to the substantial amount of genes involved in exercise response [17]. This variation in response to exercise training is primarily characterized by aerobic capacity (VO2max) and by strength and muscle mass [18].

The large discrepancy among different training protocols (i.e., frequency, duration, intensity) increases the variability among the exercise field and is a limitation to be able to compare exercise responses between studies. However, the complexity of physiological and molecular responses to aerobic and resistance training emphasizes the relevance of collaborative efforts among investigators in the known phenotypic adaptations [18]. Despite many genes having been associated with exercise responses, the vast majority have not been replicated [19]. Replication is crucial to minimise number of false positives an increases the likelihood that results are true [20, 21]. Thus, **the aim of the first study (Chapter 3) was to identify robust genetic variants and create an exercise polygenic score (EPS) in exercise responses.**

Multiple sources of variability have been determined in exercise training studies [22]. This variability is minimized when a control period in included in the study design, suggesting that any physiological or molecular changes post-exercise are attributed to the intervention and not to day-to-day variability or technical error [22]. In addition to the physiological adaptations to exercise, mitochondrial changes are one of the most apparent adaptations responding to exercise [23], with even a single bout of exercise producing mitochondrial adaptations [24]. Also known as the "power house" of the cell, mitochondria are responsible for multiple regulations in skeletal muscle [23]. In addition, skeletal muscle mitochondria promote biogenesis and adaptation in function and, therefore, contribute to maintaining cellular and whole-body health [25]. There are two main aspects to evaluate mitochondrial adaptations in exercise responses: content and quality. Even though exercise training induces physiological and mitochondrial changes, these changes may not necessarily progress together [26]. Evaluating a composite mitochondrial measure is crucial to assess mitochondrial health, as previous studies showed that high mitochondrial content may exhibit a reduction in mitochondrial function, suggesting that mitochondrial content can be increased to compensate poor mitochondrial quality [27]. Also, it is suggested that single mitochondrial markers are bad

at predicting changes in the whole organelle [28]. The mitochondrial health index (MHI) combines key markers for mitochondrial content and function; it also integrates nuclear and mitochondrial encoded measurements, and mathematically outperformed individual markers in leucocytes [29]. However, it is unknown how MHI performs in other tissues. Therefore, **the aim in chapter 4 was to investigate physiological phenotypes adaptations and mitochondrial adaptations by exploring differences in mitochondrial content and function before and after exercise training preceded by a control period.**

Exercise response studies have reported a genetic contribution. However, a key limitation of these studies is that they assessed individual genes as a single variable, irrespectively of the gene's allelic composition [30]. Also, most of these studies looked at elite athletes but not at the general population, and not at robust muscle-related molecular phenotypes. In addition, previous exercise studies reported that no single gene is responsible for a specific phenotype [16, 17], with physiological and molecular phenotypes influenced by several genetic variants with small individual effect sizes [31-33]. Also, multiple genes interact to produce the phenotype's outcome [34]. Thus, the evaluation of combined multiple genes will play an important role to identify their association with physiological and molecular phenotypes. To evaluate the genetic influence on trainability, it was important to better understand such differences at baseline in physiological and molecular phenotypes. To our knowledge, no study to date has investigated the association between an exercise polygenic score with physiological and mitochondrial phenotypes before an exercise intervention. This is crucial as adaptations to exercise responses are also dependent on initial fitness levels, and baseline fitness levels reflect a long life of exercising. Therefore, **the aim of the third study (Chapter 5) was to assess the associations of the Exercise Polygenic Score derived from robust genetic variants with physiological and mitochondrial phenotypes before and after exercise.**

With a literature review (**Chapter 2**), this thesis further comprises three experimental chapters as described above:

I. **Chapter 3**: Deciphering robust genetic variants in exercise responses in aerobic and resistance trainability.

II. **Chapter 4**: Mitochondrial markers that explain physiological responses preceded by a control period (the Gene Study).

III. **Chapter 5**: Associations between exercise polygenic score (EPS) and physiological and mitochondrial markers before and after exercise training.

The main findings of this thesis are summarised with a general discussion (**Chapter 6**), including the limitations of each study, the contribution to knowledge and recommendations for future studies

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Chapter 2: Review of the Literature

2.1 The concept of trainability

Exercise alters a vast amount of whole-body human physiological and molecular phenotypes including oxygen capacity, muscle power, muscle strength, lactate threshold and mitochondrial biogenesis. These adaptations between individuals differ in both physiological and molecular responses to any given exercise training (also called “trainability”) [1-3]. One of the key aspects to quantifying trainability is understanding the components of the variations involved. Uncovering the molecular drivers of trainability has the potential to advance our understanding of exercise adaptations to regular exercise. This generated knowledge may in turn be used to develop biomarkers that predict the ability of an individual to improve physiological markers in response to exercise training [4].

Differences in human exercise phenotypes are influenced by the individual and combined effects of our genes and environment [5]. However, the molecular mechanisms by which genetic and environmental factors interact to impact individual responses to exercises phenotypes, such as cardio-respiratory fitness, are poorly understood [6]. Previous research has indicated that the variability between individuals on response to exercise is mediated by both genetic and environmental factors [7]. However, the influence of environmental conditions on individual trainability is gaining relevance as the variability in physiological adaptation enables tbe study of the association between molecular responses to exercise and the magnitude of physiological change in humans [8, 9].

2.2 Biological factors influencing trainability

A large variability has been observed in many physiological parameters [10], including maximal oxygen uptake (VO_2max) [11, 12], resting heart rate [12], exercise heart rate [12], aerobic threshold [13], anaerobic threshold [12], muscle mass and strength [14, 15]. In addition to this, molecular phenotypes such as resting muscle glycogen content, muscle enzyme activity

[16], and in mitochondrial function have also shown large variability [17]. There are several potential biological contributors to this, including sex, genetics, and molecular markers such as mitochondrial markers [18].

2.3 Trainability between sexes

It is widely known that males and females respond significantly different to exercise. Not only anatomical, but physiological differences between males and females determine the variation in human performance between sexes [19], with a large variability shown in several physiological phenotypes between them [20]. For example, females have an increased lifespan compared to males [21], and have also shown higher increase in VO_{2max}, as well as higher lipolysis rate to an intervention in endurance training [22]. There are several examples showing that males hold greater absolute muscle strength and produce greater power output than females in multiple exercise conditions [23]. In contrast, females also have a higher heart rate (HR) response than males for similar workloads, showing a significant variability in physiological phenotypes between sexes [24].

In addition to physiological markers, sex differences also exist in molecular markers associated with skeletal muscle health and functioning. Skeletal muscle fibre-type distribution differs between sexes, with females having more type I fibres compared to males [25, 26]. In contrast, females have moderately lower mitochondrial ADP sensitivity than males [27]. Major enzymes involved in fat oxidation vary between sexes. For example, the tri-functional protein alpha (TFPα), the very long acyl-CoA dehydrogenase (VLCAD), and medium chain acyl-CoA dehydrogenase (MCAD) were found to be higher in content in females [28]. In addition to sex, biological factors that may significantly impact trainability are genetics and mitochondrial functioning.

2.4 Methodological factors influencing trainability

In addition to the biological factors influencing trainability, there are several methodological factors affecting variability in response to exercise. These include type of exercise, volume and intensity of training, and method of prescription [18], which need to be considered when attempting to untangle and characterise the effects of biological factors on exercise responses. Further, differing statistical methods must be employed to properly isolate individual biological responses from random error [29]. Common approaches to evaluate the mean response of a sample have failed to recognize the inter-individual differences in response to any exercise program [30]. Also, tissue samples and laboratory variability techniques are to be considered when quantifying the relevance of genetic variations using human muscle tissue [31]; for example, is reported that technical error and sample variability in the same biopsy area account for about 15% of the variance on the reported results [32, 33]. Approaches to classify individuals as “responders” or “non-responders” dichotomy are not a sensible way to investigate personal response [34]. A more useful approach is to study dimensions rather than categories. Thus, trainability is not a qualitative variable; it should be considered as a quantitative one, as it is a matter of more or less [35].

2.5 Genetics and Exercise

Humans are considerably different across populations in exercise traits [36] and the human variability in exercise traits is a representation of the substrate of humans genetics [37]. There is compelling evidence that genetics is a significant component in the human variation in exercise responses [1, 38]. Genes explain around 50% of the heritable aspect of exercise responses [39]. In the HERITAGE study, it was shown that exercise traits at baseline as well as exercise responses were more similar within families than between them [40]. Thus, the recognition of genetic variants has become essential to identify human variation in complex traits such as exercise responses [38].

The ultimate goal of genomic research is to identify DNA differences causing diseases or predicting the potential adaptability on a particular trait [31]. This goal has been investigated by two different approaches: candidate genes and genome-wide association (GWAS) study. A candidate gene is a gene that theoretically has relationship with a physiological or molecular trait of interest [31, 41]. Although this approach was the origin of several exercise response studies, it is also known that candidate gene studies often have small sample sizes and lack statistical power [42, 43]. In contrast to candidate genes is the GWAS approach. This method scans up to 5 million genetic variants and reported that trainability is influenced by many genes (i.e., it is polygenic) [44]. From the first GWAS study performed in 2007 [45], the exercise field developed interest in the combined influence of genetic variants in exercise responses. To date, 2339 genetic variants are associated with exercise; the catalogue of these studies and their results can be examined at http://www.genome.gov/gwastudies. Thus, GWAS have become essential to identify SNPs contributing to the variation in exercise responses studies [31].

2.5.1 Exercise Polygenic Score (EPS)

The genetic architecture of trainability is a polygenic trait. This means that many parameters such as number, frequency, relationship between and magnitude of effect of genetic variants contribute to it [46]. Despite the initial potential of GWAS to interrogate for polygenic traits, enthusiasm quickly faded due to the small effect sizes on individual alleles. This has led to the idea of testing multiple genetic loci simultaneously ("genomic profiling"), which collectively may provide superior prediction [47]. These predictions are based on whether the genetic variants overcome an arbitrary P value threshold, or the estimation of their effects in a particular sample followed by validation in an independent sample for a given phenotype [48]. Prior to undertaking a polygenic analysis a power calculation is needed to establish the boundaries of what can be achieved [49]. It was initially hoped that once the genetic architecture of a trait was identified, the observed effects of the associated alleles could be used

to construct a combined score and to predict individuals at the tail ends of the distribution [50]. In addition, new methodologies that aggregate data from a larger fraction of the genotyped variants that scored below the genome-wide significance threshold were formulated to account for undiscovered loci [47].

The use of the Exercise Polygenic Score is becoming more frequent in the field of exercise genomics. EPS can be used to gain insight into the aetiology of physiological phenotypes in exercise responses studies [51] as well as in elite athletes [52]. Although several genetic variants have been associated with trainability [51] and athletic performance [41], the results of these studies have not been replicated to date. In association analysis, the aim is to identify specific associated variants. A stringent threshold for declaring significance of individual SNPs is important, providing confidence that the identified variants are true positives.

2.5.2 Genetic and molecular contribution to exercise responses

To date, there is very little literature ascribed to understanding the interplay between genes and other molecular factors and the development of physiological traits. Much work remains to identify causal variants and functional relevance of genes associated with molecular phenotypes related to exercise responses, and how these associations link to physiological phenotypes in aerobic and resistance trainability (Figure 2.1). Few studies have investigated links between the mitochondrial genome and training adaptations; therefore, conclusive findings from such studies are limited and only focus on particular sections of the mitochondria [53]. Key molecular phenotypes explored in this project are mitochondrial markers, which have important roles in responses to exercise. For example, it has been shown that with exercise there is an increase in the mitochondria electron transport chain [54] and in mitochondrial enzymes [55]. Exercise also upregulates mitochondrial content in humans [56, 57].

2.6 Mitochondria as a key organelle in exercise response to training

Structurally, mitochondria consist of four compartments: the inner membrane, the outer membrane, the intermembrane space and the matrix (located inside the inner membrane). Mitochondria perform numerous tasks such as pyruvate oxidation, the Krebs cycle, and metabolism of amino acids, fatty acids, and steroids. However, the most crucial function is the generation of energy as adenosine triphosphate (ATP) by means of the electron-transport chain and the oxidative-phosphorylation system (the "respiratory chain") [58].

The respiratory chain consist of five multimeric protein complexes that are vital for maintaining health through their regulation of substrate metabolism and energy production [59], and are also involved in influencing skeletal muscle size and function [60]. As a result of exercise, several mechanisms have been identified such as mitochondrial biogenesis [61], gene expression [62], mitochondrial remodelling [63], mitochondrial quality increase through mitophagy [64] and more recently mitochondrial cristae density [65]. Exercise training also influences the expression of nuclear encoded mitochondrial biogenesis markers such as PGC1α, PPARD, PDK4, NFR-2, GAPDH, NAMPT [66, 67] as well as fusion markers such as MFN1, MFN2, ERRα, MFF and PARK2 [68]. During exercise there is an increase in several molecular signals in skeletal muscle that are in part responsible for the initial activation of mitochondrial biogenesis [69]. Thus, exercise can diminish mitochondrial skeletal muscle diseases induced by defects of the respiratory chain due to enzyme deficiencies, structural alterations [69] or induced by defects in any of the mitochondrial pathways [58].

Mitochondrial proteins are encoded both by nuclear and mitochondrial genomes, and changes in either the nuclear DNA or mitochondrial DNA can potentially alter mitochondrial biogenesis and result in individual variation in mitochondrial content or function [70]. Human mitochondrial DNA (mtDNA) is a circular multicopy genome coding for 13 peptides involved

in the electron chain transport with an estimated mtDNA copy number in muscle cells of ~ 3700 [71] (Figure 2.1).

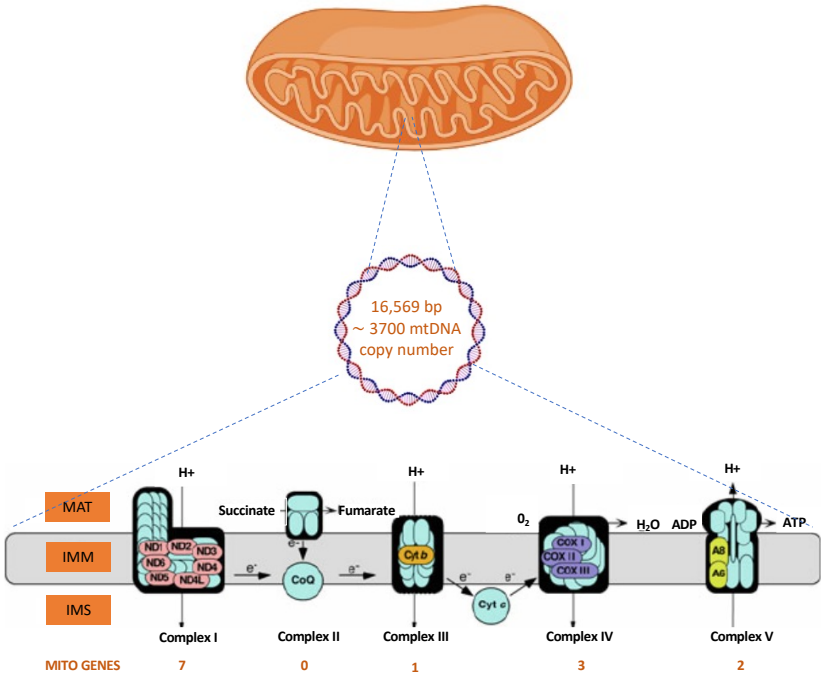

Figure 2.1 *Mitochondrial diagram of the key components involved in this study.*

The increase of mitochondrial protein content and subsequent expansion of the mitochondrial reticulum (mitochondrial biogenesis) [72] is a recognized marker for adaptive function response to training [73, 74]. Although the markers to evaluate the function or the content of the mitochondria are well known [75, 76], the measurement of their content or quality maybe insufficient to evaluate mitochondrial function as changes in their content, volume or enzyme activities may not necessarily progress together [77]. For instance, elevated mitochondrial content may cause a reduction in mitochondrial quality, and as a result, mitochondrial DNA copy number per cell can be increased as a compensatory mechanism [78].

2.6.1 Mitochondrial markers

Multiple markers have been used to evaluate mitochondrial biogenesis and dynamics in skeletal muscle [79], as it is well known that exercise training induce an increase in enzyme

activities [73]. For this thesis, we will evaluate four functional parameters, which represent nuclear and mitochondrial genomes.

There are two main known aspects in measuring mitochondrial adaptations to changes in oxidative demand induced by exercise:

1. mitochondrial content within cells: Mitochondrial DNA copy number (mtDNA-cn) is a measure of the number of mitochondrial genomes per cell [75].

2. Mitochondrial function: The main function of mitochondria is to generate ATP. Thus, the most direct way to measure mitochondrial function in the context of bioenergetics is to assess ATP synthesis rates [76]. Alternatively, mitochondrial function can be measured by the activity of 13 different enzymes normalized by mitochondrial protein content [76].

2.6.1.1 Mitochondrial DNA copy number

Mitochondrial DNA copy number (mtDNA-cn) is a measure of the number of mitochondrial genomes per cell [75] and therefore mtDNA content is often used as a marker of mitochondrial content in skeletal muscle [80]. It also reflects the net results of gene-environmental interactions between unknown hereditary factors and the level of oxidative stress [81]. In contrast, mitochondrial content decreases in skeletal muscle in disuse [82].

2.6.1.2 Cytochrome c Oxidase

Cytochrome c Oxidase (COX) is the terminal component of the mitochondrial respiratory chain complex that catalyses the conversion of redox energy to ATP. The largest COX subunits I, II and III, represent the catalytic core of the enzyme. These subunits are encoded by the mitochondrial DNA and synthesized within the mitochondria, while the rest of the smaller subunits implicated in the mitochondrial function are encoded in the nuclear DNA [83]. The proton gradient complexes consist of three proteins known as complex I (NAHD/ubiquione oxidoreductase), III (*cytochrome c reductase*), and IV (*cytochrome c oxidase*). Complex IV transfers the electrons from cytochrome c to molecular oxygen and

contributes to the proton gradient by using up to four protons per consumed oxygen molecule of water [84]. COX is used as marker of complex IV as well as the mitochondria membrane marker [85].

2.6.1.3 Succinate dehydrogenase

Succinate dehydrogenase (SDH) is the enzyme which catalyses the oxidation of succinate to fumarate in the Krebs cycle and transfers electrons to the ubiquinone pool (complex II of the mitochondrial respiratory chain). SDH represents one of the reliable marker of the mitochondrial ATP synthesis [86]. It also plays an important role at high respiration rates, as its activity is considered a good indicator of mitochondrial metabolic capacity [87, 88]. SDH activity differs from other mitochondrial dehydrogenases [87], thanks to its unique redox properties, and represents a crucial antioxidant enzyme in the mitochondria which controls superoxides' scavenging activity of the respiratory chain [89]. In addition, SDH plays an important role at high respiration rates and its activity has been considered a good indicator of the mitochondrial oxidative metabolic capacity [89].

2.6.1.4 Citrate synthase

Citrate synthase is one of the most common markers of mitochondrial content [90]. It is the first enzyme involved in the citric acid cycle, where it performs the irreversible condensation of acetyl-COA with oxaloacetate to form citrate, which in turn determines the whole tricarboxylic acid cycle rate [91]. Citrate synthase is significantly elevated by acute exercise in human skeletal muscle in both exercise-trained and untrained muscle [92]. Multiple studies have reported that the training effect on citrate synthase ranges between 0 to 100% [55, 93].

2.6.1.5 Mitochondrial Health Index

Mitochondrial health is emerging as the major determinant of healthy physiological regulation [94]. In relation to disease, mitochondria also play a determinant role in cellular life

and death [95]. The mitochondrial health index (MHI) integrates four functional markers in a simple equation with two numerators and two denominators, which equally represent the nuclear and the mitochondrial genomes. In the MHI, respiratory chain activity (SDH and COX) are mean-centred and added as numerator, and markers of mitochondrial content (CS and mtDNA copy number) are also mean-centred and added as the denominator. The quotient of both terms therefore yields a scalar index, the MHI, which reflects respiratory chain capacity per unit of mitochondrial content. This MHI measure has been previously shown to outperform individual mitochondrial functions measures in leucocytes [17]. In addition, it was demonstrated that activities of SDH and COX enzymes are likely regulated through independent mechanisms, which is consistent with the fact that SDH is entirely encoded by the nucleus whereas COX is partially encoded by the mtDNA. Thus, SDH and COX must contribute independently to mitochondrial health. However, this index has not been examined in skeletal muscle in the context of exercise responses.

2.7 Summary and study aims

Exercise response is a polygenic trait. Thus, it should be evaluated using robust genetic variants and tight-controlled study designs to minimize variability from internal (biological) or methodological (statistics, or untrained controlled period) factors. Understanding the genetic component and the mitochondrial component in exercise responses in skeletal muscle will enable a more systemic picture.

2.7.1 Aims

The overarching aim of this thesis is to determine the influence of robust genetic variants using the Exercise Polygenic Score (EPS) in mitochondrial and physiological response to exercise phenotypes to four weeks of High Intensity Interval Training (HIIT) in the gene SMART study (Figure 2.2). The specific aims are as follow:

1. To identify mitochondrial skeletal muscle markers that explain exercise physiological fitness responses.
2. To test whether exercise polygenic score (EPS) is associated with physiological and mitochondrial phenotypes at baseline (pre-exercise).
3. To test EPS association with changes in physiological and molecular phenotypes after 4 weeks of HIIT.

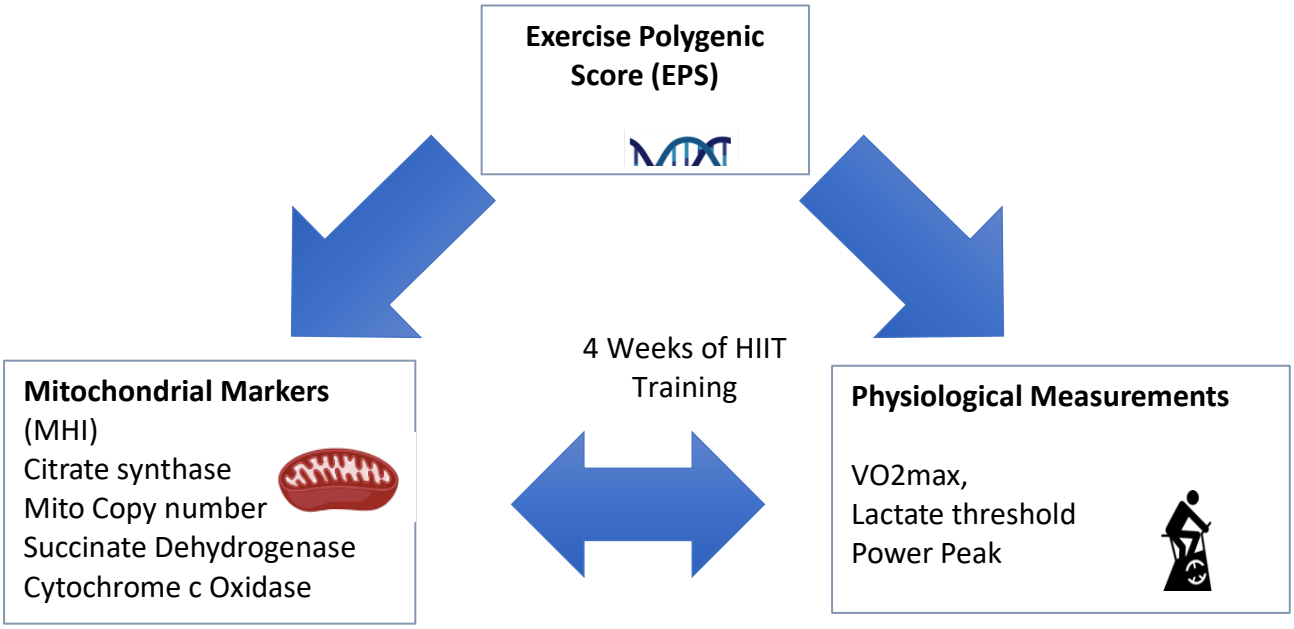

Figure 2.2 *Schematic representation of the study design.*

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Chapter 3: Exercise Polygenic Score

The following section is based on the following publication:

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1. PUBLICATION DETAILS (to be completed by the candidate)

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3.1 Genetics and Exercise

The heritable component of trainability is large, with genetics explaining 47% of the variance in VO_2 peak trainability, and around 52% in resistance variability (1). The contribution of familial factors (genetics and environment) to trainability was demonstrated in the seminal HERITAGE family study (2). This study indicated that VO_2max was more variable between families than within families at baseline (3) and in response to exercise training (4), thus suggesting that DNA sequence variations could modulate exercise responses (5, 6). Pinpointing the responsible gene variants could illuminate the fundamental mechanisms driving this heterogeneity in response to exercise training (6).

