THE EPIGENETIC BASIS OF VARIABLE RESPONSES TO EXERCISE TRAINING

Macsue Jacques

Thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

Principal supervisor: Prof. Nir Eynon

Co-Supervisors: Dr. Sarah Voisin

Dr. Xu Sean Yan

Victoria University

College of Sport and Exercise Science

Institute for Health and Sports (iHES)

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Abstract

Exercise training provides health benefits to the general population, but there is considerable variability in the individual response to similar training. Some people have limited improvements following exercise ("low responders"), while others seem to improve considerably ("high responders"). To date, most exercise studies that have claimed to identify "low" or "high" responders assumed that if the participants were to repeat the same exercise training, they would show a similar response. However, *within-subject variability* has not been tested, which might lead to inaccurate classification of exercise responses at the individual level and the waste of precious research resources. Exposing individuals to a repeated or longer training intervention can assist in identifying the magnitude of responses to exercise training with better accuracy.

Recent evidence also suggests that the response to exercise training may be influenced by epigenetic signatures. Epigenetics is a reversible process that affects how genes are expressed in cells, and it carries the memory of past cellular and environmental events. To date, no study has tested whether individual response is influenced by epigenetic marks. **Thus, the overarching aim of this thesis is to identify the physiological, molecular, and epigenetic marks of exercise responses.**

Twenty young, healthy men from the Gene SMART (Skeletal Muscle Adaptive Response to Training) study completed a repeated and a longer exercise training intervention to measure within-subject variability and to obtain individual progress curves (See Figure 3.1 for study design). Participants underwent a four-week control period followed by four weeks of High-Intensity Interval Training (HIIT), had a washout period of > 1 year, and underwent another four weeks of HIIT followed by an additional 8 weeks of HIIT. The HIIT program was adjusted to individual fitness levels that were re-assessed every four weeks during the intervention to ensure improvements. Participants' peak power output (W_{peak}), lactate threshold (LT), and maximal oxygen uptake (VO_{2max}) were assessed in duplicates at each time point. We used five known statistical methods to investigate changes in fitness and mixed models to estimate individual response. Muscle biopsies were collected at each time point to measure mitochondrial markers (i.e. mitochondrial respiration, citrate synthase, cytochrome C oxidase, succinate dehydrogenase, mitochondrial copy number, fibre typing, and myosin heavy chains

PCRs), as well as genome-wide DNA methylation profiles in skeletal muscle using the Illumina HumanMethylation EPIC array.

In Chapter 3, we show that at the group level, all physiological measures increased in a dose-response manner following HIIT ($p<0.05$). We found no changes in mitochondrial function and content or fibre type distributions. Baseline citrate synthase (CS) was associated with HIIT-induced changes in cytochrome-c oxidase (COX) and vice-versa ($p < 0.05$). At the individual level, we successfully identified trainability in physiological measurements using the repeated testing approach but failed to do so using the repeated intervention approach. We did not identify consistent individual response at the molecular level (mitochondrial function and content and fibre type distribution) using either approach, as measurements were highly variable within participants.

We then investigated the reliability of the mitochondrial respiration technique (Chapter 4) by measuring the Technical Error of measurement (TEM) and the coefficient of variation (CV) for each mitochondrial complex. While the correlation between the two chambers was good for all complexes ($R > 0.7$ $p < 0.001$), the TEM was large (7.9 to 27 pmol·s-1·mg-1), and the CV was > 15% for all complexes. We performed statistical simulations to determine the sample size that would be required to detect a range of effect sizes at 80% power. We found that duplicate measurements on 75 participants are required to detect a 6% change in mitochondrial respiration after an intervention.

Finally, Chapter 5 and 6 focus on the DNA methylation measures at the group and individual level respectively. For the first time at the group level, we have investigated DNA methylation patterns that are associated with fitness by combining three measurements of performance into a comprehensive z-score (Chapter 5). We found 12,107 DMPs that were associated with baseline fitness (z-score) (FDR < 0.005), 18.2% of which were hypomethylated and 81.8% hypermethylated with higher fitness levels. We identified 1,268 DMRs for baseline fitness, 15.3% of which hypomethylated and 85.7% hypermethylated. Hyper-DMRs were robustly over-represented in genic enhancers and flanking active TSS, and highly depleted in strong and weak candidate enhancers. Hypo and Hyper-DMRs had a moderate association with bivalent enhancers and promoters. Both hyper and hypo-DMRs presented a moderate representation in regions actively repressed by PolyComb proteins. Finally, significant DMRs were enriched for 26 GO terms, and these pathways were related to muscle system processes, actin cytoskeleton organization and regulation of actin filament and cytoskeleton processes.

Next, we investigated the effects of exercise on the methylome, and surprisingly we observed an inverse pattern of DNA methylation profile after exercise. In summary, we found 568 DMPs that significantly changed after the 4 weeks (FDR < 0.005), and out of those only 1.4% were hypermethylated and 98.6% were hypomethylated. We identified 17 DMRs associated with changes in DNA methylation in response of 4 weeks of HIIT, and 100% of DMRs were hypomethylated. Lastly, we intersected DMPs that were significant for both baseline fitness z-score and after 4 weeks of HIIT. Five DMPs were significant, and they appeared to have inverse patterns for baseline z-score (more hypermethylation) and 4 weeks of HIIT (more hypomethylation). When we transitioned to the individual level study in Chapter 6, neither of our approaches (i.e. repeated intervention and repeated testing) yielded many significant results: only one DMP was significant (cg11260483, p-value: 3.22000e-10, adj.p-value: 0.00022) after the repeated intervention, and no DMPs significant after the repeated testing approach.

The challenging experimental design of this thesis provided high resolution, longitudinal physiological and molecular profiles in skeletal muscle following repeated exercise training and testing. It yielded novel insights into the phenomenon of trainability in humans; young, healthy men displayed individual responses to HIIT at the physiological level, but not at the molecular level. This thesis also issued methodological considerations for protocols aimed at measuring individual response (Chapter 3). In particular, the high *within-subject* variability we observed led us to conclude that many repeated testings on the same individual at regular intervals during the training program, along with a moderate-to-large sample size, were necessary to estimate inter-individual variability in response to training. The mitochondrial respiration technique showed high technical variability (Chapter 4), making the measurement unreliable in our study with only n = 20 men and only two duplicates per individual. The typical sample sizes used in exercise training studies (n < 20) are likely insufficient to capture exercise-induced changes in mitochondrial respiration at the group level, let alone the individual level. Lastly, we observed a clear DNA methylation profile association with fitness levels (Chapter 5). However, when an exercise intervention was applied, we noticed a change in DNA methylation patterns that were inverse to those observed at baseline for the fitter participants. Such observations left us wondering on potential reasons to why this occurs. Thus, future research should also integrate the methylome with transcriptome and proteome to elucidate the mechanisms underlying adaptations to exercise training.

Student Declaration

"I, Macsue Jacques, declare that the PhD thesis entitled "The epigenetic basis of variable response to exercise training" is no more than 80,000 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 other 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 the Responsible Conduct of Research and Victoria University's Higher Degree by Research Policy and Procedures"

Signature  ____ Date___03/05/2021____

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisors Professor Nir Eynon and Dr Sarah Voisin for your continual support, guidance, and sharing of your extensive research experience with me throughout my PhD journey. A special thank you to Sarah Voisin, who has introduced me to R and for all your patience in teaching me how to code as well as statistics. Without you I would have never found out how much I love working with R and found the desire to become a bioinformatician one day, thank you for being such a great inspiration! To Nir Eynon who has helped me from day one to be where I am today, without your help and belief in me I probably would not be getting a PhD today, thank you for being a great supervisor and someone I can always count on. I would also like to thank Dr Xu Yean and Dr Danielle Hiam for your valuable insights and contribution to this research.

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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. **Jacques M**, Hiam D, Craig J, Barrès R, Eynon N, Voisin S. (2019) Epigenetic changes in healthy human skeletal muscle following exercise- a systematic review. Epigenetics. Jul;14(7):633-648 (Chapter Two).

2. Voisin S, **Jacques M**, Lucia A, Bishop DJ, Eynon N. (2018) Statistical considerations for exercise protocols aimed at measuring trainability. Exerc Sport Sci Rev. Jan;47(1):37-45 (Chapter Two).

3. **Jacques M**, Yan X, Bishop DJ, Romero JA, Munson F, Kuang J, Garnham A, Papadimitriou I, Voisin S*, Eynon N*. (2019) Mitochondrial respiration variability and simulations in human skeletal muscle: the Gene SMART study. FASEB J. Feb;34(2):2978-2986. doi:10.1096/fj.201901997RR (Chapter Four).

The following work has been submitted or is being prepared for publication at peer reviewed journals in support of this thesis:

1. **Jacques M**, Landen S, Romero JA, Yan X, Garnham A, Hiam D, Siegwald M, Mercier E, Voisin S, Eynon N. Individual physiological and mitochondrial responses during 12 weeks of intensified exercise. Physiological Reports (Accepted) (Chapter Three).

2. **Jacques M**, Voisin S, Xu Yan, Eynon N. Measuring true physiological responses to exercise using a repeated and longer exercise intervention. Sports Medicine (Submitted) (Chapter Three).

3. **Jacques M**, Landen S, Alvarez-Romeiro J, Hiam D, Garnham A, Schittenhelm R, Shah A, Huang C, Voisin S, Eynon N. An integrative OMICS approach to investigate changes in DNA methylation and proteomics with exercise: A 12-week time-course analyses (provisional title). In preparation. (Chapter Five).

4. **Jacques M**, Landen S, Alvarez-Romeiro J, Hiam D, Garnham A, Schittenhelm R, Shah A, Huang C, Voisin S, Eynon N Individual DNA methylation response to exercise training (provisional title) (Chapter Six).

The following work has been published in a peer reviewed journal during my candidature, and is outside the scope of this thesis:

1. Harvey N, Voisin S, Lea S, Yan X, Benton M, Papadimitriou I, **Jacques M**, Haupt L, Ashton K, Eynon N, Griffiths L. (2020) Investigating the influence of mtDNA and nuclear encoded mitochondrial variants on high intensity interval training outcomes. Sci Rep. Jul 6;10(1):11089. doi: 10.1038/s41598-020-67870-1.

2. Harvey NR, Voisin S, Dunn PJ, Sutherland H, Yan X, **Jacques M**, Papadimitriou ID, Haseler LJ, Ashton KJ, Haupt LM, Eynon N, Griffiths LR. (2019) Genetic variants associated with exercise performance in both moderately trained and highly trained individuals. Mol Gent Genomics. 20;10.1007/s00438-019-01639-8.

3. Hiam D, Smith C, Voisin S, Denham J, Yan X, Landen S, **Jacques M**, Alvarez-Romeiro J, Garnham A, Wossner M, Herrmann M, Duque G, Levinger I, Eynon N. (2019) Aerobic capacity and telomere length in human skeletal muscle and leukocytes across the lifespan. Aging. 12(1):359–369.

4. Papadimitriou ID, Eynon N, Yan X, Munson F, **Jacques M**, Kuang J, Voisin S, North KN, Bishop DJ. (2019) A “human knockout” model to investigate the influence of the α-actinin-3 protein on exercise-induced mitochondrial adaptations. Sci Rep.Sep 3;9(1):12688.

5. Hiam D, Voisin S, Yan X, Landen S, **Jacques M**, Papadimitriou ID, Munson F, Byrnes E, Brennan Speranza TC, Levinger I, Eynon N. (2019) The association between bone mineral density gene variants and osteocalcin at baseline, and in response to exercise: The Gene SMART study. Bone. Volume 123, June, Pages 23-27.

6. Yan X, Dvir N, **Jacques M**, Cavalcante L, Papadimitriou ID, Munson F, Kuang J, Garnham A, Landen S, Li J, O’Keefe L, Tirosh O, Bishop DJ, Voisin S, Eynon N. (2018) ACE I/D gene variant predicts ACE enzyme content in blood but not the ACE, UCP2, and UCP3 protein content in human skeletal muscle in the Gene SMART study. J Appl Physiol (1985). Sep 1;125(3):923-930.

7. Papadimitriou ID, Lockey SJ, Voisin S, Herbert AJ, Garton F, Houweling PJ, Cieszczyk P, Maciejewska-Skrendo A, Sawczuk M, Massidda M, Calò CM, Astratenkova IV, Kouvatsi A, Druzhevskaya AM, **Jacques M**, Ahmetov II, Stebbings GK, Heffernan S, Day SH, Erskine R, Pedlar C, Kipps C, North KN, Williams AG, Eynon N. (2018) No association between ACTN3 R577X and ACE I/D polymorphisms and endurance running times in 698 Caucasian athletes. BMC Genomics. Jan 3;19(1):13.

Pre-prints

1. Landen S, **Jacques M**, Hiam D, Alvarez J, Harvey NR, Haupt LM, Griffiths LR, Ashton KJ, Lamon S, Voisin S, Eynon N. (2021) Genome-wide DNA methylation and transcriptome integration reveal distinct sex differences in skeletal muscle. bioRxiv 2021.03.16.435733

2. Genders AJ, Kuang J, Marin EC, Saner NJ, Botella J, **Jacques M**, McConell GK, Andrade-Souza VA, Chagolla J, Bishop DJ.(2020) Changes in insulin resistance do not occur in parallel with changes in mitochondrial content and function in male rats. bioRxiv. https://doi.org/10.1101/2020.07.06.190702

3. Voisin S, **Jacques M**, Landen S, Harvey NR, Haupt LM, Griffiths LR, Gancheva S, Ouni M, Jahnert M, Ashton KJ, Coffey VG, Thompson JM, Doering TM, Gabory A, Junien C, Caiazzo R, Verkindt H, Raverdy V, Pattou F, Froguel P, Craig JM, Blocquiaux S, Thomis M, Sharples AP, Schurmann A, Roden M, Hovarth S, Eynon N.(2020) Meta-analysis of genome-wide DNA methylation and integrative OMICs in human skeletal muscle. bioRxiv. https://doi.org/10.1101/2020.09.28.315838

Book Chapters

1. **Jacques M**, Landen S, Palmer A, Eynon N. (2020) Epigenetic effects of exercise on human skeletal muscle. *Handbook of Stress Volume 4: Stress Genetics, Epigenetics, and Genomics.* Elsevier.

2. **Jacques M**, Landen S, Voisin S, Eynon N. (2018) Summary Findings on Genetics and Sport Performance. *The Routledge Handbook of Sport and Exercise System Genetics*. Taylor & Francis.

3. **Jacques M**, Landen S, Voisin S, Lamon S, Eynon N. (2018) Nurture vs Nature: The Genetics and Epigenetics of Exercise. *Research Methods in Physical Activity and Health.* Routledge.

4. **Jacques M**, Hanson ED, Eynon N. (2018) Genetic Aspects of Sprint, Strength, and Power Performance – 2017 update. *Nutrition and Enhanced Sports Performance: Muscle Building, Endurance and Strength* (2nd Edition). Elsevier.

Details of Included Papers

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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
2	Epigenetic changes in healthy human skeletal muscle following exercise- a systematic review.	Published	Jacques M, Hiam D, Craig J, Barrès R, Eynon N, Voisin S. (2019) Epigenetic changes in healthy human skeletal muscle following exercise- a systematic review. Epigenetics. Jul;14(7):633-648.
2	Statistical considerations for exercise protocols aimed at measuring trainability.	Published	Voisin S, Jacques M, Lucia A, Bishop DJ, Eynon N. (2018) Statistical considerations for exercise protocols aimed at measuring trainability. Exerc Sport Sci Rev. Jan;47(1):37-45.
3	Jacques M, Landen S, Romero JA, Yan X, Garnham A, Hiam D, Siegwald M, Mercier M, Voisin S, Eynon N. Trainability analyses of whole body and skeletal muscle molecular adaptation to 12 weeks of high-intensity exercise.	Under review	Trainability analyses of whole body and skeletal muscle molecular adaptation to 12 weeks of high-intensity exercise. Target Journal: American Journal of Physiology. Date of submission:
3	Jacques M, Voisin S, Xu Yan, Eynon N. Individual physiological and mitochondrial responses during 12 weeks of intensified exercise	Under review	Individual physiological and mitochondrial responses during 12 weeks of intensified exercise. Target journal: MSSE. Date of submission:
4	Mitochondrial respiration variability and simulations in human skeletal muscle: the Gene SMART study.	Published	Jacques M, Yan X, Bishop DJ, Romero JA, Munson F, Kuang J, Garnham A, Papadimitriou I, Voisin S*, Eynon N*. (2019) Mitochondrial respiration variability and simulations in human skeletal muscle: the Gene SMART study. FASEB J. 2020;

Declaration by [candidate name]: Macsue Jacques

Signature: [redacted]

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

%	Percent
<	Less than
>	Greater than
~	Approximately
ADP	Adenosine diphosphate
akt-mTOR	Phosphatidylinositol 3-kinase target of the rapamycin signalling pathway
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
BCAR3	Breast cancer anti-estrogen resistance 3
BCL6	B cell lymphoma 6
BMI	Body Mass Index
BP	Biological Process
CaMKII	Calcium calmodulin-dependent protein kinase II
CC	Cellular Component
CD93	Cluster of differentiation 93
chr	Chromosome
CI	Confidence Interval
CI	Complex I, electron input through CI
CI+CII	Convergent electron input through CI and CII
$CI+CII_E$	measurement of electron transport system [ETS] capacity [E] through CI+CII
$CI+CII_P$	Complex II [CII] linked respiration
CI_P	oxidative phosphorylation [OXPHOS] capacity [P] through CI
COX	Cytochrome-c Oxidase
CRF	Cardiorespiratory Fitness
CS	Citrate synthase
Ct	Cycle threshold
CV	Coefficient of variation
DMPs	Differently Methylated Positions
DMRs	Differently Methylated Regions
DNA	Deoxyribonucleic acid
DNMTs	DNA methyltransferases
DZ	Dizygotic
E	ETS capacity
END	Endurance

ETS	Electron Transport System
EWAS	Epigenome-Wide Association Studies
FC	Fold Change
FCCP	p-trifluoromethoxyphenylhydrazone
FDR	False Discovery Rate
FZD5	Frizzled-5
Gene SMART	Genes and Skeletal Muscle Adaptive Response to Training
GEO	Gene Expression Omnibus
GO	Gene Ontology
GpGs	Cytosine and Guanine separated by a Phosphate
GXT	Graded Exercise Test
H_2O_2	Hydrogen Peroxide
HDAC	Histone Deacetylase
HIIT	High Intensity Exercise Training
HOX	Homeobox
Inv-RCR	Inverse of respiratory control ratio [CIL/CI+II_P]
IPA	Incidental Physical Activity
K_2HPO_4	Dipotassium phosphate
KEGG	Kyoto Encyclopedia of Genes and Genomes
KH_2PO_4	Monopotassium phosphate
L)	Leak respiration
LCR	Leak control ratio [CIL/CI+II_E]
LT	Lactate Threshold
MEF2	Myocyte Enhancer Factor-2
MF	Molecular Function
MHI	Mitochondrial Health Index
miRNAs	microRNAs
$mL \cdot min^{-1} \cdot kg^{-1}$	Millilitres per minute per kilogram
mmol	Millimole
mRNA	Messenger RNA
mtDNA	Mitochondrial DNA
MYOM1	Myomesin-1
MZ	Monozygotic
n	Sample size
ncRNA	Non-coding RNAs
OXPHOS	Oxidative phosphorylation
P	Oxphos capacity
PCR	Phosphorylation control ratio [CI+II_P/CI+II_E]
PCR	Polymerase Chain Reaction

PDK4	Pyruvate dehydrogenase lipoamide kinase isoenzyme 4
PGC-1a	Proliferator-activated receptor gamma coactivator 1-alpha
PGC-1ß	Proliferator-activated receptor gamma coactivator 1-beta
PPAR-d	Peroxisome Proliferator Activated delta
PPO	Peak Power Output
qPCR	Quantitative Polymerase Chain Reaction
RES	Resistance
RNA	Ribonucleic acid
ROX	Residual oxygen consumption
RT-PCR	Real-Time Polymerase Chain Reaction
SCR	Substrate control ratio at constant P [CIP/CI+II_P]
SD	Standard deviation
SDH	Succinate dehydrogenase
T2D	Type 2 Diabetes
T2D	Type 2 diabetes
TCA	Tricarboxylic Acid
TE_M	Technical Error of measurement
TET enzymes	Ten-eleven translocation enzymes
TFAM	Transcription Factor A, Mitochondrial
TSS	Transcription Start Site
VO_{2max}	Maximal rate of oxygen uptake
W	Watt
W_{peak}	Power Peak
β-HAD	3-hydroxyacyl-CoA dehydrogenase
Δ	Delta change
µm	Micrometre

Chapter 1. Introduction

Exercise training results in many morphological, metabolic, and functional adaptations in the human body. The magnitude of adaptations to repeated exercise sessions (e.g. increased cardiac output, or maximal oxygen uptake (VO_{2max}) depends on many factors, such as the duration, intensity, volume, and the type of exercise training[1]. However, not everyone adapts to it to the same degree. It is becoming clear that there is large inter-individual variability in humans in response to similar exercise training stimuli[2–6]. This variability has been observed in all measurements of interest, whether physiological, health, or performance-related[4], and in both short (e.g. 2 weeks) and longer (e.g. 12 weeks) exercise training interventions[6].

Personalised exercise prescription is an appealing term for coaches, exercise physiologists, and clinicians who strive to use the best possible scientific information to prescribe exercise training to their clients and patients depending on their "trainability" (i.e. consistent individual response to exercise). Trainability lays at the core of personalised exercise prescription and it is based in the assumption that individuals have variable ability to respond to similar exercise training, with some individuals showing little to no improvement, while others significantly improve following a specific training regime[7]. However, intra-individual variability (i.e. variable response within an individual) has proven to be an often-ignored limitation on the search for reliable biomarkers of adaptive response to exercise[5,8–10]. For instance, a recent study comparing changes in muscle messenger RNA (mRNA) expression after repeatable bouts of exercise found that changes in mRNA expression were not repeatable (i.e. individuals presented a different mRNA response to the same stimulus)[9]. Additionally, the intra-individual variability in expression was not explained by technical error, indicating that sources of variability were originated from within the muscle[8,9]. Personalised exercise prescription cannot succeed if inter- and intra-individual variability are not appropriately considered for the accurate identification of individual responses profiles. Therefore, **the aim of the first study (Chapter Three) was to accurately identify individual response at the physiological and molecular level (e.g. mitochondria), using a comprehensive study design and robust statistical approaches.**

Mitochondria are essential organelles that control the regulation of the metabolic status of skeletal muscle[11]. The mitochondria exhibit remarkable plasticity in response to exercise, and among the benefits of exercise is the consistent up-regulation of mitochondrial function and content[12–17]. As little as a single bout of exercise can initiate signalling cascades in skeletal muscle, leading to increases in mitochondrial biogenesis, along with the onset of organelle turnover conducted by the mitophagy pathway. Such turnover warrants a high functioning network of mitochondria function for optimal ATP supply[11]. One of the primary ways of accessing mitochondrial function and capacity is via mitochondrial respiration measured by maximal oxygen consumption in skeletal muscle fibres[18,19]. However, previous reports in humans have shown that such technique presents a large variability between duplicated samples from the same muscle biopsy (i.e. intra-biopsy variability)[19]. Furthermore, within-biopsy variability has also been recently reported for mRNA expression[9]. Thus, such observations have driven us to shift our focus from measuring mitochondrial function to investigate the reliability of mitochondrial respiration measurements in human *vastus lateralis* muscle. In our recent publication (See Chapter 4) we have also provided guidelines for future studies regarding sample and effect sizes to achieve reliable results using the mitochondrial respiration technique (Chapter Four).

Virtually all exercise training studies suggest that there is a high inter-individual variability in response to specific training programs. This variability is partly explained by genetics, but attempts to identify genetic markers of exercise response have largely failed, primarily due to low statistical power to accurately measure individual response to exercise as well as low-throughput genetic approaches[20]. Epigenetics (literally 'on top of genetics') is another level of gene regulation that is sensitive to environmental stimuli. It allows cells to remember their past activity and primes them to respond to future environmental stresses[21]. Epigenetic marks such as DNA methylation are sensitive to exercise, and epigenetic modifications have been proposed to influence exercise adaptations. DNA methylation responds to an acute exercise session[22], and a handful of studies reported alterations in DNA methylation patterns after short-term (e.g. 4 weeks/1 acute session) exercise training[23–28]. However, research on exercise training and epigenetics is still in its infancy. Despite the accumulating evidence that exercise induces changes in the muscle methylome, there is no evidence that these changes are related to physiological improvements or *causally* involved in the physiological adaptations to

exercise training. **Thus, the aim of Chapter Five was to evaluate whether epigenetic (DNA methylation) patterns are associated with measures of physical fitness, and whether exercise-induced DNA methylation changes correlated with exercise-induced physiological improvements.**

In addition, it is still unknown whether individual DNA methylation responses contribute to the *individual* physiological adaptations to exercise training (trainability). **Thus, Chapter Six aimed to detect individual responses to exercise training at the epigenetic level**.