The genetic contribution to trainability has been investigated by two different approaches: candidate genes and genome-wide association (GWAS) study. The GWAS approach involves scanning several hundred thousand (currently up to 5 million) DNA markers across the human genome to find genetic variations associated with a particular trait. One of the advantages of the GWAS approach is that it is unbiased and hypothesis-free. In contrast, candidate gene studies require knowledge of the trait of interest and are particularly useful to validate the functional impact of gene loci such as those identified by GWAS (7). GWAS have demonstrated that trainability is polygenic (i.e., influenced by many genetic variants), and that people harbouring the same genotypes in specific gene variants respond more similarly to exercise training than people harbouring different genotypes (8-11). These variants may modulate gene expression that is essential to the molecular adaptation to exercise training, since molecular processes mediate metabolism,

angiogenesis, cardiac and skeletal myofiber hypertrophy, and other processes that lead to better fitness (12).

While many SNPs have been associated with exercise response and trainability, the vast majority of the genes previously identified have not been replicated (13). Replication in an independent cohort is important as it increases the likelihood that results are true and reduces the number of false positives (14, 15). In this chapter we summarised SNPs associated with both resistance and aerobic trainability which have been replicated in two independent cohorts. In addition, we have screened these SNPs with the goal of identifying SNPs at trainability-associated loci that may have functional relevance. Further, we discussed future directions of performing large-scale exercise studies to elucidate the functional relevance of the discovered genomic markers. This approach will allow more rigour and reproducible research in the field of exercise genomics.

3.1.1 SNPs Selection criteria

To provide a robust and comprehensive narrative review, a semi-structured search was performed (July 2019) to identify all studies relating to genetic variants and exercise trainability. Three electronic databases (PUBMED, MEDLINE and SCOPUS) were used to identify relevant articles using the following keywords “genes”, “genome”, “exercise”, “physical activity”, “aerobic capacity”, “resistance”, “strength”, “power”. We excluded studies where the sole focus was on populations with a diagnosed medical condition such type 2 diabetes mellitus, any inflammatory conditions, and cardiovascular disease. Articles were separated in two categories: genetic variants associated with either aerobic or resistance trainability (Table 2.1 and 2.2).

Finally, we selected SNPs that were classified as robust and separated them according to whether they were related to the aerobic trainability or resistance trainability. We chose this criterion as it reflects the reliability of the findings and increases the likelihood that there is true association of the SNP with trainability (15). It also allows us to identify and summarise SNPs with biological relevance, which is useful for researchers to 'select' candidate SNPs to identify causality and purpose of gene (16).

SNPs were considered robust if:

1) They showed consistent association with a given phenotype in at least *two independent* cohorts, and/or;

2) SNPs were shown to have functional relevance in an animal model or cell culture, with gene expression/DNA methylation Quantitative Trait Loci (QTLs) analysis or network, and enrichment analysis.

***Table 3.4**. Gene Variants associated with aerobic trainability*

Author, Date	Sample Size	Sex (% Males)	Age	Ancestry/ Country / ethnicity	Chromosome	Annotated gene	Variant	Genotype and training response (+/−/0)	Intervention (if any)/ exercise	Duration	Type of study
Alves (2009) (1)	N= 83	100%	20-35yrs	Brazil	17 1	*ACE* *ATG*	rs4340 rs699	ACE (2) VO_{2max} TT (+) LVM	Moderate intensity endurance training	3 days/week 16 weeks	Candidate Gene
Bouchard 2011 (3)	N= 742	Males and Females	17-65yrs	HERITAGE study Caucasian and African-American USA	4 6 9 3 9 3 1 1 20 11 14 15 11 14 2 4 11 3 22 11 6	*ACSL1* *PRDM1* *GRIN3A* *KCNH8* *C9orf27* *ZIC4* *CAMTA1* *RGS18* *BIRC7* *DBX1* *DAAM1* *NDN* *CXCR5* *TTC6* *LOC400950* *LOC100289626* *LOC100130460* *NLGN1* *MN1* *CD44* *ENPP3*	rs6552828 rs10499043 rs1535628 rs4973706 rs12115454 rs11715829 rs884736 rs10921078 rs6090314 rs10500872 rs1956197 rs824205 rs7933007 rs12896790 rs4952535 rs2053896 rs2198009 rs2030398 rs738353 rs353625 rs10452621	(+) VO2max	Endurance training Moderate: at 55% HR first two weeks and intense: last 6 weeks 75% HR	20 weeks	GWAS
Dionne (1991)(4)	Males N=46	Males 100%	17-29yrs	Canada, USA	Mitochondria	*MTND2* *MTND5*		MTN2 (-) VO_{2max} MTND5 (+) VO_{2max}	Endurance training at 85% of HRR	3-5 days/week 20 weeks	Candidate gene
Hautala et al. 2007 (5)	N= 478	Males=48.3% Females	17-65yrs	HERITAGE study Caucasian and African-American Canada, USA	22	*PPARD*	rs2016520 rs2076167	African American only rs2016520 CC (-) $VO_{2max,}$ PPO rs2076167 (2)	Endurance training moderate 55% of VO2 and absolute 75% of VO2 intensity	20 weeks	Candidate gene
He et al. 2008 (6)	N= 181	Males 100%	19± 1	Han Chinese	7	*NRF-1*	rs2402970	rs2402970 CC (+) VT, RE	Endurance training	18 weeks	Candidate gene

					15	*NRF-1* *NRF-2*	rs6949152 rs6949152	rs6949152 AA (+) VT, RE rs6949152 AA (+) VO2max	95% to 105% ventilatory threshold		
He et al. 2006 (7)	N= 181	100%	19± 1	Han Chinese	11	*HBB*	rs10768683	C (+) RE	Endurance training 95% to 105% ventilatory threshold	18 weeks	Candidate gene
He et al. 2007 (8)	N= 181	Males= 100%	19± 1	Han Chinese	15	*NRF-2* *NRF-2* *NRF-2*	rs12594956 rs8031031 rs7181866	ATG haplotype (+) RE	Endurance training 95% to 105% ventilatory threshold	18 weeks	Candidate gene
He et al. 2008 (9)	N= 181	100%	19± 1	Han Chinese	4 4 4	*PPARGC1A* *PPARGC1A* *PPARGC1A*	rs17847357 rs8192678 rs6821591	rs17847357, rs8192678 (2) VO2max rs6821591 G (+) VO2max	Endurance training High intensity 95% to 105% HR	18 weeks	Candidate gene
He et al. 2010 (10)	N= 181	100%	19± 1	Han Chinese	4 4 4 2 9	*PPP3CA* *PPP3CA* *PPP3CA* *PPP3R1* *PPP3R2*	rs2850965 rs3804423 rs3804358 rs4671887 rs3739723	G (+) VO2max G (+) VO2max G (+) VO2max A (+) VO2max A (+) RE	Aerobic endurance 95% to 105% of ventilatory threshold	18 weeks	Candidate gene
He et al. 2010 (11)	N= 181	100%	19± 1	Han Chinese	8 8 8 8 8	*PPP3CC* *PPP3CC* *PPP3CC* *PPP3CC* *PPP3CC*	rs1879793 rs1075534 rs7430 rs2461483 rs10108011	CC (+) SV AA (+) SV, CO GG (+) SV CC (+) SV GG (+) SV	Aerobic endurance 95% to 105% of ventilatory threshold	18 weeks	Candidate gene
Leon et al. 2004 (12)	N= 766	Males (43%) and Females	17-65yrs	HERITAGE study Caucasian and African-American USA	19	*APOE*	E2, E3, E4	(2)VO2max	Endurance training Moderate: at 55% HR first two weeks and intense: last 6 weeks 75% HR	20 weeks	Candidate Gene
McKenzie 2011 (13)	N= 109	46.7% Males	50-75yrs	Caucasian USA	14	*AKT1*	rs1130214	Men: GG (+) VO2max Females: (2)	Aerobic training moderate 50-70%	24 weeks	Candidate gene
McPhee et al 2011 (14)	N=58	Females only	Age 18-37yrs	Caucasian UK	14	*HIF1A*	rs11549465	T (+) VO2max	Aerobic 75-90% of HRmax	6 weeks	Candidate gene

Pickering et al 2018 (15)	N=42	Males only	16-19 yrs	European (UK)	4	*PPARGC1A* *VEGF* *ADBR2* *ADBR2* *CRP*	rs8192678 rs2010963 rs1042713 rs1042714 rs1205	Endurance genotype (+) Yo-Yo Test	Aerobic training moderate to intense	8 weeks	Candidate gene
Prior et al. 2003 (16)	N=233	Males 39.3% and Females	50-75 yrs	Caucasian and African-American USA	14	*HIF1A*	rs28708675 rs11549465	African American cohort: rs28708675 AA (+) VO2max Caucasian cohort: rs11549465 CC (+) VO2max	Aerobic training moderate 50-70%	24 weeks	Candidate gene
Prior et al. 2006 (17)	N=146	Males (42%) and Females (58%)	50-75 yrs	Caucasian and African-American USA	6	*VEGF*	rs699947 rs1570360 rs2010963	AAG & CGC haplotypes (+) VO2max	Aerobic training moderate 50-70%	24 weeks	Candidate gene
Rankinen et al 2000 (18)	N= 472	Males and Females	Age 17-65yrs	HERITAGE study Caucasian USA	1	*ATP1A2*	Polymorphisms at exon 1 and 21-22	2α haplotype (+) VO2max and PP	Endurance training Moderate: at 55% HR first two weeks and intense: last 6 weeks 75% HR	20 weeks	Candidate Gene
Rankinen et al 2000 (19)	N= 472	Males (48.7%) and females	Age 17-65yrs	HERITAGE study Caucasian USA	17 1	*ACE* *ATG*	rs4340 rs699	Males: ACE I/D (2) ATG M (+) reduced diastolic blood pressure. Females: ACE I/D (2) ATG M/T (2)	Endurance training Moderate: at 55% HR first two weeks and intense: last 6 weeks 75% HR	20 weeks	Candidate Gene
Rico-Sanz et al 2003(20)	N= 779	Males and Females	Age 17-65yrs	HERITAGE study Caucasian and African-American USA	1	*AMPD1*	rs17602729	TT (-) VO2max	Endurance training Moderate: at 55% HR first two weeks and intense: last 6 weeks 75% HR	20 weeks	Candidate Gene

Ring-Dimiriou et al 2014 (21)	**N=24**	Males	45-65yrs	Austria	4	*PPARGC1A*	rs8192678	GG (+) VO_{2peak}	70-90% of Vo2peakk	3 days/week 10 weeks	Candidate Gene
Rivera et al 1997 (22)	N= 240	Males and Females	17-65yrs	HERITAGE study Caucasian and African-American USA	19	*CKMM*	rs8111989	CC (-) VO2max	Endurance training Moderate: at 55% HR first two weeks and intense: last 6 weeks 75% HR	20 weeks	Candidate Gene
Sonna et al 2001 (23)	N=147	Males (42.2%) and Female	Age 21.7 ± 3.6yrs	USA: 57% Caucasians, 25% African-Americans, 14% Hispanics, 3% Asians, and 1% Native American	17	*ACE*	rs1799752	ACE I/D (2) VO_{2max}	2 aerobic days and 2 strength training days per week	8 weeks	Candidate Gene
Stefan et al (2007) (24)	N= 136	Males and Females	Age 19-67 yrs	Germany	22 22 22 22 4	*PPARD* *PPARD* *PPARD* *PPARD* *PPARGC1A*	rs2267668 rs6902123 rs2076167 rs1053049 rs8192678	rs2267668 G (-) AT, VO_{2peak} rs6902123 (2) rs2076167 (2) rs1053049 (2) rs8192678 A (-) AT	Unsupervised: 3h of moderate sports per week	9 months	Candidate Gene
Steinbacher et al. 2015 (25)	**N=28**	Males Only	50-69yrs	Austria	4	*PPARGC1A*	rs8192678	AA (-) decreased fibre type 1 transformation	70-90% of Vo2peakk	3 days/week 10 weeks	Candidate Gene
Woods el al. (26)2001	N= 108	No percentage mentioned	Age 18.9 ±0.4yrs	Caucasian	17	*ACE*	rs1799752	ACE I/D (2)	Aerobic Submaximal training at 75%	11 weeks	Candidate gene
Yoo et al 2016 (27)	N= 123	Males and Females	Age 30-60yrs	Korea	12 18 2 3 6 2 2	*AMN1* *CDH2* *ASB3* *SRGAP3* *UST* *PUM2* *KCNH7*	rs11051548 rs2542729 rs1451462 rs13060995 rs6570913 rs11096663 rs12613181	(+) VO2 max (+) VO2 max (+) VO2 max (+) VO2 max (+) VO2 max (+) VO2 max (+) VO2 max	HIIT 60%-84% of VO2max	9 weeks	GWAS
Yu et al. 2014 (28)	N= 360	Males (50%) and Females	Age 18-40yrs	China	19	*APOE*	E2, E3, E4	E2/E3 (+) VO2max E3/E4 (+) VO2max	Aerobic 60%-85%	6 months	Candidate gene
Zarebska et al 2014 (29)	N=66	Females only	Age 19-24yrs	Caucasian Poland	11	*GSTP1*	rs1695	G (+) VO2max and VEmax	Aerobic training	12 weeks	Candidate gene

									50% to 70% of HRmax		
Zhou et al. 2006 (30)	N=102	Males Only	18.8 ± 0.9yrs	China	19	*CKMM*	rs1803285	AG (-) RE	Distance running program 95-105% of VT	18 weeks	Candidate Gene

Table 3-5. Gene variants associated with resistance trainability.

Author, Date	Sample Size	Sex (% Males)	Age	Ancestry/ Country of origin/ ethnicity	Chromosome	Gene	Variant	Genotype and training response (+/−/0)	Intervention	Duration	Type of study
Ash (2) (31)	N=602	Males and Females	Age 18-40yrs	FAMuSS study: Predominantly European-American Ancestry	5	*NR3C1*	*rs10482614* *rs10482616* *rs4634384*	Females: rs4634384 T (+) Hypertrophy Males: rs10482616 GG (+) MVC rs10482614 AA (+) MVC	Upper arm, Unilateral resistance program	12 weeks	Candidate Gene
Charbonneau (2008) (32)	N=243	Males =35.3% and Females	Age 50-85yrs	U.SA. Caucasian	17	*ACE*	*rs1799752*	Females: ACE (2) Males: ACE (2)	Knee Extension unilateral resistance program	10 weeks 3days/weeks	Candidate Gene
Clarkson (2005) (33)	N=602	Males =41% And Females	Age 18-40yrs	FAMuSS study: Predominantly European-American Ancestry	11	*ACTN3*	*rs1815739*	Females: ACTN3 XX (+) Maximal dynamic strength (1RM). Males: ACTN3 (2)	Upper arm, Unilateral resistance program	12 weeks	Candidate Gene
Delmonico (2007) (34)	N=157	Males=45.2% And Females	Age=50-85yrs	Caucasian USA	11	*ACTN3*	*rs1815739*	Females: ACTN3 RR (+) PP Males: ACTN3 (2)	Knee Extension unilateral resistance program	3days/week 10 weeks	Candidate Gene
Erskine (2012) (35)	N=51	Males: 100%	Age 20.3± 3.1yrs	Caucasian UK	8	*PTK2*	*rs7843014* *rs7460*	rs7843014 AA (+) Strength (MVC) *rs7460* TT (+) Strength (MVC)	Knee Extension unilateral resistance program	3days/week 9 weeks	Candidate Gene
Erskine (2013) (36)	N=51	Males: 100%	Age 20.3± 3.1yrs	Caucasian UK	17 11	*ACE* *ACTN3*	*rs1799752* *rs1815739*	ACE (2) ACTN3 (2)	Knee Extension unilateral resistance program	3days/week 9 weeks	Candidate Gene
Folland (2000) (37)	N=33	Males: 100%	Age 18-30yrs	UK	17	*ACE*	*rs4646994*	Isometric training: ACE DD/ID (+) Isometric strength (MVC) Dynamic training: ACE DD/ID (2)	Isometric Training	3days/week 9 weeks	Candidate Gene

									Dynamic training		
Giaccaglia (2006) (38)	N=213	Males and Females	Age>60yrs	Predominantly Males and Females of European-American Ancestry	17	*ACE*	*rs4646994*	ACE DD (+) strength (MVC)	Light resistance training	3days/week 18 months	Candidate Gene
Harmon (2010) (39)	N=874	Male 41.1%	Age 18-40yrs	FAMuSS study: Predominantly European-American Ancestry	17 3	*CCL2* *CCR2*	*CCL2 (rs17652343), (rs1860189), (rs3917878), (rs2857654), (rs1024611), (rs1024610), (rs3760396), (rs2857656), (rs2857657), (rs4586), (rs13900)* *CCR2 (rs17141010), (rs768539), (rs3918358), (rs1799864), (rs1799865).*	Females: CCL2 (2) and CCR2 (2) Males: CCL2 T (rs1024610) (+) Maximal Isometric strength (MVC) Males and Females CCR2 (AA) rs3918358 and (TT) rs1799865 (+) Isometric strength (MVC)	Upper arm, Unilateral resistance program	2 days/week 12 weeks	Candidate Gene
He (2019) (40)	N=40	(Females only)	Age 53-66yrs	Chinese, Beijing	17	*ACE*	*rs4646994*	ACE DD (+) Maximal Isometric strength (MVC), muscle hypertrophy and grip strength	Whole body resistance training	3 days/week 8 weeks	Candidate Gene
Hong (2014) (41)	N=83	100%	Age 22.6 ± 1.4 yrs	South Korean	11	*CNTF*	*rs1800169*	CNTF G/A (2)	Resistance training of the upper extremities	3 days/week 8 weeks	Candidate Gene
Jamshidi et al (2002) (42)	N=144	Males only	19.6 (2.4) yrs	UK	6	*PPARA*	*rs425778*	C (+) LV mass	Upper and lower body training program	10 weeks	Candidate Gene
Jones (2006) (43)	Study 1, N=28. Study 2 N=39	100%	18-20 yrs	Caucasian UK	17	*(Power-related polygenic*	*ACE D (rs1799752) ACTN3 (rs1815739)*	Power genotype (+) Power (CMJ) after high intensity resistance training but not low intensity resistance training.	Low intensity (~30% of 1 RM and high	8 weeks of high or low	Polygenic Score

					11	*risk score)*	*ADRB2 C (rs1042714) AGT C (rs699) IL-6 G/C (rs1800795) PPARA C (rs4253778) TRHR G (rs8192676) VDR A (rs1544410)*		repetitions) and high-intensity (~70% of 1 RM and low repetitions) resistance training	resistance training 1 to 2 days per week	
Keogh (2015) (44)	N=58	Not percentage mentioned	Age 69.8 ± 5.3	New Zealand (European ancestry)	17	*ACE* *UCP2*	*rs4646994* *rs7109266*	ACE ID (2) UCP2 GG (+) Lower body strength (8ft Up and Go time)	Resistance training light to moderate intensity	2days/week, 12 weeks	Candidate Gene
Kostek (2005) (45)	N=67	Males= 47.7%	50-85yrs	U.S.A Caucasian	12	*IGF1*	*IGF1 192*	IGF1 192/192 + 192/- (+) dynamic (1RM) muscle strength	Unilateral resistance program	10 weeks 3days/wk	Candidate Gene
Li (2014) (46)	N=94	100%	Age 18-22years	Han Chinese	2	*MTSN*	*rs1805086 rs1805065*	MTSN KR (+) Hypertrophy in Biceps and Quadriceps MTSN AT + TT (+) Hypertrophy in Biceps	Arm and Leg resistance training	3-4 days/wk 8 weeks	Candidate Gene
Pereira (2013) (47)	N=139	(Females only)	Age 65.5 (8.2)	Portugal, Caucasian	17 11	*ACE* *ACTN3*	*rs1799752* *rs1815739*	ACE D/D (+) maximal dynamic strength 1RM, power (CMJ), functional capacity (STS) ACTN3 RR (+) maximal dynamic strength (1RM), power (CMJ), functional capacity (STS)	High-speed power training	12 weeks 3days/week	Candidate Gene
Pescatello (2006) (48)	N=631	Males=42 %	Age 18-40yrs	FAMuSS study: Predominantly European-American Ancestry	17	*ACE*	*rs4646994*	Trained Arm Post Intervention: ACE II/ID (+) Maximal Isometric strength (MVC) Untrained Arm Post Intervention: ACE DD/ID (+) maximal dynamic strength (1RM), muscle size (CSA of Type II fibres).	Upper arm, Unilateral resistance program	12 weeks, 2days/week	Candidate Gene
Pistilli (2008) (49)	N= 748	Males (40.2%) Females	18-40yrs	Caucasian	10	*IL15RA*	*rs2296135*	*rs2296135* CC (+) MVC	RT program	12 weeks 2 day/week	Candidate gene

Reichman (2004) (50)	N= 153	Males 49.6% Females	Aged 18-31 years	Predominantly European-American Ancestry	10	*IL15RA*	*rs3136617* *rs3136618* *rs2296135*	rs3136617 C (+) muscle hypertrophy rs2296135 C (+) muscle hypertrophy	Whole body resistance training @75% of 1RM	10 weeks, 3days/week	Candidate Gene
Sprouse (2014) (51)	N= 874	Males= 50%	Age: 18-40 years	FAMuSS study: Predominantly European-American Ancestry	8	*SLC30A8*	*rs13266634*	Females: SCL30A8 (2) Males: SCL30A8(2)	Upper arm, Unilateral resistance program	Acute and 12-week Intervention	Candidate Gene
Thomis (2004) (52)	N=57	100%	22.4 (3.7) yrs	Flemish Brabant, Belgium	17 2	*ACE* *MTSN*	*rs4646994* *rs1805086* *rs1805065*	ACE (I/D) (2) strength, isometric and concentric torque or arm muscle cross-sectional area MTSN: Unable to be determined	High resistance training program	10 weeks, 3days/week	Candidate Gene
Walsh (2009) (53)	N=745	Males 40%	Age 18-40yrs	FAMuSS study: Predominantly European-American Ancestry	11	*CNTF*	*rs1800169*	Females: CNTF GG (+) isometric (MVC) and dynamic (1RM) muscle strength Males: CNTF (2)	Upper arm, Unilateral resistance program	12 weeks, 2 days/wk	Candidate Gene
Walsh (2012) (54)	N=560	Males and Females No percentage mentioned	Age 18-40yrs	FAMuSS study: Predominantly European-American Ancestry	1	*LEP* *LEPR*	*rs2167270* *rs1137100* *rs1137101* *rs1805096* *rs8179183*	LEP (GG/GA) rs2167270 (+) Muscle hypertrophy LEPR (2)	Upper arm, Unilateral resistance program	12 weeks, 2 days/wk	Candidate Gene

Table 3.6. *Robust SNPs associated with aerobic or resistance trainability.*

Aerobic Trainability			**Resistance trainability**		
SNP	**Nearest Gene**	**Beneficial allele**	***SNP***	**Nearest Gene**	**Beneficial allele**
rs6552828	*ACSL1*	G	rs4646994*	*ACE*	D
rs699	*AGT*	T	rs1799752*	*ACE*	D
rs6090314	*BIRC*	A	rs1815739	*ACTN3*	R
rs12580476	*C12orf36*	TBC	rs2296135	*IL15 RA*	C
rs884736	*CAMTA1*	G	rs4253778	*PPARA*	C
rs353625	*CD44*	TBC			
rs1956197	*DAAM1*	G			
rs17117533	*NDN*	A			
rs8192678	*PPARGC1A*	G			
rs10921078	*RGS18*	A			
rs7531957	*RYR2*	TBC			
rs11715829	*ZIC4*	G			

*Linkage Disequilibrium above 80 % according to ensemble LD calculator.

3.1.2 Aerobic Trainability

Twin and family studies indicate that ~22–57% of aerobic fitness variability between individuals can be explained by genetics and therefore plays an important role in the range of aerobic phenotypes observed in a population (1). Here, we briefly describe some of the robust SNPs that have been associated with aerobic trainability, which means they were replicated in at least 2 independent cohorts and were shown to have functional relevance.

A bioinformatic analysis study conducted by Ghosh *et al.* found that the greatest number of SNPs were annotated to the PPAR signalling pathway suggesting its importance in VO_{2max} trainability (2). As such, the most widely studied genes within this pathway are the peroxisome proliferator-activated receptors (*PPARA, PPARG,* and *PPARD*) and their transcriptional coactivators (*PPARGC1A* and *PPARGC1B*). These genes have been linked to multiple aerobic phenotypes, including muscle morphology, aerobic capacity and endurance performance (3, 4). *PPARD* is expressed predominantly in adipocytes and skeletal muscle

where it promotes fatty acid oxidation (5). In the HERITAGE family study, the rs2016520 SNP located in *PPARD* was associated with VO_{2max} and maximal power output after a 20 week endurance training intervention in African-Americans, but not in Caucasians (6). *In vitro* and animal studies show that the minor allele (C allele) in this SNP (rs2016520) results in higher *PPARD* transcriptional activity, which in turn promotes lipid accumulation and alters normal regulation of lipid uptake and storage (5, 7, 8). In a European cohort it was shown that the *PPARD* rs2267668 SNP was associated with VO_{2peak} and anaerobic threshold after a 9-month lifestyle intervention (9). They then confirmed that in human primary cell lines that those carrying the minor allele at rs2267668 (G allele) were associated with lower mitochondrial activity, demonstrating a potential functional effect (9). Taken together, the *PPARD* locus may play a role in aerobic trainability, but larger cohorts of different ancestries and more in depth functional studies to determine causal SNP are needed to confirm this.

The transcriptional co-activator *PPARGC1A* interacts with *PPARD* and regulates mitochondrial biogenesis, angiogenesis, lipolysis and adipogenesis (10). Four candidate gene studies, predominantly in men, found consistent associations of rs8192678 within *PPARGC1A* and aerobic capacity in Europeans (9, 11-13). While in the Han Chinese cohort another nearby SNP (rs6821591) was associated with VO_{2max} specifically, the G allele was associated with increased VO_{2max} compared to those carrying the A allele (14). Work conducted in a Han Chinese cohort found that the *PPARGC1A* rs6821591 SNP had functional significance as gene expression was altered; this was dependent on genotype (A v G allele), with the G allele displaying increased PGC-1α gene expression (15). Overexpression of PGC-1α in an animal model showed increased Type 1 fibres in muscles that are normally Type II fibre-dense and this induced increases in resistance to fatigue, inferring increased aerobic capacity (16). These population-specific results indicate that it is the *PPARGC1A* locus itself, rather than individual SNPs located within that locus, may be important for trainability (14, 17).

Currently 26 SNPs associated with VO_{2max} trainability were identified in a GWAS and were validated in 2 separate cohorts (detailed in **Table 2**) (18). They accounted for 49% of VO_{2max} trainability and were able to classify responders and non-responders (18, 19). Whether these SNPs are directly involved in gene function or regulation of genes is the next step to validate these findings. The most robust is the SNP rs6552828 located near the *ACSL1* gene, which was the strongest predictor (~6%) of aerobic trainability (VO_{2max}) (18). It has subsequently been validated in a bioinformatics pathway analysis and found to be strongly correlated to the aerobic electron transport chain phenotype and the PPAR signalling pathway, providing a robust candidate gene in VO_{2max} trainability (2). ACSL1 regulates lipid metabolism by facilitating the transport of long chain fatty acids into the mitochondria and is an essential step in fatty acid oxidation (20). Timmons *et al.* integrated RNA profiles with genetic variants and found the following genes *CD44,* and *DAAM1,* also discovered in the Bouchard et al. GWAS, were associated with gene expression changes (21). Gene expression of *CD44* was up-regulated in response to endurance training (21) and was strongly associated with phenotypic terms associated with aerobic exercise, such as cardiovascular physiological processes, muscle contraction, physical fitness and aerobic electron transport chain (2). This indicates that this gene and any alterations to its function (i.e. via SNPs) may play in important role in aerobic trainability. While these genes certainly provide robustness, there are still limitations in determining the causality of these particular SNPs in the molecular mechanisms affecting aerobic trainability.

Many candidate gene and GWAS studies have been conducted and this chapter highlights the large collection of candidate genes that have been associated with aerobic trainability. Only 12 SNPs have been robustly associated with aerobic trainability (**Table 3**), meaning that they have been validated in at least 2 independent cohorts and were shown to have some functional

relevance. Subsequent studies should focus on understanding the functional role of the SNPs that have been replicated, as this chapter highlights the lack of understanding of the molecular mechanism and limits our understanding of aerobic trainability.

3.1.3 Resistance Trainability

Muscular strength and power show a heritability estimated around 52% (22). Skeletal muscle strength is defined as the force produced by muscle contraction. A variety of measures have been investigated, including muscle strength, maximal voluntary contraction (MVC), 1 repetition maximum (1RM) and handgrip strength. While the production of skeletal muscle power is defined as how much force can be produced and the velocity at which it is produced, the production of power can be measured at the by undertaking tests such as Wingate's, counter movement jumps (CMJ) and vertical jumps.

The *ACE I/D and ACTN3 R/X* SNPs are two of the most extensively studied gene loci. We have chosen not to discuss *ACTN3* here as it has recently been reviewed in detail by Del Coso et al. (24) and instead focus on the *ACE* I/D SNP. The *ACE* gene encodes the angiotensin-converting enzyme that is a central component of the renin-angiotensin-system (25). The *ACE I/D* results in either an insertion (26) or deletion (26) of a 287-basepair region in intron 16 of the gene (27) and can alter the levels of ACE in the blood (27). It has recently been shown that polymorphism can manipulate the activity of the C- and N-terminal domain in the enzyme (28). Further, exercise can decrease enzyme activity in the C-terminal domain and increase the activity in the N- terminal domain, which results in improved blood flow and proliferation of red blood cells (28). The I allele is assumed to confer enhanced endurance performance while the D allele is thought to confer increased muscle power and strength (29). The D allele was consistently shown across 6 separate candidate gene studies to be associated with greater gains

in strength after resistance training, and this was consistent across sex and age (30-35). While the literature is consistent regarding muscular strength, the association with muscular power is less convincing (30, 36-38). The D allele in *ACE* was associated with CMJ in older females after a 12-week power training program (33) and in young males after a high intensity training program (39). However, it was the I allele in *ACE* that was associated with a higher baseline VJ at baseline in males and females (37). Another two studies did not find any association between the *ACE* I/D and skeletal muscle power at baseline or in response to resistance training (36, 38). *ACE* provides a robust candidate gene for explaining variation in muscular strength but not muscular power, suggesting that this gene loci may only explain some of the inter-individual resistance variability dependent on type of resistance exercise.

Many of the candidate genes in resistance trainability came from a large multi-centre trial (FAMuSS) which aimed to identify nonsynonymous SNPs with functional effects on muscle power and strength (40). These include: *Glucocorticoid receptor (NR3C1)* (41)*, alpha-actinin 3 (ACTN3)* (42), *Chemokine (C-C motif) ligand 2 (CCL2)* (43)*, Chemokine (C-C motif) ligand 2 Receptor (CCR2)* (43), *ACE* (35)*, Solute carrier family 30 (zinc transporter), member eight gene (SLC30A8)* (44), *Leptin (LEP)* and *Leptin receptor (LEPR)* (45). The FAMuSS study was conducted in young (18-40 years old) males (N=247) and females (N=355) of predominantly European-American ancestry. Participants underwent a 12-week unilateral resistance program consisting of upper arm exercises in the non-dominant arm (35). Only *IL-15RA, ACTN3* and *ACE* from this series of studies were replicated in separate cohorts and have functional relevance. In the *IL-15RA* locus the rs2296135 SNP was associated gains in muscular strength and replicated in two different studies in European ancestry cohorts (46, 47). When the gene *IL-15RA* is knocked down in an animal model it alters the contractile properties and fatigability in skeletal muscle fibres (48). While the locus is important, it is not yet clear which SNPs is responsible for altering the function of *IL-15RA* protein. Although SNPs within

CCL2, CCR2 and *CNTF* have not been replicated, they interestingly showed sex-specific associations with muscle strength. *CTNF* polymorphisms were associated with strength gains only in females (49), which was subsequently confirmed in a South Korean cohort (50). SNPs in *CCL2* and *CCR2* were associated with strength gains in males only (43). This indicates potential sex-specific differences in the genetic architecture of complex traits and should be incorporated into study design (51, 52). In addition, PTK2, CNTF, IL-6, PPARA and VDR candidate genes have been replicated with functional relevance (39, 53).

In total 5 SNPs (**Table 3)** were robustly associated with resistance variability. While there is a plethora of candidate gene studies, no GWAS have been conducted that specifically focuses on resistance trainability.

3.1.4 Functional Validation

We have identified 12 SNPs and 5 SNPs that are robustly associated with variance in aerobic and resistance trainability respectively. The next steps are to a) identify the causal SNP, b) annotate the causal SNP to the correct gene and then c) to establish the functional relevance of the gene (19). The overall evidence from literature connecting causal genes to trainability is relatively low (2). If we hope to identify the causal variants or genes it is vital that we begin to integrate 'omic' technologies, from genome and epigenome, to transcriptome, to proteome and metabolome, which can capture a complete picture of complex human traits such as aerobic and resistance trainability (54, 55).

There have been attempts to associate molecular pathways or 'molecular phenotypes' with physiological phenotypes of aerobic and resistance trainability (56-58). Sarzynski et al. applied this systems biology approach by combining the 21 SNP identified in a GWAS from

the HERITAGE study cohort (Table 2) (18, 59) and examined the joint contributions of these SNPs to exercise response (19). This approach identified potential pathways in calcium signalling, energy sensing and partitioning, mitochondrial biogenesis, angiogenesis, immune functions, and regulation of autophagy and apoptosis, which can be investigated more closely (19). Another integrative approach is expression quantitative trait loci (eQTLs) analysis, which leverages gene loci identified from GWAS and integrates them with gene expression data to identify differential gene expression levels, and uncover the 'molecular phenotype' that leads to these variations in exercise response (60, 61). Willems *et al.* identified the rs6565586 SNP in *ACTG1* as a strong candidate gene in inter-individual variability in the resistance-related phenotype (hand grip strength) and correlated this with a lower expression of mRNA in skeletal muscle. *ACTG1* encodes Actin Gamma 1 and is involved in the structure and function of skeletal muscle fibres. Interestingly, in a knockout mouse model, animals displayed overt muscle weakness (62). This type of analysis presented an ideal candidate gene to begin understanding the molecular mechanisms in human skeletal muscle.

The type of follow-up experiment will depend on the location of SNP within the gene. For SNPs within coding regions, ideally experiments are performed to study the effect of the SNP has on protein structure and function. For SNPs in non-coding regions it more difficult to determine, as they may not directly affect a gene but alter/regulate transcription factors and mediate alterations in genes this way (54). However, with the introduction of the large epigenetic database ENCODE (Encyclopaedia of DNA elements) we can now identify the transcription factor association, chromatin structure and histone modification of target genes (63), and more recently enhancers providing candidate gene targets for follow up analysis (64). With the discovery of CRISPR Cas-9 genome-editing tool in 2012 (65), this has paved the way for establishing causality of SNPs and the functional effects of them. This has been successfully used for establishing causal genes implicated in insulin resistance, whereby they were able to

determine the causal effect of 12 candidate genes that had previously been identified in a GWAS (66). To date no experiments have been conducted using this gene-editing tool to establish the function and causality of candidate genes of trainability beyond association analysis.

There is still much work to do before personalised exercise prescription (both in a clinical and elite athlete setting) can be based on an individual's genetics. However, there are concerted efforts taking place to make this possible, such as the Athlome Project Consortium and the Gene SMART (Skeletal Muscle Response to Training), recently launched with the aim of uncovering the genetic variation underlying athletic performance, adaptation to exercise training, and exercise-related musculoskeletal injuries (67, 68). These, and other initiatives will allow for population-based approach to understand the role of genes and environmental factors contributing to the complex exercise response phenotype (69).

3.2 References

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Chapter 4: Variability in physiological fitness and skeletal muscle mitochondria-related phenotypes in response to HIIT

This chapter is based on the following article currently under review in a peer-reviewed journal. Alvarez Romero, J., et al., Physiological and mitochondrial phenotypes in response to high intensity interval training in male and female skeletal muscle: The Gene SMART Study. Medicine & Science in Sports & Exercise 2022.

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I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

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3. CO-AUTHOR(S) DECLARATION

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4.1 Introduction

Significant inter-individual differences exist in both physiological and molecular responses to any given exercise training [1-3]. Considerable variability is observed in many physiological parameters [4], including maximal oxygen uptake (VO_{2max}) [5, 6], resting heart rate [6], exercise heart rate [6], aerobic and anaerobic thresholds (LT) [6, 7], and muscle mass and strength [8, 9]. Molecular phenotypes such as resting muscle glycogen content, muscle enzyme activity [10], and mitochondrial function have also shown large variability [11].

Variability identified in exercise training studies stems from multiple sources, including technical and biological factors as well as different baseline levels between individuals [12]. This variability is minimised when a control period is included in the study design and compared to the intervention period [13, 14]. A control period refers to exposing the same individual to a pre-intervention period, in which data is collected before and after [13, 14]. This data can then be correlated against the baseline data (immediately before starting the intervention), ensuring no significant changes in the tested phenotype. Thus, we can conclude that any phenotypic alteration post-exercise is attributed to the exercise intervention and not due to day-to-day variability or technical error [12]. Previous studies have addressed the changes in molecular and physiological phenotypes after the intervention, e.g. the mean individual-difference in pre-post exercise responses [15]. In addition, multiple studies have compared exercise responses for the training group vs a control group [16]. However, it is unknown if these changes would remain when a control period is applied.

Skeletal muscle mitochondria are highly responsive to exercise [17], with improvements in quantity (content) and quality (structure and function) as typical outcomes of an exercise

intervention [18, 19]. Prior research has examined changes in individual mitochondrial markers in response to exercise [20-25]; however, examining a composite measure is crucial to assess mitochondrial health, as changes in mitochondrial content are a compensatory mechanism for changes in mitochondrial quality [26, 27]. Thus, mitochondrial health may reveal insights around mitochondrial adaptations in response to exercise. The mitochondrial health index (MHI) mathematically outperformed individual markers of mitochondrial function and content in relation to caregiver status [11]. This study confirmed that combining mitochondrial markers in leucocytes had higher effect size (0.63) than individual components of the mitochondrial energy production machinery (0.23 ± 0.12). Thus, we aim to validate these results in skeletal muscle. The MHI combines four measures of mitochondrial function and content into a composite measure that integrates nuclear and mitochondrial DNA encoded measurements. Mitochondrial function is represented by key respiratory chain enzymes activity, including succinate dehydrogenase (SDH) and cytochrome C oxidase (COX) [28]. Mitochondrial content is represented by ii) citrate synthase (CS) [29] activity and mitochondria (mtDNA) copy number [30].

The aim of this study was to identify changes in physiological and mitochondrial markers in relative large Australian adult cohort (n=112) following four weeks of high-intensity interval training (HIIT), relative to a four weeks of a control period.

4.2 Methods

4.2.1 Participants

This study was performed on participants from the Gene SMART (Skeletal Muscle Adaptive Response to Training) cohort. A detailed methodology of the Gene SMART study has been previously published [31]. Briefly, 92 healthy males and 20 females aged 18 to 45 years with a body mass index (BMI) of 25.46 ± 3.29kg m^2 were recruited to complete a four weeks of HIIT intervention. The study was approved by the Human Ethics Committee at

Victoria University (HRE13-223, and HRE 21-122), and written consent was obtained from each participant. A detailed medical history was obtained by questionnaire. Participants were excluded from the study if they had definite or possible heart disease, chronic respiratory condition, musculoskeletal problems that would restrict cycling, uncontrolled endocrine and metabolic disorders, or diabetes requiring insulin or other therapies.

4.2.2 Study design

The study design consisted of six steps **(Figure 1)**: 1) screening and familiarisation, 2) baseline testing before control period, 3) control period, 4) end of control and baseline testing pre-exercise training intervention, 5) exercise intervention (HIIT), and 6) post-training testing. Throughout the study participants were asked to maintain their habitual dietary and physical activity patterns and refrain from strenuous exercise, caffeine, or alcohol consumption for at least 24 hours before the tests.

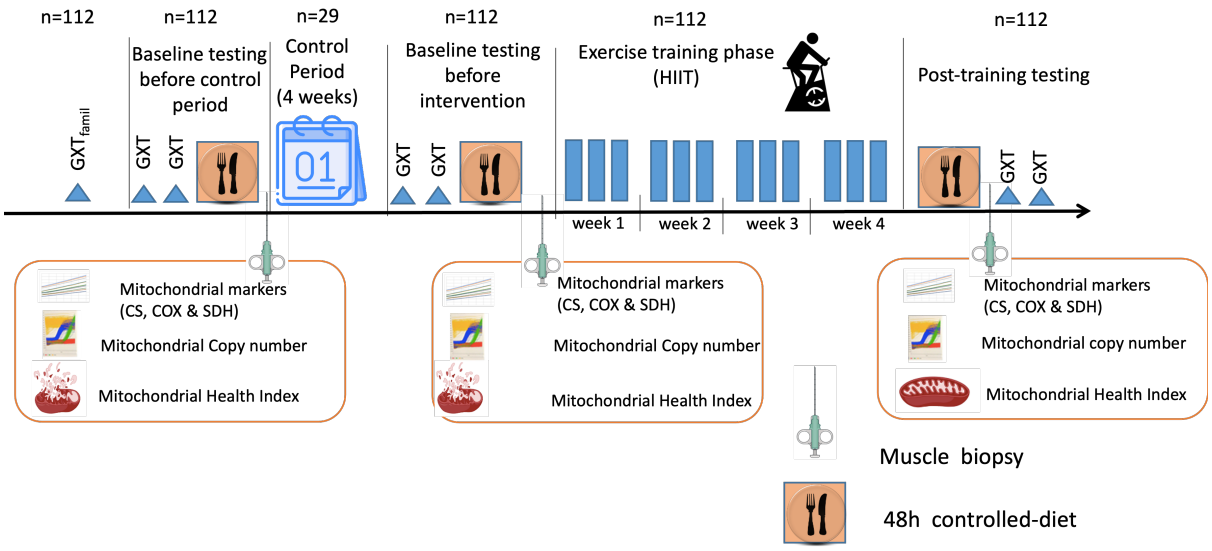

Figure 4.1 **Study design.** Abbreviations; graded exercise test (GXT), high-intensity interval training (HIIT), citrate synthase activity (CS), cytochrome c oxidase activity (COX), succinate dehydrogenase activity (SDH).

4.2.3 Graded-Exercise Tests (GXT) tests

Prior to commencing a four-week HIIT intervention, a subset of Gene SMART participants (n=29, 10 females and 19 males) completed a four weeks of a control phase, in which participants were not prescribed any training and were asked to continue with any regular exercise, diet, and sleep routine. Before commencing this control phase, a pre-control baseline assessment was completed involving two GXTs. Tests were performed on an electronically braked cycle ergometer (Lode-Excalibur sport, Groningen, the Netherlands) and consisted of 4-min stages separated by 30-sec rest periods until exhaustion. W_{peak} was determined from the GXTs as 105% of the maximum power reached in the last stage of the GXT [32]. The test started at 50 and 60 watts and increased by 25 or 30 watts in each subsequent stage for males and females respectively. When females were not able to get six stages in the first GXT, they started the tests at 25 watts. Capillary blood samples were taken at rest after each completed stage and immediately following exhaustion, and analysed by the YSI STAT plus system (Yellow Springs, Ohio, USA). LT was calculated by the modified DMAX method, which is determined by the point on the polynomial regression curve that yields the maximum perpendicular distance to the straight line connecting the first increase in lactate concentration above resting value and the final lactate point. When the difference between replicate GXT was less than 5%, the average of the two GXT tests were used to individualise exercise intensities; otherwise, the highest value was used. Following a 5-min rest, peak oxygen consumption (VO_{2max}) was measured using a calibrated Quark CPTE metabolic system (COSMED, Rome, Italy). In this test, participants wore the COSMED face mask, and cycled for 3-min at a starting intensity of 50 or 60W (females and males, respectively) and then cycled at W_{peak} until exhaustion.

4.2.4 Muscle biopsies

During pre-control baseline testing, muscle samples were taken from the *vastus lateralis* by an experienced medical doctor. Following injection of a local anaesthetic (5 mL, 1% Xylocaine), incisions were made, and the biopsy needle was inserted. Muscle samples were collected with manual suction applied. Following collection, the samples (50–200 mg) were immediately blotted on filter paper to remove excess blood. The muscle was immediately snap frozen in liquid nitrogen and stored at −80 °C for subsequent analyses.

4.2.5 Dietary control (48h prior to muscle biopsies)

Standardised meals were provided to participants for 48-hours before muscle and blood sampling. These diets were based on the Australian National Health and Medical Research Council NHMRC guidelines (15-20% protein, 50-55% carbohydrates, <30% fat and <10% of saturated fat) and adjusted for dietary restrictions and preferences. Participants were also asked to fast for a minimum of eight hours prior to the biopsy.