Commencing with a literature review (Chapter Two), this thesis further comprises four experimental chapters:

I. Chapter Three: Deciphering the true physiological and molecular responses to exercise training using a repeated and a longer exercise intervention approach.
II. Chapter Four: Mitochondrial respiration variability and simulations in human skeletal muscle: The Gene SMART study.
III. Chapter Five: Association between DNA methylation patterns, exercise training and aerobic fitness.
IV. Chapter Six: Individual responses to exercise training at the DNA methylation level.

The main findings of this thesis are summarised with a general discussion (Chapter Seven), including limitations of each study presented, and recommendations for future research.

Chapter 2. Literature Review

This chapter is based on the following publications:

1. **Jacques M**, Hiam D, Craig J, Barrès R, Eynon N, Voisin S. (2019) Epigenetic changes in healthy human skeletal muscle following exercise- a systematic review. Epigenetics. Jul;14(7):633-648.

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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

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

Title of Paper/Journal/Book:	Epigenetic changes in healthy human skeletal muscle following exercise– a systematic review Macsue Jacques, Danielle Hiam, Jeffrey Craig, Romain Barrès, Nir Eynon & Sarah Voisin (2019) Epigenetic changes in healthy human skeletal muscle
Surname: Jacques	First name: Macsue
Institute: Institute for Health and Sport	Candidate's Contribution (%): 65
Status:	
Accepted and in press: ☐	Date:
Published: ☑	Date: 2019

2. CANDIDATE DECLARATION

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.

Signature	Date
Macsue Jacques Digitally signed by Macsue Jacques Date: 2021.04.06 16:17:09 +10'00'	06/04/2021

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following **location(s)**:

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Danielle Hiam	10%	Contributed to data interpretation & assisted withwriting and revising the manuscript	[redacted]	10/04/2021
Jeffrey Craig	5%	Contributed to data interpretation & assisted withwriting and revising the manuscript		08/04/2021
Romain Barres	5%	Editing and approval of final manuscript		08/04/2021
Sarah Voisin	10%	Editing and approval of final manuscript		12/04/2021
Nir Eynon	5%	Editing and approval of final manuscript		12/04/2021

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PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

2. Voisin S, **Jacques M**, Lucia A, Bishop DJ, Eynon N. (2018) Statistical considerations for exercise protocols aimed at measuring trainability. Exerc Sport Sci Rev. Jan;47(1):37-45.

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2. CANDIDATE DECLARATION

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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Macsue Jacques Digitally signed by Macsue Jacques Date: 2021.04.06 16:19:43 +10'00'	06/04/2021

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PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

Victoria University ABN 83776954731
CRICOS Provider No. 00124K (Melbourne),
02475D (Sydney), RTO 3113

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Sarah Voisin	45%	Draft and manuscript editing.	[redacted]	0/04/2021
Alejandro Lucia	5%	Editing and approval of final manuscript.		0/04/2021
David Bishop	5%	Editing and approval of final manuscript.		0/04/2021
Nir Eynon	5%	Editing and approval of final manuscript.		0/04/2021

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2.1 Chapter outline

The literature review opens with an introduction to the concept of trainability and a comprehensive review of statistical methods proposed to accurately identify trainability following an exercise intervention. We then provide an overview of epigenetics and epigenetic marks. Finally, we provide a comprehensive overview of the most updated literature on epigenetics and exercise.

2.2 Statistical and methodological considerations to accurately identify trainability in exercise studies

Exercise challenges whole-body homeostasis and promotes positive adaptations in the musculo-skeletal system. Skeletal muscle is a plastic tissue rapidly reacting and adapting to internal (e.g. hormones) and external (e.g. mechanical loads) stimuli. Exercise training can be broadly classified into two major categories; 1) Endurance (END), which primarily promotes endurance-specific metabolic adaptations, including increases in cardiorespiratory fitness (CRF) and mitochondrial function; and 2) Resistance (RES), which primarily promotes mechanical adaptations, including muscle remodelling and increases in muscular mass and strength. Physiological adaptations are specific to the training modality, intensity, and duration of exercise. Furthermore, responses to exercise both END and RES training varies between individuals, resulting in individuals being classified based on their degree of response to a specific training regime (i.e. "high" or "low" responders)[29].

2.2.1. Responders, non-responders, and the concept of trainability

The terms responders, non-responders, and adverse responders[10,29] to exercise training are relevant when a threshold, whether purely theoretical or empirically determined, is defined to split individuals into those categories[10]. Using the concepts of high/extreme, modest, and low-responders implies that no formal threshold has been used to classify individuals into categories, but individuals are instead grouped depending on their relative degree of response and those groups are then compared with each other[10,30].For some, "responders" are simply those who show a positive response after training (post- minus pre training (Δ) > 0), as opposed to non-responders who show no response or a negative response ($\Delta \leq 0$), regardless of its magnitude[2,31]. Others have

considered individuals to be responders if they show a response with a magnitude beyond the random error in individual measurement, but there is no uniform formula to calculate this threshold[29,32–34]. This random error in individual measurement is distinct from within-subject variability and consists of natural biological day-to-day and technical variability. Finally, some have used practically meaningful thresholds, such as the "smallest worthwhile difference"[35,36] or the "minimum clinically important difference"[37], above which individuals are considered to be responders[6,38]. Studies that are more clinical or performance-oriented require target outcomes, such as a significant improvement in a patient's survival rate or an athlete's personal best time to judge the efficacy of an intervention for a particular individual. However, such dichotomization (i.e., responder/ non-responder) is not appropriate in studies aiming to uncover modulators of the variable response to exercise training. Indeed, the transformation of the continuous spectrum of exercise responses into a dichotomous variable leads to unnecessary loss of information and statistical power[39,40].

The adaptation of a given individual to specific exercise training at any given occasion is modulated by the interaction between intrinsic factors (e.g. genetics, epigenetics, age, sex) that dictate the *potential* for improvement, and extrinsic factors (e.g. sleep, diet, stress, physical activity) that bring about the *actual* improvement. The potential for improvement is commonly referred to as *trainability*, and can be defined as the *consistent* response of a given individual to a specific intervention[5,30,40,41]. However, the presence of random variability in data collected during exercise training studies in humans hinders the identification of such components[5,6,10,35,38,42]. The key to quantifying trainability is to isolate sources of variation in exercise training responses. The extrinsic factors that largely modulate exercise responses are challenging to measure in humans, since they fluctuate constantly and vary *within subjects*. Specific study designs and methods[10,40,43] have been proposed to overcome this barrier, and each will be discussed in detail by the following section (Section 2.2.4.). To date, only one study has implemented some of those designs, looking only at VO_{2max} response to exercise intervention[10].If exercise physiologists wish to use physiological and molecular data to prescribe personalized exercise programs, well-designed exercise studies addressing all sources of variability should be implemented. Specifically, repeating the intervention on the same participants (for short interventions), or assessing the outcome multiple times during the intervention at regular intervals (for long intervention), allows measuring

within-subject variability and disentangle it from true trainability. This is both challenging and costly, but it is guaranteed to save a large amount of resources and effort as the obtained results are reliable. Given the prime importance of study design to successfully estimate trainability, we will now provide a comprehensive overview of the sources of variability that can be present in exercise studies.

2.2.2. Sources of variability

The observed variability in collected data is always a mix of true variability between individuals and random variability due to experimental and environmental factors. The key is to separate the variability of interest from the unwanted variability but obtaining a high signal-to-noise ratio can be difficult. We first describe hereinafter the different sources of variability that are typically present in exercise training studies, and then discuss some of the methods used to distinguish trainability from noise.

From previous work on the topic[5,6,10,35,38,42] we have identified six sources of variability that contribute to the observed variance in a typical exercise training dataset (**Figure 2.1**). Note that these sources of variability are used from a statistical perspective, meaning that they contribute to the variability observed not only in individual training responses but also in the whole dataset.

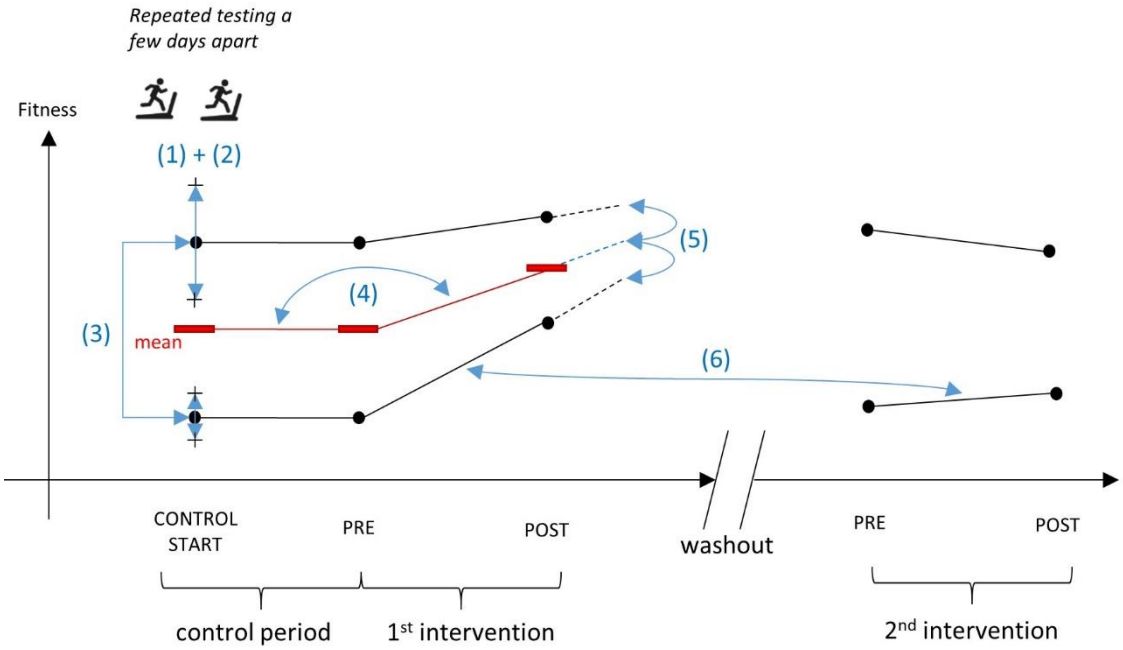

Figure 2.1. Sources of variability in exercise training studies.

In this exercise training study, we have represented the hypothetical case where individuals underwent a control period, an exercise intervention, and a second intervention after an adequate washout period. We plotted the fitness levels of two individuals (black dots), as well as their mean (black bars). At the beginning of the control

period, we also represented the scenario where their fitness levels were tested twice a few days apart (black crosses) and the black dots are the average of these repeated tests. We have represented each source of variability with blue arrows and a number. 1) Technical variability, due to machine and experimenter errors; 2) day-to-day biological variability, due to fluctuations in life components (sleep, diet, circadian rhythms) between individuals. 1) and 2) are illustrated by the difference between the black crosses; 3) variability due to different baseline values between subjects and measured with a random intercept in a linear mixed model; 4) variability due to the intervention, which is measured with a fixed effect in a linear mixed model (control vs intervention); 5) variability in response between individuals (trainability), which is measured with a random slope for each individual in a linear mixed model; 6) within-subject variability, which is the variability observed when a subject undergoes the same intervention again, and it can be estimated by comparing the slopes in the first and second interventions. The slopes can be made more accurate by doing multiple tests a few days apart for a given time point, or by doing repeated tests at regular time points during the course of the exercise intervention.

1. *Technical variability*[10,29,32,44]: This variability derives from differences in machine calibration, protocol, and experimenter. Its magnitude depends on the measured parameter(e.g., small magnitude for VO_{2max}[45] and large magnitude for mitochondrial respiration[19]); it is theoretically identical for all individuals. It can be illustrated by the question: what would have happened if the outcome had been measured on a different machine, with a different protocol, or by a different person?

2. *Biological day-to-day variability*[4,10,29,32]: This variability derives from differences in environmental factors, such as sleep quality, diet, weather, circadian time, psychological stress, or menstrual cycles between individuals, influencing the outcome. It is individual-specific. For instance, shift workers may display particularly large variability in performance during a test, as their sleep patterns often are erratic[46]. It can be illustrated by the question: what would have happened if the outcome had been measured on a different day, at a different time of the day, or after a different meal?

3. *Variability due to exercise training, regardless of the individual:* This source of variability is due to the intervention, as opposed to no intervention at all (i.e., control condition). It corresponds to the mean effect of the exercise intervention on all the individuals but does not contribute to the variability in individual training responses. It can be illustrated by the question: what would have happened to the phenotype if it had been measured after a similar period as the exercise-training program but without any intervention (i.e., control period)?

4. *Variability due to the individual, regardless of exercise training*: This source of variability is due to individuals having different mean levels. In a heterogeneous cohort (e.g., large age or fitness range), it can be a major source of variability and needs to be taken into consideration, by adding a random intercept to a statistical linear mixed model, for example. It is a source of variability that is independent from the exercise training (i.e., it is not an interaction between individuals and exercise training and does

not correspond to trainability). It can be illustrated by the question: what would have happened if the outcome had been measured on individual A instead of individual B?

5. *Variability in responses to the same exercise training between individuals*: This variability corresponds to the interaction between each individual and the training and should not be mistaken for the abovementioned variability. Even if the exercise-training program had an average effect on all individuals, each individual showed a consistently better (or worse) response than the average response. This is the variability of interest (trainability, individual training response[5,10] and its magnitude is debated[10,38], as it often is impossible to disentangle from within-subject variability. It can be illustrated by the question: what would have happened to the changes in outcome following the exercise-training program, if it was individual A instead of individual B?

6. *Within-subject variability*: This source of variability is difficult to capture; however, Hecksteden et al. have discussed possible approaches to account for it[5,10]. We currently do not know the magnitude of this variability, as it requires implementing repeated interventions or repeated tests during the intervention. It can be illustrated by the question: what would have happened to the changes in outcome in individual A if we applied the same training again?

The ideal exercise training protocol allows separating all these sources of variability, but it can be very resource- and time-consuming[5,35,42]. Although we can easily estimate the magnitude of technical and biological day-to-day variability by performing a reliability trial, we currently have little information on the relative magnitudes of trainability and within-subject variability, which means that all observed between-subject differences in response to exercise training could be just noise. Next we will review the only two studies we are aware of that provided insights into the magnitude of within-subject variability[10,47].

2.2.3. Within-subject variability is an important yet overlooked source of error

Two recent studies have elegantly provided some insights into the magnitude of within-subject variability[10,47]. In the first study, 12 young (28.5 ± 3.8 years old), moderately fit (VO_{2max} = 40.3 ± 4.3 $mL \cdot min^{-1} \cdot kg^{-1}$) men and women underwent two training periods separated by a washout period of 9 months. During the first training period, participants performed three sessions of knee extensions per week for 12 weeks

with one leg only, whereas in the second training period (after a 9-month washout period), they trained both legs[47]. This design is close to a crossover study with repeated intervention where participants are assigned successively to the control condition (the untrained leg in the first training period), and then in the intervention condition (the trained leg in the first training period) with a repetition of the intervention (the trained leg in the second training period). This approach makes it possible to quantify most sources of variability, including between-subject variability, subject-by-training interaction (trainability), and within- subject variability. This study has an advantage compared with a crossover study, because here, any lifestyle-related event that could affect muscle responses during either the "control" or the "intervention" periods would have had the same effect on both legs, thus significantly reducing random variation[4]. Although the focus of this study was on skeletal muscle memory at the transcriptional level[47], it was interesting to observe individual responses to the two repeated training periods. Using the Digitizelt software (Köln, Germany), we extracted individual values from the Supplementary Data of the study[47] and quantified within-subject variability (**Figure 2.2, Table 2.1**). As noted by the authors[47], changes in performance at a 15-min optimal performance test, in 3-hydroxyacyl-CoA dehydrogenase (β-HAD) activity and in citrate synthase (CS) activity, were surprisingly poorly correlated between the two training periods, which was reflected in our linear mixed model (**Table 2.1**). The magnitude of residual error (containing within-subject variability) was large compared with the magnitude of trainability (see 90% confidence interval in **Table 2.1**), and we did not detect significant trainability for any outcome (all p-values >0.05 in likelihood ratio test).

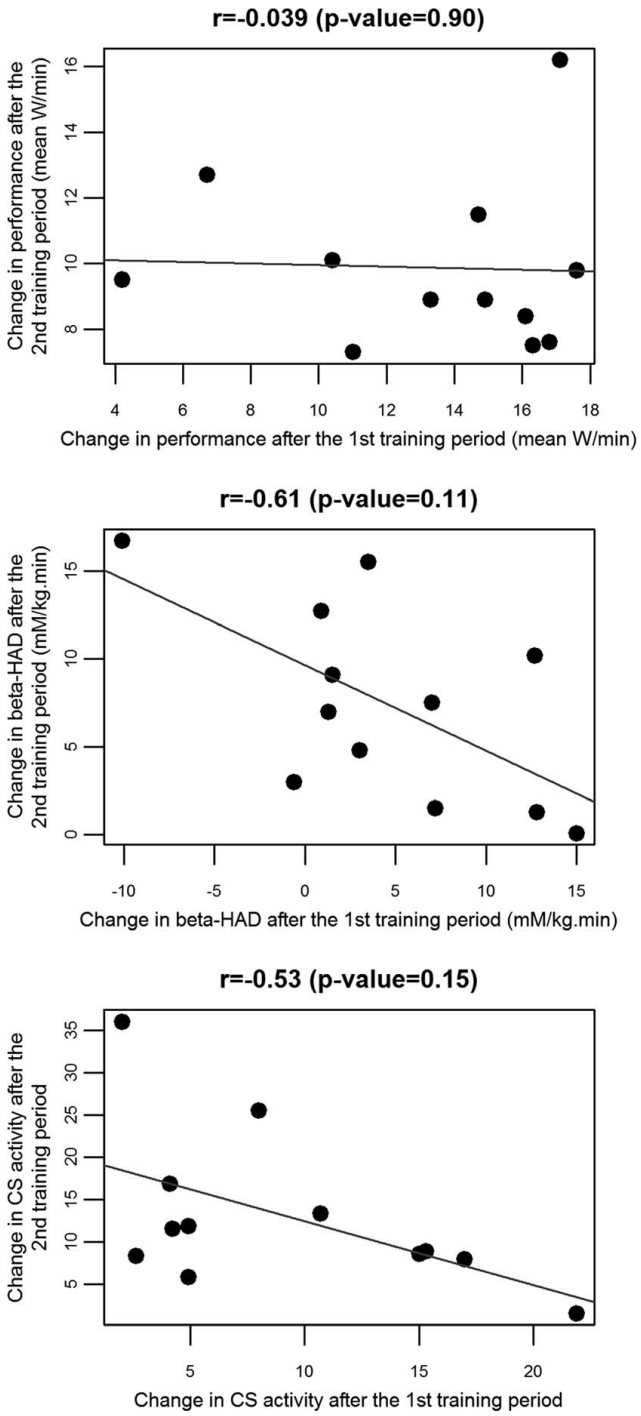

Figure 2.2. Correlation between changes in performance, citrate synthase (CS), and 3-hydroxyacyl-CoA dehydrogenase (β-HAD) in the same leg of the same individuals after the first and second training periods in Lindholm *et al.* (n= 12)[47].

	ΔPerformance (W)		Δβ-HAD activity (mM/kg/min)		ΔCS activity (AU)	
	Estimate (90% CI)†	p-value*	Estimate (90% CI)†	p-value*	Estimate (90% CI)†	p-value*
Random effect: ID x Condition (trainability)	2.33 (1.30; 5.59)	0.91	2.94×10^{-12} (1.64×10^{-12}; 7.06×10^{-12})	1	9.44×10^{-15} (5.28×10^{-15}; 2.27×10^{-14})	1
Residual error (incl. within-subject variab)	10.5 (5.87; 25.3)	NA	37.7 (21.1; 90.7)	NA	59.9 (33.5; 144.1)	NA

Table 2.1. Estimates of trainability and within-subject variability in Lindholm *et al*., 2016

Individual changes in performance, in 3-hydroxyacyl-CoA dehydrogenase (β-HAD) activity, and citrate synthase (CS) activity were extracted from the Supplementary data of Lindholm *et al*.[47]. Data were analysed in R (R Core Team, 2017) using the *lmerTest* package[48] to estimate the relative magnitudes of trainability (i.e. subject-by-training interaction) and within-subject variability (i.e. included in the residual error). We used the Δ in outcome as the dependent variable; we used condition (training/no training) as fixed effect; we used random intercepts and random slopes (subject-by-training interaction) as random effects. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality.
*P-values for significance of random effects, obtained with a likelihood ratio test as implemented in the ranova function of the *lmerTest* package.
†Estimates of the variances were given in the output of the lmer function in R, and 90% confidence intervals were obtained with the following formula where n = sample size and assuming a χ^2 distribution of sampling variance as per Hecksteden *et al*.[10]:

$$90\%\ CI: \frac{(n-1)*Var}{\chi_{0.05}} < Var < \frac{(n-1)*Var}{\chi_{0.95}}$$

However, this analysis has limitations because of the study design. Contrary to a 2x2 classic crossover design, the "control" condition was only administered once and in a specific order, making it impossible to formally test for potential carry-over effects. Although there was no evidence for a training-induced skeletal muscle global transcriptome memory in the original study[47], there could be a residual effect at the epigenetic level that was not investigated. Furthermore, there could also be cross-education from one leg to the other[49]. Finally, we could not separate random variability in individual measurements (i.e., technical, and biological day-to-day variability) from trainability, as the reliability of performance measures in this study is unknown. It should however be noted that β-HAD and CS activities have technical variability <2 units; samples were all run in duplicates, and rerun if necessary until a reliable measure was obtained, thereby reducing the possible influence of technical variability[26], but not excluding the possibility of biological variability.

In the second study, 20 men and women underwent a one-year exercise-training program that consisted of walking or jogging 3 days·week^{-1} for 45 min with a constant

heart rate prescription. Participants were tested for VO_{2max} at baseline, 3, 6, 9, and 12 months, which allowed building a progress curve to analyse each individual slope (trainability). Trainability was estimated accurately for each individual, but the SDs of the segmental changes (within-subject variabilities) were large compared with the overall progress they made[10].

The insights provided by the aforementioned studies suggest that within-subject variability is an important source of variability. We argue that most exercise studies do not yield trainability estimates that are accurate enough to warrant further investigation. Some protocols have been proposed to estimate individual responses to exercise training, but they do not all address the key issue of within-subject variability.

2.2.4. Methods to estimate trainability

The following list of methods have been proposed recently and discussed by others[5,10,35,42,43]. In this section, we discuss these methods and highlight their strengths and weaknesses. In addition, we have added a method that has not been discussed thoroughly in the past, which involves a control period before the intervention in the same participants.

1. Separate Control Group: The presence of a separate, independent control group provides an estimate of the variability because of the exercise intervention[42]. Involving a control group allows estimating the magnitude of interindividual variability in the absence of exercise training and is essential to know whether the interindividual variability in the presence of exercise training is indeed due to the training[6,35,38]. The variability in change scores in the control group is subtracted from the variability in change scores in the exercise group to obtain the true variability in change scores between individuals, according to the following formula:

$$SD_{true} = \sqrt{SD_{inter}^2 - SD_{control}^2}$$

where SD_{true} is the true interindividual variability in response to the intervention, SD_{inter} is the observed interindividual variability in change scores in the exercise group, and $SD_{control}$ is the observed interindividual variability in change scores in the control group (**Figure 2.3**). This equation is based on the assumption that 1) both SD_{inter} and $SD_{control}$ contain between-subject variability (i.e., the variation between participants given the same condition) and within-subject variability (i.e., the variation from occasion to

occasion when the same individual is given the same condition) and 2) the only extra source of variability contained in SD_{inter} is the true interindividual variability in response to the intervention.