4.2.6 Control period

Following pre-control baseline testing, the defined subset of Gene SMART participants (n=29) completed a four-week control period, where no exercise interventions were conducted and participants were told to continue with their habitual day-to-day lifestyle for two weeks. Following this period, duplicate GXTs and a muscle biopsy were conducted following procedures defined above.

4.2.7 Pre-HIIT baseline testing and HIIT intervention

All participants (n=112) completed two GXTs and muscle sampling baseline assessments before beginning the HIIT intervention as described above. Participants then

commenced a four-week HIIT program, where they trained in the laboratory, under supervision, three times a week for four weeks. All training sessions were completed on an electronically braked cycle ergometer (Velotron, Racer Mate Inc, Seattle, USA) and were preceded by a 5-min warm-up at 50 W. Each session consisted of six to twelve 2-min intervals performed at different intensities ranging from 40 to 70% of (W_{peak} - LT) above LT, and interspersed by 1-min recovery periods (work-to-rest ratio of 2:1). The number of intervals and the intensity were progressively increased to maintain progression. Testing and training sessions were interspersed over 48 hours to avoid overtraining. At the completion of the four weeks of HIIT, two post-intervention GXTs were performed and a further muscle sample was collected.

4.2.8 Muscle derived mitochondrial health index (MHI)

We present a comprehensive index of mitochondrial health that includes either changes in mitochondrial content as well as mitochondrial functional capacity (quality). Collected muscle samples were used to assess MHI, which was derived from separate analyses of succinate dehydrogenase (SDH), cytochrome c oxidase (COX), citrate synthase (CS) activity, and mitochondrial copy number (mtDNA) for each participant. SDH using a colorimetric SDH activity Assay kit (Abcam #228560) was measured according to the manufacturer's instructions. Samples were analysed in duplicate, and the absorbance was measured in kinetic mode at 600nm for 10-30min at 25°C. COX was assessed using a COX assay kit (Abcam, #ab239711). and manufacturer's instructions were followed The muscle lysate from the SDH analysis was used in the COX activity analysis. Samples were measured in triplicates and the plate was immediately read at a 500nm wavelength and continuously measured over a period of 30-45min. CS activity (mol/h/kg of protein) was measured in triplicates at 30°C and pH 7.5. Muscle samples were lysed in an ice-cold buffer (KH_2PO_4 & $KHPO_4$) using a TissueLyser II

(Qiagen). In all analyses described above if the coefficient of variance [33] was >10% for the duplicate or triplicate results, values were removed.

Mitochondrial copy number (mtDNA) was determined in quadruplicates using multiplex qPCR. This method allows for simultaneous amplification of a mitochondrial (ND1) and a nuclear amplicon (RNAseP, Thermofisher Scientific #4403328) to verify their relative abundance (Krishnan et al. 2007; Picard et al. 2018). The assay was run on a QuantStudio™ 7 Flex Real-Time PCR System. The average CV for mtDNA Cts was 1.02%. Data was manually curated, and cases where samples yielded a standard deviation > 0.3 were removed.

The sequences for the ND1 amplicon (IDT) are as follows:
Forward primer (300nM), 5'CCCTAAAACCCGCCACATCT3';
Reverse primer (300nM): 5'GAGCGATGGTGAGAGCTAAGGT3'; and
Probe (100nM): 5'FAMCCATCACCCTCTACATCACCGCCC-TAMRA3'.

To calculate MHI [11], the following formula was used:

$$\left[\frac{Energy\ production\ capacity}{Mitochondrial\ content}\right] = \left[\frac{Compex\ II\ (SDH) + Complex\ IV(COX)}{CS + mtCN}\right] * 100$$

4.2.9 Statistical analysis

Statistical analysis was performed using R version 4.1.0 [34] using a linear mixed model of the form:

$$Outcome = Delta\ change \sim sex + age + timepoint + (1|ID)$$

where the outcome variable was either the change in physiological measure of fitness (W_{peak} or LT or VO_{2max}) or the mitochondrial markers (CS activity, mtCN, SDH, COX) and the

composite measure (MHI), the timepoint was a numeric variable (0 (PRE intervention) or 1 (post intervention)). The mixed model estimates whether there were changes in outcome at the group level after four weeks of control vs. intervention period. Pearson correlations between changes in physiological variables and mitochondrial makers were also formulated to investigate any associations between them.

4.3Results

4.3.1 Changes in physiological and mitochondrial markers following a control period

No significant changes were observed in any physiological measurements following the control period (**Figure 2**). Further, when examining changes between the end of the control phase and pre-HIIT baseline testing, there were also no differences in any of the physiological variables. For mitochondrial markers, when comparing changes across the end of control Vs HIIT baseline we did not observe changes in mtCN and MHI (**Supplementary fig. 1**); however, we observed changes in SDH, CS and COX (**Supplementary fig. 1**).

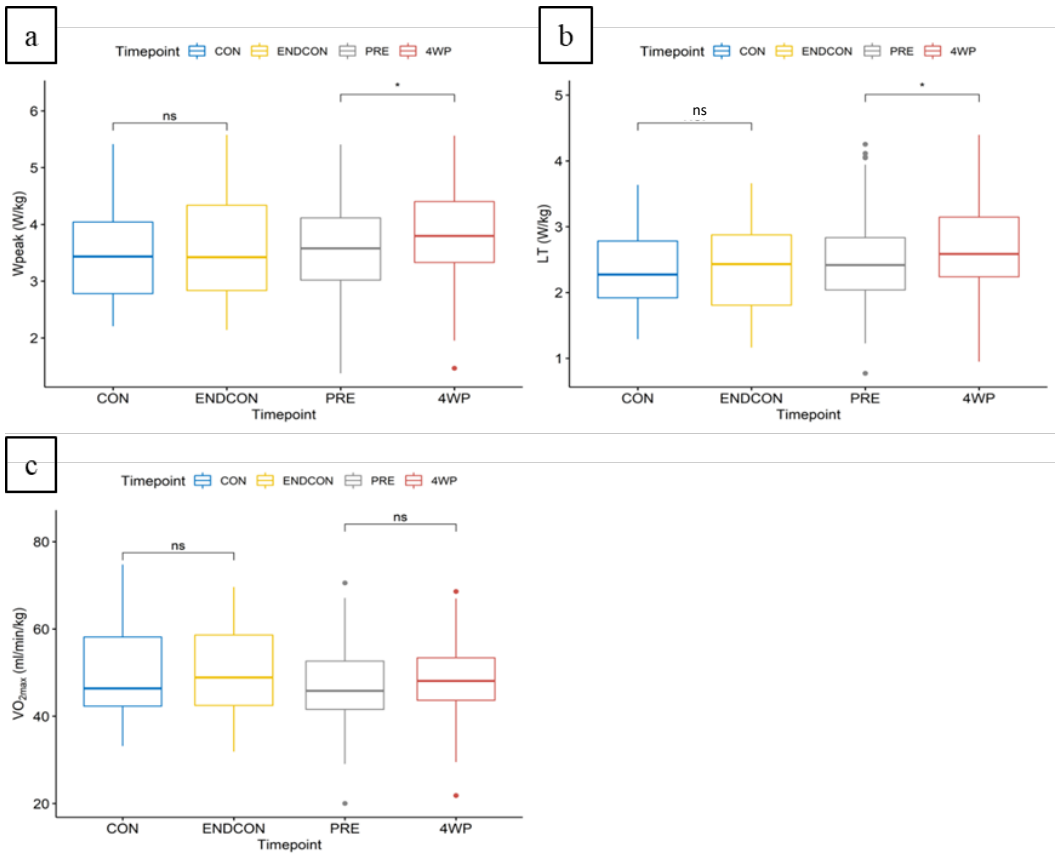

Figure 4.2 **Changes in physiological variables over four weeks of control and HIIT exercise periods.** Box plots show the mean changes over four weeks of the control period and four weeks of HIIT for a) power peak, b) lactate threshold and c) VO_{2max}. Asterisks represent p-value < 0.05 and 'ns' represents non-significant results. Abbreviations: Lactate threshold (LT), power peak (W_{peak}), start of control period (CON), end of control period (ENDCON), start of HIIT intervention (PRE), and following HIIT intervention (4WP).

4.3.2 Changes in physiological variables following HIIT

Following four weeks of HIIT, W_{peak} (p= 0.001, **Figure 2a**) and LT (p = 0.001, **Figure 2b**) was significantly increased, with no significant change found in VO_{2max} (p < 0.05, **Figure 2c**).

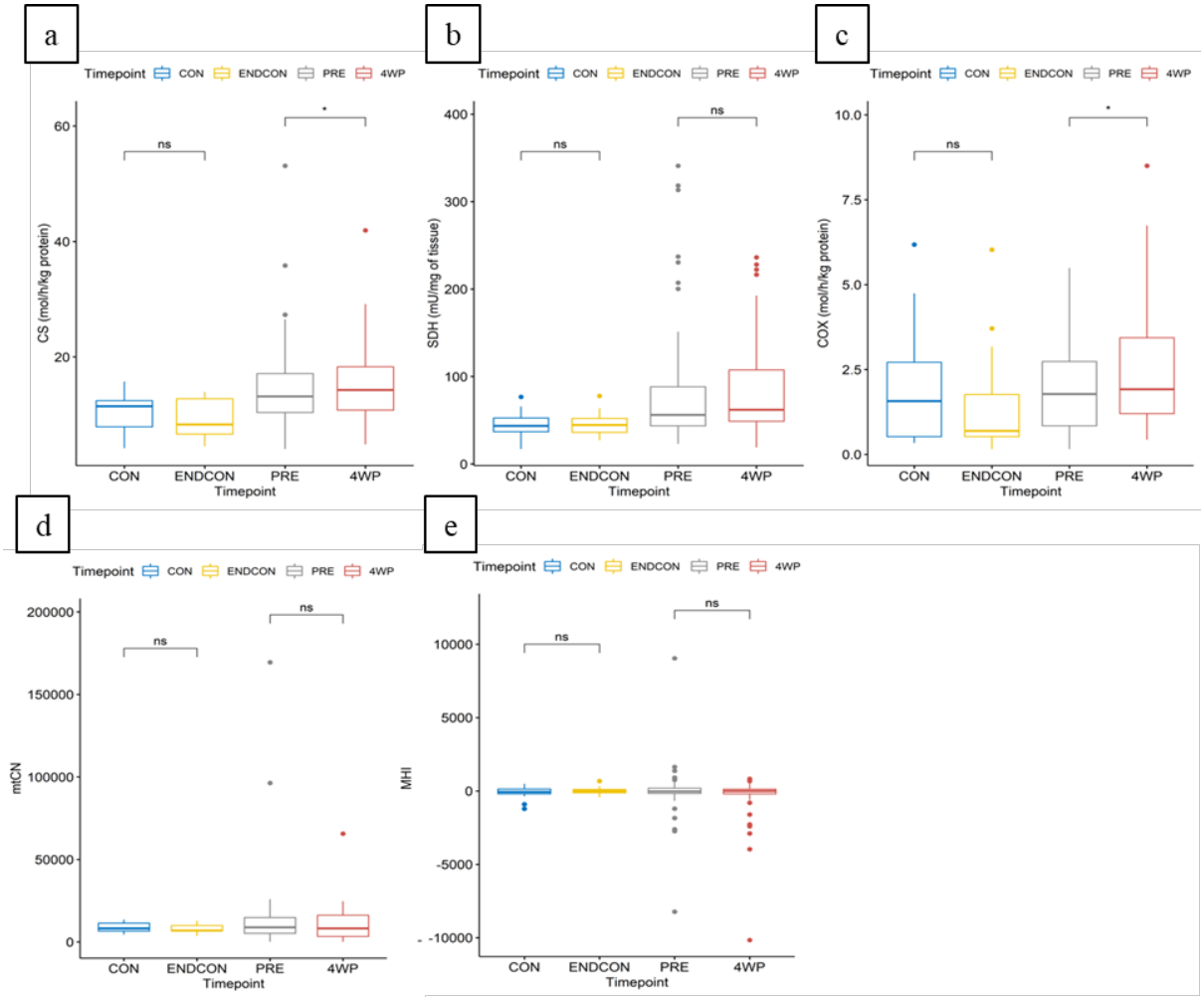

Figure 4.3 **Changes in mitochondrial markers over four weeks of control and four weeks of high intensity interval training (HIIT).** Box plots show the mean changes over four weeks of the control period and four weeks of HIIT for a) CS, b) SDH, c) COX, d) mtCN, and e) MHI. Asterisks represent p-value < 0.05 and "ns" represents non-significant results. Abbreviations: Citrate synthase activity (CS), cytochrome c oxidase activity (COX), succinate dehydrogenase activity (SDH), mitochondria copy number (mtCN), mitochondrial health index (MHI), start of control period (CON), end of control period (ENDCON), start of HIIT intervention (PRE), and following HIIT intervention (4WP).

4.3.3 Changes in mitochondrial markers following HIIT

We did not observe significant changes in the MHI following four weeks of HIIT (**Figure 3e**). When changes in mitochondrial markers were analysed separately, there was a significant increase in CS ($p < 0.05$, **Figure 3a**) and COX ($p < 0.05$, **Figure 3c**).

4.3.4 Associations between changes in physiological and mitochondrial markers

We investigated whether there were associations between changes in the physiological and mitochondrial markers following four weeks of HIIT. Changes in LT were found to be positively correlated with changes in both CS (r = 0.2, p = 0.014, **Figure 4a**) and COX (r = 0.19, p = 0.019, **Figure 4b**) following four weeks of HIIT. Significant correlations were found between increased changes in VO_{2max} and increased changes in two mitochondrial markers, CS (r = 0.24, p = 0.0025, **Figure 5a**) and SDH (r = 0.20, p = 0.011, **Figure 5c**), following four weeks of HIIT. Finally, a significant correlation was found between increased changes in W_{peak} and changes in CS following four weeks of HIIT (r= 0.24, p = 0.0024, **Figure 6a**). No significant associations were found between W_{peak} changes and changes in the other assessed mitochondrial markers **Figure 6b-e**.

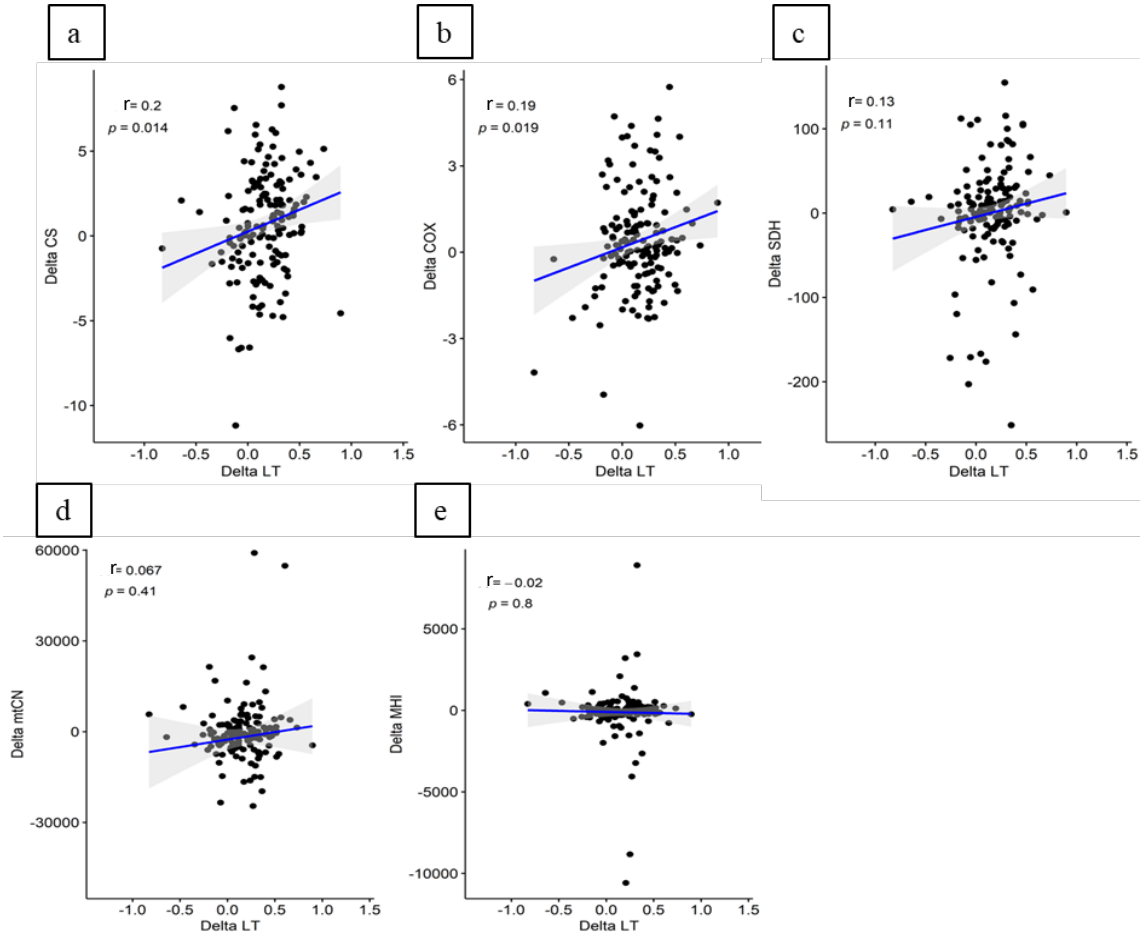

***Figure 4.4* Correlations between changes in mitochondrial markers and changes in lactate threshold (LT) following four weeks of high intensity interval training (HIIT).** Scatter plots of correlations between changes in lactate threshold and changes in A) CS, B) COX, C) SDH, D) mtCN and E) MHI following four weeks of HIIT. Abbreviations: lactate threshold (LT), citrate synthase activity (CS), cytochrome c oxidase activity (COX), succinate dehydrogenase (SDH), mitochondria copy number (mtCN), and mitochondrial health index (MHI).

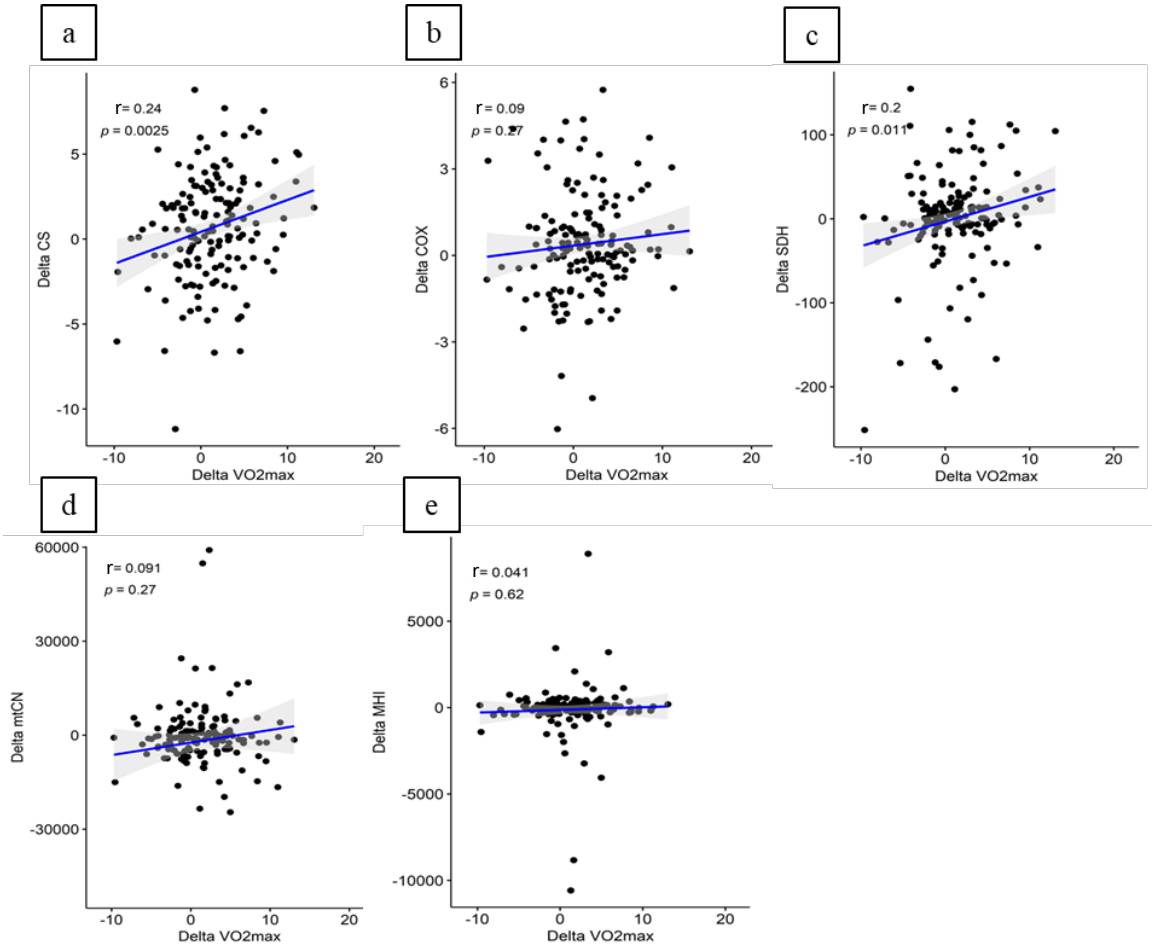

***Figure 4.5* Correlations between changes in mitochondrial markers and changes in VO_{2max} following four weeks of high intensity interval training (HIIT).** Scatter plots of correlations between changes in maximal VO_{2max} and changes in a) CS, b) COX, c) SDH, d) mtCN and e) MHI following four weeks of HIIT are shown. Abbreviations: maximal consumption capacity (VO_{2max}), citrate synthase activity (CS), cytochrome c oxidase activity (COX), succinate dehydrogenase (SDH), mitochondria copy number (mtCN), and mitochondrial health index (MHI).