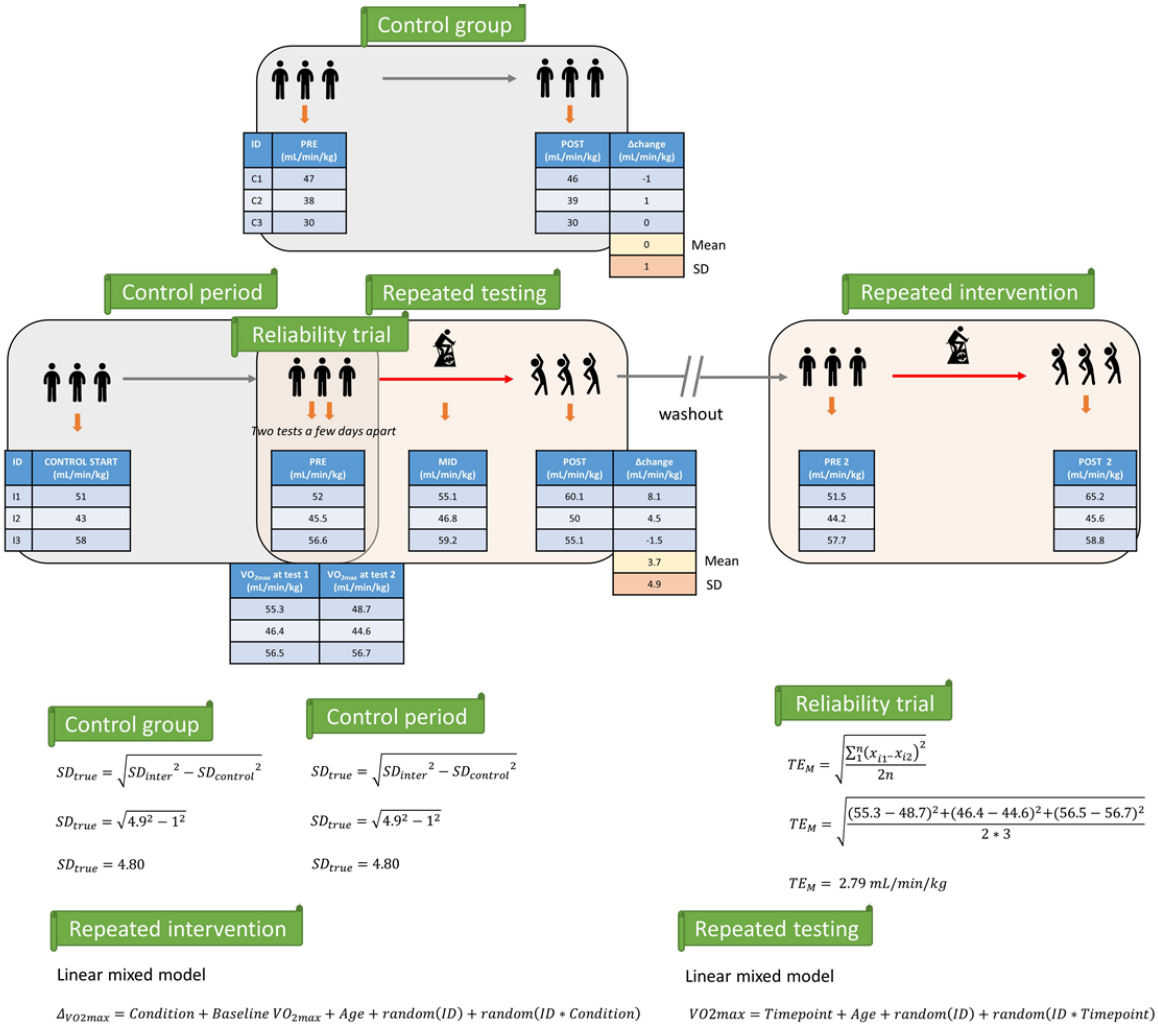

Figure 2.3. Protocols to quantify interindividual variability in response to exercise training.

Using maximum oxygen uptake (VO_{2max}) as an example phenotype, we have represented the different methods to estimate trainability, namely the control group, control period, reliability trial, repeated testing, and repeated intervention methods. We also have written down the statistical calculations associated with each method to obtain trainability estimates at the group level or the individual level.

It should, however, be noted that SD_{true} estimated with this approach may overestimate trainability because of residual within-subject variability in the exercise training group. Indeed, the response to training may vary from individual to individual (trainability), but the response to training also may vary from occasion to occasion for a given individual (within-subject variability), and this would lead to an inflation of variance within the exercise group[42]. The control group method is nonetheless useful in medium (3–6 months) to long (>6 months) interventions, where it is possible to run both

the exercise and control groups at the same time, but it significantly increases the required sample size. Moreover, the use of a control group in long exercise training studies can pose ethical issues when individuals are required to remain inactive for a long period of time[5].

2. Control Period Before the Intervention: One study design that has not been discussed thoroughly is the possibility to ask the participants to undergo a control period before starting the exercise program. This is slightly different from a crossover trial because the "treatments" (control/ exercise) are administered in a particular order (first control, then exercise). Indeed, the appropriate washout period for exercise training studies is difficult to estimate, so this would be the only way to avoid the potential carry-over effects of exercise training. This method is similar to the separate control group, but the same participants undergo a control period before commencing the exercise intervention. The "true" variability in exercise response is then calculated using the formula [40]:

$$SD_{true} = \sqrt{SD_{inter}^2 - SD_{control}^2}$$

Where SD_{inter} is the observed inter-individual variability in change scores after the exercise period and $SD_{control}$ is the observed inter-individual variability in change scores after the control period. This method removes any variability between the control group and exercise group due random sampling of individuals, but this method suffers from the same shortcomings as the control group method.

3. Reliability Trial: When neither a control group nor a control period is available, some resort to reliability trials that consist in repeating the same exercise tests a few times and a few days apart (e.g., exercise test to exhaustion), repeating the same test multiple times in a row on the same machine (e.g., mitochondrial respiration), or running biological samples in technical duplicates or triplicates (e.g., gene expression) (**Figure 2.3**). All these tests provide estimates for technical variability, and the exercise tests also include biological day-to-day variability. Although repeated tests are not needed at each time point, averaging duplicates or triplicates increases the accuracy of individual measurements. Second, even if this method cannot directly estimate SD_{true}, between-test variability can be used to calculate a threshold above which individuals may be classified as "responders." It should be noted that the accuracy of classification in the responder and

non-responder categories highly depends on the reliability of the test (i.e., a noisy test cannot detect true changes with certainty when they are small). All the calculated cut-offs are based on the so-called typical error of measurement (TE_M), calculated with the following formula [50]:

$$TE_M = \sqrt{\frac{\sum_1^n (x_{i1} - x_{i2})^2}{2n}}$$

where n is the sample size and x is the measurement of interest.

TE_M could also be called "within-subject standard deviation," as it corresponds to the square root of the sum of the squared differences of replicates divided by twice the number of pairs of replicates. Importantly, TEM includes the variability due to machine calibration and human error, so it is likely to be specific to a given laboratory; for physiological and performance tests, it also includes day-to-day biological variability, so it is likely to be specific to the studied population (e.g., young/old, trained/untrained). Therefore, we suggest that studies including a reliability trial should assess TEM instead of extracting it from the literature.

4. Repeated Intervention with a Control Period: Theoretically, the best method to separate trainability from within-subject variability is to repeat the exercise intervention on the same participants, after an adequate washout period (**Figure 2.3**). This is achieved by making the participants undergo 1) a control period, 2) an exercise period, 3) a washout period, 4) another exercise period, and fitting the following linear mixed model to the data:

$$\Delta = Condition + Covariates + random\,(ID) + random\,(ID * Condition)$$

Δ is the change score in the measure outcome, Condition is a dichotomous variable corresponding to control/exercise, Covariates is any relevant covariate that can influence Δ change (such as age), random ID is a random effect that allows each individual to have his or her own intercept, and random ID*Condition is a random effect that allows each individual to have his or her own slope (trainability). Each individual slope corresponds to the trainability estimate for each individual, separated from within-subject variability that is contained in the residual variability of the model. The residual variability also will contain technical and biological day-to-day variability, and only a reliability trial can estimate it.

Although this is the most compelling way to obtain individualized trainability estimates, it has many practical limitations. First, it is extremely time- and resource-consuming, because participants are required to remain in the study for two training periods separated by a washout for whose duration there is no guidelines. Second, the high risk of participant dropout would not allow to achieve a large sample size and the sample could end up being biased if low-responders were more likely to quit. Third, a single repetition of the exercise training may not be sufficient to obtain good estimates of trainability if within-subject variability is large, and there may be long-lasting effects of the first intervention (e.g., at the epigenetic level) that are difficult to account for. A repetition of the exercise training program is therefore recommended for short exercise interventions (<2 months), where participant attrition is kept to a reasonable amount and a short washout period is likely to be sufficient.

5. *Repeated Tests During the Exercise Training Program*: An elegant, recently proposed way to circumvent the need for a repeated intervention is to perform additional tests on subjects during the exercise-training period, provided that the training period is long enough to allow for repeated assessments (**Figure 2.3**). This permit building a slope of the progress for each individual and examining segmental changes to partition trainability from within-subject variability. The distance between individual points and the slope represents this within-subject variability, and the further the points are from the slope, the greater within-subject variability is (and the less accurate the slope is). A linear mixed model is an appropriate way to analyse these data, as follows:

$$Outcome = Timepoint + Covariates + random\,(ID) + random\,(ID * Condition)$$

This random intercept and slope model estimate the time course of outcome changes for each individual. Of note, nonlinear mixed models also are available when the change in phenotype with time is nonlinear (such as when a plateau is reached)[5], and the autocorrelation between measurements can be accounted for[51]. A brilliant twist of this protocol is that without repeating the intervention, trainability (the magnitude of individual slopes) and within-subject variability (variability between different segments of individual slopes) can be partitioned. This method is more time- and resource-efficient than any of the abovementioned methods, but it seems only appropriate for medium to long interventions (e.g., >2 months). Repeated tests are hardly feasible during short interventions where they are likely to interfere with the training itself.

Finally, random noise is prevalent in exercise training studies, but elegant protocols have been proposed recently to isolate individual responses to exercise training. The existence of the terms "responders" and "non-responders" to exercise is increasingly and rightfully being challenged, as individuals originally identified as non-responders following a specific training protocol show actual improvements if the type of training is changed[33], if the frequency of training is increased[52], or if the training intensity is increased[53,54]. The terms "responders" or "non- responders" are not fundamentally wrong, but because most studies aim to uncover the genetic, epigenetic, and molecular modulators of trainability, such dichotomous classification is not actually precise and reduces statistical power. To our knowledge, no study has performed yet a qualitative comparison of the different methods to quantify trainability. Hecksteden et al. have shown great discrepancy between different approaches to classify individuals into responders and non-responders to exercise training[10], future studies are necessary to perform simulations with known trainability and variability parameters to help clarify which protocols are best adapted to estimate trainability. Uncovering the modulators of trainability would generate relevant and progressive knowledge[55,56], but it is of paramount importance to ensure first that our protocols are accurate enough to measure trainability devoid of within-subject variability.

2.3 Epigenetics

Epigenetics has recently gained much attention as it is both sensitive to environmental stimuli and can affect how our genes are read (**Figure 2.4**). In this section we will present what epigenetics is, and how it might influence exercise training adaptations.

Epigenetics can be defined as the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states[57]. Epigenetics can alter gene expression, without changing the DNA sequence, while being remodelled by environmental factors. For example, exposure to heavy metals and pesticides, regular exercise, diet, smoking, and obesity can all remodel the epigenome in a tissue-specific manner[58–60]. One interesting feature of epigenetic modifications is that they are not confined to the initial cells that have been affected, but they can be passed on to daughter cells during mitosis and meiosis[58]. Epigenetic modifications often involve the addition of chemical groups to the DNA or to proteins, such as methylation, acetylation,

phosphorylation, ubiquitylation and sumoylation[58]. Since Waddington first coined the term "epigenetics" in 1956[61], the definition of epigenetics has considerably evolved and there are ongoing discussions regarding which marks should be considered "epigenetic" because some marks are not heritable through cell divisions[62,63], and some epigenetic modifications quickly disappear once the initial stimulus is gone[64–66]. However, it is generally accepted that epigenetic modifications fall broadly into three categories: DNA methylation, histone modification, and microRNAs (miRNAs).

An important epigenetic modification and perhaps the most studied one is **DNA methylation**[67]. *DNA methylation* is the covalent modification of a cytosine base usually located in the dinucleotide sequence 5'CpG3' (cytosine and guanine separated by a phosphate)[56]. Global DNA methylation patterns are established during embryogenesis in mammals[68], and is accurately replicated after cell divisions, and therefore it is often considered a form of cell memory[21]. The enzymes DNA methyltransferases (DNMTs) specifically DNMT3A and DNMT3B are responsible for the addition of methyl group to the cytosine base during de novo methylation. The enzyme DNMT1 is then responsible for maintaining the methyl marks during subsequent cell divisions[69]. Whether DNA methylation alters gene expression is highly dependent on the genomic location within a gene (i.e. promoter, gene body, or enhancer), and the density of CpGs. For example, increased DNA methylation at CpG-dense promoters tends to lead to a decrease in gene transcription[70]. In addition, the silencing of a gene can lead to the accumulation of DNA methylation at the promoter of said gene, further locking it into a silent state[71–76]. DNA methylation can also be removed actively (demethylation) by Ten-eleven translocation (TET) enzymes[75,77]. Further, there is a cross-talk between DNA methylation and other epigenetic processes such as histone lysine methylation and acetylation[69]. DNA methylation levels are altered in several diseases, including cancer[58] and metabolic syndrome[60].

In eukaryotes, DNA tightly coils around proteins called *histones* to form the chromatin[78–80]. Histones have (N)- and (C)- terminals tails that protrude from the centre of the nucleosome and can interact with adjacent nucleosomes and linker DNA[80]. These histone tails can undergo post-translational modifications (acetylation, phosphorylation, methylation and ubiquitylation) that alters chromatin structure and modifies the accessibility of transcription factors and machinery to the DNA[81]. When chromatin is tightly coiled in heterochromatin, gene expression is almost non-existent[82]. When

chromatin uncoils and become less tightly folded in euchromatin, portions of DNA become more accessible for transcription. Epigenetic modifications are responsible for the transformation of chromatin structure, inhibiting genes or opening frames for expression. Histone tails can also serve as a binding site for other proteins (non-histones) to chromatin[80].Active genes typically display high levels of lysine acetylation on the tails of histones H3 and H4, trimethylation of H3 lysine's 4, 36 and 79, and ubiquitylation of H2B[80]. Conversely, gene that have been silenced typically display trimethylation of H3 lysine 9 and 27, and ubiquitylation of H2A lysine 119[80].

Gene expression can also be regulated by ncRNAs[83]. The best characterized ncRNAs are microRNAs (miRNAs) that are ~22 nucleotides long and mediate post-transcriptional gene silencing[84]. miRNAs are non-protein coding molecules that act by base-pairing to the 3'-untranslated regions of the target mRNAs and repress protein synthesis[85]. Approximately 50% of protein-coding genes are regulated by miRNAs[86].

Contrary to DNA mutations, epigenetic modifications can be reverted. This was demonstrated in study by Barrès (2012) and colleagues, where L6 myotubes cells were treated with caffeine. Caffeine exposure decreased promoter methylation of *PGC1-α, TFAM, MEF2a*, CS, and *PDK4*, inhibiting gene expression on those sites. However those changes were reverted when cells were treated with H_2O_2, eliciting hypermethylation[22]. Exposure to pollutants, drugs and lifestyle components (diet, exercise, smoking) have a major impact on epigenetics, which was demonstrated in a twin studies[87,88]. Monozygotic (MZ) twins have very similar DNA methylation patterns. However, epigenetic variability amongst twins increases with age and the greatest differences are observed in twins who differed most in lifestyle[88]. Differences in epigenetic patterns between twins can be observed in several tissues, such as lymphocytes, epithelial mouth cells, intra-abdominal fat and skeletal muscle[87]. Furthermore, rates of disease discordance among MZ twins are usually over 50%[88], which suggests that epigenetics may be a significant contributor to a twin's phenotype. While epigenetic patterns are partly heritable89–91, they are also influenced by environmental factors. Thus epigenetics can be considered the crossroads between genetics (nature) and the environment (nurture)92. Epigenetics holds great promise to explain exercise-related phenomena such as the inter-individual variability to similar exercise training, and skeletal muscle memory.

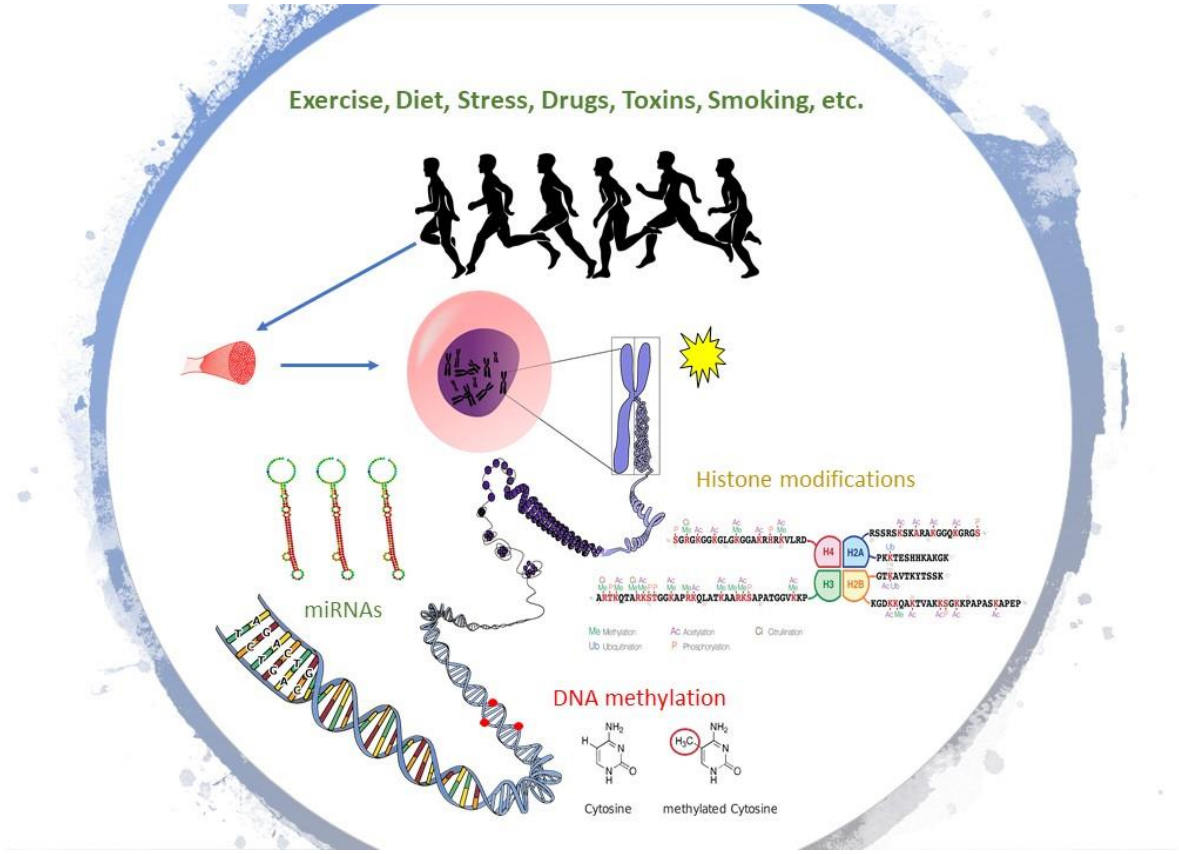

Figure 2.4. Epigenetic modifications after environmental stimuli (e.g. exercise).

2.4 Epigenetics and exercise

Repetitive muscle contractions result in increases in mitochondrial size and number, changes in substrate metabolism, enhanced angiogenesis and hypertrophy of cardiac and skeletal muscle fibres[93]. Adaptations to exercise occur in a coordinated time frame and are mediated by a plethora of transcriptional, translational, and post-translational regulators[94,95]. In the last two decades, there have been significant advances in research regarding the cellular and molecular adaptations to exercise training in muscle[1]. However, despite ongoing investigations, the molecular mechanisms responsible for these adaptations are yet to be fully understood[1,96]. Specifically it is not well understood if and how epigenetic signals can mediate physiological adaptations to exercise training[56,97]. Next, we will review what has been done so far in the field of epigenetics and exercise. For easy comprehension we have subdivided each section based on epigenetic markers.

2.4.1. Histone modifications and exercise

Only one study focused on histone modifications following exercise. This study investigated changes in H3K36 and H3K9/14 acetylation in 9 men following an acute bout of endurance exercise[98]. While H3K9/14 acetylation was not altered, H3K36 acetylation increased by 64% from baseline ($P<0.05$) immediately after exercise. As H3K36 acetylation regulates transcriptional elongation, these results suggest that exercise-induced chromatin remodelling is associated with enhanced transcription. While there was no change in global HDAC activity ($P =0.31$), two kinases that can induce nuclear export of HDAC4 and 5 (AMPK and CaMKII) showed signs of activation. This data delineates a signalling pathway that might mediate gene transcription in human skeletal muscle in response to exercise[98]. However, since this information is based on one study, further investigation is required to validate these findings and to uncover novel exercise-related histone modifications.

2.4.2. MiRNAs and exercise – candidate gene approach

MiRNAs act in a tissue-specific manner and when exclusively expressed in skeletal muscle are called myomiRs. A total of 10 miRNA studies were included and divided into candidate and high-throughput studies (**Table 2.3**). A vast majority of studies focused on candidate miRNA and expression following exercise training as it is simple and cost-effective way to analyse miRNAs. Six of the papers focused on the effect of an acute bout of exercise (endurance[99–101], resistance[102,103] and concurrent exercise[104]), two studies conducted both an acute intervention and chronic exercise training intervention[99,101] (10 days and 12 weeks of training, respectively). In addition three studies investigated the effect of chronic exercise training on miRNA expression[105–107], and one study compared powerlifting athletes to healthy controls[108]. Keller *et al.*[105] conducted 6 weeks of endurance training, while Zhang *et al.*[106] and Mueller *et al.*[107] conducted 20 weeks and 12 weeks of resistance training, respectively. Seven studies were done in men, in addition Zhang *et al.*[106] and Mueller *et al.*[107] included women in their cohorts. Each study had a small sample size (8-28 participants), with the largest combined cohort of 35 participants undergoing resistance training[106,107]. MiRNA expression changes following exercise training were dependent on the mode and length of the intervention. After acute exercise, only miR-1 and miR-133a, known modulators of muscle proliferation and differentiation[109], were consistently upregulated in candidate

miRNA studies ($p<0.05$)[99,101,103]. However after chronic exercise miR-1 and miR-133b were downregulated in a majority of studies[99,105–107]. Only two studies reported increased expression of miR-133b and miR-181 which is thought to be associated with increased glucose homeostasis[101,110], and another study found a decrease in miR-23[100] involved in myogenic processes[111]. A case-control study comparing powerlifters to healthy controls[108] reported a unique miR expression profiles that was able to distinguish powerlifters from healthy controls based on a five miR signature (miR-126, -23b, -16, -23a, -15a). While multiple miRNAs were identified to be associated with exercise, the results were heterogeneous. Discrepancies between studies could be due to variability in biopsy time, low statistical power, and differences in exercise intensity and duration. In addition, variable amount of total RNA can influence cDNA synthesis efficiency[112], and the use of different housekeeping genes (small RNA to 18s) for normalisation in studies could also generate variable findings[113].

Very few studies attempted to link changes in miRNA expression to exercise trainability. Russel *et al*.[101] reported correlations between VO_{2peak} and Peak Power Output (PPO) with changes in miRNA expression. Baseline VO_{2peak} positively correlated with miR-181 ($r= 0.70$, $P=0.03$), while baseline PPO negatively correlated with miR-23a ($r= -0.79$, $P=0.012$) and post training PPO negatively correlated with miR-31 ($r= -0.74$, $P=0.042$). Zhang *et al.*[106] reported a strong positive correlation between the change in knee strength following resistance training and the change in miR-133a, miR-133b and miR-206 expression ($p<0.01$). In summary, expression changes of candidate miRNAs were more consistent in chronic than acute studies, and appear to be dependent on the exercise modality, intensity, and duration. Changes in expression of miRNA following exercise training may underlie training adaptations, but more work is needed to confirm this.

2.4.3. MiRNAs and exercise – high-throughput analyses

With advances in technology, high-throughput miRNA expression analysis has become more readily available (**Table 2.4**) with technologies ranging from microarrays, digital multiplex to miRNA-seq, allowing hundreds of miRNAs to be analysed simultaneously. However, with such different platforms, results may yield differences in expression that might simply be due to variability between techniques. All high-throughput studies have been conducted after an acute bout of exercise. Of the 4 studies,

3 used targeted miRNA arrays[114–116] and 1 study conducted miRNA sequencing[117]. Three of the studies were based on resistance exercise (men, n=26)[115,116,118], and one following endurance exercise (men and women, n=6)[117]. The range of miRNAs that were differentially expressed across 3 studies varied from 26 to 102 after resistance exercise[115,116,118]. Interestingly, Zacharewicz *et.al.*[116] identified that 7 of the 26 miRNAs may regulate cellular growth and proliferation pathways and another 9 miRNAs may regulate the Akt-mTOR signalling pathway, a central regulator of muscle protein synthesis and muscle growth[119]. McLean *et.al.*[117] found 13 miRNAs that increased after endurance exercise ($p<0.001$), and several of those miRNAs belonged to the miR-378 family. This family of miRNAs is embedded in the first intron of *PGC-1β*[120]. Ogasawara et al. was the only study that investigated genome-wide miRNA expression changes following chronic exercise and found that the expression levels of 102 miRNAs were altered after chronic resistance exercise training ($p <0.05$)[115]. Interestingly 26 miRNAs were differentially regulated in high vs low responders for hypertrophy[115]. This further consolidates the candidate miRNA studies that specific miRNAs change following acute (and perhaps chronic) exercise, although the specific function of the miRNAs in exercise trainability remains to be elucidated.

Acute Exercise										
Candidate Gene Approach										
Author	**Year**	**Age**	**BMI**	**Sex**	**Sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
D'Souza *et al.*	2017	24.6 ± 4.9	28.1 ± 3.3	M	9	Resistance Training	1 session (acute)	Baseline, 2h and 4h	TaqMan RT-PCR (10ng of total RNA)	30 miRNAs were analysed, of which 6 were significantly altered by exercise (miRNA -24a, -133a, -146a, -206, -378b and 486) p=<0.05.
Fyfe *et al.*	2016	27 ± 4	26.5 ± 2.8	M	8	Concurrent Training	1 session (acute)	Baseline, 1h and 3h	TaqMan RT-PCR (20ng of total RNA)	4 miRNAs were analysed, of which miRNA -133a decreased in expression after RE and after HIIT+RE. In addition differences between types of training (HIIT+RE vs RE) and miRNA expression for miRNA -133a, -378 and -486 were also observed. miRNA -1 didn't show any changes.
Rivas *et al.*	2014	22 ± 1	NA	M	8	Resistance Training	1 session (acute)	Baseline and 6 hrs after exercise	miRNA PCR array from Qiagen (total RNA amount not described)	Of the 60 miRNAs analysed 17 were significantly altered by resistance exercise in young men. Only one miRNA was increased in expression both in young and old subjects after exercise (miR-423-5p)
Russell *et al.*	2013	23 ± 5		M	9	Endurance training + HIIT	1 session (acute) + 10 days of training	Acute: 0h, 3h	TaqMan RT-PCR (10ng of total RNA)	Of the 12 miRNAs analysed miR-1, -133a, 133b, -181 were significantly increased after acute endurance exercise (60%, 35%, 40% and 35% respectively). miR-9, -23a, -23b, and -31 reduced after exercise (50%, 24%, 27% and 28%). Short term training increased

										miR-29b by 210% and decreased miR-31 by 35%.
Nielsen *et al.*	2010	30.5 ± 5.5	<30	M	10	Endurance training	1 session (acute) + 12 weeks	Baseline, +1hr, +3h. Before and After intervention	TaqMan RT-PCR (10ng of total RNA)	After the acute exercise miR-1 and -133a increased in expression($p<0.05$). After chronic exercise all miRNAs showed a decrease in expression. (mir-1 (32%, $P<0.05$), mir-133a (23%, $P<0.01$), mir-133b (19%, $P<0.05$) and mir-206 (49%, $P<0.01$)). Such levels returned to baseline after 2-weeks.
Ringholm *et al.*	2011	26.2 ± 5.3	22.8 ± 2.7	M	12	Endurance	1 session (acute)	Baseline, immediately after, +3h	TaqMan RT-PCR (total RNA amount not described)	Only miR-23a had a decrease of 27% ($p<0.05$) in response to exercise. miR-1, -29b, -133a did not change in response to exercise.

EWAS										
Author	**Year**	**Age**	**BMI**	**Sex**	**Sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Russell *et al.*	2017	24.2 ± 0.9	23.3 ± 0.9	M	10	Resistance training	1 session (acute)	NA	TaqMan Array Human MicroRNA A+B Cards version Set v3.0 (total RNA amount not described) GEO:NA	26 miRNAs expression were significantly changed by age, exercise, or a combination of both.
Ogasawara *et al.*	2016	21.4 ± 1.1	22.4 ± 1	M	6	Resistance training	12-week	Baseline, +3hr, after 12weeks	Digital multiplex Nano string nCounter human miRNA expression	Analysed patterns of 800 miRNAs before and after acute exercise and 12-week exercise. Expression levels of 85 and 102 miRNAs were

									assay (10-30ng of total RNA) GEO: NA	altered after acute and chronic exercise respectively ($p<0.05$).
Zacharewicz *et al.*	2014	24.2 ± 0.9	23.3 ± 0.9	M	10	Resistance training	1 session (acute)	NA	TaqMan Array Human MicroRNA A+B Cards version Set v3.0 (350ng of total RNA) GEO: NA	26 miRNAs were regulated by age or exercise. Of those 7 were differentially regulated by age and exercise, and other 7 miRNAs were regulated by exercise in either young or older subjects.
McLean *et al.*	2015	33 ± 2	27.2 ± 0.8	9M/ 5F	6	Aerobic exercise (cycling)	1 session (acute)	Baseline, 30min post exercise	microRNA sequencing - Illumina HiSeq 2000 (total RNA amount not described) GEO: GSE66334	13 miRNAs increased in expression after exercise. Only 2 miRNAs decreased expression after exercise miRNA -144-5p and -144-3p, but such decrease was not statistically significant.
Chronic Exercise										

Candidate Gene Approach										
Author	**Year**	**Age**	**BMI**	**Sex**	**sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Russell *et al.*	2013	23 ± 5		M	9	Endurance training + HIIT	1 session (acute) + 10 days of training	Acute: 0h, 3h	TaqMan RT-PCR (10ng of total RNA)	Of the 12 miRNAs analysed miR-1, -133a, 133b, -181 were significantly increased after acute endurance exercise (60%, 35%, 40% and 35% respectively). miR-9, -23a, -23b, and -31 reduced after exercise (50%, 24%, 27% and 28%). Short term training increased miR-29b by 210% and decreased miR-31 by 35%.

Keller *et al.*	2010	29 ± 6		M	8	Endurance training	6 weeks	Before and after intervention	TaqMan RT-PCR (10ng of total RNA)	21 miRNAs were investigated. There was a trend for reduced in miRNA expression after exercise (14 miRNAs vs 7 miRNAs respectively) >1.5FC, FDR <15%.
Nielsen *et al.*	2010	30.5 ± 5.5	<30	M	10	Endurance training	1 session (acute) + 12 weeks	Baseline, +1hr, +3h. Before and After intervention	TaqMan RT-PCR (10ng of total RNA)	After the acute exercise miR-1 and -133a increased in expression($p<0.05$). After chronic exercise all miRNAs showed a decrease in expression. (mir-1 (32%, $P <0.05$), mir-133a (23%, $P <0.01$), mir-133b (19%, $P <0.05$) and mir-206 (49%, $P <0.01$)). Such levels returned to baseline after 2-weeks.
Zhang *et al.*	2015	70.5 ± 2.5		3M/ 4F	7	Resistance training	5 months	Before and after intervention	TaqMan RT-PCR (total RNA amount not described)	After RE all miRNAs tended to decrease, however the only significant one was miR-133b ($-26.5 \pm 27.5\%$; $p = 0.043$; ES = -1.46).
Mueller *et al.*	2011	80.1 ± 3.7		14 M/1 4F	28	Resistance training	12 weeks	Before and after intervention	Taqman MicroRNA assays (total RNA amount not described)	miRNA 1 expression was decreased after training.
D' Souza *et al.*	2017	25.1 ± 5.8		M	28	Powerlifters	Powerlifting athletes (resistance training) vs controls	Biopsy in athletes and controls	TaqMan RT-PCR (10ng of total RNA)	17miRs species were analysed. 12 were differently expressed ($p < 0.05$) between groups with 7 being more abundant in powerlifters and five having lower expression. The unique miR expression profiles between groups allow for categorization of individuals as either powerlifter or healthy controls based on a five

										miR signature (miR-126, -23b, -16, -23a, -15a).

EWAS										
Author	**Year**	**Age**	**BMI**	**Sex**	**sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Ogasawara *et al.*	2016	21.4 ± 1.1	22.4 ± 1	M	18	Resistance training	12-week	Baseline, +3hr, after 12weeks	Digital multiplex Nano string nCounter human miRNA expression assay (10-30ng of total RNA) GEO: NA	Analysed patterns of 800 miRNAs before and after acute exercise and 12-week exercise. Expression levels of 85 and 102 miRNAs were altered after acute and chronic exercise respectively ($p<0.05$).

Table 2.2. Summary of miRNAs study's findings.

Studies highlighted in yellow had both acute and chronic intervention applied.

ACUTE EXERCISE - Candidate Genes				
Author	**Type of exercise**	**No. of participants**	**miRNAs upregulated**	**miRNAs downregulated**
D'Souza, 2017	Resistance	9	**miR-133a**, miR-206, miR-486, miR-146a	miR-378, **miR-23a**
Fyfe, 2016	Concurrent	11	NA	**miR-133a**
Rivas, 2014	Resistance	8	miR-423-5p	miR-16-5p, miR-23b-3p, miR-24-3p, miR-26a-5p, miR-26b-5p, miR-27a-3p, miR-27b-3p, **miR-29a-3p**, miR-29c-3p, **miR-30a-5p**, **miR-30d-5p**, miR-95-3p, miR-107, miR-126-3p, miR-133b, miR-140-3p, miR-181a-5p, miR-324-3p, **miR-378a-5p**
Russell, 2013	Endurance	9	**miR-1**, **miR-133a**, miR-133b, miR-181a	miR-9, **miR-23a**, miR-23b, miR-31
Nielsen, 2010	Endurance	10	**miR-1**, **miR-133a**	NA
Ringholm, 2011	Endurance	12	NA	**miR-23a**
ACUTE EXERCISE - High-throughput				
Russell, 2017 & Zacharewicz, 2014	Resistance	10	**miR-486-3p**, miR-518b	miR-1201, miR-149, miR-520g, miR-99b
Ogasawara, 2016	Resistance	6	**miR-29a-3p**, miR-146a-5p	let-7b-5p, miR-18a-5p, miR-95, miR-99a-5p, miR-139-5p, miR-146b-3p, miR-147b, miR-188-5p, miR-192-5p, miR-219-1-3p, miR-298, miR-320a, miR-323a-3p, miR-323a-5p, miR-326, miR-330-3p, miR-338-5p, miR-362-5p, miR-369-3p, miR-369-5p, miR-378b, miR-380-3p, miR-382-5p, miR-429, miR-429a, **miR-486-3p**, miR-491-5p, miR-494, miR-506-3p, miR-520a-3p, miR-524-3p, miR-539-5p, miR-548ad, miR-548ag, miR-548ah-5p, miR-548k, miR-548x-3p, miR-550b-3p, miR-553, miR-556-3p, miR-559, miR-561-3p, miR-566, miR-577, miR-582-3p, miR-584-5p, miR-585, miR-587, miR-590-3p, miR-602, miR-627, miR-653, miR-664a-3p, miR-744-5p, miR-759, miR-802, miR-875-3p, miR-888-5p, miR-943, miR-1179, miR-1204, miR-1233, miR-1251, miR-1265, miR-1267, miR-

				1269a, miR-1275, miR-1284, miR-1285-3p, miR-1290, miR-1291, miR-1301, miR-1321, miR-1322, miR-1323, miR-1324, miR-1468, miR-2113, miR-2278, miR-3180, miR-3190-5p, miR-3934, miR-4792
McLean, 2015	Endurance	6	miR-10a-5p, **miR-30a-5p**, **miR-30d-5p**, **miR-22-3p**, miR-128, miR-378a-3p, **miR-378a-5p**, miR-378f, miR-378g, miR-378i, miR-422a, miR-532-5p	NA
CHRONIC EXERCISE - Candidate Genes				
Russell, 2013	Endurance training	9	**miR-29b**, **miR-1**, **miR-133b**	miR-31
Keller, 2010	Endurance training	8	miR-125a, miR-183, miR-189, miR-432, miR-575, miR-616, **miR-637**	miR-101, miR-133, miR-144, miR-15b, miR-26b, miR-28, **miR-29b**, miR-338, miR-455, miR-92, miR-98, miR-451, miR-589, **miR-1**
Nielsen, 2010	Endurance training	10	NA	**miR-1**, **miR-133a**, **miR-133b**, **miR-206**
Zhang, 2015	Resistance training	7	NA	**miR-133b**
Mueller, 2011	Resistance training	28	NA	**miR-1**
D'Souza, 2017	Powerlifting	28	**miR-206**, miR-15a, miR-16, miR-451a, miR-23a, miR-23b, miR-30b	miR-486, miR-499a, **miR-133a**, **miR-1**, miR-126
CHRONIC EXERCISE - High-throughput				
Ogasawara, 2016	Resistance	6	miR-19b-3p, miR-21-5p, miR-126-3p, miR-136-5p, miR-376a-3p, miR-663b	miR-7-5p, let-7b-5p, let-7c, **miR-22-3p**, miR-30d-5p, miR-99a-5p, miR-141-3p, miR-146b-3p, miR-147b, miR-192-5p, miR-198, miR-222-3p, miR-297, miR-323a-5p, miR-325, miR-338-5p, miR-361-5p, miR-362-5p, miR-369-3p, miR-369-5p, miR-371a-5p, miR-378d, miR-380-3p, miR-422a, miR-424-5p, miR-429, miR-449a, miR-450b-3p, miR-483-5p, miR-485-5p, miR-496, miR-499a-3p, miR-502-3p, miR-502-5p, miR-512-5p, miR-526b-5p, miR-548ag, miR-548an, miR-548x-3p, miR-549, miR-550a-5p, miR-551b-3p, miR-552, miR-555, miR-559, miR-574-3p, miR-577, miR-581, miR-582-3p, miR-583, miR-584-5p, miR-596, miR-603, miR-627, miR-628-3p, **miR-637**, miR-640, miR-653, miR-

				664-3p, miR-744-5p, miR-802, miR-875-5p, miR-877-5p, miR-888-5p, miR-891a, miR-920, miR-937, miR-1185-5p, miR-1202, miR-1206, miR-1233, miR-1247-5p, miR-1251, miR-1267, miR-1269a, miR-1272, miR-1273f, miR-1275, miR-1283, miR-1284, miR-1285-3p, miR-1290, miR-1291, miR-1301, miR-1827, miR-1908, miR-1915-3p, miR-2053, miR-3123, miR-3151, miR-3168, miR-3182, miR-3190-5p, miR-3934, miR-4458, miR-4532

Table 2.3. Summary of miRNAs that were up- or down-regulated after exercise.

miRNAs highlighted in red presented discordant results between studies, while those highlighted in blue presented concordant results.

2.4.4. DNA methylation and exercise – candidate gene approach

Early studies on DNA methylation, and exercise investigated candidate genes involved in exercise adaptations (**Table 2.2**). Most candidate studies focused on peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC-1a*), the master regulator of mitochondrial biogenesis and fat metabolism[95]. Alibegovic et al. investigated the effect of forced bed rest (10 days) on DNA methylation levels at three CpG sites located in the promoter of *PGC-1a,* in the *vastus lateralis* muscle of 20 participants[121]. DNA methylation at two of those sites negatively correlated with *PGC-1a* mRNA expression at baseline (site 816: r =-0.65, P=0.03; site 783: r=-0.59, P=0.04), and methylation at site 816 increased by ~7% following bed rest (P=0.04). After four weeks of aerobic retraining, DNA methylation levels decreased, but did not return to their baseline levels[121]. Barres et al. investigated DNA methylation in *vastus lateralis* biopsies after an acute bout of exercise and identified the *PGC-1a* promoter as differentially methylated after an exercise intervention. In addition, the promoter regions of key genes involved in exercise response (i.e. *PGC-1a*, *TFAM*, *MEF2A* and *PDK4*) were hypomethylated by ~10% immediately after a strenuous bout of cycling and became remethylated 3 hours after exercise (n=14, $p < 0.05$). It should be noted that some genes showed a delayed hypomethylation (i.e. *PPAR-d*, 3h after exercise), and these changes were exercise intensity dependent. Hypomethylation of the promoters was accompanied by increases in mRNA levels either immediately after or 3h after exercise[22]. Bajpeyi et al. divided 11 healthy young men into high- and low-responders based on their DNA methylation response at an important regulatory region in *PGC-1a* following an single

bout of exercise[122]. Only high-responders, who had decreased DNA methylation after exercise, showed nucleosome repositioning in the promoter of *PGC-1a* along with an associated increase in *PGC-1a* mRNA expression (1.05 ± 0.08 to 1.29 ± 0.11-fold-change). The dichotomising of only 11 subjects included in this study was arbitrary which reduced statistical power and leads to the question whether the conclusions would have been similar using a continuous spectrum of responses. Nonetheless it can be concluded that these candidate-gene studies demonstrated that exercise alters DNA methylation levels at genes involved in muscle metabolism and are associated with a concomitant change in mRNA expression.

2.4.5. DNA methylation and exercise – genome wide approach

Four studies conducted Epigenome-Wide Association Studies (EWAS) investigating genome-wide DNA methylation changes following an exercise training intervention in healthy populations[25–27,123] (**Table 2.2**). The studies consisted of a 6-month endurance training intervention in 28 middle-aged men with and without family history of Type 2 Diabetes (T2D)[25], a 3-month unilateral endurance training in 17 young men and women [26]; a 7-week resistance training intervention in 8 young men[123]; 3 months of endurance, resistance or combined training in 34 young and 26 old men and women[27]. Results from these studies were highly heterogeneous, due to differences in exercise mode (resistance[27,123], endurance[25–27] or both[27]), length of intervention (from 7 weeks to 6 months), genome coverage (gene promoters only[25,27] or all genomic regions[47,123]), density coverage (2-4% of all CpGs with Illumina 450k or 850k arrays[25–27] or 8% of all CpGs with MeDIP-chip[25]), sample size, age and sex.

Two studies reported more hypomethylation than hypermethylation at the Differentially Methylated Positions (DMPs)[25,123] following an exercise training intervention, one study reported a similar number of hypo- and hyper-methylated DMPs[26], and the last study did not find any DMP[27] after 3 months of exercise. Interestingly, a moderate effect size was consistent across the studies (< 10% methylation change after the intervention[25–27,123]), suggesting that exercise training may alter the DNA methylation state of multiple genes, in an exercise dose-dependent manner. While the biological relevance of such small changes in methylation is questionable, a direct correlation between DNA methylation levels and the resulting expression level of mRNA of selected genes was demonstrated by gene reporter assay[25]. Three of the studies

identified a consistent inverse relationship between DNA methylation and gene expression changes[25,26,123]. Robinson *et al.*, who used an absolute cut-off of 5% methylation change following exercise found no DMPs however only focused on promoter regions[27]. Lindholm et al. investigated the distribution of these DMPs and reported an enrichment of DMPs in enhancers and regulatory regions which may give an explanation to why no DMPs were observed in the Robinson et al. study[26]. It is worth noting that the magnitude of DNA methylation changes following training was smaller than after acute exercise[22], indicating that DNA methylation changes in response to exercise is a dynamic process activated in the early phase of gene expression. Yet residual DNA methylation changes are retained after the training stimulus is gone, indicating that these changes are accumulated over multiple exercise sessions.

Acute Exercise										
Candidate Gene Approach										
Author	**Year**	**Age**	**BMI**	**Sex**	**Sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Bajpeyi *et al.*	2017	24.6 ± 1 years	23.2 ± 0.5	M	11	Cycling until 650 Kcal	1 session (acute)	Before and immediately after	Pyrosequencing	Repositioning of -1N within the PGC1a promoter, which in turn increased fold change of PGC1a gene expression after acute exercise (1.05 ± 0.08 to 1.29 ± 0.11-fold change).
Barrès *et al.*	2012	NA	NA	M/F	14	Endurance exercise	1 session (acute)	Before, immediately after and +3h	Pyrosequencing	HIIT reduced DNA methylation of PGC-1a, TFAM, MEF2A, and PDK4 immediately after exercise, whereas PPAR-d methylation was decreased 3hr after exercise. Decreases in DNA methylation were associated with increase in gene expression at the same or following time point.
EWAS										
Author	**Year**	**Age**	**BMI**	**Sex**	**Sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Seaborne *et al.*	2018	27.6 ± 2.4	<30	M	8	Resistance exercise	1 session (acute) + 7-week	Before, immediately after and post 7-week	Illumina EPIC array GEO: GSE114763	17,365 CpG sites were significantly ($P < 0.05$) differentially epigenetically modified following 7-week resistance training compared to baseline, with a larger number being hypomethylated (9,153) compared to

										hypermethylated (8,212). Following a period of 7-week without training, another 7-week of resistance exercise was conducted, and it was observed an increase in the number of epigenetically modified sites (27,155) and an enhanced number of hypomethylated DNA sites (18,816). Hypermethylation remained stable during second 7-week of training (8,339) versus untraining period (8,638) and initial 7-week (8,212).
Chronic Exercise										
Candidate Gene Approach										
Author	**Year**	**Age**	**BMI**	**Sex**	**Sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Alibegovic *et al.*	2010	25 ± 1	24.1 ± 2.3	M	20	Endurance training	4 weeks	Before and After intervention	Sequenced with the ABI 3130xl genetic analyser.	General increased DNA methylation of PPARGC1A gene after bed rest (only one site of 3 being significant -816 p=0.04), tending to reversibility after retraining but not achieving same levels as before.

EWAS										
Author	**Year**	**Age**	**BMI**	**Sex**	**Sample size**	**Type of intervention**	**Length of intervention**	**Biopsy time**	**Technique**	**Results**
Lindholm *et al.*	2014	27 ± 0.8	24 ± 0.8	12M/ 11F	23	One-legged knee extension	3 months	Rest, before and after training	Illumina Infinium HumanMethylation450 array GEO: GSE60655	Endurance training induced significant changes in 4919 sites across the genome of the trained leg. The corresponding transcriptional analyses resulted in 4076 differentially expressed genes.
Nitert *et al.*	2012	37.5 ± 5.2	27.85 ± 3.0	M	28	Endurance exercise	6 months	Before and after intervention	MeDIP-Chip GEO: Not Available	134 individual genes changed amount of methylation after exercise (115 hypomethylation and 19 hypermethylation). See supplementary data - has complete data set.
Robinson *et al.*	2017	23-26	24.6-25.6	M/F	34	HIIT	3 months	Before and After intervention	Infinium 450K array (Illumina). GEO: GSE97084	Relatively small changes (<10%) in DNA methylation in comparison to a more substantial increase in gene expression following exercise training.
Seaborne et al.	2018	27.6 ± 2.4	<30	M	8	Resistance exercise	1 session (acute) + 7-week	Before, immediately after and post 7-week	Illumina EPIC array GEO: GSE114763	17,365 CpG sites were significantly ($P < 0.05$) differentially epigenetically modified following 7-week resistance training compared to baseline, with a larger number being hypomethylated (9,153) compared to hypermethylated (8,212). Following a period of 7-week without training, another 7-

										week of resistance exercise was conducted, and it was observed an increase in the number of epigenetically modified sites (27,155) and an enhanced number of hypomethylated DNA sites (18,816). Hypermethylation remained stable during second 7-week of training (8,339) versus untraining period (8,638) and initial 7-week (8,212).

Table 2.4 Summary of DNA methylation findings.

Studies highlighted in yellow had both acute and chronic intervention applied.

Differentially methylated genes were enriched for pathways such as retinol metabolism and calcium signalling[25], structural remodelling of the muscle, inflammatory/immunological processes and transcriptional regulation[26]. Three of the EWAS studies found DMPs enriched for pathways linked to glucose and/or insulin metabolism[25–27,123]. However, whether DNA methylation changes result in downstream changes in phenotype has not been investigated in depth. In fact only one study found that a higher number of hypomethylated sites was associated with hypertrophy in the muscle following a repeated intervention of 7 weeks[123]. This indicates DNA methylation changes might correlate with exercise trainability.

2.5 Summary and study aims

To summarize, individual responses to exercise training should be properly assessed using robust study designs and adequate statistical methods, all of which involve repeated measurements on individuals to account for within-subject variability. These study designs can accurately measure individual responses to exercise. Understanding exercise-induced physiological (e.g. $VO2_{max}$) and molecular markers (e.g. mitochondrial function) at the individual level is tedious and requires optimal study designs as well as large sample sizes.

The epigenome is highly sensitive to environmental stimuli such as exercise, and the best characterised epigenetic mark is DNA methylation. Exercise epigenetics is a new and fascinating research field, and, to date, most studies have been limited to *a single bout of exercise*, have mostly focused on *candidate genes*, and are all plagued by *small sample size*. This thesis aims to draw a much more comprehensive picture of the DNA methylation sites, genes, and pathways associated with exercise training.