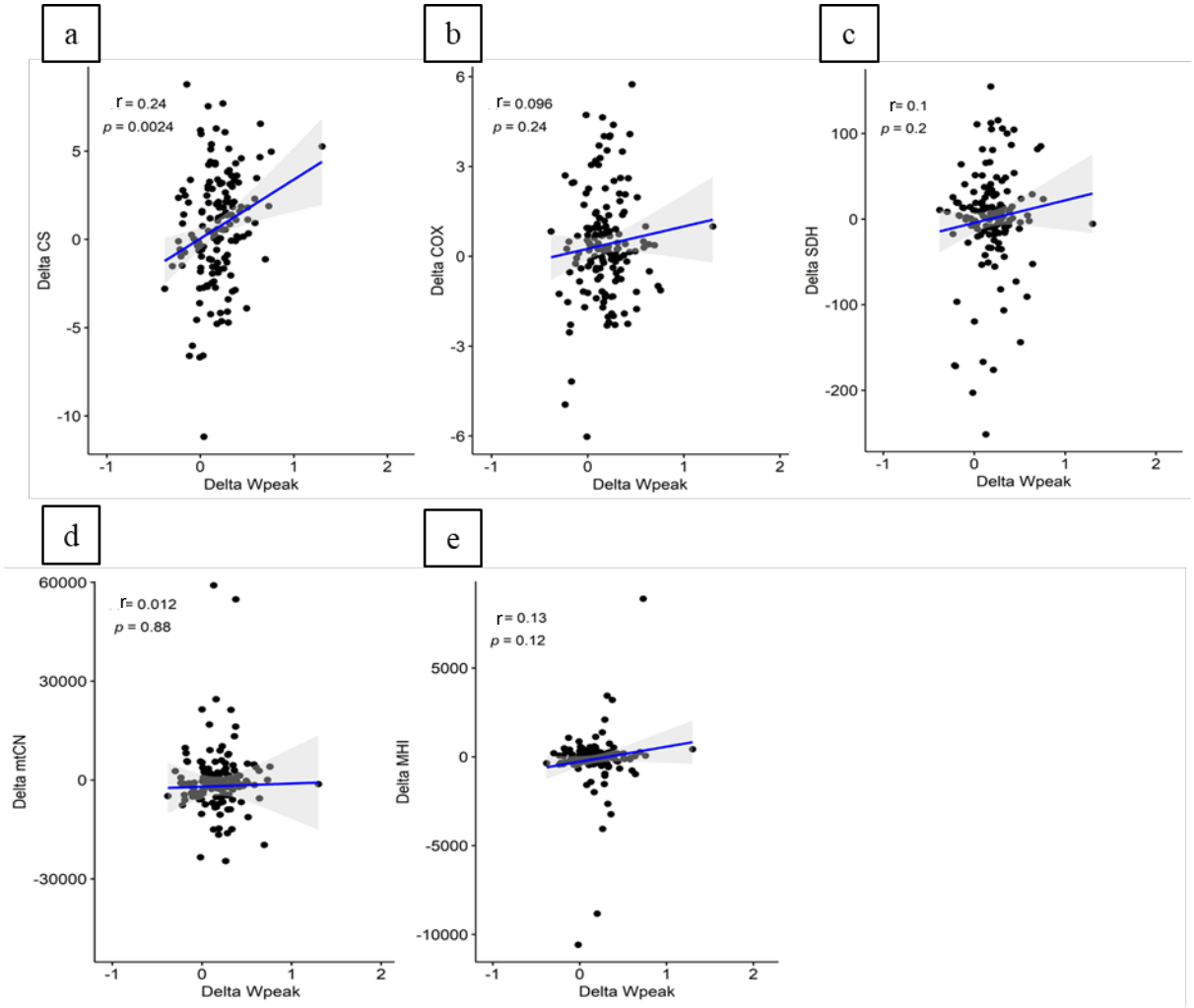

Figure. 4.6. **Correlations between changes in mitochondrial markers and changes in peak power (W_{peak}) following four weeks of high intensity interval training (HIIT).** Scatter plots of correlations between changes in W_{peak} and changes in a) CS, b) COX, c) SDH, d) mtCN and e) MHI following 4-weeks of HIIT are shown. Abbreviations: peak power (W_{peak}), citrate synthase activity (CS), cytochrome c oxidase activity (COX), succinate dehydrogenase (SDH), mitochondria copy number (mtCN), and mitochondrial health index (MHI).

4.4 Discussion

In a relatively large sample of males and females (n=112) we have provided an extensive analysis of changes in physiological variables and mitochondrial markers following four weeks of control vs HIIT intervention. We report a significant increase in two physiological phenotypes, LT and W_{peak}, following four weeks of HIIT. This suggests that observed changes in physiological variables, LT and W_{peak}, were induced by the HIIT intervention and not due

to external factors. In addition, increases were observed in two mitochondrial markers, CS and COX, after four weeks of HIIT. We then integrated key mitochondrial measurements into MHI to detect broader mitochondrial responses after exercise. However, we did not observe changes in MHI after four weeks of the HIIT intervention. We found positive correlations between some physiological and molecular markers. Specifically, increases in LT were correlated with increases in CS and COX. This correlation suggests that training near the lactate threshold affects also mitochondrial markers, and affects them in an independent manner [35]. Increases in VO_{2max} were correlated with increases in CS and SDH. Thus, these results suggests that increases in mitochondrial skeletal muscle COX activity are not necessary for increases in VO_{2max}. Finally, increases in W_{peak} were correlated with an increase in CS activity following four weeks of HIIT. This correlation is supported for a previous HIIT study where CS was also increased [36]. In general, these correlations suggests that HIIT affects physiological measurements and mitochondrial enzymes independently and not in parallel, as suggested previously [37].

The most common method to investigate exercise response variability is to compare an intervention group with an independent/separate control group [16]. However, there are limitations to this approach as observed differences cannot be truly attributed to the exercise intervention alone, as they may be due to individual variability between the control and exercise group. [16]. We aimed to address this limitation by including a four week control period, followed by four weeks of HIIT in the same individuals to minimise *inter-individual* variability during the control phase and in response to a HIIT training intervention.

In line with prior studies, we observed changes in measures of mitochondrial content following HIIT [38]. Here, we examined the usefulness of measuring mitochondrial health via

a composite measure MHI in skeletal muscle, as it was previously reported to be useful in blood [11]. Following four weeks of HIIT, we did not observe significant changes in skeletal muscle MHI. This finding was unexpected, given that measures used to derive MHI, mitochondrial function and content, are shown to upregulated in skeletal muscle by exercise [20, 39]. When examining each mitochondrial measurements individually, we found an increase in CS activity and COX [40, 41]; however, we did not observe increases in either SDH or mtCN. This may be due to the length of the training intervention, as previous studies with a training period of 8 weeks found SDH activity increased more than 27% after an interval training intervention, and more than 20% increase after a continuous exercise intervention [42].

We did not observe changes in mtCN after four weeks of HIIT. Our findings are supported because mtCN is weakly correlated with mitochondrial function or mitochondrial content [43]. We observed significant increases in mitochondrial markers (CS, SDH and COX) when comparing changes across the end of control period vs HIIT baseline testing (**supplementary figure 4.1**). This finding could be because the analysis was performed separately (possible batch effect). Nevertheless, we addressed the potential impact for these outcomes, putting the timepoint and batch as covariables in our statistical model. In addition, we did not see changes in the CON period, suggesting that changes after the HIIT intervention are accurate and not due to day-to-day variability. It is also known that enzymatic activity varies considerably within subjects according to the intracellular enzyme state [44]. We also minimized the possible technical variability, removing any variance >10% in duplicate results.

In this study we noted that HIIT intervention consistently affects mitochondrial content and physiological markers. Increases in CS activity were consistently associated with increases in VO_{2max}, LT and W_{peak}. This finding is interesting given CS is recognised as a robust marker

for mitochondrial content [20, 38]. In addition, we found that increase in LT is associated with an increase in COX activity, with this finding plausible given COX is the primary site of cellular oxygen consumption and is essential for aerobic energy generation in the form of ATP [45]. In addition this finding is supported due to skeletal muscle mitochondrial adaptations being largely dependent on exercise intensity [46]. These findings are consistent with previous results when using CS or COX activity as biomarkers of the skeletal muscle oxidative adaptation to a training intervention and a relationship between changes in aerobic capacity [40, 41]. In addition, CS activity has been associated with mitochondrial content, and is shown to be influenced by the oxidation of substrates and complex IV [43]. These findings may explain the presented relationships between CS and COX and physiological measures of aerobic capacity (VO_{2max}, LT, W_{peak}) Finally, we found a correlation between SDH and VO_{2max}. This is consistent with previous studies where increases in VO_{2max} were clearly correlated with increases in SDH in heart failure patients, healthy participants, and professional cyclists [47, 48].

A limitation of this study is that we found significant differences in mitochondrial markers when comparing CONEND vs PRE (supplementary figure 4.1), and although we conducted the analysis in separated batches that may explain this, we also considered that batch and time point are covariables and we cannot explore this further. Also, not all participants undertook the control period; hence, the number of participants in each analysis was different (29 for CON group and 112 for PRE).

4.5 Conclusion

We found significant changes in several physiological and mitochondrial variables following four weeks of HIIT. We also identified consistent positive correlations between

mitochondrial markers (CS, SDH, and COX) and physiological variables (VO_{2max}, W_{peak}, LT). Including a control period in the same individuals that completed the HIIT intervention allowed us to perform a direct comparison of the physiological and mitochondrial measurements and we can conclude that changes are likely due to the HIIT intervention. This study design highlights the value of including a control period in exercise studies. Compensatory mechanisms may affect the variability across the mitochondrial variables as reported here with COX and CS. However, further studies are needed to establish such mechanisms in exercise responses.

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Chapter 5: Genetic Variants and its association with physiological and molecular phenotypes in skeletal muscle

5.1 Introduction

There is a wide variability across individuals in response to the same exercise intervention [1] Post-exercise intervention variability in fitness phenotypes is well known, for example, maximal oxygen consumption (VO_{2max}) [2-11], lactate threshold [12] [9, 10] and power peak (W_{peak}) [3, 13]. Variability is also seen with molecular traits such as mitochondrial functional markers [14]. The factors that drive this inter-individual variability in exercise responses remain largely unclear, however there is strong evidence that a genetic component contributes to this variability [15]. Genetic background plays an important role in sporting potential, with physiological and molecular traits such as VO_{2max} and mitochondrial markers known to be polygenic traits which are influenced by several genetic variants [16-19]. Many studies focus on a limited number of genes, which individually may contribute only small effects, and explain only a small proportion of overall variability. However, the polygenic nature of these traits implies there may be benefit investigating the combined effect of multiple genetic variants to stablish the overall influence of genetics on exercise responses at both a physiological and molecular level.

Genetic variants identified in GWAS studies may be combined into a polygenic risk score that captures an individuals' total genetic tendency for a specific trait, with the use of polygenic risk scores used initially in clinical research for disease and risk prediction [20]. Polygenic scores may also be used in the context of fitness, by using genetic variants identified through several GWAS studies for fitness traits such as VO_2max [21], heart rate and recovery of autonomic nervous system [22]. However, only one study has explored creating a polygenic

score in this context [23], albeit considering only one fitness trait, VO_{2max}, and no other physiological or underpinning molecular fitness phenotypes.

In examining the role of genetics in exercise responses it should be considered that someone's baseline fitness level (before an exercise intervention is implemented) is a key factor that influences their degree of response to that intervention. For example, it has been reported that the degree of change in phenotypes, such as VO_2max [24, 25] and strength [26], following exercise interventions is dependent on the initial fitness levels of the individual, prior to the intervention. For example, has been reported that individuals with lower VO2max at baseline have the highest increases in it after the intervention [27]. In addition, changes in VO_2max following resistance training is dependent of the individual´s VO_2max baseline levels [28]. Noting this, the vast majority of the literature in this area explores how genetic variants may predict response to exercise training [29] with little known around how they may predict baselines fitness levels, which reflects long-life and cumulative exposures to exercise.

In this study we therefore assessed the association between exercise polygenic scores, derived from genetic variants identified in Chapter 3 of this thesis, and physiological and mitochondrial markers at baseline and following a four-week high intensity interval training (HIIT) intervention.

5.2 Methods:

5.2.1 Participants and study design

This chapter utilises data collected for the Gene SMART study cohort, which has been described in detail previously in Chapter 3 and in a prior publication [30].

Briefly, the Gene SMART cohort study is a controlled exercise study involving a 4-week HIIT intervention, with familiarisation and baseline assessments completed before this intervention and a further study assessment conducted after it (**Figure 5.1**). This chapter reports on data gathered from 96 males and 20 females (18-45 years, BMI: 25.46±3.29kg m^2) who completed this study. This study was approved by the Humans Ethics Research Committee at Victoria University (HRE13-223 and HRE 21-122), and we obtained a written consent form from each participant.

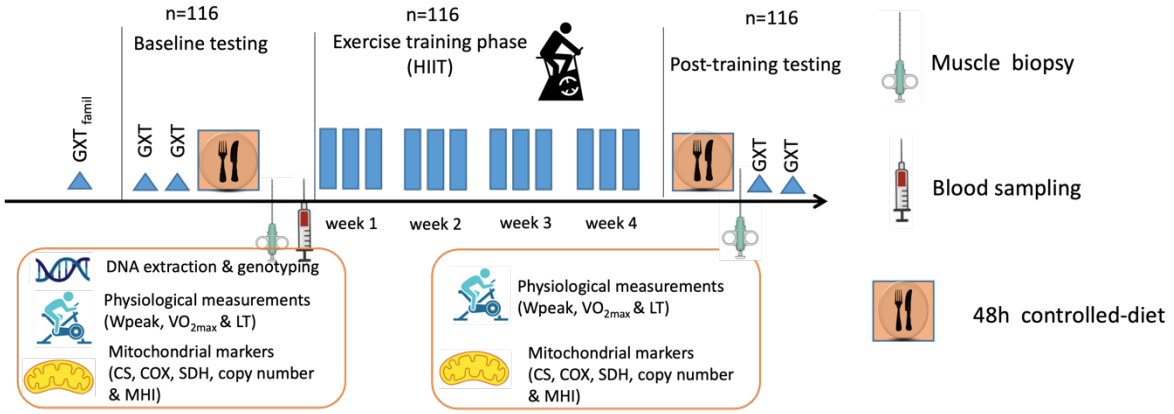

Figure 5.1. *Study design*

5.2.2 Familiarization study visit

Prior to completing the 4-week HIIT intervention, participants completed a familiarisation exercise session that consisted of one graded exercise test (GXT). Physiological measurements (lactate threshold, peak aerobic power and $VO2_{max}$) were taken during this familiarisation GXT and then used to set the initial intensity for the following baseline GXTs.

5.2.3 Baseline testing

Following a familiarisation session, all participants then completed two baseline GXTs. On a separate examination day, a muscle biopsy and blood sample were also taken. This was preceded by a 48-hour standardised diet, following procedures described in Chapter 4.

5.2.4 Exercise intervention (HIIT) and post-intervention testing

Following baseline visits, participants trained three times/week under supervision for four weeks. Each session consisted of six to twelve 2-min intervals performed at different intensities ranging from 40 to 70% of (W_{peak} - LT) above LT and interspersed by 1-min recovery periods (work-to-rest ratio of 2:1). The number of intervals and the intensity were progressively increased to maintain progression. Testing and training sessions were at intervals of 48-hours to avoid overtraining. Following 4 weeks of HIIT, participants completed post-intervention study assessments, comprised of two GXTs and collection of a muscle and blood sample, as at baseline.

5.2.5 Mitochondrial measures

Analyses of mitochondrial measures succinate dehydrogenase (SDH), cytochrome C oxydase (COX), citrate synthase (CS), and mitochondria copy number (mtDNA) were performed using muscle samples collected at baseline and following the 4-week HIIT intervention, following methods described previously in Chapter 4. Values for these measures were used to derive the mitochondrial health index (MHI) using the following formula:

$$\left[\frac{Energy\ production\ capacity}{Mitochondrial\ content}\right] = \left[\frac{Complex\ II\ (SDH) + Complex\ IV\ (COX)}{CS + mtCN}\right] * 100$$

5.2.6 DNA extraction and genotyping

Genomic DNA was extracted from 350ul of blood samples using the MagSep Blood gDNA kit (Eppendorf, Germany), using the epMotion M5073 automated pipetting system (Eppendorf, Germany) and reversible absorption of nucleic acids to paramagnetic beads as per manufacturer instructions.

Isolated DNA samples (~200ng) were then genotyped using the Illumina Genome-Wide Genotyping array-24 Version 3.0 Beadchip following the illumine Infinitum HTS assay GGTNM00263). Quality assessment of the samples was performed by QuantiFluor at the Australian Genome Research Facility, Melbourne, Australia, with samples removed if they showed any deficiency in staining, extension, hybridization, stringency, non-specific binding or non-polymorphic controls. All samples from this project met the Illumina expectations of <0.3 for Log SD.

5.2.7 Exercise Polygenic Score

Genotype data generated using the Illumina Genome-Wide Genotyping array-24 Version 3.0 Beadchip was used to derive additive exercise polygenic scores, using the list of robust SNPs linked to exercise responses outlined in Chapter 3. Two exercise polygenic scores were created, with the first comprised of seven SNPs linked to changes aerobic fitness, and the second comprised of these seven SNPs plus two linked to resistant training (**Table 5.1**). Three SNPs not covered by the array were replaced by SNPs in high linkage disequilibrium (LD): 1) rs6090314 with rs6090327 (pairwise r^2= 1), 2) rs884736 with rs11120796 (pairwise r^2=0.98), and 3) rs1956197 with rs12891759 (pairwise r^2= 1). Several SNPs identified in Chapter 3 were omitted from the polygenic scores due to their beneficial allele being unknown or the genotyping array not covering this SNP or their close proxies: ACE rs4646994, rs1799752, rs4340, and rs13447447; IL15RA rs2296135; C12orf36 rs12580476; CD44 rs353625; NDN rs17117533; RYR2; rs7531957; and ZIC4 rs11715829. The remaining included SNPs were summed to generate additive polygenic scores, with participants scoring a 1 for each beneficial allele they carried. For example, the G allele for the rs6552828 (A/G) SNP was identified to be beneficial to aerobic fitness in Chapter 3, with individuals who carry two copies of this G allele

scoring 2 towards their exercise polygenic score, with a heterozygous genotype corresponding to a score of 1 and a 0-score representing no copies of the G allele.

Table 5.1 *Lists of SNPs used to generate exercise polygenic scores.*

	Exercise Polygenic Score 1 (EPS1)		**Exercise Polygenic Score 2 (EPS2)**	
Gene Name	*Variant*	*Beneficial allele*	*Variant*	*Beneficial allele*
ACSL1	rs6552828	G	rs6552828	G
AGT	rs699	A	rs699	A
BIRC	rs6090327[1]	G	rs6090327[1]	G
DAAM1	rs12891759[2]	G	rs12891759[2]	G
PPARGC1A	rs8192678	G	rs8192678	G
CAMTA1	rs11120796[3]	G	rs11120796[3]	G
RGS18	rs10921078	A	rs10921078	A
PPARA			rs4253778	G
ACTN3			rs1815739	G

1: replaces rs6090314, 2: replaces rs1956197, 3: replaces rs884736

5.2.8 Statistical analysis

Data analyses were conducted using R software version 4.0.2 [31] (packages: *dplyr [32] readxl [33]*, *mice [34]*, and *miceadd [34, 35]*). Descriptive statistics were generated to describe cohort characteristics and allele frequencies; continuous data included means and standard error (SE), and categorical data included counts (n), frequencies (%). Data missing at random was imputed using multiple imputations using the *mice* package [34], and results were pooled from all imputed iterations for models with the *miceadds* package [35]. Findings derived from the unimputed dataset are also reported. Linear regression was used to assess relationships between exercise polygenic scores and physiological and mitochondrial variables, with relationships between individual SNPs and outcome variables also explored. All models were corrected for age and sex, with models assessing changes in outcome variables over 4 weeks of HIIT also adjusting for baseline levels of that variable. Post-hoc comparisons were used to investigate pairwise significance between genotypes. The level of significance was set

at α=0.00028 based on the Bonferroni method adjustment for multiple (176 overall models) testing [36].

5.3 Results

5.3.1 Cohort phenotypes before and after HIIT.

The mean age of the cohort was 31.6 years and had mean exercise polygenic scores of 4.8 (out of possible 14) for EPS1 and 8.4 for EPS2 (out of possible 18). Fitness phenotypes were on average higher following 4-weeks of HIIT, compared to baseline; VO_{2max} (47.6 (SD 8.6) vs. 46.6 (SD 8.8)), LT (2.6 (SD 0.7) vs. 2.4 (SD 0.7)) and Wpeak (3.7 (SD (0.8) vs 3.5 (SD 0.8)), however this increase was only significant ($p<0.05$) for LT and Wpeak. In addition, there were significant increases in mitochondrial markers, CS (13.6 (SD 6.3)vs 14.6 (SD 6.7)) ($p<0.05$) and COX (1.9 (SD 1.3) vs 2.1 (SD 1.6)) ($p<0.05$), after 4-weeks of HIIT. No significant changes were found in VO_{2max}, SDH, mtCN and MHI after 4-weeks of HIIT (**Table 5.2**).

Table 5.2 *Cohort phenotypes before and after HIIT.*

	Baseline *Mean (SD)*	**After 4-weeks of HIIT** *Mean (SD)*	**Change Δ** *Mean (SD)*
Age	31.6 (8.1)	-	-
EPS1	4.8 (1.8)	-	-
EPS2	8.4 (2.1)	-	-
VO_2max	46.6 (8.8)	47.6 (8.6)	1.4 (4.3)
LT	2.4 (0.7)	2.6 (0.7)	***0.2 (0.2)****
WPeak	3.5 (0.8)	3.7 (0.8)	***0.2 (0.2)****
CS	13.6 (6.3)	14.6 (6.7)	***1.0 (3.0)****
SDH	85.0 (69.9)	78.9 (55)	13.0 (45.5)
COX	1.9 (1.3)	2.1 (1.6)	***0.2 (1.6)****
mtCN	12664.9 (21085.5)	10456.8 (6534.4)	-5022.8 (28870.0)
MHI	166.3 (703.4)	-189 (1236)	-192.3 (1142.7)

(*) represents significant differences between baseline and 4 weeks measures. Abbreviations: Exercise polygenic score 1 and 2 (EPS1 and EPS2 respectively), maximal oxygen uptake (VO_2max), lactate threshold [12], power peak (Wpeak) citrate synthase (CS), succinate dehydrogenase (SDH), cytochrome oxidase (COX), mitochondria copy number (mtCN), and mitochondrial health index (MHI).

5.3.2. Allele frequency distribution in the Gene SMART cohort

The distribution of examined SNPs (rs6552828, rs699, rs6090327, rs12891759, rs8192678, rs11120796, rs10921078, rs4253778, and rs1815739) are outlined in **Table 5.3.** Genotype frequencies were consistent with Hardy-Weinberg Equilibrium ([38], P-value>0.05) for all SNPs except for rs8192678 (P=0.04).

Table 5.3 *Allele distribution in cohort.*

Variant	N (%)	Beneficial allele	HWE (P-value)
rs6552828		G	
AA	17 (17.3)		0.56 (0.44)
AG	52 (53.1)		
GG	29 (29.6)		
rs699		T	
CC	18 (18.3)		0.12 (0.72)
CT	50 (51.1)		
TT	30 (30.6)		
rs6090327		A	
AA	66 (67.3)		0.44 (0.50)
AG	30 (30.6)		
GG	2 (2.1)		
rs12891759		G	
AA	67 (68.3)		0.31 (0.57)
AG	29 (29.6)		
GG	2 (2.1)		
rs8192678		G	
AA	17 (17.3)		3.92 (0.04)
AG	36 (36.7)		
GG	45 (46)		
rs11120796		G	
AA	16 (16.3)		0.42 (0.51)
AG	51 (52)		
GG	31 (31.7)		
rs10921078		A	
GG	61 (62.2)		1.43 (0.23)
AG	30 (30.6)		
AA	7 (7.2)		
rs4253778		C	
GG	6 (6.1)		1.42 (0.23)
GC	28 (28.5)		
CC	64 (65.3)		
rs1815739		C	
TT	6 (6.1)		1.42 (0.23)
TC	28 (28.5)		
CC	64 (65.3)		

Abbreviations: HWE; Hardy-Weinberg Equilibrium

5.3.3 Associations between exercise polygenic scores and physiological and mitochondrial phenotypes.

No associations were found between exercise polygenic scores (both EPS1 and EPS2) and physiological and mitochondrial phenotypes either before or after 4-weeks of HIIT in the Gene SMART cohort (**Table 5.4).** These results did not change when models were run on unimputed data **(Appendix, Supplementary Table 5.1).**

Table 5.4 *Associations between Exercise Polygenic Scores and physiological and mitochondrial phenotypes.*

Outcome	EPS1			EPS2		
	Estimate	**SE**	**P value**	**Estimate**	**SE**	**P value**
VO_{2MAX} BL	-0.53	0.52	0.31	-0.55	0.44	0.22
VO_{2MAX} 4W-Δ	-0.29	-1.08	0.28	-0.21	0.23	0.36
Wpeak BL	-0.04	0.04	0.33	-0.05	0.04	0.18
Wpeak 4W-Δ	-0.00	0.01	0.90	0.0004	0.01	0.96
LT BL	-0.03	0.03	0.34	-0.04	0.03	0.15
LT 4W-Δ	0.003	0.01	0.81	0.002	0.01	0.83
CS BL	-0.18	0.33	0.58	-0.24	0.28	0.38
CS 4W-Δ	0.05	0.20	0.78	0.11	0.19	0.56
COX BL	0.08	0.08	0.29	0.02	0.07	0.70
COX 4W-Δ	0.008	0.08	0.92	0.03	0.07	0.57
mtCN BL	-1080.94	1029.95	0.29	-760.67	884.37	0.39
mtCN 4W-Δ	-314.24	0.04	0.51	-1.01	0.04	0
SDH BL	1.19	4,52	0.79	-1.31	3.90	0.73
SDH 4W-Δ	1.73	3.51	0.62	0.25	3.06	0.93
MHI BL	535.96	4534.74	0.90	-620.25	3879.86	0.87
MHI 4W-Δ	-55.70	0.78	0.48	-70.97	0.76	0.35

Each line represents a separate model, with EPS1 and EPS2 also examined separately. All models were corrected for age and sex, with models exploring change over 4 weeks also adjusting for baseline levels of the appropriate outcome variable. Abbreviations: SE: standard error, BL: baseline, 4W-Δ: change over 4 weeks, EPS1 and EPS2: exercise polygenic score 1 and 2 respectively, VO_2max: maximal oxygen uptake, LT: lactate threshold, Wpeak: power peak, CS: citrate synthase, SDH: succinate dehydrogenase, COX: cytochrome oxidase, MCN: mitochondria copy number, and MHI: mitochondrial health index.

5.3.4 Associations between individual SNPs and physiological variables

Given no associations were found between composite exercise polygenic scores and physiological phenotypes, potential associations between individual SNPs in these scores and these phenotypes were further explored. No significant differences were found between SNP genotypes and VO_2max, LT and Wpeak measures at baseline and following 4-weeks of HIIT

(Table 5.5). These results did not change when models were run on unimputed data (**Appendix, Supplementary Table 5.2**).

Table 5.5 *Least square means estimates by genotype for physiological phenotypes before and following HIIT.*

Variant (*Gene*)	Least square means (SE)[1]		
rs6552828 (*ACSL1*)	***AA***	***AG***	***GG***
VO_{2MAX} (mL/kg/min) BL	43.9 (2.31)	46.1 (1.36)	45.3 (1.85)
VO_{2MAX}(mL/kg/min) 4W-Δ	2.01 (1.29)	1.01 (0.69)	0.71 (0.94)
Wpeak (W/kg) BL	3.40 (0.22)	3.43 (0.12)	3.35 (0.17)
Wpeak (W/kg) 4W-Δ	0.24 (0.05)	0.15 (0.03)	0.27 (0.04)
LT (W/kg) BL	2.37 (0.17)	2.33 (0.10)	2.29 (0.14)
LT (W/kg) 4W-Δ	0.28 (0.07)	0.23 (0.04)	0.29 (0.07)
rs699 (*AGT*)	***CC***	***CT***	***TT***
VO_{2MAX}(mL/kg/min) BL	48.4 (2.22)	44.8 (1.38)	44.9 (1.76)
VO_{2MAX} (mL/kg/min) 4W-Δ	1.08 (1.16)	1.72 (0.67)	0.09 (0.97)
Wpeak (W/kg) BL	3.51 (0.21)	3.39 (0.13)	3.37 (0.16)
Wpeak (W/kg) 4W-Δ	0.19 (0.05)	0.20 (0.03)	0.19 (0.04)
LT (W/kg) BL	2.43 (0.17)	2.31 (0.10)	2.30 (0.13)
LT (W/kg) 4W-Δ	0.24 (0.07)	0.25 (0.04)	0.25 (0.06)
rs6090327 (*BIRC*)	***GG***	***AG***	***AA***
VO_{2MAX} (mL/kg/min) BL	45.9 (1.25)	44.7 (1.80)	41.1(6.39)
VO_{2MAX} (mL/kg/min) 4W-Δ	0.95 (0.63)	1.28 (0.87)	5.81 (2.98)
Wpeak (W/kg) BL	3.40 (0.11)	3.44 (0.16)	3.23 (0.59)
Wpeak (W/kg) 4W-Δ	0.20 (0.03)	0.19 (0.04)	0.13 (0.14)
LT (W/kg) BL	2.32 (0.09)	2.37 (0.13)	2.06 (0.48)
LT (W/kg) 4W-Δ	0.25 (0.04)	0.26 (0.05)	0.20 (0.19)
rs12891759 (*DAAM1*)	***AA***	***AG***	***GG***
VO_{2MAX} (mL/kg/min) BL	45.4 (1.28)	45.8 (1.73)	37.0 (6.26)
VO_{2MAX} (mL/kg/min) 4W-Δ	1.27 (0.66)	0.85 (0.87)	-1.44 (3.66)
Wpeak (W/kg) BL	3.39 (0.12)	3.45 (0.16)	2.73 (0.58)
Wpeak (W/kg) 4W-Δ	0.18 (0.03)	0.22 (0.04)	0.17 (0.20)
LT (W/kg) BL	2.33 (0.09)	2.34 (0.13)	1.92 (0.47)
LT (W/kg) 4W-Δ	0.25 (0.04)	0.26 (0.05)	0.21 (0.26)
rs8192678 (*PPARGC1A)*	***AA***	***AG***	***GG***
VO_{2MAX} (mL/kg/min) BL	48.0 (2.39)	44.9 (1.59)	45.3 (1.44)
VO_{2MAX}(mL/kg/min) 4W-Δ	1.03 (1.24)	1.56 (0.79)	0.79 (0.73)
Wpeak (W/kg) BL	3.72 (0.22)	3.39 (0.14)	3.34 (0.13)
Wpeak (W/kg) 4W-Δ	0.21 (0.06)	0.19 (0.03)	0.19 (0.03)
LT (W/kg) BL	2.58 (0.18)	2.30 (0.12)	2.29 (0.10)
LT (W/kg) 4W-Δ	0.34 (0.07)	0.26 (0.05)	0.22 (0.05)
rs11120796 (*CAMTA1)*	***AA***	***AG***	***GG***
VO_{2MAX} (mL/kg/min) BL	47.8 (2.43)	44.4 (1.39)	46.1 (1.68)
VO_{2MAX} (mL/kg/min) 4W-Δ	2.06 (1.28)	1.00 (0.72)	0.94 (0.86)
Wpeak (W/kg) BL	3.64 (0.22)	3.37 (0.13)	3.37 (0.15)
Wpeak (W/kg) 4W-Δ	0.18 (0.06)	0.20 (0.03)	0.19 (0.04)
LT (W/kg) BL	2.48 (0.19)	2.32 (0.10)	2.28 (0.12)
LT (W/kg) 4W-Δ	0.22 (0.08)	0.23 (0.04)	0.30 (0.05)
rs10921078 (*RGS18)*	***GG***	***AG***	***AA***
VO_{2MAX}(mL/kg/min) BL	45.4 (1.34)	45.3 (1.74)	46.6 (3.39)
VO_{2MAX}(mL/kg/min) 4W-Δ	1.07 (0.68)	1.48 (0.86)	0.02 (1.88)

Wpeak (W/kg) BL	3.38 (0.12)	3.39 (0.16)	3.63 (0.31)
Wpeak (W/kg) 4W-Δ	0.20 (0.03)	0.21 (0.04)	0.07 (0.09)
LT (W/kg) BL	2.30 (0.10)	2.34 (0.13)	2.51 (0.25)
LT (W/kg) 4W-Δ	0.25 (0.04)	0.28 (0.05)	0.17 (0.14)
rs4253778 *(PPARA)*	***GG***	***GC***	***CC***
VO_{2MAX} (mL/kg/min) BL	47.1 (4.03)	46.5 (1.74)	44.9 (1.31)
VO_{2MAX} (mL/kg/min)4W-Δ	1.78 (2.16)	0.57 (0.84)	1.36 (0.68)
Wpeak (W/kg) BL	3.47 (0.37)	3.57 (0.16)	3.32 (0.12)
Wpeak (W/kg) 4W-Δ	0.22 (0.13)	0.18 (0.04)	0.20 (0.03)
LT (W/kg) BL	2.35 (0.29)	2.52 (0.13)	2.23 (0.09)
LT (W/kg) 4W-Δ	0.26 (0.19)	0.19 (0.05)	0.28 (0.04)
rs1815739 (*ACTN3)*	***TT***	***TC***	***CC***
VO_{2MAX} (mL/kg/min)BL	47.1 (4.03)	46.5 (1.74)	44.9 (1.31)
VO_{2MAX} (mL/kg/min)4W-Δ	1.78 (2.16)	0.57 (0.84)	1.36 (0.68)
Wpeak (W/kg) BL	3.47 (0.37)	3.57 (0.16)	3.32 (0.12)
Wpeak (W/kg) 4W-Δ	0.22 (0.13)	0.18 (0.04)	0.20 (0.03)
LT (W/kg) BL	2.35 (0.29)	2.52 (0.13)	2.23 (0.09)
LT (W/kg) 4W-Δ	0.25 (0.19)	0.19 (0.05)	0.28 (0.04)

All models were adjusted for sex, age, and baseline levels of appropriate phenotype (for models on 4W-Δ outcomes only) and performed on imputed data. Values within the same row that are bolded and denoted with different letters represent significant differences. (<0.05). Abbreviations: SE: standard error, BL: baseline, 4W-Δ: change over 4 weeks, VO_2max: maximal oxygen uptake, LT: lactate threshold, Wpeak: power peak

5.3.5 Associations between individual SNPs and mitochondrial variables

Potential associations between individual SNPs and mitochondrial variables were also explored. A significant association between baseline mtCN and the rs8192678 SNP was found (p=0.02), with individuals with the AA genotype having significantly higher mtCN levels on average (10579 (SE: 4912)) compared to individuals with AG (9631 (SE: 3137)) or GG (9762 (SE: 2813)) genotypes (**Supplementary Table 5.3).** We did not observe any further significant associations and mitochondrial phenotypes when models were performed using imputed data (**Supplementary Table 5.3).** However, when potential associations between individual SNPs and mitochondrial variables were examined in non-imputed data, several additional significant associations were found **(Table 5.6).**

Firstly, an association was found between baseline mtCN and the rs8192678 SNP (P=0.02), as shown in imputed data, with a further relationship between change mtCN

following 4 weeks of HIIT and the rs6090327 (P= < 0.01 add). For the rs6090327 variant, the AG genotype was associated with significantly greater decreases in mtCN following 4 weeks of HIIT (mean -10783 (SE: 1667)) when compared to the GG genotype (mean -3060 (SE: 1120), **Table 5.6**). A further association was found between change in CS following 4 weeks of HIIT and the rs699 variant (p=0.03). The greatest mean change was seen with the CC genotype (1.85 (SE: 0.76)) compared to CT (0.06 (SE: 0.47)) and TT genotypes (0.37 (SE: 0.61), **Table 5.6**).

Significant associations were also observed between change in SDH after 4-weeks of HIIT and rs6090327 (p=0.03) and rs12891759 (p=0.03) variants **(Table 5.6).** The AG genotype for rs6090327 was associated with significant increases in SDH following HIIT (mean increase 38.85 (SE: 14.98)), with decreases observed on average for GG (-0.03 (SE: 9.24)) and AA (-31.91(SE: 44.7) genotypes. Relationships were further found between baseline MHI and three genetic variants: rs4253778, rs10921078 and rs1815739. For rs4253778, MHI was significantly higher in individuals with GG genotype (mean 1452 (SE: 686)), when compared to the CC and GC (mean -153 (SE: 172) genotype (**Table 5.6**). Similarly, the GG genotype for rs10921078 was related to the highest MHI at baseline (mean 245.1 (SE: 146)) when compared the AG genotype (mean -312.7 (SE: 219)) for this variant. Further, for variant rs1815739, MHI was significantly higher in individuals with the CC genotype (mean 1452 (SE: 686)), when compared to the TC genotype (mean -153 (SE: 172), **Table 5.6**). No further associations were found between SNPs and mitochondrial markers at baseline or after 4-weeks of HIIT.

Table 5.6 *Least square means estimates for mitochondria phenotypes before and after the HIIT intervention, by genotype (non-imputed data)*

Variant (gene)	**Least square means (SE)[1]**		
rs6552828 (*ACSL1*)	***AA***	***AG***	***GG***
CS (mol/kg/protein) BL	11.0 (1.57)	11.4 (0.86)	10.5 (1.15)
CS (mol/kg/protein) 4W-Δ	1.25 (0.81)	0.13 (0.45)	0.88 (0.65)
COX (mol/kg/protein) BL	1.70 (0.37)	1.60 (0.20)	2.04 (0.27)

COX(mol/kg/protein) 4W-Δ	0.47 (0.49)	-0.15 (0.30)	0.23 (0.40)
mtCN BL	14342 (7286)	10018 (3998)	16817 (5607)
mtCN 4W-Δ	-5721 (2633)	-4782 (1492)	-6126 (2556)
SDH (mol/kg/mg tissue) BL	59.9 (20.4)	75.7 (13.3)	71.3 (17.8)
SDH(mol/kg/mg tissue)4WΔ	8.08 (16.6)	8.22 (11.7)	12.00 (17.1)
MHI BL	-309 (290)	-16.6 (164)	181 (247)
MHI 4W-Δ	-228 (247)	-169 (210)	-205 (397)
rs699 (*AGT*)	***CC***	***CT***	***TT***
CS (mol/kg/protein) BL	11.4 (1.43)	11.4 (0.87)	10.3 (1.13)
CS (mol/kg/protein) 4W-Δ	**1.85 (0.76)[a]**	**0.06 (0.47)[b]**	**0.37 (0.61)[b]**
COX (mol/kg/protein) BL	1.49 (0.35)	1.90 (0.21)	1.58 (0.27)
COX(mol/kg/protein) 4W-Δ	0.23 (0.50)	0.32 (0.29)	-0.51 (0.40)
mtCN BL	8515 (7313)	14820 (3784)	8196 (6122)
mtCN 4W-Δ	-6719 (2645)	-5858 (1383)	-1712 (2460)
SDH(mol/kg/mg tissue) BL	67.2 (20.0)	78.0 (13.4)	60.8 (17.8)
SDH(mol/kg/mg tissue)4WΔ	-8.42 (16.7)	5.61 (12.6)	28.31 (13.7)
MHI BL	102 (289)	164 (161)	-185 (273)
MHI 4W-Δ	-250.6 (307)	-41.4 (185)	-444.3 (337)
rs6090327 (*BIRC*)	***GG***	***AG***	***AA***
CS(mol/kg/protein) BL	11.6 (0.76)	9.51 (1.71)	10.6 (3.85)
CS (mol/kg/protein) 4W-Δ	0.49 (0.42)	0.39 (0.67)	0.22 (2.69)
COX (mol/kg/protein) BL	1.72 (0.18)	1.85 (0.27)	-0.28 (1.26)
COX(mol/kg/protein) 4W-Δ	0.09 (0.27)	0.05 (0.37)	-
mtCN BL	10539 (3556)	18680 (5635)	7844 (16101)
mtCN 4W-Δ	**-3060 (1120)[a]**	**-10783 (1667)[b]**	**3600 (5464)[b]**
SDH(mol/kg/mg tissue) BL	73.4 (11.7)	71.1 (18.5)	18.3 (50.9)
SDH(mol/kg/mg tissue)4WΔ	**-0.03 (9.24)[a]**	**38.85 (14.98)[b]**	**-31.91(44.7)b**
MHI BL	-27.7 (135)	454.8 (244)	1100.5 (689)
MHI 4W-Δ	-204 (147)	-172 (288)	-
rs12891759 (*DAAM1*)	***AA***	***AG***	***GG***
CS (mol/kg/protein) BL	11.17 (0.82)	11.09 (1.088)	6.82 (3.83)
CS (mol/kg/protein) 4W-Δ	0.41 (0.45)	0.57 (0.59)	0.51 (2.75)
COX (mol/kg/protein) BL	1.65 (0.19)	1.91 (0.26)	-
COX(mol/kg/protein) 4W-Δ	-0.11 (0.27)	0.45 (0.37)	-
mtCN BL	14461 (3750)	8529 (5659)	2118 (15807)
mtCN 4W-Δ	-5896 (1319)	-2654 (2211)	-18002 (6383)
SDH(mol/kg/mg tissue) BL	71.4 (11.7)	70.9 (18.3)	-
SDH(mol/kg/mg tissue)4WΔ	**-0.23 (9.05)[a]**	**36.02 (14.66)[b]**	-
MHI BL	85.3 (149)	108.3 (252)	-
MHI 4W-Δ	-252.8 (140)	79.6 (370)	-
rs8192678 (*PPARGC1A)*	***AA***	***AG***	***GG***
CS (mol/kg/protein) BL	10.0 (1.48)	10.6 (0.99)	11.8 (0.92)
CS (mol/kg/protein) 4W-Δ	0.66 (0.81)	0.33 (0.56)	0.52 (0.50)
COX (mol/kg/protein) BL	1.52 (0.41)	1.79 (0.24)	1.74 (0.21)
COX(mol/kg/protein) 4W-Δ	-0.10 (0.58)	-0.31 (0.35)	0.37 (0.30)
mtCN BL	**27286 (6618)[a]**	**9869 (4679)[b]**	**10033 (4106)[b]**
mtCN 4W-Δ	-2828 (3149)	-6747 (1825)	-4645 (1657)
SDH(mol/kg/mg tissue) BL	59.1 (21.9)	88.2 (15.4)	62.2 (13.5)
SDH(mol/kg/mg tissue)4WΔ	6.16 (19.4)	16.64 (13.5)	0.09 (10.1)
MHI BL	459.0 (302)	40.1 (198)	30.4 (177)
MHI 4W-Δ	1949 (1221)	-338 (232)	-385 (153)
rs11120796 (*CAMTA1)*	***AA***	***AG***	***GG***
CS (mol/kg/protein) BL	11.0 (1.60)	10.9 (0.87)	11.5 (1.08)
CS(mol/kg/protein) 4W-Δ	-0.82 (0.87)	0.88 (0.47)	0.24 (0.58)

COX (mol/kg/protein) BL	1.97 (0.39)	1.75 (0.21)	1.64 (0.25)
COX (mol/kg/protein) 4W-Δ	-0.53 (0.63)	0.35 (0.29)	-0.15 (0.36)
mtCN BL	12556 (7442)	13531 (4030)	10414 (5372)
mtCN 4W-Δ	-3023 (3030)	-5289 (1450)	-6217 (2224)
SDH(mol/kg/mg tissue) BL	86.9 (25.4)	63.3 (13.3)	78.9 (15.5)
SDH(mol/kg/mg tissue)4WΔ	40.55 (24.7)	7.72 (11.7)	4.87 (12.8)
MHI BL	92.7 (344)	175.3 (163)	-72.5 (217)
MHI 4W-Δ	-211 (425)	-352 (143)	120 (203)
rs10921078 (*RGS18)*	***GG***	***AG***	***AA***
CS (mol/kg/protein) BL	11.64 (0.82)	10.50 (1.10)	8.82 (2.21)
CS (mol/kg/protein) 4W-Δ	0.33 (0.45)	0.43 (0.60)	1.86 (1.21)
COX(mol/kg/protein) BL	1.66 (0.20)	1.64 (0.25)	2.61 (0.47)
COX(mol/kg/protein) 4W-Δ	0.15 (0.29)	0.07 (0.38)	-0.50 (0.91)
mtCN BL	15059 (4009)	8688 (4733)	12286 (11212)
mtCN 4W-Δ	-4831 (1441)	-5717 (2045)	-7638 (4946)
SDH (mol/kg/mg tissue) BL	66.1 (12.3)	68.4 (18.0)	110.2 (28.5)
SDH(mol/kg/mg tissue)4WΔ	8.88 (10.9)	13.19 (16.8)	-6.68 (34.2)
MHI BL	**245.1 (146)[a]**	**-312.7 (219)[b]**	**86.2 (396)[b]**
MHI 4W-Δ	-279.4 (133)	90.5 (260)	-
rs4253778 *(PPARA)*	***GG***	***GC***	***CC***
CS (mol/kg/protein) BL	14.9 (2.50)	11.8 (1.09)	10.6 (0.82)
CS (mol/kg/protein) 4W-Δ	-0.81 (1.39)	0.61 (0.59)	0.49 (0.47)
COX (mol/kg/protein) BL	2.62 (0.67)	1.90 (0.25)	1.62 (0.19)
COX (mol/kg/protein) 4W-Δ	-0.57 (1.11)	-0.28 (0.34)	0.36 (0.28)
mtCN BL	15667 (15708)	8169 (5069)	14905 (3934)
mtCN 4W-Δ	-2026 (6958)	-4773 (2255)	-5568 (1499)
SDH(mol/kg/mg tissue) BL	52.3 (33.8)	82.8 (15.4)	64.3 (13.2)
SDH(mol/kg/mg tissue)4WΔ	13.68 (27.5)	23.53 (13.1)	-3.82 (11.9)
MHI BL	**1452 (686)[a]**	**-153 (172)[b]**	**293 (167)[b]**
MHI 4W-Δ	-93.4 (179)	-368.6 (251)	-
rs1815739 (*ACTN3)*	***TT***	***TC***	***CC***
CS (mol/kg/protein) BL	14.9 (2.50)	11.8 (1.09)	10.6 (0.82)
CS (mol/kg/protein) 4W-Δ	-0.81 (1.39)	0.61 (0.59)	0.49 (0.47)
COX (mol/kg/protein) BL	2.62 (0.67)	1.90 (0.25)	1.62 (0.19)
COX (mol/kg/protein) 4W-Δ	-0.57 (1.11)	-0.34 (0.34)	0.36 (0.28)
mtCN BL	15667 (15708)	8169 (5069)	14905 (3934)
mtCN 4W-Δ	-2026 (6958)	-4773 (2255)	-5568 (1499)
SDH(mol/kg/mg tissue) BL	52.3 (33.8)	82.8 (15.4)	64.3 (13.2)
SDH(mol/kg/mg tissue)4WΔ	13.68 (27.5)	23.53 (13.1)	-3.82 (11.9)
MHI BL	**1452 (686)[a]**	**-153 (172)[b]**	**293 (167)[b]**
MHI 4W-Δ	-93.4 (179)	-368.6 (251)	-

All models were adjusted for sex, age, and baseline levels of appropriate phenotype (for models on 4W-Δ outcomes only) and performed on the non-imputed dataset. Values within the same row that are bonded and denoted with different letters represent significant differences. (<0.05). Data is missing in cases where no outcome data was available for individuals with particular genotypes. Abbreviations: SE: standard error, BL: baseline, 4W-Δ: change over 4 weeks, VO_2max: maximal oxygen uptake, LT: lactate threshold, Wpeak: power peak, (CS) citrate synthase, (COX) Cytochrome C oxidase, (mtCN) mitochondrial copy number, (SDH) succinate dehydrogenase, (MHI) mitochondrial health index.