Therefore, the overarching aim of this thesis is two-fold: 1) to accurately estimate trainability using two distinct study designs (repeated intervention, and repeated testing during the intervention); and 2) to uncover the DNA methylation marks in skeletal muscle associated with responses to exercise training. For easy comprehension this work has been subdivided into research studies presented here as chapters.

2.5.1. Chapter Three

Currently most exercise studies have failed to overcome the difficulties in isolating sources of variability and accurately identifying trainability. Only few studies

have applied some of the methods described previously and have used too few measures of fitness. To overcome these barriers, we modified and extended the original protocol of the Gene SMART (Skeletal Muscle Response to Training) study to test the five above-mentioned methods to comprehensively and accurately estimate trainability. By implementing all five in a single study, we provide a comprehensive comparison of these methods to estimate trainability and direct future studies to better study protocols. Furthermore, we have extended the protocol that was most efficient in our case to measure trainability at the physiological level to build a comprehensive time-course of mitochondrial and fibre type changes following 12 weeks of HIIT. In addition, we investigated to what extent physiological markers of fitness correlate with skeletal muscle mitochondrial ones within the same individuals as they progress through the 12-week HIIT intervention.

2.5.2. Chapter Four

Mitochondria function is highly modulated by exercise, and initially we aimed on using respiration protocols to measure such changes. However, high intra-biopsy variability was observed with this protocol, which left us wondering how reliable such measurements were. Therefore, the aim of the present study was to investigate the reliability of mitochondrial respiration measurements in human *vastus lateralis* muscle using a large number of duplicate fibre bundles (160 pairs) collected from a range of participants at different time points.

2.5.3. Chapter Five

Exercise can modify DNA methylation profile in individuals; however, we are unaware of the link between DNA methylation and fitness measures. Thus, the aim of this chapter was to investigate this relationship and what DNA methylation changes would be associated with 4 weeks of HIIT. Finally, we intersected baseline fitness measures with changes in DNA methylation following 4 weeks of exercise intervention to test if it would change towards the profile of more fit individuals.

2.5.4. Chapter Six

Following investigation of DNA methylation at a group scale, we also investigated if DNA methylation consistently changed at the individual level after a repeated and longer intervention.

Chapter 3. Deciphering the true physiological and molecular responses to exercise training using a repeated and longer intervention

This paper is based on the following publications that are being prepared:

1. **Jacques M**, Voisin S, Xu Yan, Eynon N. Implementation of multiple statistical and exercise training methods to measure trainability. Target Journal: MSSE

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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

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

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Surname: Jacques First name: Macsue

Institute: Institute for Health and Sport Candidate's Contribution (%): 70

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2. CANDIDATE DECLARATION

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.

Macsue Jacques — Digitally signed by Macsue Jacques Date: 2021.04.06 16:26:55 +10'00' — 2021

Signature **Date**

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

The undersigned certify that:

1. They meet criteria for authorship in that they have participated in the conception, execution or interpretation of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

Victoria University ABN 83776954731
CRICOS Provider No. 00124K (Melbourne),
02475D (Sydney), RTO 3113

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3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following **location(s)**:

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Shanie Landen	5%	Data collection and revised manuscript	[redacted]	
Javier Alvarez Romeiro	5%	Data collection and revised manuscript		
Xu Yan	5%	Data collection and revised manuscript		
Danielle Hiam	5%	Data collection and revised manuscript		
Nir Eynon	5%	Manuscript Editing and final approval		
Sarah Voisin	5%	Guided student through statistical analyses and revised manuscript		

Updated: September 2019

PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

Victoria University ABN 83776954731
CRICOS Provider No. 00124K (Melbourne),
02475D (Sydney), RTO 3113

1. **Jacques M**, Landen S, Romero JA, Yan X, Garnham A, Hiam D, Siegwald M, Mercier M, Voisin S, Eynon N. (2020) Individual physiological and

mitochondrial responses during 12 weeks of intensified exercise. (Accepted) – Physiological Reports.

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

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Surname: Jacques	First name: Macsue
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Status:	
Accepted and in press: ✔	Date: 2021
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2. CANDIDATE DECLARATION

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.

Macsue Jacques (Digitally signed by Macsue Jacques Date: 2021.04.06 16:21:58 +10'00')	2021
Signature	**Date**

3. CO-AUTHOR(S) DECLARATION

In the case of the above publication, the following authors contributed to the work as follows:

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PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following **location(s)**:

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Shanie Landen	2%	Data collection, lab analyses and revised manuscript	[redacted]	
Javier Alvarez Romeiro	2%	Data collection		
Xu Yan Andrew Garnham	2% 2%	Data collection and analyses Performed Muscle biopsies		
Danielle Hiam Melina Siegwald	2% 2%	Data collection and revising manuscript Data collection and analyses		
Emma Mercier Anne Hecksteden	2% 5%	Data collection and analyses Manuscript editing and final approval		
Sarah Voisin Nir Eynon	10% 6%	Guided student to successful completion of analysis and reviewe manuscript. Editing and final approval of manuscript		

PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

3.1 Introduction

Exercise training leads to physiological adaptations, such as increased maximal oxygen uptake (VO_{2max}) as well as molecular adaptations, such as mitochondrial biogenesis[93]. The magnitude of these adaptations depends on the duration, intensity, volume, and type of exercise training[1]. Although the benefits of exercise are well described, large inter-individual variability in the response to apparently similar exercise training exists[2–6] for all exercise-related phenotypes[4], independently of the intervention duration[6]. Individual response (also known as subject-by-training interaction) relies on the assumption that consistent training changes occur for each individual[5,30,40,41]. However, we and others, have shown that measuring individual response for any given variable is more complex than previously assumed, and exercise studies often fail to robustly measure it[5,6,10,38,40,43,124].

The key to quantifying individual responses to exercise is to isolate sources of variation by first quantifying the magnitude of variation in training response, given that if subject-by-training interaction is low then assessing response of individuals is futile; and only then quantifying individual responses. In exercise studies, two stances are commonly observed as sources of variation: 1) day-to-day or biological variability (i.e. sleep, nutrition, etc), and 2) statistical variance such as random error. In order to isolate such sources of variation and obtain true effects of exercise training, specific study designs and methods[10,40,43] have been proposed. For instance, measuring gradual adaptations at consecutive timepoints during the intervention, to obtain individual progress curves, or by utilizing a cross-over repeated intervention. With the reference standard being a replicated cross-over design, and repeated testing measuring gradual adaptations at consecutive timepoints being a relative substitute. To date, two studies to our knowledge has implemented the repeated testing design solely for VO_{2max} measurement[10,125], and no molecular markers have been investigated thus far. Furthermore, physical exercise leads to cellular metabolic stress; however, it remains unclear whether improvements in physiological phenotypes (i.e. VO_{2max}, lactate threshold (LT)) from exercise interventions mirror improvements in molecular phenotypes in skeletal muscle (i.e. mitochondrial markers).

Among the many molecular changes that are led by exercise (i.e. fibre type switch, glucose uptake, etc.), the mitochondria is known to be heavily regulated by exercise

training [95,126–128]. The mitochondrion is responsible for energy production to the cells, and mitochondrial deficiency can lead to both physical and psychological disorders [129–132]. Exercise studies often rely on isolated mitochondrial markers measures; however, one human cell contains multiple copies of mitochondria and consequently mitochondrial DNA (mtDNA). MtDNA encodes critical components of the respiratory complexes and is necessary for ATP production. An increase in mtDNA copy number (mtCN) does not necessarily equate with an increase in mitochondrial capacity and could simply be a consequence of compensatory mechanisms (i.e. reduction in mitochondrial quality and elevated mitochondrial content)[133,134]. Thus, mitochondrial markers measured in isolation do not provide the full picture of mitochondrial health[135]. Combining measures of mitochondrial content and quality is essential to access mitochondrial health. A functional index of mitochondrial health in blood has been recently proposed (Mitochondrial Health Index), by mathematically integrating biochemical enzymatic activities and mtCN into a single score, that may represent an optimized measure of mitochondrial functional capacity[135]. This method successfully captured a reduction in mitochondrial health in blood as a result of chronic psychological stress[135]. However, this approach has not been explored in skeletal muscle, either in the basal state, or following a chronic physiological stimulus, such as exercise training. Furthermore, variability across mitochondrial measures is not well described, and no study to date has estimated subject-by-training interaction by the mitochondria.

To overcome these barriers, and comprehensively and accurately estimate individual response, we modified and extended the original protocol of the Gene SMART (Skeletal Muscle Response to Training) study[136] to test the five methods described in our literature review, namely; separate control group, applying control period before the intervention, reliability trial, repeated intervention and repeated tests during exercise training program. By implementing these five methods in a single study, we provide a comprehensive comparison of these methods to estimate trainability to direct future studies to better study protocols. In addition, we report a comprehensive time-course of mitochondrial and physiological changes during 12 weeks of HIIT in 16 healthy young men. We hypothesised that measuring multiple physiological and molecular components at regular intervals would allow to account for sources of variability and identification of true individual responses to exercise. Furthermore, for the first time, we investigated to what extent whole body physiological markers correlate with skeletal muscle

mitochondrial markers within the same individuals as they progress through the 12-week HIIT intervention.

3.2 Methods

3.2.1. Participants

This study had a variable number of participants as not all participants completed all components of the full intervention. The number of participants used by each test is highlighted in the methods/results (**Figure 3.1**). In brief, the study was subdivided into two parts, the Gene SMART study and second repeated intervention in the same participants. The Gene SMART participants recruitment and selection criteria has been previously described in detail [136]. 95 participants commenced the Gene SMART study, and 78 of them completed the entire intervention period (Testing at baseline, 4 weeks of HIIT and testing after 4 weeks). Following at least one-year of washout period, participants who had previously completed the Gene SMART study were contacted and invited to participate in a repeated and longer intervention (12 weeks of HIIT). All participants who completed the first intervention were contacted to avoid selection bias, and 20 of them agreed to take part in the repeated intervention. Participants were apparently healthy, moderately trained men, aged 18 to 45 years (**Table 3.1**). The study was approved by the Victoria University Human Ethics Committee (HRE13-223) and written informed consent was obtained from each participant. Participants were excluded from the study if they had a past history of definite or possible coronary heart disease, significant chronic or recurrent respiratory condition, significant neuromuscular, major musculoskeletal problems interfering with ability to cycle, uncontrolled endocrine and metabolic disorders or diabetes requiring insulin and other therapies[136].

3.2.2. Study design

Participants from the Gene SMART study[136] (T_{10}) completed a control period of 4 weeks (T_{11}), followed by 4 weeks of HIIT (T_{12}). After a washout period of 12 months (T_{21}), participants that agreed to return for a follow up intervention, repeated the 4-week HIIT intervention (T_{22}), followed by another 4 weeks (T_{23}), and another 4 weeks (T_{24}), totalling 12 weeks of HIIT. Two graded Exercise Tests (GXTs) were conducted at each time point to determine peak power output (W_{peak}), the lactate threshold (LT) and maximal oxygen consumption (VO_{2max}) **(Figure 3.1**).

Graded exercise test to exhaustion (GXT)

Participants underwent GXTs, for baseline determination of the LT and W_{peak} and VO_{2max}. This test was performed on an electronically-braked cycle-ergometer (Lode-excalibur sport, Groningen, the Netherlands) and was consisted of 4-min stages separated by 30-s rest periods until exhaustion. The test started at 60W and was increased by 30W in each subsequent stage. Capillary blood samples were taken at rest, and after each completed stage, and immediately following exhaustion, and was analysed by a YSI 2300 STAT Plus system (Yellow Springs, Ohio, USA). During the GXT, the 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. The average of the two GXT tests were used to individualise exercise intensities, if the difference were more than 5%, otherwise the highest value was used.

After 5 min of rest following the GXT, VO_{2max} was measured using a calibrated Quark CPET metabolic system (COSMED, Rome, Italy). Briefly, participants wore the Cosmed face mask and VO_2 values were collected at stationary for 2 min, while exercising for 3 min at the intensity of the first stage of GXT (60W), and during exercise to exhaustion at 105% of W_{peak} measured during the previous GXT. VO_{2max} was considered the highest value in 1 min obtained during the test. The HIIT phase commenced 48–72 h after the last baseline exercise test.

Control period (4 weeks)

Prior to commencing the study, participants were familiarised with a graded exercise test (GXT). Participants were instructed to wear an activity monitor for two weeks to estimate incidental physical activity (IPA) during their normal daily life. The control period was key to quantifying inter-individual variation in fitness occurring regardless of the intervention[6].

HIIT – first intervention (4 weeks)

Participants trained 3 times/week under supervision. 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 training intensity initiated at 40% of LT threshold for the first week (1st training 8 intervals, 2nd training 9 intervals and 3rd training 10 intervals). Onthe second week the intensity increased to 50% of LT threshold (4th training 10 intervals, 5th training 12 intervals, 6th interval 11 intervals). On thethe third week the intensity increased to 60% of LT threshold (7th training 11 intervals, 8th training 12 intervals, 9th training 14 intervals), and on the fourth week the intensity increased to 70% of LT threshold (10th training 11 intervals, 11th training 9 intervals, 12th training 6 intervals).

	Control			First Intervention (4 weeks)			Second Intervention (12 weeks)				
	Start	End	Δ	Pre	4WP	Δ	Pre	4WP	8WP	12WP	Δ
N	30	29	-	95	78	-	20	19	18	17	-
Age (years)	33.20±8.33	-	-	31.56±8.24	-	-	33.07±8.96	-	-	-	-
BMI (kg.m-2)	26.18±3.98	26.20±3.98	-	25.43±3.29	25.29±3.39	-	26.40±4.23		-	26.42±3.78	-
W_{peak} (W/kg)	3.44±0.90	3.45±0.91	0.00±0.16	3.64±0.76	3.88±0.77	0.23±0.21	3.48±0.97	3.76±0.96	3.88±0.95	4.06±0.94	0.56±0.28
LT (W/kg)	2.39±0.69	2.34±0.68	0.00±0.28	2.54±0.63	2.74±0.67	0.20±0.19	2.38±0.74	2.63±0.79	2.71±0.76	2.76±0.70	0.37±0.35
VO_{2max} (mL.min-1.kg-1)	48.53±10.57	49.60±10.27	-0.80±9.12	47.19±7.97	48.65±8.09	1.50±3.84	51.0±10.6	53.1±10.3	54.5±11.1	55.3±10.7	4.43±6.39

Table 3.1. Group characteristics for each period (Control, 1st intervention and 2nd intervention) with Delta Changes.

Values are presented as mean ± SD. Δ = 12WP-Pre measurements.

HIIT – second intervention (4 weeks + 8 weeks of additional training)

After a washout of at least 1 year, participants repeated the HIIT described above, and continued training for a total of 12 weeks. To ensure progression, training intensity was re-adjusted every 4 weeks based on the newly determined W_{peak} and LT from the GXTs. Training length and intervals were repeated in each week of the intervention, as described above, and only resistance was changed to reflect the participants' progression.

We used the duplicate measurements from the two GXTs conducted at each time point to calculate the typical error of measurement (TE_M) that encompasses both technical variability due to machine and experimenter error, and day-to-day biological variability in test performance[40,50]: $TE_M = \sqrt{\frac{\sum_1^n (x_{i1} - x_{i2})^2}{2n}}$, where n is the number of pairs of duplicates and x is the specific measurement (i.e. W_{peak}, LT or VO_{2max}). The value of a given individual at a given time point was calculated as follows: if the values from two GXTs differed by > TE_M, the maximum value was taken; otherwise, the average of the two values was taken.

From the 20 returning participants, **19 completed 4 weeks of HIIT** (1 dropout)**, of these 18 completed 8 weeks** (1 dropout)**, and 16 completed the full 12-weeks of HIIT** (1 dropout and 1 exclusion due to inconsistent results, i.e., duplicate tests provided more than 10% difference) (see Figure 3.1B).

3.2.3. Muscle biopsies

Muscle biopsies were collected at timepoints T_{21}-T_{24} for comprehensive analyses of mitochondrial markers, including citrate synthase, succinate dehydrogenase, mitochondrial copy number, cytochrome oxidase and fibre type composition (**Figure 3.1**). Due to dropouts previously described, all statistical analyses for mitochondrial measures were based on 16 participants.

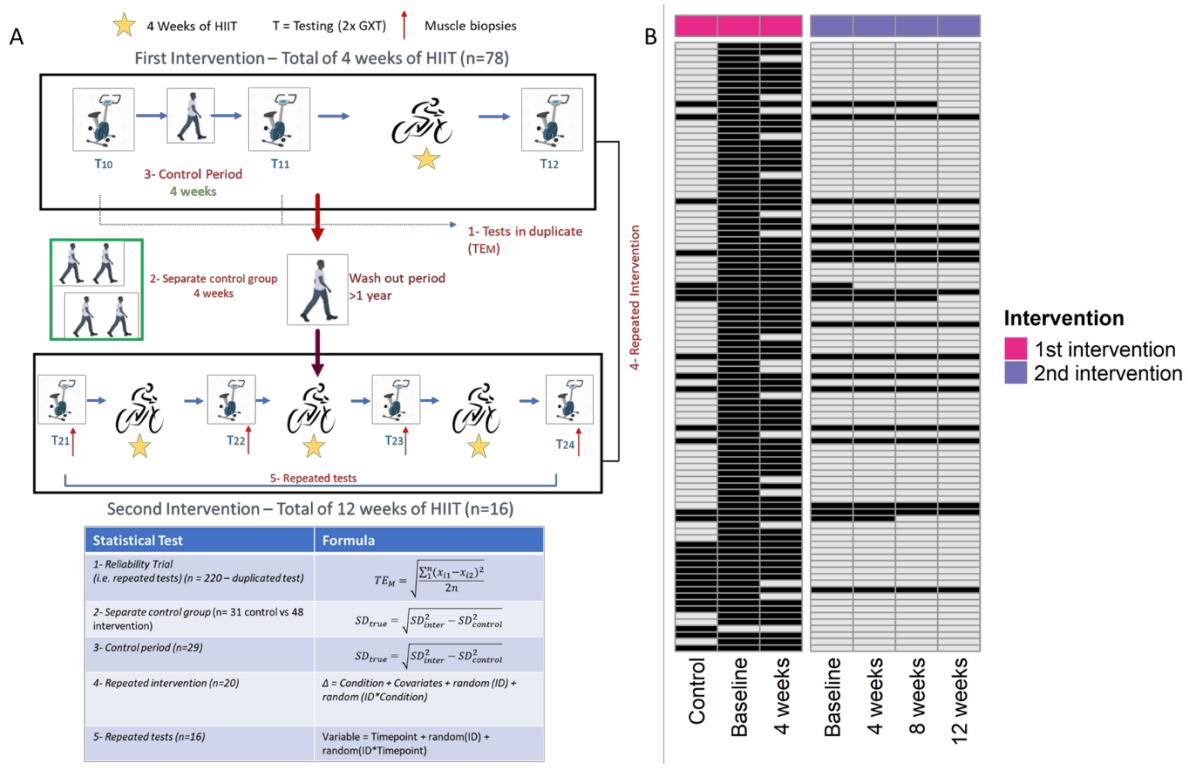


Figure 3.1. A: Study design and statistical methods with indication of how many participants/tests were used for each approach. B: Number of participants in each timepoint. Blocks highlighted in black indicate that participant has completed the respective timepoint.

3.2.4. Molecular analyses and immunohistochemistry

Energy production capacity markers (mitochondrial function)

Succinate Dehydrogenase Activity (Complex II activity). We utilised the Succinate Dehydrogenase (SDH) Activity Assay Kit (Colorimetric) (#ab228560). Muscle was lysed according to kit's protocol and 15ul of muscle lysate was used for reaction. Assay was performed in duplicates. In this assay, SDH converts succinate to fumarate, and transfers the electron to an artificial electron acceptor (Probe), which changes the colour from blue to a colourless product (depending upon the sample enzymatic activity). The assay kit was able to detect less than 0.1mU Succinate Dehydrogenase activity in our muscle samples. Protocol was followed according to kit user guide. SDH activities (mU/mg of tissue) were averaged and if CV >10% for the duplicate results values were removed.

Cytochrome C Oxidase Activity (Complex IV activity). For Cytochrome C Oxidase Activity (COX), we utilised the assay kit (#ab239711). Muscle was lysed using SDH buffer described above and 10ul of lysate was used for each reaction. The activity of the enzyme was determined calorimetrically in triplicates by following the oxidation

of reduced Cytochrome c as an absorbance decrease at 550 nm, according to kit user guide. COX results were averaged and if CV >10% for the triplicate divergent results were removed. COX results ate presented as mol/h/kg of protein.

Mitochondrial content

Intrinsic changes in mitochondria reflect changes in mitochondrial content and are commonly used to normalise global measurements of mitochondrial function.

Citrate Synthase Activity. The most commonly used measurement of mitochondrial content is the citrate synthase (CS) enzyme activity[137]. Complete enzyme extractions, from small pieces of frozen tissues, were performed in an ice-cold buffer (KH_2PO_4 & K_2HPO_4) using a TissueLyser II (Qiagen, Hilden, Germany). Protein concentration was assessed using the bicinchoninic acid assay. Total CS activity (mol/h/kg of protein) was measured in triplicates (30°C, pH 7.5) using standard spectrophotometric assays. Results for CS activity were manually curated, and values that presented a Coefficient of Variance (CV) > 10% were removed.

Mitochondrial Copy Number. Mitochondrial DNA copy number also reflects the content of mtDNA, and it is usually associated with mitochondrial gene stability and mitochondrial biogenesis. Mitochondrial copy number (mtDNA) was determined in quadruplicates, using multiplex qPCR. This method allows for simultaneous amplification of a mitochondrial (ND1) and a nuclear (RNAseP) amplicon to verify their relative abundance[135,138]. 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'.

We utilised the RNAseP assay kit (Thermofisher Scientific #4403328). Taqman Universal Mastermix (Thermofisher #4304437) was used, and the assay ran 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, the divergent sample was removed.

Mitochondrial Health Index (MHI)

The calculated MHI as previously reported in blood[135] was obtained using the following equation:

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

Fibre Typing (immunohistochemistry), and Expression (RT-PCR). Exercise is known to affect fibre type composition and expression. Shifts in fibre type composition might not be achieved so easily in human interventions, however changes in myosin heavy chain expression patterns might occur even after short interventions[139]. Thus, in our study we measured both parameters to see if trainability could be observed in either measurement.

Immunofluorescence analyses of muscle fibre types were performed on frozen muscle tissue sections. Primary and secondary antibodies information have been previously described somewhere else[140]. In summary, preserved muscle in optimum cutting temperature (O.C.T.) were sectioned at 5-8 μm and immediately fixed in 4% formaldehyde (10 min). After 10 min, excess formaldehyde was removed, and slides were washed 3x1min in ddH2O. 10% goat serum was using for blocking (1hr). Primary antibodies in 10% goat serum (ThermoFisher #50062Z) (1:25) incubated slides overnight in the dark. Primary antibody was then removed, and slides washed 3x5min in ddH2O. Secondary antibodies in 10% goat serum (1:500) were then used to incubate slides for 2 hr in the dark at 4 degrees. Slides were washed once more in ddH2O and then mounted with PBS for imaging. Fibre type distribution was quantified using Fiji software and values are presented in percentage distribution.

RNA was extracted using the AllPrep DNA/RNA FFPE Kit (#80234 Qiagen). 10ng of RNA was then diluted into 50ul and reverse transcription was conducted using the iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad) with a thermomixer. Primers for myosin heavy chain I, IIa and IIx used for this experiment have been described elsewhere[139]. RT-PCR was conducted using the QuantumStudio-7 (bio-rad). mRNA expression levels were quantified by real-time PCT using SYBR green fluorescence. Cycle threshold (Ct) values were normalized to a housekeeping gene,

Cycl1. Primers used were described elsewhere[139]. Samples were analysed in triplicates and data was manually curated. In cases where samples yielded a standard deviation > 0.4, the divergent sample was removed.

3.2.5. Statistical analyses

Response to training at the group level

Responses at the group level were investigated using 2 different mixed models:

Control period vs intervention period. We fit a linear mixed model using changes after the 4-week control period and after the 4-week intervention: $\Delta \sim \text{condition} + \text{IPA} + \text{age} + \text{random intercept (ID)}$, where Δ is the change in physiological measurement (W_{peak}, LT or VO_{2max}), condition is either "control" or "intervention", IPA is Incidental Physical Activity, and the ID corresponds to the participants' individual ID, which allows each individual to have his own intercept. The fixed effect for "condition" estimates whether there were changes in physiological measurements at the group level after the intervention period beyond that of the control period.

Repeated testing during intervention: We fit a linear mixed model[48] using changes after 4, 8 and 12 weeks:

$$\begin{aligned}\text{Physiological fitness outcome} \\ = \text{timepoint} + \text{random intercept (ID)} \\ + \text{random slope (ID x Timepoint)}\end{aligned}$$

where outcome is W_{peak} or LT or VO_{2max}, timepoint is a numeric variable (0, 4, 8 or 12), the random intercept accounts for baseline differences between individuals. The fixed effect for "timepoint" estimates whether there were changes in physiological measurements at the group level over time during the 12-week intervention period. We applied a similar model for the molecular markers as outcome:

$$\begin{aligned}\text{Molecular marker outcome} = \text{Physiological variable} + \text{timepoint} + \\ \text{random intercept (ID)} + \text{random slope (ID x Timepoint)}.\end{aligned}$$

Response to training at the individual level (trainability)

We conducted five distinct statistical analyses, as previously suggested[40]:

1. Reliability Trial (i.e. duplicate tests). This method uses multiple measurements of the outcome of interest (in our study, W_{peak}, LT and VO_{2max}) at each

timepoint to estimate technical variability due to machine and technician error, and day-to-day biological variability in test performance[40,50]. TE_M, or a multiple of it, is then used as a threshold to determine whether an individual has indeed improved after training, with a sufficient degree of certainty[40,50]. Some studies have used 1 x TE_M as a threshold, which gives ~95% confidence that the response of a given individual is non-null (i.e. >0 or <0)[43,53], while other studies have chosen a more stringent threshold of 2 x TE_M, which gives ~98% confidence the response of a given individual is positive[10,29,43]. The coefficient of variance (CV) is derived from TE_M and is usually presented as a percentage. This measurement has been widely used as to identify and classify response to training[34,141]. It is important to note that this method does not quantify trainability at the group or individual levels but provides a threshold to tell with a certain degree of confidence that an individual has shown a non-null response (i.e. >0 or <0).

2. Separate control group. This method estimates the variability in changes following a control period (i.e. no exercise training). The variability under the control condition is then subtracted from the variability under the intervention condition. The "true" variability in exercise response is then calculated using the formula[10,40,43]: $SD_{true} = \sqrt{SD_{inter}^2 - SD_{control}^2}$, where SD_{inter} is the observed inter-individual variability in change scores in the exercise group and $SD_{control}$ is the observed inter-individual variability in change scores in the control group. While this method insinuate the amount of variability in response that is induced by the exercise training itself, it does not quantify individual response, but rather represents the difference in variability between exercise and control groups[43].

3. Control period. This method is similar to the separate control group, but the same participants undergo a control period before commencing the exercise intervention. The "true" variability in exercise response is then calculated using the formula [40]: $SD_{true} = \sqrt{SD_{inter}^2 - SD_{control}^2}$, where SD_{inter} is the observed inter-individual variability in change scores after the exercise period and $SD_{control}$ is the observed inter-individual variability in change scores after the control period. This method removes any variability between the control group and exercise group due random sampling of individuals, but this method suffers from the same shortcomings as the control group method.

4. Repeated intervention. 19 participants successfully repeated the 4-week HIIT program after a washout period > 1 year. We fitted a linear mixed model of the form: $\Delta = IPA + age + random\ intercept\ (ID)$, were Δ is the change score in the measured outcome (W_{peak}, LT or VO_{2max}), IPA (kcals) is measured by an activity monitor worn for 1 week during the intervention, and the random intercept corresponds to trainability. The residuals of the linear mixed model contain the unwanted source of variability, namely within-subject variability in response to the same exercise training. We tested the significance of the random intercept (i.e. trainability) with a likelihood ratio test. This method directly estimates within-subject variability in response to training.

5. Repeated tests during the intervention. 16 participants successfully completed 12 weeks of HIIT with measurements at four timepoints (at 0, 4, 8 and 12 weeks). We estimated the slope of progress for each individual (i.e. trainability), with the variance around the slope corresponding to within-subject variability. These segmental changes partitioning trainability from within-subject variability were examined with a linear mixed model: Outcome = timepoint + random intercept (ID) + random slope (ID x Timepoint), where outcome is W_{peak}, LT, VO_{2max}, CS, COX, SDH, mtCN, MHI or fibre type composition/expression, timepoint is a numeric variable (0, 4, 8 or 12), the random intercept accounts for baseline differences between individuals, and the random slope corresponds to trainability (subject-by-training interaction). The individual segmental changes from this method estimate within-subject variability, acknowledging that response to training may not be linear.

Finally, a series of bivariate latent growth curve models[142] were used to test whether the slopes of fitness variables were correlated with the slopes of molecular variables. We did not have enough muscle for some individuals so mitochondrial data was missing, and the pattern was missing completely at random. Thus, we used multiple imputations using the *mice* package[143], and results were pooled from all imputed iterations for both mixed models as well as parallel growth models with the *miceadds* package[144]. All analyses were performed using the R software version 4.0.2. (packages: *dplyr*[145], *readxl*[146], *ggpubr*[147], *lmerTest*[48], *ggplot2*[148]).

3.3 Results

3.3.1. The repeated and longer exercise training intervention improved aerobic fitness in a dose-response manner.

The exercise training triggered positive physiological adaptations, compared with a control group or a control period (**Table 3.1**). After the first intervention, W_{peak} increased by 7% ($p < 0.005$), LT by 8% ($p < 0.005$) and VO_{2max} by 3% ($p = 0.001$). After the repeated intervention, W_{peak}, LT and VO_{2max} increased by 8%, 12% and 4% respectively after 4 weeks, 11%, 15% and 6% after 8 weeks, and by 17%, 18% and 8% after 12 weeks of HIIT compared with baseline ($p<0.05$ for all variables, **Figure 3.2 & Table 3.2**).

	Fixed Effects				Random Effects			
	Variable	Regression coefficient	Standard error	p-value	Variable	Variance	Standard error	p-value
W_{peak} (W/kg)	Timepoint	0.162	0.035	**0.00028**	ID x Timepoint	0.019	0.138	**0.00046**
					Residual	0.141	0.119	
LT (W/kg)	Timepoint	0.116	0.028	**0.00068**	ID x Timepoint	0.009	0.094	**0.052**
					Residual	0.027	0.164	
VO_{2max} (mL/min/kg)	Timepoint	1.385	0.513	**0.015**	ID x Timepoint	3.809	1.952	**0.00019**
					Residual	4.543	2.131	

Table 3.2. Repeated Testing.

Linear mixed model – Variable ~ Timepoint + random intercept (ID) + random slope (ID x Timepoint). Age and Kcals were omitted from results as those have not shown any significant values. Where Variable = Measurement value, Timepoint = PRE – 4WP – 8WP – 12WP.

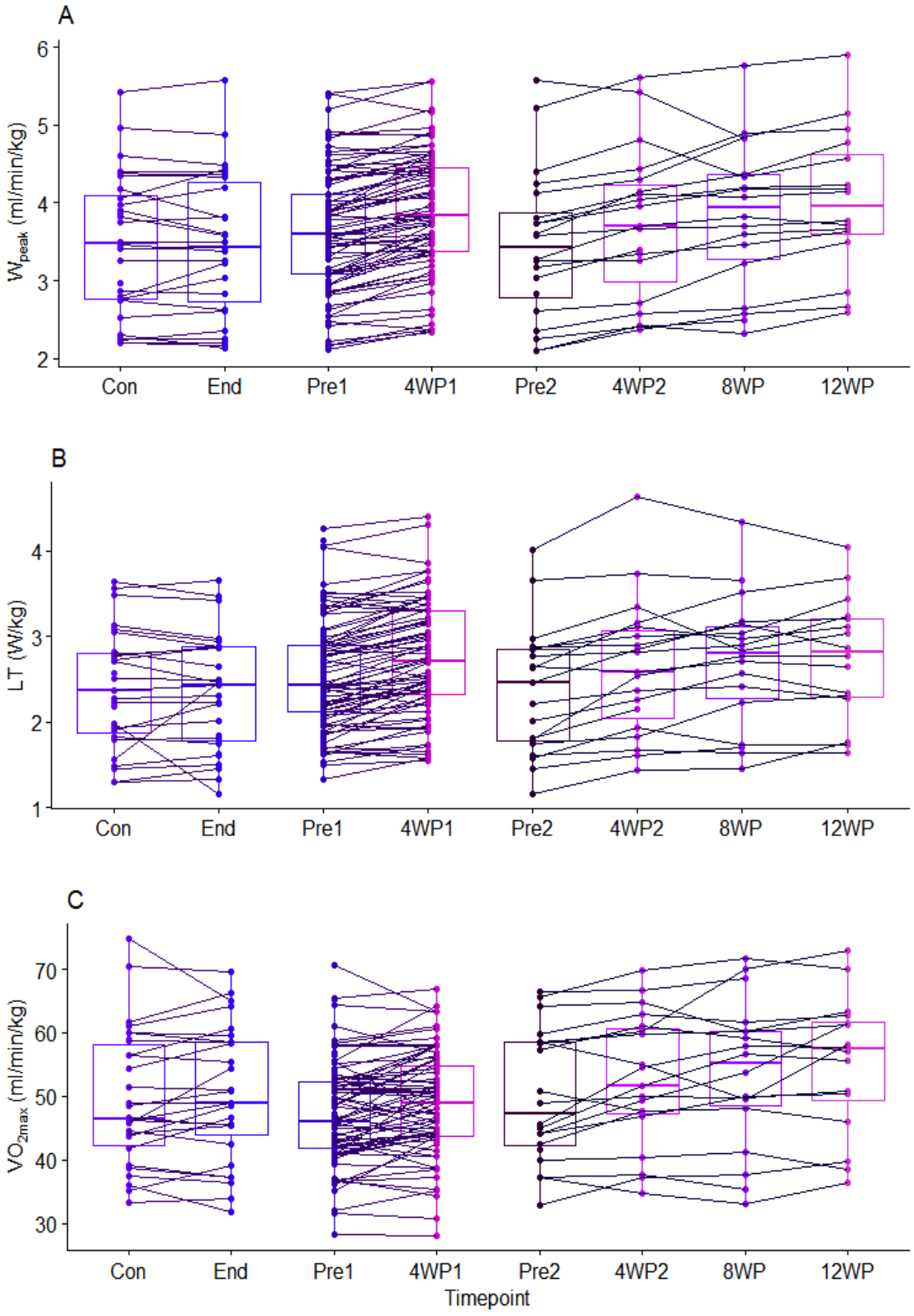


Figure 3.2. Individual changes in peak power output (W_{peak}), the lactate threshold (LT) and maximal oxygen uptake (VO_{2max}) after 4 weeks of control (Con/End), first intervention (4 weeks of HIIT, Pre1/4WP), and second intervention (12 weeks of HIIT, Pre2/12WP).

3.3.2. Analysis of inter-individual variation in response to exercise training

Technical Error of Measurement (TE_M) to assert non-null response at a certain level of confidence

GXTs were conducted a few days apart for each participant at each time point. Therefore, we estimated the TE_M for each physiological measurement using all duplicate pairs of GXTs (**Table 3.3**). Multiples of this TE_M were then used as a threshold above which an individual may be considered to have responded positively to the training. After the first four weeks of HIIT, 31 out of 73 participants increased their W_{peak} by more than 2 x TE_M (~ 98% CI), 5 participants for LT and 8 participants for VO_{2max} (**Figure 3.3**). After the second intervention, 14 of 17 participants included in the analyses surpassed 2 x TE_M for W_{peak}, 6 for LT and 4 for VO_{2max}.

	TE_M	CV (%)	number of paired GXTs
W_{peak} (W/kg)	0.13	3%	224
LT (W/kg)	0.25	9%	217
VO_{2max} (mL/min/kg)	3.33	7%	209

Table 3.3. Technical error of measurement (TE_M) and coefficient of variation (CV) for peak power output (W_{peak}), lactate threshold (LT) and maximum oxygen uptake (VO_{2max}).

GXTs with errors such as software breakdown during test, mask fall off, etc. that might have occurred during the test were removed.

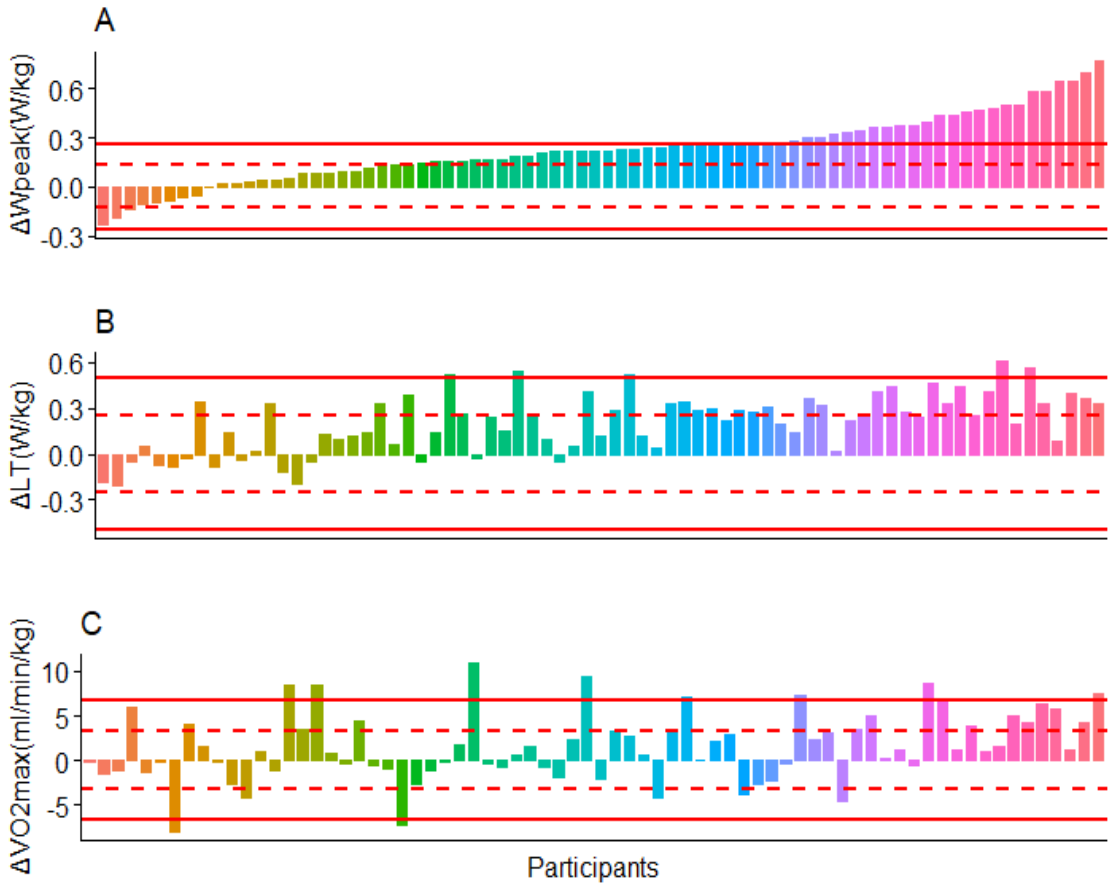


Figure 3.3. Participants ordered by peak power output (W_{peak}) response after 4 weeks of HIIT

Same colour corresponds to the same participant. Dotted line represents 1 x TE_M (~95% confidence), solid line represents 2 x TE_M (~98% confidence)

Control group (True SD)

We evaluated physiological changes after 4 weeks in a separate control group (n = 30) and compared them to that of the exercise group who underwent 4 weeks of HIIT (n = 48 – participants from first intervention that did not underwent a control period prior to intervention). This determined whether the observed inter-individual variability in training response was indeed due to exercise training itself[35,38,42]. Age, baseline fitness and IPA did not differ between control and intervention groups **(Table 3.4).** We calculated the true inter-individual response to exercise training (SD_{true}) as $SD_{true} = \sqrt{SD^2_{inter} - SD^2_{control}}$. The amount of inter-individual variability that was left after subtracting that of a control group was $SD_{true} = \sqrt{0.21 - 0.16} = 0.22\ W/kg$ for W_{peak} and $SD_{true} = \sqrt{3.93 - 3.65} = 1.46\ mL/min/kg$ for VO_{2max} (**Figure 3.4A**). We were

unable to calculate SD_{true} for LT as we observed a larger variability in the control group than the exercise group ($SD_{control} > SD_{inter}$).

	Mean±SD control group	Mean±SD intervention group	p-value
Kcal	637±217	714±295	0.341
Age	33.1±8.55	32±8.28	0.451
BMI	25.9±4.2	25.1±2.97	0.259
Wpeak (W/kg)	3.44±0.937	3.71±0.740	0.199
LT (W/kg)	2.36±0.717	2.6±0.642	0.18
VO_{2max} (ml/min/kg)	49.6±11.0	47.5±7.31	0.334

Table 3.4. Baseline comparison between control group and intervention group.

P-values are non-significant between groups, indicating homogeneity between groups.

Control period

29 participants who completed the 4-week HIIT intervention also completed a 4-week control period prior to the intervention, and we used this control period in the same way as the control group to estimate the variability in training response that is indeed due to the exercise training itself. Participants did not change their levels of IPA between the control and intervention periods (p= 0.609). Similarly, to the control group, we were unable to calculate SD_{true} for any physiological measurements because there was more variability in the control period than in the intervention period (**Figure 3.4B**).

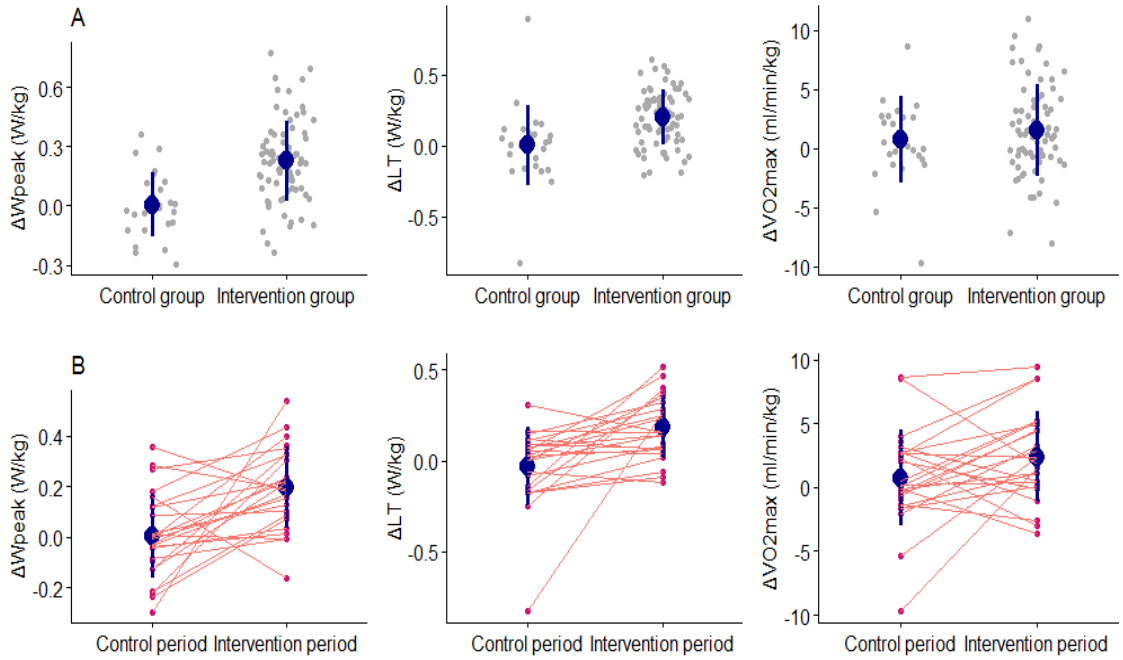


Figure 3.4. Changes in peak power output (W_{peak}), lactate threshold (LT) and maximal oxygen uptake (VO_{2max}).

Data is presented with mean and SD for each variable. A) Control group (different subjects in each group); B) Control period (same subjects).

Repeated intervention

Before quantifying within-subject variability in response to the same intervention, we first investigated potential selection bias (for example, participants who accepted to come back for the second intervention may also be keener exercisers and would be fitter than the group average from the first intervention). Returning participants had similar baseline W_{peak} and LT levels as the whole group from the first intervention, but they showed higher VO_{2max} (+ 4.09 mL/min/kg, p = 0.000656). We also tested whether returning participants had similar baseline fitness levels before starting the first and second interventions (i.e. after >1 year of washout). There was a strong correlation between baseline fitness levels before the first and second interventions (**Figure 3.5A**), and participants showed similar baseline W_{peak} and LT levels at the first and second intervention (W_{peak} p = 0.279; LT p = 0.078). However, participants had higher baseline VO_{2max} (+ 4.4 mL/min/kg, p = 0.000893). Levels of IPA were similar during the first and second interventions (p = 0.91).

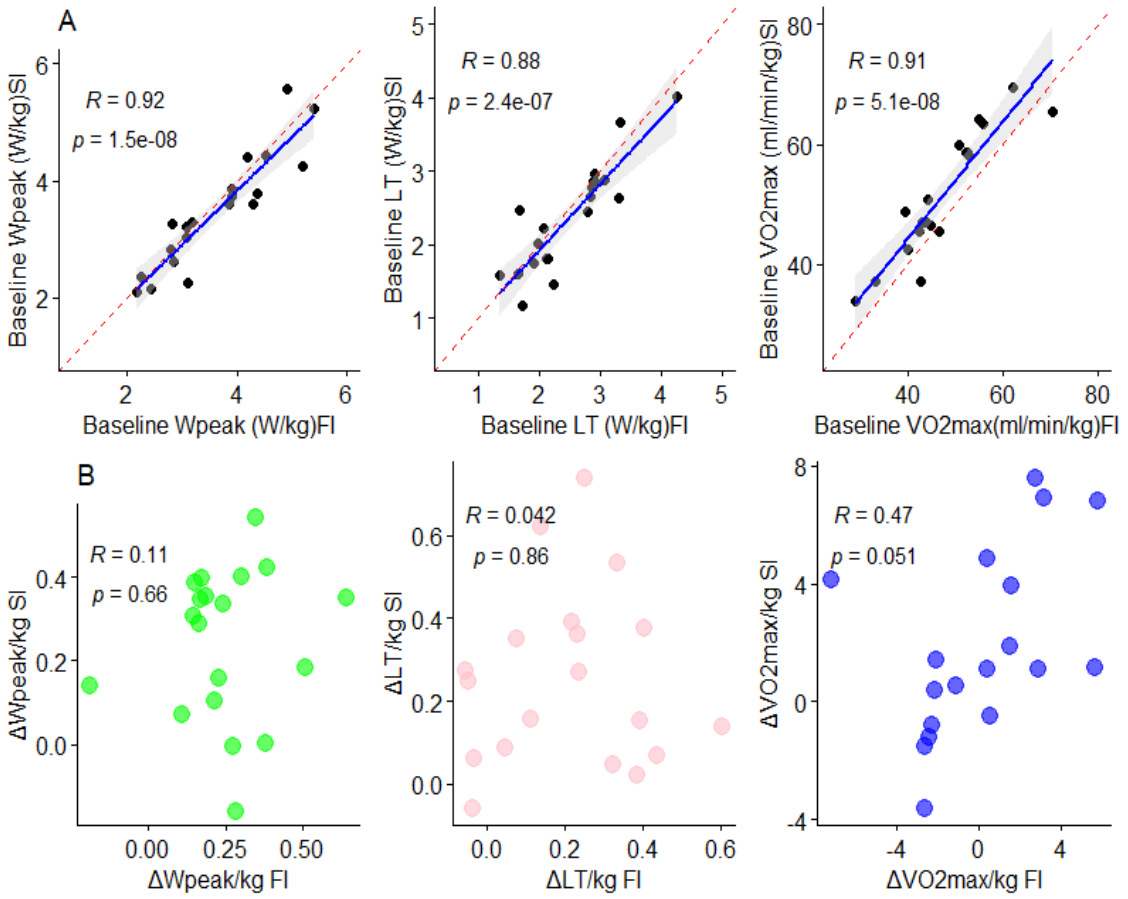


Figure 3.5. A: Correlation between baseline in peak power output (W_{peak}), lactate threshold (LT) and maximal oxygen uptake (VO_{2max}) before the first (x-axis) and second (y-axis) interventions (Pearson's correlation coefficient). B: Within-subject correlation between response to first (x-axis) and second (y-axis) interventions

The repeated intervention allows testing directly whether there is within-subject variability in response to the same intervention. Participants did not show a consistent response to the same exercise training **(Figure 3.5B)** and no subject-by-training interaction was detected in the linear mixed model **(**random effect p-value Wpeak = 0.49, LT = 0.99, VO_{2max} = 0.27 **Table 3.5**).