5. 4 Discussion

This study investigated the relationships between exercise polygenic scores and physiological and mitochondrial markers before and after 4-weeks of HIIT. The examined polygenetic scores were derived from robust genetic variants linked to exercise responses (Chapter 3), with relationships between these individual genetic variants and physiological and mitochondrial markers also explored. The present study comprehensively looked at this in a tightly controlled exercise study cohort. This study found no associations between derived exercise polygenetic scores and physiological and mitochondrial markers before or after 4-weeks of HIIT. However, several associations were found between individual genetic variants (rs699, rs8192678, rs6090327, rs12891759, rs10921078, rs4253778 and rs1815739) and baseline and post-4-week HIIT measures of mitochondrial function (SDH and MHI) and mitochondrial content.

Previous efforts to detect the genetic influence in exercise responses have had limited success [39]. In our study, a lack of association between exercise polygenic scores and physiological and mitochondrial phenotypes may be due to four weeks not being enough time to detect the influence of genetics on changes in physiological and mitochondrial phenotypes. We further observed no associations between exercise polygenic scores and baseline fitness levels, which may be explained by a small and likely underpowered sample set, or a different set of genes underpinning lifelong fitness traits. In addition, exercise responses are modulated by many genes with small and different effect sizes, thus, the proposed exercise polygenic score has equal SNPs effect seizes for the studied phenotypes. While our interventional study was done in the general population with different fitness levels, cross-sectional studies have reported a genetic influence on physiological exercise responses in elite athletes [40, 41]. Other

studies including intervention have reported a genetic influence after 20 weeks of continuous exercise in sedentary individuals [21, 42] vs the 4 weeks of HIIT intervention proposed in this study. Although we condensed in the EPS a group of genetic variants robustly associated with exercise responses, the relationship between genes and fitness traits remain inconclusive, given that those findings are derived from varied cohorts (e.g. varied ethnicities, elite and sedentary populations) of small samples sizes, contributing to a large heterogeneity across studies [39]. In addition, the baseline heterogeneity in our study could be considered as limitation as the impact of the HIIT intervention on individuals who were already trained differ within our cohort. However, this heterogeneity is also an advantage as the findings of this study are applicable to the general population. As highlighted in Chapter 3, no single study has exceeded 900 participants, which makes all the genetics studies gravely underpowered. We could not overcome this limitation with 116 individuals involved in our study. This is one of the major reasons for the large body of contradictory results reported in exercise science literature [43]. We tried to overcome this limitation by including several genetic variants from different sources (candidate genes and GWAS) studies, but according to our results this was not achieved. Although previously demonstrated that exercise training is as polygenic trait, undoubtedly many genetic variants remain to be identified.

In the current study, seven genetic variants (rs8192678, rs699, rs6090327, rs12891759, rs4253778, rs1815739 and rs10921078) were associated with measures of either mitochondrial function (SDH and MHI) or mitochondrial content (CS and mtCN). Firstly, a significant association was found between rs8192678 with baseline mtCN levels and also with mtCN changes after 4 weeks of HIIT. The causality of this finding cannot be explored here. However, this finding is supported by previous studies relating this SNP to training-induced adaptations of clinical relevance, with increases in mitochondrial function and several cardiovascular

phenotypes [44]. It also has been consistently associated with an increased aerobic capacity in Europeans [45, 46]., However, these results were in contrast to a Chinese cohort [47] where no increases were detected after 18-weeks of training for GG allele carriers. Significant associations were found between rs699 and change in CS following 4 weeks of HIIT, rs12891759 and change in SDH after HIIT, and between rs6090327 and changes in both mtCN and SDH after HIIT. The rs699 variant (also known as M235T) is located at amino acid 235 in the angiotensinogen (*AGT*) gene, which encodes pre-angiotensin. It is associated with elite power as it upregulates angiotensinogen II and increases serum *AGT* concentrations [48], which acts as a growth factor in skeletal muscle and thus promotes hypertrophy, power or speed [48, 49]. In the present study, the rs699 genetic variant was associated with citrate synthase activity in skeletal muscle, which has not been previously reported. This finding may be explained by the mitochondrial function of the *AGT*, with previous studies demonstrating that the stimulation of angiotensin receptors enhances mitochondrial biogenesis and increases mitochondrial content markers [50].

This study further reports an association between rs12891759 and increases in SDH after HIIT for the G allele carriers, which has not been previously reported. In this study, we replaced the rs12891759 genetic variant with one in high LD with the rs1956197 (pairwise r^2= 1), which has been linked to increases in VO_{2max} in other studies [51], however this relationship was not reported here. The rs6090327 variant was further linked to decrease SDH after HIIT for the AA genotype carriers. It also was also linked to increase mtCN in this cohort fort the AA genotype carriers, with this variant located in the *BIRC* gene linked to apoptosis and mitochondrial signalling pathways [52, 53]. rs6090327 is in high LD with exercise-related rs6090314, and acted as a proxy for this SNP in the current study given it was covered by the genotyping array used. rs6090314 has been associated with increases in VO_{2max} after 20-weeks

of continuous exercise [21]; however, it was not related to this phenotype in the current study, likely due to a shorter exercise intervention. Nevertheless, it was linked to changes in SDH and mtCN after 4-weeks of HIIT, suggesting this genetic variant is a prominent candidate gene for future molecular pathways studies in exercise responses.

Three genetic variants (rs4253778, rs1815739 and rs10921078) were associated with an increased MHI for the TT genotype. Interestingly, none of the three SNPs were linked to individual mitochondria (CS, SDH, mtCN and COX) in the current study, highlighting the potential value of examining a composite measure of mitochondrial function and content, and subsequently considering the combined effect of a single genetic variant gene on multiple mitochondrial markers.

rs4253778 is located in the intron of the *PPARA* gene and appears to influence gene expression by disrupting mRNAs site and consequently altering encoded PPARα proteins [54]. The observed relationship between this variant and MHI is supported by a prior study linking the C allele of rs4253778 to the downregulation of mitochondrial enzymes [55]. rs1815739 and rs10921078 variants were also associated with MHI in the current study. The rs1815739 variant is located in the gene that codes for alpha-actinin-3 (ACTN3), which has been related to many aspects of exercise responses (especially speed and power [56]) as well as mitochondrial content [57]. In a prior study, a variant in high LD with rs10921078, rs17581162 (pairwise r^2 = 0.84), was found to be associated with increases in VO_{2max} [21]; however, it has not been previously linked to markers of mitochondrial function or content. No relationships were found between SNPs and physiological variables (VO_2max, LT and Wpeak), with this likely due to the 4-week HIIT intervention having limited effect on these measures, with a significant but small change only reported for LT.

This study has several limitations that should be considered when interpreting findings. Firstly, we investigated a relatively small sample size, with limits power and the ability the capture small genetic effects, even when combined into polygenic scores. Further, the cohort examined was predominately Caucasian, with the generalisability of our findings therefore limited. However, the study design had strengths in using repeated GTXs and controlled diets to reduce variability in physiological and mitochondrial phenotypes. Also, the study derived a mitochondrial health index for all individuals, to assess the effects of genetics on a composite measure of mitochondrial function and content. Models also adjusted for sex, age and baseline measures of outcome where appropriate.

5.5 Conclusion

In conclusion, we found no association between exercise polygenic score and physiological and mitochondrial phenotypes. However, several associations between individual SNPs (rs8192678, rs699, rs6090327, rs12891759, rs4253778, rs1815739 and rs10921078) and mitochondrial markers (CS, SDH, mtCN and MHI) were found, which have not been reported previously. Further investigations combining cohorts to improve sample size, paired with validation and functional experiments, are needed to improve understanding of the links between genetics and fitness.

5.6 References

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Chapter 6: General discussion, contribution to knowledge, limitations, and future research

6.1 General discussion

Regular exercise is crucial for good health and healthy ageing, with physical inactivity associated with many chronic diseases [1, 2]. The benefits provided by exercise stem from skeletal muscle adaptations in response to exercise, which subsequently influence other tissues at a whole-body level. However, our understanding of molecular and physiological phenotypes that may explain or underpin these exercise adaptations and the factors that influence these adaptations, such as genetics, is still in its infancy [3]. Advancing our understanding in these areas may provide new avenues to use exercise interventions to further support our health and combat chronic diseases.

The overarching aim of this thesis was to investigate the influence of robust genetic variants using the Exercise Polygenic Score (EPS) in mitochondrial and physiological response to exercise phenotypes after four weeks of High Intensity Interval Training (HIIT) in the gene SMART study. Chapter 1 provided an overview of this thesis. Chapter 2 then outlined the relevant background literature for this project, including the concept of trainability and the key biological elements (e.g., genetics, mitochondria), as well as methodological and statistical factors that influence trainability. Following this, the aim of this thesis was addressed over three experimental chapters (Chapters 3-5). Firstly, in Chapter 3, prior studies exploring links between genetic variants and fitness variables were systematically reviewed to identify genetic variants robustly linked to exercise responses. In Chapter 4, we examined changes and correlations between physiological and mitochondrial markers in response to 4 weeks of HIIT in the Australian Gene SMART cohort (n=116). Finally, in Chapter 5, we brought together the genetic variants identified in Chapter 3 and data from the Gene SMART study to create exercise polygenic scores, which were then assessed against baseline and HIIT changes in physiological

and mitochondrial markers to explore how they may be used to predict trainability. In this Chapter (Chapter 6), the key findings of these experimental chapters will be discussed, as well as strengths, limitations, and potential future directions of this work.

I. Which genetic variants are most robustly and consistently linked to exercise responses? (Chapter 3)

Multiple studies have identified a plethora of genetic variants associated with exercise responses [4]. However, it remains unclear which genetics variants are key drivers in trainability, with the majority of genetic variants previously associated with exercise based on findings in a single cohort, or residing within genes with unclear functional relevance to exercise [5]. Evidence of findings between replicated in other independent cohorts and/or functional relevance of the identified genetic variants is important, as a large limitation of genetic studies, -- which sometimes examine 1000s of SNPs – is the risk of false positives, where genetic variants are falsely associated with traits. In Chapter 3, we screened and summarised the genetic variants associated with either resistance or aerobic trainability in at least two independent cohorts, and reviewed where these SNPs were located as well as the biological factors influenced by these loci. We identified 12 and 5 genetic variants robustly associated with aerobic or resistance trainability, respectively. The use of thorough selection criteria ensured that these SNPs held a true association with exercise responses. The identified genetic variants resided in genes within pathways related to multiple trainability phenotypes. For example, the genetic variant rs4253778 (annotated to peroxisome proliferator-activated receptor *PPARA*) and rs8192678 (annotated to peroxisome proliferator transcriptional coactivator *PPARGC1A*) are linked to muscle morphology, aerobic capacity, endurance performance and mitochondrial biogenesis [6]. In addition, the rs6552828 genetic variant located near the *ACSL1* is reported to be strongly correlated toe the aerobic electron transport

chain as well as to the transport of fatty acids into the mitochondria [7]. Moreover, rs353625 (annotated to the *CD44* gene) and rs1956197 (annotated to *DAAM1* gene) were associated with up-regulated gene expression in response to endurance training [8]. Genetic variants annotated to the ACE gene (rs4646994, rs1799752, rs4340 and the rs13447447) are reported to be associated with improved blood flow and proliferation of red blood cells [9]. Also, rs1815739 (annotated to the ACTN3 gene) is reported to be associated to muscle strength [10]. These findings highlighted key genetic variants and biological pathways influencing trainability and provided direction for genetic variants to be explored in the Gene SMART cohort (Chapter 5).

II. Are physiological and skeletal muscle mitochondria adaptations seen after 4-weeks of HIIT, relative to a 4-week untrained control period? (Chapter 4).

In Chapter 4, the response of multiple physiological and mitochondrial markers following four weeks of high-intensity interval training (HIIT), relative to four weeks of an untrained control period, was assessed. This study assessed the value of using a composite measure of mitochondrial content and quality, the mitochondrial health index (MHI). The MHI had previously been used with great success in leucocytes [11], but had not yet been explored in skeletal muscle in the context of exercise and neither had its potential relationships with changes in physiological variables.

In Chapter 4 we showed significant increases in physiological variables (W_{peak} and LT) as well as increases in mitochondrial markers (CS and COX) after 4 weeks of HIIT. However, changes in the MHI were not seen, and significant increases were also seen in CS and COX during the untrained control period. Our findings were unexpected given that mitochondrial function and content are upregulated in skeletal muscle by exercise [12, 13]. This, may in part, be explained by the length of the HIIT intervention potentially being too short, with prior studies showing effects of 6-8 weeks [13, 14]; or it may be due to an batch effect, given muscle

samples from different time points were analysed separately. Chapter 4 also reports positive correlations found between changes in CS activity following 4-weeks of HIIT and $VO_{2max,}$, LT, and Wpeak. These findings are supported by similar studies that show HIIT interventions lead to changes in mitochondrial and physiological phenotypes [15, 16]. Correlation findings are also, in part, supported by a study by Guillen et al. [17], where increase of CS activity in skeletal muscle mitochondria as well as increase in VO_{2max} were reported after 12 weeks of sprint interval training in sedentary males. Prior studies also suggests a relationship between lactate production and defects in mitochondrial function in individuals with Huntington's Disease [18]. Finally, data from Chapter 4 revealed the importance of using a control untrained period to more robustly detect true changes in response to exercise.

III. *Which physiological phenotypes and mitochondrial markers are associated with Exercise Polygenic Score (EPS)? (Chapter 5)*

Previous studies have reported a genetic influence in exercise responses in general populations, elite athletes, and disease-specific groups [19-22]. The limitations of prior studies have been in investigating the association between single genetic variants, which have small effect sizes on fitness phenotypes. In this thesis, a combined genetic score (exercise polygenetic score) was devised from several genetic variants shown to be robustly linked to exercise responses (Chapter 3), with relationships between this score and physiological and mitochondrial variables prior to and following a 4-week HIIT intervention then examined. Prior to this thesis, no studies had examined the association between genetic variants and fitness markers before an exercise intervention (i.e., at baseline).

We found no significant associations between the devised exercise polygenic scores and mitochondrial and physiological markers before and after 4-weeks of HIIT. When examining for potential relationships between mitochondrial and physiological markers and the individual

SNPs making up the exercise polygenic scores, no association were shown between the latter and the physiological variables, with limited associations found between the SNPs and the mitochondrial markers. Reasons for these findings may be that four weeks of HIIT might be a relative short training intervention to detect the genetic influence in exercise, specifically for our moderately-active cohort. Furthermore, although a handful of studies have reported that multiple genetic variants are associated with exercise responses [7, 23, 24], these studies have either bigger cohorts or the exercise intervention was longer than the 4 weeks proposed in the present study. Based on this, it appears this work may have been underpowered to be able to associate genotypes with phenotypes.

However, several associations were found between individual genetic variants and responses in muscle function and mitochondrial content before and after 4-weeks of HIIT. For example, we detected associations between the rs4253778 and rs1815739 with the MHI at baseline, with this supported by prior literature that found an association between rs4253778 and mitochondrial enzymes [25] and between rs1815739 and mitochondrial content [26]. We report several associations between SNPs (rs10921078, rs6090314, rs699, rs8192678) and mitochondrial markers, which have not been reported previously. However, many of these SNPs have been linked to physiological outcomes, such as changes in VO_{2max} after 20 weeks of continuous exercise (SNP: rs6090314) [7], power in elite athletes (rs699) [27] and aerobic capacity in women (SNP: rs8192678) [28]. This data suggests these genetic variants influence these exercise phenotypes by altering mitochondrial functioning, with relationships between fitness physiological and mitochondrial measures supported by correlations reported between several physiological measures and CS in Chapter 3.

6.2 Contribution to Knowledge

This thesis contributes to the existing body of knowledge by compiling a list of robust genetic variants associated with exercise. We leveraged this list to create and assess the usefulness of using an exercise polygenic score to predict exercise responses. Although this investigation had limited findings, likely due to a limited sample size, this list of SNPs and our approach may provide important direction for future investigations exploring the role of genetics in trainability in larger cohorts. Further studies may use this list of SNPs to refine their genetic targets, and increase their ability to capture genetic effects through the use of polygenic scores.

This thesis sought to provide new understanding of the exercise adaptations in skeletal muscle as well as skeletal muscle mitochondria, with robust genetic variants involved in these molecular and physiological adaptations. It presents new evidence of relationships and potential interplay between genetic and mitochondrial factors and changes in physiological traits during exercise. In addition, this work shows the value of using an untrained exercise period in exercise trials to minimise variability in human studies. This study design may provide direction for further investigations, and will increase the field's ability to identify true responses to exercise interventions, which may be overlooked or currently under/over-estimated.

6.3 Strengths

Strengths of this thesis include the use of a systematic approach to defining robust SNPs linked to exercise responses and further devise exercise polygenic scores to be explored in later parts of the thesis. Further, the Gene SMART (Chapter 4-5) is a well-controlled cohort study, which utilised uncontrolled training periods, repeated exercise tests, and controlled diets and muscle analyses to minimise variability in data. For example, we performed the muscle biopsies consistently at the same time of the day to mitigate changes in circadian cycles. In

addition, our experiments at the mitochondrial level were conducted using pieces of the same biopsy, as intra-biopsy variability is substantial [29] and may affect analysis. This tight-controlled study was suitable to detect physiological and mitochondrial phenotypes changes after the intervention.

6.4 Limitations

A limitation of this study is the small number of participants and the length of the intervention in the Gene SMART study (n= 116). Whilst as a cohort size this is quite substantial for an exercise study, it is likely to be underpowered to detect small effect sizes, such as the effect of genetic variants on phenotypes, or changes in physiological and mitochondrial markers over a 4-week exercise period. Although we adjusted our model for multiple testing and many variables were included, we were not able find significant results when comparing genotypes with phenotypes. Further, while we identified significant changes in physiological and mitochondrial markers, a larger cohort is needed to identify the genetic influence in exercise responses. A further limitation of this work is that not all Gene SMART participants undertook the control untrained period. Further, although we included a control period before intervention and we provided a 48-hour diet before biopsies, we could not control participants' lifestyle (diet, sleep, physical activity and many others) which may impact the effect of the intervention, especially on mitochondrial analysis. Further limitations are mitochondrial analyses being performed in different batches, and therefore a risk of batch effect in the data. In addition, this Gene SMART study cohort is predominately Caucasian, which means that our results are less applicable to other ethnic cohorts.

6.5 Future research

There remains several knowledge gaps and questions around what underpins variability in exercise responses. There are discordant or inconclusive findings across studies, with the vast majority of findings for associations between genetic variants and exercise responses

based on one study [30]. Further independent studies are needed to validate findings linking genetic variants to fitness traits, to provide a clearer picture around the contribution of genetics to exercise. It is likely that future work will need to explore combining and collating genetic data from multiple global cohorts to increase statistical power and the generalisability and robustness of findings. Many prior studies in this field focus on physiological changes seen with exercise, and neglect to explore potentially underpinning changes in mitochondria or broader effects on wider tissues. As mitochondrial health is an important regulator of cellular function across different tissues, further work is needed exploring the influence of exercise interventions not only in skeletal muscle mitochondria, but also in other tissues. This work may provide insights into how exercise supports healthy ageing and prevents chronic diseases [31], by identifying mechanisms that influence exercise adaptations across tissues and uncovering molecular targets to guide disease prevention interventions and treatment.

To develop further insights in the mechanisms that may link genetic and molecular processes to exercise responses, future studies should include other “omics” layers such as methylome, transcriptome, and metabolome. Integrating omic data from skeletal muscle will provide a more comprehensive and improved understanding of exercise adaptations, as genetic composition alone does not determine fitness phenotype, and changes in many of these omic are more responsive to external factors such as exercise [32].