	Fixed Effects				Random Effects			
	Variable	Regression coefficient	Standard error	p-value	Variable	Variance	Standard error	p-value
ΔW_{peak} (W/kg)	Intercept	4.117e-01	1.019e-01	**0.000183**				
	Age	-6.406e-03	2.734e-03	**0.024053**	Interaction Condition x ID	0.008906	0.09437	0.4951
	Kcal	5.586e-05	8.401e-05	0.508609	Residual	0.028549	0.16896	
ΔLT (W/kg)	Intercept	3.278e-01	1.037e-01	**0.00267**				
	Age	-5.333e-03	2.771e-03	0.06123	Interaction Condition x ID	5.854e-05	0.007651	0.9948
	Kcal	8.768e-05	8.524e-05	0.30821	Residual	4.053e-02	0.201317	
ΔVO_{2max} (ml/min/kg)	Intercept	3.7438575	2.1463597	0.087				
	Age	-0.0808566	0.0559901	0.156	Interaction Condition x ID	4.733	2.175	0.273
	Kcal	0.0003136	0.0016881	0.853	Residual	9.683	3.112	

Table 3.5. Results of the linear mixed model for the repeated intervention after 1 year of washout.

(Changes~Covariates+random(ID)+random(ID*Condition). Where Changes = change in measurement (PRE & POST), Covariates= Age, Activity monitor (kcals consumed), rand (ID) = random intercept corresponding to individual IDs.

Repeated testing during the exercise intervention (12 weeks of HIIT)

The use of multiple measurements at different time points allows estimating within-subject variability between multiple segments during the training period. We applied mixed modelling with a random intercept corresponding to individual baseline levels and a random slope corresponding to individual trainability (**Table 3.2 –** random effects). We separated trainability from within-subject variability that corresponds to the error surrounding the segmental changes of the slope. We were able to successfully identify trainability, meaning each participant responded differently to the intervention, with some participants showing rapid and large increases in fitness while others showed slower improvements **(Figure 3.6)**. The highest responder improved in VO_{2max} by an additional +3.28 (ml/min/kg), compared with the group average +0.03 (ml/min/kg); conversely, the lowest responder declined in VO_{2max} by -2.29 (ml/min/kg) less than the group average.

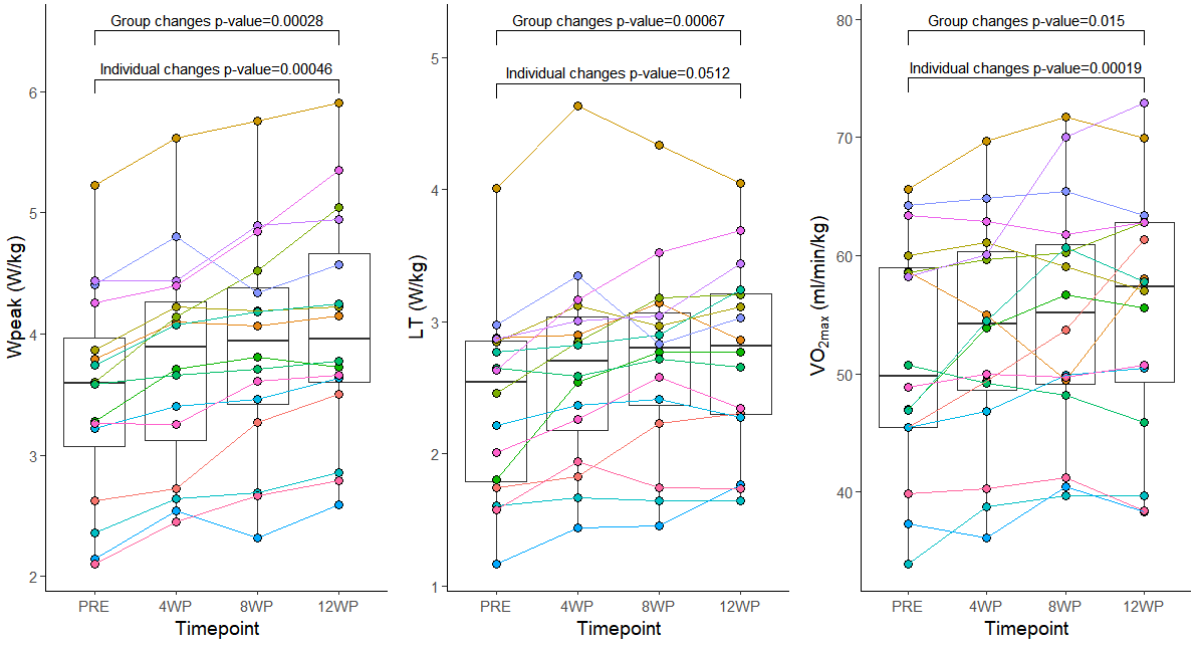


Figure 3.6. Individual changes in physiological measures (W_{peak}, LT, and VO_{2max}) after 4, 8 and 12 weeks of HIIT.

This figure is a recreation of Figure 3.2 highlighting only second intervention and individual participants.

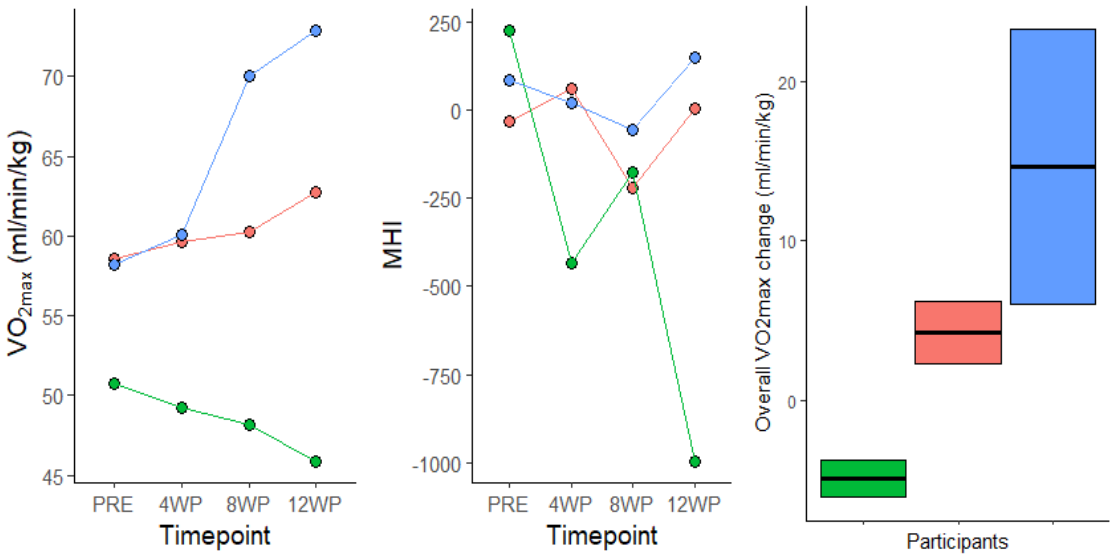


Figure 3.7. Example of trainability and comparison between whole body and molecular markers for high, average, and low responder for VO2max and MHI measurement after 12 weeks of HIIT.

The highest responder is represented in blue, the group average in pink and the lowest responder in green. It can also be noted in the last graph that if confidence intervals (CI) were calculated according to the formula proposed by Hecksteden et al. 2018 (segmental_changes±1.96*SE_segmental changes), participant in green would have a small CI, meaning his changes are more consistent overtime than compared with participant in blue.

We failed to detect any change in CS, SDH, COX, mtCN, or MHI at the group or individual level following 12 weeks of HIIT **(Figure 3.8, Table 3.6).** In addition, mitochondrial markers were not associated with physiological fitness measures ($p>0.05$ – for complete results summary see **Table 3.6**). Of note, mtCN was strongly associated with age in all models ($p<0.005$) (**Table 3.6**), which is in accordance with the literature[149,150].

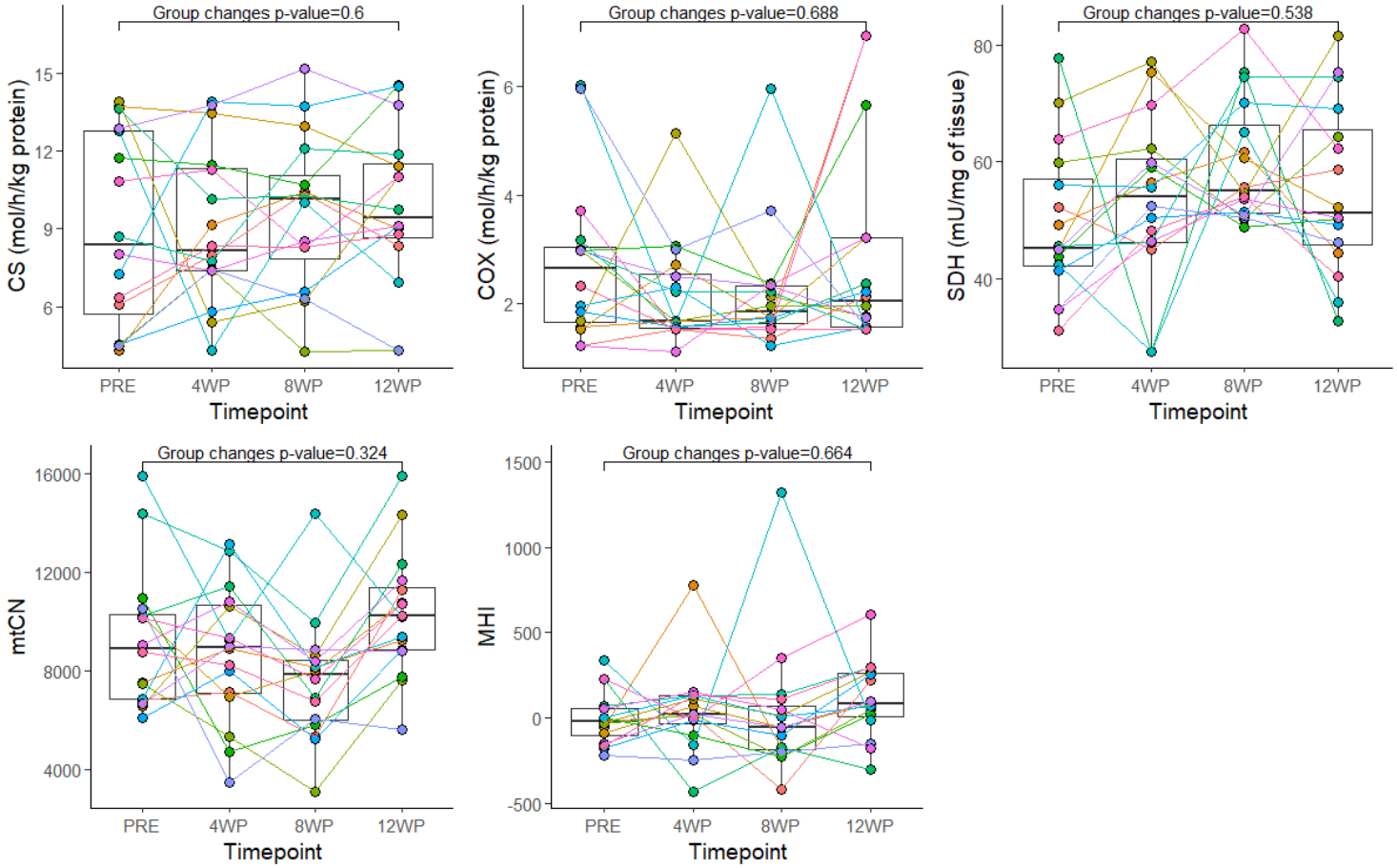


Figure 3.8. Individual changes in Citrate Synthase (CS), Cytochrome-C Oxidase (COX), Succinate Dehydrogenase (SDH), Mitochondrial Copy Number (mtCN), and Mitochondrial Health Index (MHI) for each 4 weeks up to 12 weeks.

Next, we investigated whether the training led to changes in fibre type distributions and whether those changes were associated with physiological or molecular changes (**Tables 3.6 & 3.7**). First, we tested whether fibre type proportion (as a percentage of number of fibres that were counted) and expression of myosin heavy chains (MHC) were correlated. Fibre type proportion and MHC expression presented small but significant correlation (**Figure 3.9**). We did not detect any shifts in fibre type percentage distributions or MHC expression after 12 weeks of HIIT at either the group or individual level (p-value >0.05) (**Figure 3.10, Table 3.7**). However, the proportions of types I and IIa, but not IIx, were associated with physiological markers (W_{peak}, LT and VO_{2max}, $p<0.05$) (**Table 3.7**). Finally, fibre type proportion and expression were not associated with any of the mitochondrial markers after adjusting p-values ($p>0.05$, data not shown).

	Fixed Effects - Group Level				Random Effects - Individual Level			
	Variable	Estimate	Standard error	p-value	Variable	Variance	Standard error	p-value
CS	Timepoint	0.321	0.274	0.241	ID x Timepoint	0.5216	0.448	0.246
	Age	0.03	0.069	0.658	Residual	1.807	0.277	
COX	Timepoint	0.088	0.196	0.654	ID	0.422	8.315	0.959
	Age	-0.002	0.027	0.933	Residual	1.403	0.199	
SDH	Timepoint	1.62	1.415	0.253	ID	4.7	2.344	**0.05**
	Age	-0.248	0.217	0.254	Residual	11.539	1.232	
mtCN	Timepoint	311.242	263.178	0.237	ID	775.159	535.213	0.148
	Age	130.091	41.251	**0.0016**	Residual	2232.516	248.248	
MHI	Timepoint	21.197	35.669	0.559	ID x Timepoint	6671	81.68	**0.0964**
	Age	4.105	4.645	0.884	Residual	68412	261.56	

Table 3.6. Results of the linear mixed model for the mitochondrial measurements.

Linear mixed model – Variable ~ Timepoint + random intercept (ID) + random slope (ID x Timepoint). Where Variable = Measurement value, Timepoint = PRE – 4WP – 8WP – 12WP. Fixed effects represent changes at the group level, while random effects represent changes at the individual level (trainability)

	Fixed Effects - Group Level				Random Effects - Individual Level			
	Variable	Regression coefficient	Standard error	p-value	Variable	Variance	Standard error	p-value
Fibre Type I %	Timepoint	-0.969	1.266	0.453	ID x Timepoint	2.672	1.635	0.792
	Wpeak	4.67	2.406	0.07	Residual	94.83	9.738	
	Timepoint	-0.876	1.192	0.471	ID x Timepoint	0.905	0.951	0.833
	LT	6.392	2.767	**0.032**	Residual	96.378	9.817	
	Timepoint	-0.85	0.206	0.458	ID x Timepoint	0.262	0.512	0.938
	VO2max	0.582	0.206	**0.01**	Residual	93.323	9.66	
Fibre Type IIa %	Timepoint	1.863	1.36	0.178	ID x Timepoint	0.841	0.917	0.792
	Wpeak	-6.034	1.908	**0.005**	Residual	127.62	11.297	
	Timepoint	1.858	1.165	0.117	ID x Timepoint	0.385	0.62	0.907
	LT	-5.66	2.714	**0.051**	Residual	94.505	9.721	
	Timepoint	1.81	1.141	0.119	ID x Timepoint	0.292	0.54	0.9289
	VO2max	-0.5	0.198	**0.02**	Residual	94.015	9.696	
Fibre Type IIx %	Timepoint	-0.981	4.4609	0.1964	ID x Timepoint	1.008	0.909	0.268
	Wpeak	0.433	1.2175	0.7217	Residual	5.856	0.704	
	Timepoint	-0.944	0.781	0.227	ID x Timepoint	0.977	0.919	0.287
	LT	-0.312	1.45	0.829	Residual	5.87	0.705	
	Timepoint	-0.89	0.778	0.253	ID x Timepoint	0.97	0.887	0.274
	VO2max	-0.068	0.114	0.549	Residual	5.827	0.702	
Fibre Type I (log expression)	Timepoint	-0.005	0.085	0.945	ID x Timepoint	0.004	0.064	0.952
	Wpeak	-0.005	0.111	0.959	Residual	0.527	0.726	
	Timepoint	-0.007	0.084	0.931	ID x Timepoint	0.003	0.059	0.959
	LT	0.003	0.136	0.979	Residual	0.529	0.727	

	Timepoint	-0.014	0.083	0.866	ID x Timepoint	0.001	0.039	0.981
	VO2max	0.005	0.01	0.611	Residual	0.53	0.728	
Fibre Type IIa (log expression)	Timepoint	0.116	0.114	0.318	ID x Timepoint	0.021	0.146	0.891
	Wpeak	-0.184	0.141	0.205	Residual	0.882	0.939	
	Timepoint	0.116	0.112	0.309	ID x Timepoint	0.02	0.143	0.881
	LT	-0.286	0.172	0.106	Residual	0.865	0.93	
	Timepoint	0.103	0.111	0.363	ID x Timepoint	0.012	0.109	0.966
	VO2max	-0.015	0.013	0.248	Residual	0.906	0.952	
Fibre Type IIx (log expression)	Timepoint	-0.048	0.156	0.757	ID x Timepoint	0.033	0.181	0.572
	Wpeak	0.227	0.028	0.455	Residual	1.545	1.243	
	Timepoint	-0.017	0.152	0.902	ID x Timepoint	0.026	0.163	0.654
	LT	0.093	0.359	0.798	Residual	1.571	1.253	
	Timepoint	-0.024	0.15	0.872	ID x Timepoint	0.027	0.166	0.624
	VO2max	0.013	0.026	0.625	Residual	1.561	1.249	

Table 3.7. Results of the linear mixed model for fibre type % and log expression.

Linear mixed model - Variable ~ Timepoint + random intercept (ID) + random slope (ID x Timepoint). Where Variable = Measurement value, Timepoint = PRE – 4WP – 8WP – 12WP. Fixed effects represent changes at the group level, while random effects represent changes at the individual level (trainability).

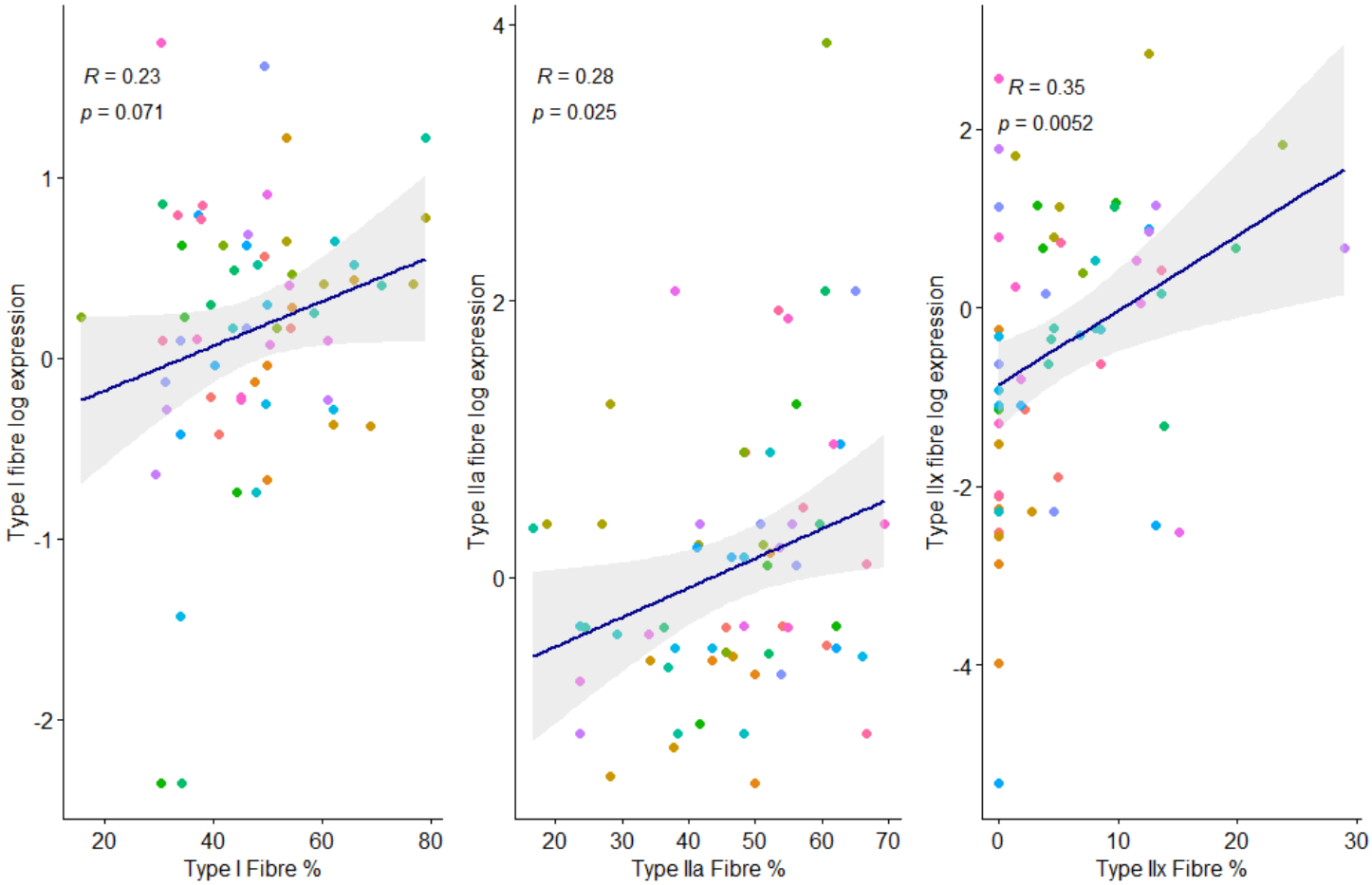


Figure 3.9. Fibre type % and log expression correlations

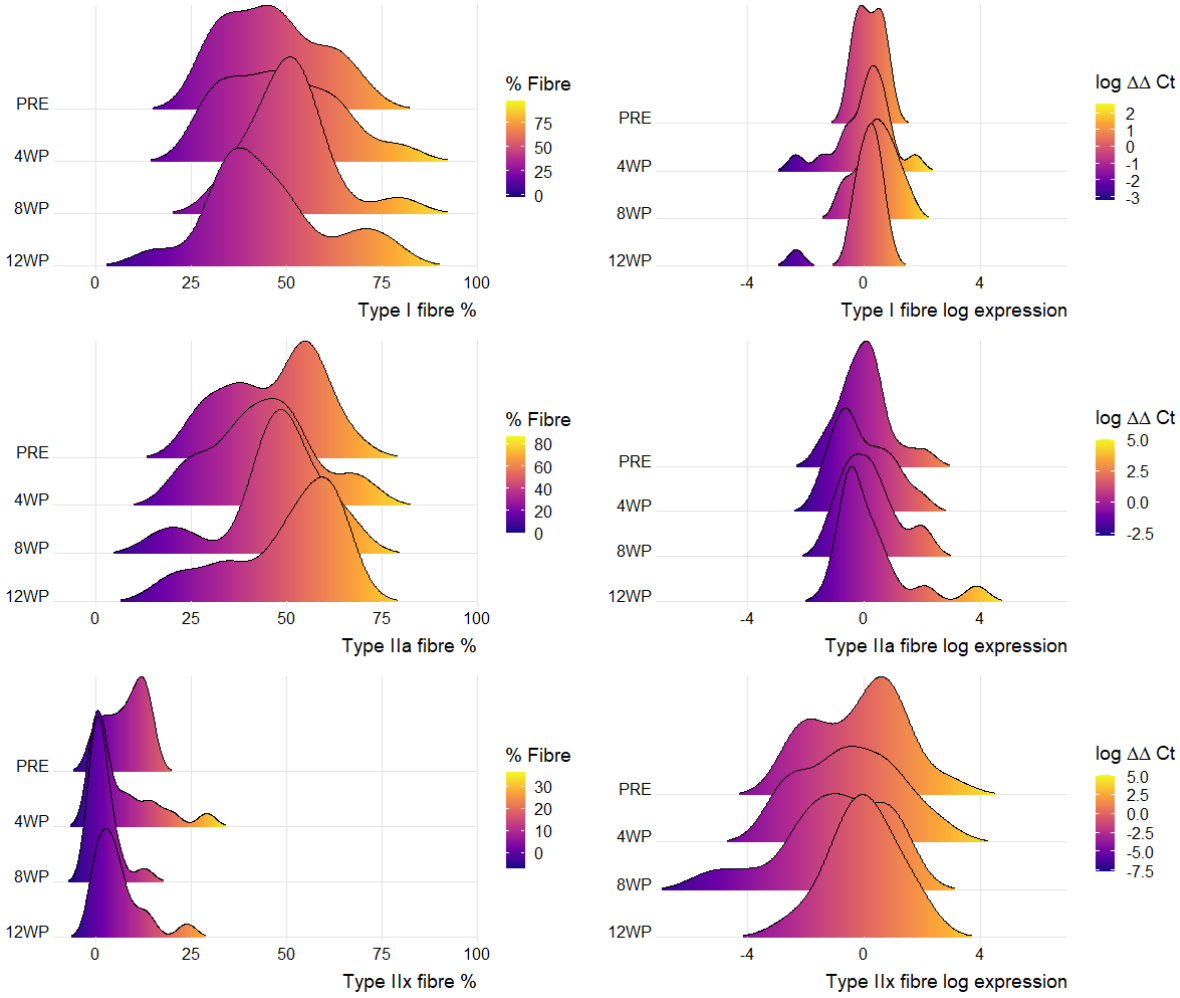


Figure 3.10. Distribution of fibre type separated by timepoint.