6.6 References

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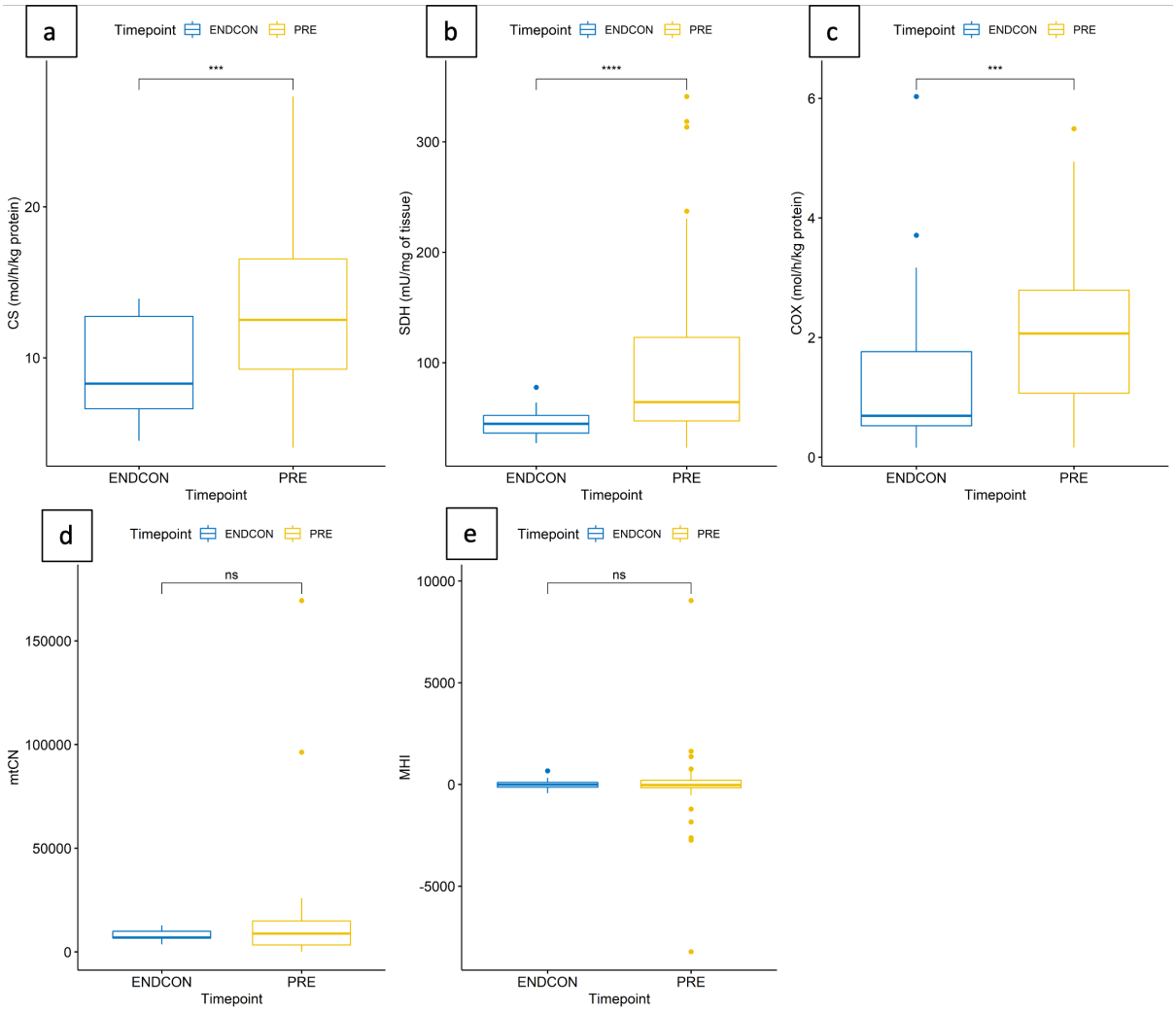
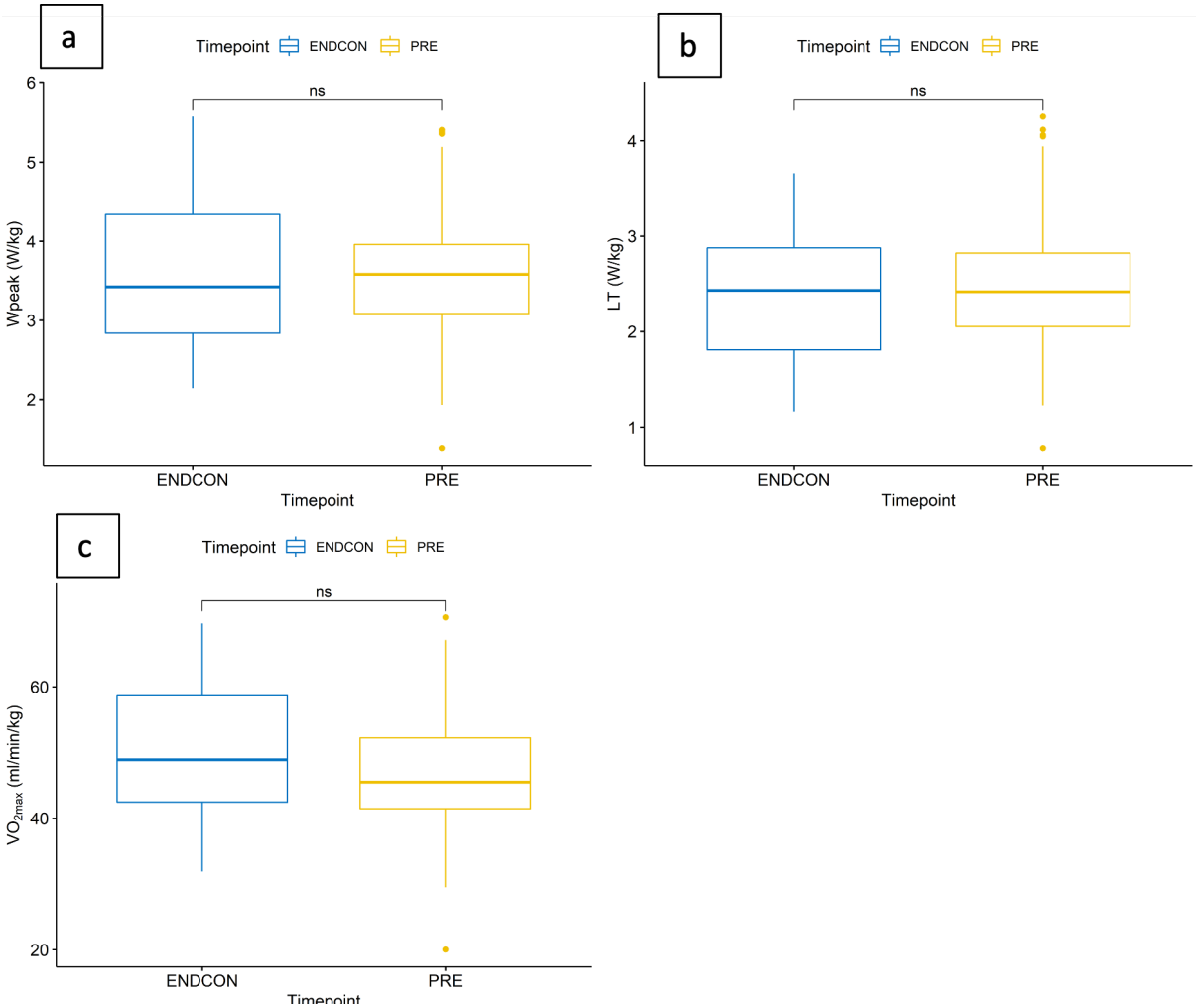
Appendix: Supplementary figure and tables

Supplementary figures



Supplementary figure 4.1 **Changes in mitochondrial markers over four weeks of control period.** Box plots showing the mean changes over 4-weeks of the untrained control period for a) CS, b) SDH, c) COX, d) mtCN, and e) MHI. Abbreviations; citrate synthase activity (CS), cytochrome c oxidase activity (COX), succinate dehydrogenase activity (SDH), mitochondria copy number (mtCN), mitochondrial health index (MHI), start of control period (CON), and and start of HIIT intervention (PRE). * Represents p-value <0.05 and ns represents non-significant results.



Supplementary figure 4.2 **Changes in physiological variables over four weeks of control period.** Box plots showing the mean changes over four weeks of the control period for a) power peak, b) lactate threshold and c) $VO2_{max}$ are shown. Abbreviations; lactate threshold (LT), power peak (Wpeak), start of control period (CON), and start of HIIT intervention (PRE), ns represents non-significant results.

Supplementary Table 5.1 *Associations between Exercise Polygenic Scores and physiological and mitochondrial phenotypes (non-imputed data)*

Outcome	EPS1			EPS2		
	Estimate	**SE**	**P value**	**Estimate**	**SE**	**P value**
VO_{2MAX} BL	-0.60	0.53	0.26	-0.65	0.46	0.16
VO_{2MAX} 4W-Δ	-0.17	0.25	0.49	-0.15	0.22	0.50
Wpeak BL	-0.05	0.50	0.30	-0.06	0.04	0.14
Wpeak 4W-Δ	0.003	0.01	0.77	0.004	0.01	0.64
LT BL	-0.40	0.40	0.31	-0.05	0.03	0.11
LT 4W-Δ	0.007	0.01	0.59	0.004	0.01	0.70
CS BL	-0.32	0.33	0.33	-0.34	0.29	0.23
CS 4W-Δ	-0.34	0.29	0.23	0.11	0.16	0.46
COX BL	0.07	0.08	0.35	-0.0008	0.07	0.99
COX 4W-Δ	-0.01	0.12	0.88	0.04	0.11	0.68
mtCN BL	-2485.3	1773.83	0.16	-1707.3	1455	0.24
mtCN 4W-Δ	-0.06	0.07	0.38	-0.03	0.05	0.59
SDH BL	0.57	5.09	0.91	-3.13	4.48	0.48
SDH 4W-Δ	7.09	5.13	0.17	1.82	4.20	0.66
MHI BL	-85.6	76.8	0.27	-39.05	73.1	0.59
MHI 4W-Δ	142.5	0.01	0.38	4.4	0.01	0.79

Each line represents a separate model, with EPS1 and EPS2 also examined separately. All models were corrected for age and sex, with models exploring change over 4 weeks also adjusting for baseline levels of the appropriate outcome variable. Abbreviations: SE: standard error, BL: baseline, 4W-Δ: change over 4 weeks, EPS1 and EPS2: exercise polygenic score 1 and 2 respectively, VO_2max: maximal oxygen uptake, LT: lactate threshold, Wpeak: power peak, CS: citrate synthase, SDH: succinate dehydrogenase, COX: cytochrome oxidase, MCN: mitochondria copy number, and MHI: mitochondrial health index.

Supplementary Table 5.2 *Least square means estimate for physiological variables before and after 4-weeks of HIIT intervention by each genotype (non-imputed data)*

Variant	Least square means (SE)[1]		
rs6552828	***AA***	***AG***	***GG***
VO_{2MAX} BL	43.1 (2.42)	46.1 (1.38)	45.2 (1.87)
VO_{2MAX} 4W-Δ	1.09 (1.13)	1.10 (0.64)	1.03 (0.89)
Wpeak BL	3.33 (0.22)	3.43 (0.12)	3.34 (0.17)
Wpeak 4W-Δ	0.24 (0.04)	0.14 (0.02)	0.28 (0.03)
LT BL	2.32 (0.18)	2.33 (0.10)	2.28 (0.14)
LT 4W-Δ	0.32 (0.05)	0.23 (0.03)	0.315 (0.04)
rs699	***CC***	***CT***	***TT***
VO_{2MAX} BL	48.6 (2.30)	44.7 (1.41)	44.6 (1.79)
VO_{2MAX} 4W-Δ	0.57 (1.08)	1.72 (0.63)	0.04 (0.86)
Wpeak BL	3.48 (0.21)	3.38 (0.13)	3.34 (0.16)
Wpeak 4W-Δ	0.19 (0.05)	0.20 (0.03)	0.18 (0.04)
LT BL	2.41 (0.17)	2.30 (0.10)	2.28 (0.13)
LT 4W-Δ	0.25 (0.05)	0.27 (0.03)	0.29 (0.04)
rs6090327	***GG***	***AG***	***AA***
VO_{2MAX} BL	45.7 (1.27)	44.7 (1.85)	41.1(6.47)
VO_{2MAX} 4W-Δ	0.81 (0.58)	1.40 (0.83)	5.64 (2.80)
Wpeak BL	3.37 (0.11)	3.44 (0.17)	3.23 (0.60)
Wpeak 4W-Δ	0.19 (0.02)	0.20 (0.03)	0.11 (0.13)
LT BL	2.30 (0.09)	2.37 (0.13)	2.06 (0.48)
LT 4W-Δ	0.26 (0.03)	0.30 (0.04)	0.19 (0.14)
rs12891759	***AA***	***AG***	***GG***
VO_{2MAX} BL	45.3 (1.32)	45.7 (1.75)	36.8 (6.33)

VO_{2MAX} 4W-Δ	1.26 (0.61)	0.78 (0.81)	-3.59 (3.94)
Wpeak BL	3.38 (0.12)	3.44 (0.16)	2.71 (0.59)
Wpeak 4W-Δ	0.18 (0.02)	0.23 (0.03)	0.20 (0.18)
LT BL	2.31 (0.10)	2.33 (0.13)	1.90 (0.48)
LT 4W-Δ	0.27 (0.03)	0.27 (0.04)	0.27 (0.21)
rs8192678	***AA***	***AG***	***GG***
VO_{2MAX} BL	48.0 (2.46)	44.8 (1.62)	45.2 (1.47)
VO_{2MAX} 4W-Δ	0.75 (1.10)	1.62 (0.74)	0.69 (0.70)
Wpeak BL	3.71 (0.22)	3.37 (0.15)	3.32 (0.13)
Wpeak 4W-Δ	0.24 (0.05)	0.20 (0.03)	0.19 (0.03)
LT BL	2.57 (0.18)	2.29 (0.12)	2.28 (0.11)
LT 4W-Δ	0.34 (0.05)	0.27 (0.03)	0.24 (0.03)
rs11120796	***AA***	***AG***	***GG***
VO_{2MAX} BL	48.1 (2.55)	44.3 (1.41)	46.0 (1.71)
VO_{2MAX} 4W-Δ	2.69 (1.22)	1.05 (0.65)	0.56 (0.79)
Wpeak BL	3.65 (0.23)	3.35 (0.13)	3.35 (0.16)
Wpeak 4W-Δ	0.19 (0.05)	0.20 (0.03)	0.19 (0.03)
LT BL	2.50 (0.19)	2.31 (0.10)	2.25 (0.13)
LT 4W-Δ	0.27 (0.06)	0.22 (0.03)	0.34 (0.04)
rs10921078	***GG***	***AG***	***AA***
VO_{2MAX} BL	45.3 (1.37)	45.2 (1.76)	46.5 (3.43)
VO_{2MAX} 4W-Δ	0.83 (0.63)	1.63 (0.82)	0.70 (1.77)
Wpeak BL	3.36 (0.12)	3.38 (0.16)	3.62 (0.31)
Wpeak 4W-Δ	0.20 (0.02)	0.21 (0.03)	0.06 (0.08)
LT BL	2.28 (0.10)	2.34 (0.13)	2.50 (0.25)
LT 4W-Δ	0.26 (0.03)	0.29 (0.04)	0.23 (0.09)
rs4253778	***TT***	***CT***	***CC***
VO_{2MAX} BL	46.8 (4.20)	46.4 (1.76)	44.8 (1.35)
VO_{2MAX} 4W-Δ	0.32 (2.02)	0.82 (0.80)	1.26 (0.63)
Wpeak BL	3.40 (0.38)	3.57 (0.16)	3.29 (0.12)
Wpeak 4W-Δ	0.18 (0.09)	0.19 (0.03)	0.19 (0.02)
LT BL	2.29 (0.31)	2.51 (0.13)	2.21 (0.09)
LT 4W-Δ	0.20 (0.10)	0.21 (0.04)	0.30 (0.03)
rs1815739	***TT***	***TC***	***CC***
VO_{2MAX} BL	46.8 (4.20)	46.4 (1.76)	44.8 (1.35)
VO_{2MAX} 4W-Δ	0.32 (2.02)	0.82 (0.80)	1.26 (0.63)
Wpeak BL	3.40 (0.38)	3.57 (0.16)	3.29 (0.12)
Wpeak 4W-Δ	0.18 (0.09)	0.19 (0.03)	0.19 (0.02)
LT BL	2.29 (0.31)	2.51 (0.13)	2.21 (0.09)
LT 4W-Δ	0.20 (0.10)	0.21 (0.04)	0.30 (0.03)

All models were adjusted for sex, age, and baseline levels of appropriate phenotype (for models on 4W-Δ outcomes only) and performed on non-imputed data. Values within the same row that are bolded and denoted with different letters represent significant differences. (<0.05). Abbreviations: SE: standard error, BL: baseline, 4W-Δ: change over 4 weeks, VO_2max: maximal oxygen uptake, LT: lactate threshold, Wpeak: power peak

Supplementary Table 5.3 *Least square means estimates for mitochondria phenotypes before and after the HIIT intervention, by genotype (imputed data)*

Variant	Least square means (SE)[1]		
rs6552828	***AA***	***AG***	***GG***
CS BL	11.7 (1.54)	11.4 (0.86)	11.0 (1.15)
CS 4W-Δ	0.64 (0.92)	0.17 (0.55)	0.74(0.71)
COX BL	1.67 (0.35)	1.62 (0.19)	2.04 (0.26)
COX 4W-Δ	0.28 (0.34)	-0.12 (0.20)	0.07 (0.28)
mtCN BL	11877 (4586)	9841 (2776)	13968 (3753)
mtCN 4W-Δ	-174 (2363)	-207 (1364)	-1073 (1945)
SDH BL	69.4 (22.2)	74.7 (11.3)	75.7 (15.4)
SDH 4W-Δ	-4.15 (14.58)	-1.35 (9.09)	2.13 (12.09)
MHI BL	-6544 (24509)	12914 (14535)	-4754 (18632)
MHI 4W-Δ	-8190 (352)	-8299 (225)	-8672 (386)
rs699	***CC***	***CT***	***TT***
CS BL	11.3 (1.47)	11.7 (0.88)	10.9 (1.14)
CS 4W-Δ	0.86 (0.98)	0.25 (0.54)	0.38 (0.77)
COX BL	1.51 (0.32)	1.86 (0.20)	1.65 (0.26)
COX 4W-Δ	0.08 (0.33)	0.18 (0.20)	-0.36 (0.25)
mtCN BL	8492 (4492)	13819 (2805)	8422 (3548)
mtCN 4W-Δ	-305 (2176)	-432 (1391)	-442 (1739)
SDH BL	69.6 (19.2)	77.2 (11.4)	70.7 (15.0)
SDH 4W-Δ	-16.32 (15.68)	0.03 (9.83)	6.24 (11.48)
MHI BL	-5570 (24058)	11961 (13986)	-1170 (20300)
MHI 4W-Δ	-8464 (470)	-8419 (252)	-8227 (283)
rs6090327	***GG***	***AG***	***AA***
CS BL	11.9 (0.79)	10.0 (1.13)	10.6 (3.93)
CS 4W-Δ	0.36 (0.49)	0.65 (0.79)	-1.24 (2.62)
COX BL	1.71 (0.17)	1.88 (0.26)	0.386 (1.01)
COX 4W-Δ	-0.01 (0.18)	0.07 (0.26)	-0.19 (1.10)
mtCN BL	10248 (2499)	14100 (3691)	9215 (12918)
mtCN 4W-Δ	695 (1397)	-3983 (1709)	7566 (5018)
SDH BL	77.0 (10.6)	69.0 (15.7)	21.6 (50.3)
SDH 4W-Δ	-1.90 (8.7)	2.13 (13.5)	-10.71 (45.9)
MHI BL	45.9 (12301)	-3957 (21910)	-2260 (64341)
MHI 4W-Δ			
rs12891759	***AA***	***AG***	***GG***
CS BL	11.49 (0.83)	11.31 (1.088)	7.37 (3.90)
CS 4W-Δ	0.40 (0.54)	0.39 (0.67)	1.05 (2.92)
COX BL	1.66 (0.18)	1.90 (0.25)	1.40 (1.13)
COX 4W-Δ	-0.09 (0.19)	0.2157 (0.26)	-0.57 (1.00)
mtCN BL	12807 (2587)	8292 (3509)	1985 (12655)
mtCN 4W-Δ	-183 (1396)	-669 (1781)	-6769 (6229)
SDH BL	74.9 (10.9)	72.3 (15.1)	60.1 (63.8)
SDH 4W-Δ	-4.96 (8.66)	8.03 (11.85)	-21.44 (48.07)
MHI BL	9265 (13487)	-3886 (18189)	-6533 (63318)
MHI 4W-Δ	-8390 (223)	-8357 (268)	-7461 (2180)
rs8192678	***AA***	***AG***	***GG***
CS BL	10.5 (1.51)	10.6 (1.007)	12.2 (0.91)
CS 4W-Δ	0.71 (0.92)	0.17 (0.64)	0.48 (0.57)
COX BL	1.45 (0.40)	1.78 (0.23)	1.77 (0.20)
COX 4W-Δ	-0.02 (0.37)	-0.22 (0.24)	0.18 (0.21)
mtCN BL	**22501 (4666)[a]**	**9631 (3137)[b]**	**9762 (2813)[b]**
mtCN 4W-Δ	707 (2669)	-1132 (1597)	-130 (1375)
SDH BL	63.0 (19.7)	82.9 (13.7)	69.6 (12.3)
SDH 4W-Δ	-11.62 (16.0)	0.75 (10.3)	0.09 (10.1)

MHI BL	-6632 (23861)	-3684 (15917)	14281 (16057)
MHI 4W-Δ	-8195 (399)	-8364 (281)	-8415 (259)
rs11120796	***AA***	***AG***	***GG***
CS BL	11.3 (1.63)	11.1 (0.88)	11.8 (1.07)
CS 4W-Δ	0.64 (0.92)	0.17 (0.55)	0.74(0.71)
COX BL	1.89 (0.38)	1.74 (0.20)	1.67 (0.24)
COX 4W-Δ	-0.23 (0.38)	0.11 (0.21)	-0.07 (0.253)
mtCN BL	10579 (4912)	12318 (2868)	9872 (3427)
mtCN 4W-Δ	-308 (2381)	-495 (1471)	-325 (1717)
SDH BL	77.2 (20.7)	69.3 (11.9)	79.6 (14.6)
SDH 4W-Δ	7.029 (18.27)	-0.687 (9.27)	-4.504 (10.91)
MHI BL	528 (31697)	11807 (14027)	-3893 (17389)
MHI 4W-Δ	-8216 (531)	-8405 (236)	-8373 (308)
rs10921078	***GG***	***AG***	***AA***
CS BL	11.81 (0.85)	10.89 (1.09)	9.88 (2.29)
CS 4W-Δ	0.27 (0.55)	0.39 (0.68)	1.61 (1.40)
COX BL	1.66 (0.19)	1.67 (0.24)	2.62 (0.47)
COX 4W-Δ	0.02 (0.19)	0.04 (0.25)	-0.31 (0.55)
mtCN BL	12402 (2666)	8622 (3511)	12142 (6935)
mtCN 4W-Δ	-586 (1313)	-384 (1738)	883 (3479)
SDH BL	70.7 (11.0)	72.5 (14.6)	105.3 (28.0)
SDH 4W-Δ	-1.12 (9.6)	2.52 (11.5)	-12.98 (24.4)
MHI BL	-7499 (38339)	-618 (21075)	8216 (13279)
MHI 4W-Δ	-8394 (237)	-8137 (278)	-9094 (525)
rs4253778	***TT***	***CT***	***CC***
CS BL	14.9 (2.51)	12.0 (1.095)	10.9 (0.83)
CS 4W-Δ	-0.73 (1.57)	0.65 (0.74)	0.35 (0.56)
COX BL	2.20 (0.62)	1.93 (0.25)	1.62 (0.19)
COX 4W-Δ	-0.30 (0.58)	-0.20 (0.25)	0.12 (0.19)
mtCN BL	10678 (7925)	8489 (3592)	12632 (2650)
mtCN 4W-Δ	-1059 (3516)	238 (1871)	-703 (1329)
SDH BL	66.0 (32.6)	83.0 (14.1)	69.8 (10.8)
SDH 4W-Δ	16.87 (28.73)	8.23 (11.12)	-6.59 (8.87)
MHI BL	-7499 (38339)	-618 (21075)	8216 (13279)
MHI 4W-Δ	-7974 (624)	-8164 (282)	-8490 (230)
rs1815739	***TT***	***TC***	***CC***
CS BL	14.9 (2.51)	12.0 (1.09)	10.9 (0.83)
CS 4W-Δ	-0.73 (1.57)	0.65 (0.74)	0.35 (0.56)
COX BL	2.20 (0.62)	1.93 (0.251)	1.62 (0.19)
COX 4W-Δ	-0.30 (0.58)	-0.20 (0.25)	0.12 (0.19)
mtCN BL	10678 (7925)	8489 (3592)	12632 (2650)
mtCN 4W-Δ	-1059 (3516)	238 (1871)	-703 (1329)
SDH BL	66.0 (32.6)	83.0 (14.1)	69.8 (10.8)
SDH 4W-Δ	16.87 (28.73)	8.23 (11.12)	-6.59 (-6.59)
MHI BL	-7499 (38339)	-618 (21075)	8216 (13279)
MHI 4W-Δ	-7974 (624)	-8164 (282)	-8490 (230)

All models were adjusted for sex, age, and baseline levels of appropriate phenotype (for models on 4W-Δ outcomes only) and performed on imputed data. Values within the same row that are bonded and denoted with different letters represent significant differences. (<0.05). Abbreviations: SE: standard error, BL: baseline, 4W-Δ: change over 4 weeks, VO_2max: maximal oxygen uptake, LT: lactate threshold, Wpeak: power peak, (CS) citrate synthase, (COX) Cytochrome C oxidase, (mtCN) mitochondrial copy number, (SDH) succinate dehydrogenase, (MHI) mitochondrial health index.