Although minor changes are observed in the distribution overtime, those were not significant for either fibre type proportion or expression following 4, 8 and 12 weeks of HIIT. Cycle threshold (Ct).

Relationship between physiological and mitochondrial variables – bivariate latent growth models

To understand the relationship between changes in molecular variables and changes in physiological variables, we built a bivariate latent growth model. **Table 3.8** summarises the interaction between each bivariate model. As expected, W_{peak}, LT & VO_{2max} were correlated at baseline, which means that participants with high W_{peak} at baseline also had high LT and VO_{2max} at baseline ($p<0.05$). A similar correlation was observed between baseline CS and COX ($p=0.049$). Unsurprisingly, W_{peak} and LT showed similar increases over time across participants ($p=0.05$). However, no other associations were observed between physiological and mitochondrial measures. Finally,

participants with higher baseline CS displayed smaller changes in COX following training (p=0.042), and vice versa, participants with higher baseline COX had smaller changes in CS following training (p=0.026) **(Table 3.8).**

Comparison of methods for measurement of trainability

The different methods provided various types and degrees of information. The control group and control period methods could not attribute the observed trainability to the exercise training itself, as the variability in changes in the control group (or after a control period) was at least as large as the variability in changes in the exercise group (or after the intervention period). The reliability trial did not allow a comparison of the training responses of different individuals, as it only ascertained response/non-response at a certain level of confidence. In addition, the CI at the individual level during the repeated intervention revealed that some people present a positive response at some later point during the intervention but present no positive response if only assessing the 4 weeks' time point or if simply assessing a pre- and post-response (**Table 3.9**).

	Covariates								
	Intercept & Intercept			Slope & Slope			Intercept & Slope		
Variable	Estimate	Std. Error	P-Value	Estimate	Std. Error	P-Value	Estimate	Std. Error	P-Value
Wpeak & LT	**0.574**	**0.209**	**0.006**	**0.012**	**0.006**	**0.052**	-0.009	0.039	0.826
Wpeak & VO2max	**7.445**	**2.718**	**0.006**	0.06	0.063	0.344	-0.107	0.542	0.844
Wpeak & CS	0.919	0.728	0.207	0.002	0.024	0.081	-0.156	0.221	0.481
Wpeak & COX	0.446	0.361	0.217	-0.015	0.047	0.755	0.114	0.411	0.782
Wpeak & SDH	1.956	2.218	0.378	0.133	0.14	0.344	76.867	43.992	0.081
Wpeak & mtCN	0.178	0.413	0.667	-0.002	0.018	0.891	0.016	0.173	0.926
Wpeak & MHI	0.416	0.293	0.156	0.026	0.037	0.484	-0.006	0.031	0.848
LT & VO2max	**6.025**	**2.256**	**0.008**	0.116	0.136	0.395	-0.245	0.498	0.624
LT & CS	0.754	0.612	0.218	0.042	0.052	0.411	-0.152	0.188	0.418
LT & COX	0.538	0.324	0.096	0.02	0.09	0.825	-0.004	0.344	0.991
LT & SDH	1.999	1.861	0.283	0.055	0.259	0.83	-0.591	0.942	-0.079
LT & mtCN	0.323	0.99	0.322	0.045	-0.786	0.434	0.196	-0.816	0.415
LT & MHI	0.227	1.162	0.245	0.008	-0.163	0.87	0.035	0.128	0.898
VO2max & CS	3.777	8.112	0.641	0.877	0.755	0.246	-1.477	2.584	0.568
VO2max & COX	3.354	3.831	0.381	-1.598	1.403	0.255	3.699	4.567	0.418
VO2max & SDH	13.69	22.645	0.546	1.023	1.511	0.499	-7.738	12.254	0.528
VO2max & mtCN	1.168	4.783	0.807	0.25	0.684	0.716	-2.101	2.946	0.491
VO2max & MHI	2.649	2.93	0.366	-0.066	0.221	0.766	0.288	0.793	0.717
CS & COX	**2.872**	**1.461**	**0.049**	0.914	0.798	0.252	**-3.578**	**1.762**	**0.042**
CS & SDH	6.966	8.449	0.41	0.884	1.451	0.543	76.867	48.169	0.111
CS & mtCN	0.493	1.44	0.733	0.077	0.137	0.577	-0.495	0.461	0.285
COX & SDH	6.795	4.465	0.128	-1.026	2.241	0.647	-0.532	4.724	0.91
COX & mtCN	0.085	0.055	0.126	-0.126	0.095	0.188	-0.087	0.067	0.195
SDH & mtCN	-0.094	0.374	0.801	-0.006	0.072	0.933	0.285	0.219	0.194

Table 3.8. Bivariate growth models.

The Intercept & Intercept informs if variables have similar baseline values, Slope & Slope informs if variables have similar rate of changes, and Intercept & Slope informs if baseline values of one variable affects the rate of change of the other variable.

	1st intervention			2nd intervention						W_{peak} (W/kg)				LT (W/kg)				VO_{2max} (mL/min/kg)			
	W_{peak} (W/kg)	LT (W/kg)	VO_{2max} (mL/min/kg)	Wpeak (W/kg)		LT (W/kg)		VO2max (mL/min/kg)		1xTEM (0.13 W/kg)		2xTEM (0.26 W/kg)		1xTEM (0.25 W/kg)		2xTEM (0.5 W/kg)		1xTEM (3.33 W/kg)		2xTEM (6.66 W/kg)	
ID	Δ (4 weeks)	Δ (4 weeks)	Δ (4 weeks)	Δ (12 weeks)	Slope	Δ (12 weeks)	Slope	Δ (12 weeks)	Slope	1st inter	2nd inter*	1st inter	2nd inter*	1st inter	2nd inter*	1st inter	2nd inter*	1st inter	2nd inter*	1st inter	2nd inter*
SG110	0.23	0.11	-2.10	NA	0.14	NA	0.13	NA	-0.26												
SG112	0.21	0.05	1.63	0.88	0.30	0.56	0.18	15.92	4.73												
SG125	0.15	0.39	-2.59	0.35	0.11	-0.01	0.06	-0.59	-0.28												
SG129	0.15	0.14	-7.18	0.68	0.20	0.03	0.02	4.39	1.63												
SG131	0.19	0.24	2.94	0.35	0.16	0.26	0.08	-2.99	0.11												
SG133	0.35	0.22	5.68	1.45	0.34	0.75	0.20	4.25	1.04												
SG134	0.38	0.25	3.16	0.44	0.15	0.97	0.24	8.62	3.22												
SG138	0.34	0.21	-6.11	NA	0.19	NA	0.12	NA	1.47												
SG139	0.11	-0.03	-2.60	0.19	0.07	0.00	0.05	-4.84	-0.88												
SG140	0.28	0.44	-2.24	NA	-0.24	NA	0.05	NA	1.29												
SG144	0.24	0.32	2.75	0.50	0.15	0.47	0.13	10.81	3.50												
SG149	0.17	-0.03	0.41	0.50	0.16	0.04	0.06	5.84	1.82												
SG152	0.51	0.39	-2.05	0.41	0.15	0.06	0.06	5.02	2.03												
SG154	0.18	-0.05	-2.41	0.45	0.15	0.60	0.17	1.02	-0.24												
SG160	0.30	0.40	-1.13	0.17	0.02	0.06	0.02	-0.85	-0.20												
SG162	0.38	0.61	1.50	0.51	0.24	0.57	0.15	14.63	4.69												
SG172	-0.19	0.34	0.54	1.10	0.33	1.06	0.26	-0.63	0.97												
SG173	0.27	-0.05	0.45	0.40	0.16	0.33	0.13	1.83	0.67												
SG174	0.64	0.08	5.80	NA	0.24	NA	0.16	NA	2.49												
SG185	0.17	0.23	NA	0.69	0.19	0.16	0.07	-1.44	-0.09												

*accounting for individual CI

Table 3.9. Methods comparison classification based on responsiveness.

Each participant was colour coded according to response (green: responder for the method, red: non-responder for the method, and yellow: inconclusive response for the method).

3.4 Discussion

In the present study, we provide a comprehensive picture of the statistical methods and their applications in an interventional study to isolate sources of variability. All methods provided different information and may be used complementarily. For example, a control period or control group determined whether inter-individual variability in response to training was due to the training itself, but it does not estimate trainability. $TE_{M,}$ is useful to determine the reliability and intra-test variability as well as to establish thresholds (i.e. minimum change necessary for biological meaning), but also does not quantify trainability. Studies investigating individual responses to exercise training often assume that if the same intervention was re-applied or extended to the same participants, their responses would be consistent[10,40]. This assumption has been rightfully challenged[5,10,40,43], but innovative methods to measure trainability have only recently been proposed and only one study has implemented some of them[10]. Here we have applied repeated intervention and repeated testing during the intervention to quantify trainability at the individual level. The repeated test approach was successful in estimating trainability while the repeated intervention did not.

Since our repeated testing approach was successful in quantifying trainability, we have extended the analyses to include mitochondrial and fibre type profiles analyses during the 12-week HIIT intervention. We found that physiological measurements improved more consistently than molecular measurements from the 12-week HIIT intervention. While there were clear changes in physiological measurements at the group level and we were able to identify high and low responders to exercise training based on these measurements, we were unable to do so with mitochondrial markers as they were highly variable both within and between participants. Type I and IIa fibres that were associated with some physiological variables (W_{peak}, LT and VO_{2max}). Baseline fitness did not influence magnitude of change for any physiological, molecular or fibre type related measurement. Finally, changes at the physiological level were not associated with changes at the molecular level.

To explore the results from each model in more depth we have subdivided the following sections according to statistical tests applied.

3.4.1. Control group/period

In line with many other studies, we found that inter-individual variability in physiological changes after the control group/period was on par with that of the intervention group/period, suggesting that the observed trainability after 4 weeks of HIIT was not due to the training itself and was in fact just random error[43]. However, this method assumes that any difference in physiological measures between groups/periods is caused by the exercise intervention alone[43], and that the groups/periods have identical environments (sleep, diet, etc.). However, participants' habits may change when they start an exercise training intervention. For example, participants may lower their IPA when commencing the intervention, replacing some of the habitual physical activity they were already doing with the new intervention[151]. This would effectively lead to an underestimation of the training effect at the group level, and an underestimation of trainability. While we did not observe any difference in IPA between the control and intervention groups/periods, we did not monitor sleep, diet, or any other environmental factor that may influence training responses. In addition, the use of a control group/period does not determine the presence of trainability, as within-subject variability in response to training may still be present.

3.4.2. Technical error of measurement (TEM)

Common measures reported by exercise studies that are used as thresholds for classifying individuals as responders (or adverse responders) include the technical error of measurement (TE_M) and coefficient of variance (CV)[4,29,32]. Although this method is based on a clear rationale (categorise individuals based on their level of response), it cannot *quantify* trainability at the individual level, resulting in a loss of statistical power as highly informative continuous data (i.e. change scores) is reduced to two or three categories. In our study, only one participant was a systematic non-responder (fitness changes $< 2 \times TE_M$) for all physiological measurements. Unsurprisingly, some global non-responders from the first intervention (n= 10) did respond after a second intervention (4 weeks), and some non-responders (n= 6) from the first intervention responded after the longer intervention (> 4 weeks of HIIT). It is important to reiterate that this method is not recommended to continuous measures (i.e. response to exercise), or classification of response. Unless a clear rationale is proposed for the establishment of thresholds of changes, or to evaluate reliability of measurements.

3.4.3. Repeated intervention

A repeated intervention on the same participants after an adequate washout period is theoretically the most straightforward approach to estimate trainability[5,6,10,35]. This method assumes that participants have not significantly changed between the first and second interventions (i.e. no major life changes between interventions). However, there are factors that may influence how well each individual respond to training in the repeated intervention, such as carry-over effects from the first intervention or ageing significantly. In our study, participants were given a relatively long washout period (>1 year) compared with the length of the first intervention (4 weeks of training), yet not long enough for participants to age significantly, limiting carry-over and age-related effects. In addition, participants showed very similar fitness levels before the first and second intervention, thus suggesting limited lifestyle changes between the first and second interventions. We found no evidence of recruitment bias for the second intervention using W_{peak} or LT, yet returning participants showed higher VO_{2max} than the group of the first intervention taken as a whole (p=0.0006). Using a repeated intervention, we could not detect trainability after 4 weeks of HIIT, as the responses to the first vs second intervention were very different within participants. This may be explained by the short length of training (4 weeks) that triggered relatively small adaptations, which are harder to detect in the "noise" of within-subject variability. We could not apply the repeated testing method on the 4 weeks of training since it was a relatively short intervention. We performed repeated testing in the longer intervention (12 weeks of training), which naturally triggered larger adaptations and made it easier to detect trainability in the "noise" of within-subject variability. Therefore, we suggest studies measuring trainability to design exercise training studies that are long enough to trigger physiological adaptations that are larger than any technical and biological day-to-day variability, to increase the "signal-to-noise" ratio. Finally, repeated interventions are challenging and costly to implement, which might limit their applicability. Repeated interventions may be used to investigate the sex and genetic basis of trainability, as these factors are stable over time. While the repeated testing method discussed in the following subsection may be better suited for linking trainability to molecular profiles that fluctuate with age and other environmental factors, and that may differ at baseline before the first and second interventions (e.g. epigenetic profiles, gene, and protein expression).

3.4.4. Repeated measurements

Including repeated measurements during the intervention is an emerging approach to estimate trainability[5,10,40]. Continuous, repeated measures during exercise interventions constitute a cost-effective approach to estimate individual exercise responses[5,10,40]. Using this method, we successfully quantified trainability for W_{peak}, LT and VO_{2max}, and identified individuals who responded to the training better (or worse) than the group. This methodology should therefore be the gold standard for studies aiming at uncovering the genetic or epigenetic basis of individual responses to exercise training. Due to the success of this application at the physiological level we have expanded our analyses in order to include molecular markers.

This study has assessed, for the first time in skeletal muscle, a comprehensive mitochondrial health "score", MHI, originally assessed in blood[135]. Although biologically relevant, mitochondrial markers measured in isolation are hard to interpret as compensatory mechanisms may be occurring among variables. Thus, the novel MHI measurement integrating biochemical and molecular mitochondrial measures, aims to obtain higher sensitivity to mitochondrial responses as it accounts for any relationship between variables[135]. During the 12-week HIIT intervention, the mitochondrial enzyme maximal activity and therefore the MHI were highly variable, and no consistent changes were observed at either the group or individual level. This was surprising given that mitochondrial content and function are upregulated by exercise[126–128]. To ensure that the variance was not due to technical variability, we removed any duplicate results that presented a variance >10%. However, it is known that enzyme activity is highly dynamic and the timeframe in which enzymes are fired may vary both within as well as between subjects[152]. In addition, we cannot rule out the potential involvement of other enzymes in similar pathways, or enzyme Km and not the maximal activity could be different between people[153], but these hypothesis remains to be tested. Furthermore, due to the nature of skeletal muscle (i.e. multi-nucleated) we could not account for cell number as suggested by Picard *et al*. The multi-nucleated characteristic of skeletal muscle promotes the possibility that each myonuclear differ in transcriptional rates and are independently regulated and distinctive from each other, to the extent that local differences in skeletal muscle (i.e. two pieces of same biopsy) might be present following exercise[9,154,155], and thus potentially explaining part of the variability observed between and among measures.

3.4.5. Associations between physiological and molecular markers

Adaptive mechanisms that improve muscle function and enhance response to exercise are initiated at the transcriptional level[9,156]. However, apart from correlational observations there is no direct evidence linking changes in mRNA expression to training induced adaptations[9,157,158]. Here, we investigated whether fibre type gene expression was associated with fibre type composition. The proportions of fibre type have been previously reported to be associated with different modalities of exercise[159–161]. For example, a high proportion of type I fibres are beneficial to endurance athletes as they are slow twitch, oxidative and relatively more resistant to fatigue. We found significant associations between the proportions of type I and IIa fibres with physiological markers. Fibre type I was positively associated with LT and VO_{2max} while fibre type IIa was negatively associated with all physiological measurements (W_{peak}, LT and VO_{2max}) ($p<0.05$). However, neither fibre type composition nor MHC mRNA expression significantly changed with 12-weeks of HIIT at either the group or individual level. It is recommended that for better accuracy a minimum of 150 fibres to be used for estimation of fibre type proportions[162], and these guidelines were followed whenever possible (i.e. large enough sample) in our study, and the absolutely minimum number of fibres considered was 100. However, even though we were careful to reduce variability within the data whenever possible, it is possible that the large noise to ratio observed by these techniques might have hindered any significant changes in our cohort[163]. Further, we hypothesise that, the apparent lack of association between composition and expression may be due to the confounding influence of random error (i.e. technical error of measurement and/or biological variability)[9]. A recent study investigating repeatability of exercise-induced changes, has shown a large intra-biopsy variation, most like to due to slight changes in the site for sampling, for fibre type distribution as well as gene expression[9], this could potentially explain the poor correlation observed between our results for fibre type composition and expression, and even the mitochondrial enzyme analysis.

Growth models allowed testing as to whether an individual who started with higher baseline values had lower improvements after training, without suffering from the statistical artefact of regression to the mean that often plagues exercise training studies[6]. We found that baseline values did not affect the rate of change of any of the physiological or molecular variables. We also hypothesised that changes at the physiological level are

a consequence of changes at the molecular level, and therefore physiological changes should be associated with changes molecular changes. Surprisingly, only W_{peak} and LT changed similarly over time, meaning that changes in most variables were independent from one another, and improvement in one variable did not necessarily mean improvement in another variable. Finally, we found an interesting relationship between CS and COX. CS and COX levels were correlated at baseline, and baseline CS values were associated with changes in COX and vice versa. CS activity is closely associated with mitochondrial content, while COX activity is strongly associated with mitochondrial oxidative phosphorylation capacity[135,137]. CS activity influences the oxidation of substrates in some respirations protocols, and complex IV is part of the mitochondrial substrate oxidation[137], which could potentially explain the relationship observed in our results. COX/CS ratio has been previously reported to be a biochemical marker of mitochondrial dysfunction related to obesity in blood[164]. An increase of this ratio (i.e. energy-coupled substrate oxidation) could potentially lead to increase of ATP synthesis which in turn may be channelled toward lipid formation[165]. Based on our results exercise might be acting as a regulator of CS/COX ratio, which might represent an important mechanism regulating adipocyte formation and reducing the risk of obesity. This is an important finding, which require further exploration and validation.

3.4.6. Summary and suggestions for future studies

In summary, we successfully accounted for sources of variability that are often common to exercise studies by applying an exercise protocol of repeated measurements at regular intervals during exercise training to quantify individual responses. However, we could only estimate trainability for physiological measures of fitness, while mitochondrial markers were highly variable both between and within participants over time. We also reported a low correlation between physiological and molecular markers of fitness. Further studies utilizing the repeated testing approach in larger cohorts are needed in order to clarify the relationship between molecular and physiological responses to training. Variability between and within variables might be due to compensatory molecular mechanisms, and other associations might be occurring such as the one reported here by CS/COX, however further studies in the field are necessary to elucidate such networks.

Finally, based on our comprehensive approach, we suggest that future exercise training studies carefully consider the strengths and weaknesses of all these methods and choose the most suitable protocol addressing their scientific question. We propose to consider the following before embarking on an exercise training study:

1) *Ensure trainability is **reproducible** within the same participant* by quantifying within-subject variability using either a repeated intervention, or repeated testing during the intervention. We found that repeated testing at regular time points during the course of the intervention was the most feasible and cheapest method to implement.

2) *Ensure the **magnitude** of individual adaptations is larger than the magnitude of technical and day-to-day variability* by conducting at minimum duplicate measurements at each timepoint. Duplicate measurements quantify technical and day-to-day variability and allow obtaining accurate measurements for each individual at each time point. Then, apply an intervention with the optimum duration and intensity to trigger physiological improvements that are detectable above random "noise." Here, increasing training length from 4 to 12 weeks, with duplicate graded exercise tests every 4 weeks, was sufficient to quantify trainability.

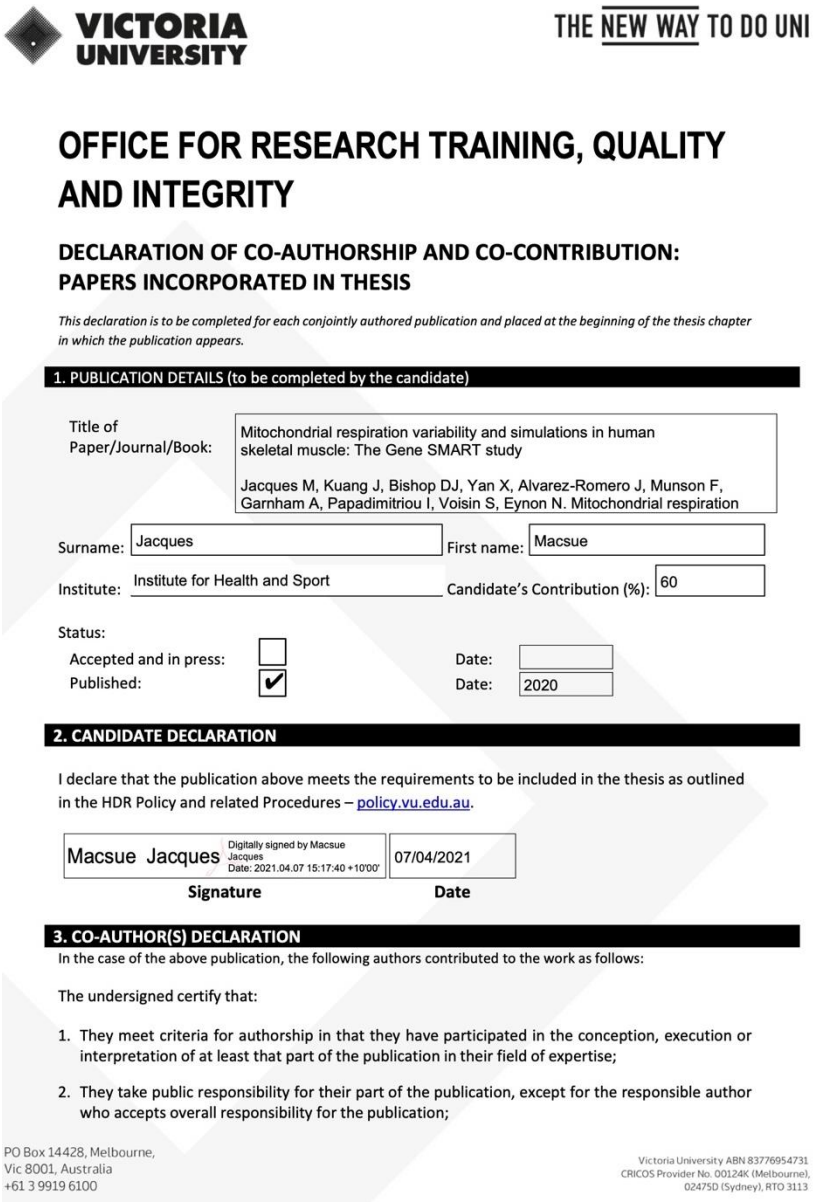
3) If possible, *use a **control group** or **control period*** (same participants) to ensure inter-individual differences in response to training are due to the training intervention. Depending on the length of the intervention, this may not be feasible for ethical reasons[10]. Both models presented similar results with SD_{true} (i.e. null or close to 0), strengthening the fact that that interventions need to be intense and long enough to overcome biological noise.

4) *Keep **statistical power** to a maximum* by avoiding dichotomising or categorising continuous, informative data into "responders"/"non-responders"/"adverse responders" and embrace continuous measures. Future studies should implement non-linear, more complex modelling of individual training responses to further increase statistical power in detecting trainability.

Chapter 4. Mitochondrial respiration variability and simulations in human skeletal muscle: The Gene SMART study

This paper is based on the following publication:

Jacques M, Yan X, Bishop DJ, Romero JA, Munson F, Kuang J, Garnham A, Papadimitriou I, Voisin S*, Eynon N*. (2019) Mitochondrial respiration variability and simulations in human skeletal muscle: the Gene SMART study. FASEB J. 2020;10.1096/fj.201901997RR.



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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

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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.

Macsue Jacques Digitally signed by Macsue Jacques Date: 2021.04.07 15:17:40 +10'00' 07/04/2021

Signature **Date**

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PO Box 14428, Melbourne, Vic 8001, Australia +61 3 9919 6100

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3. There are no other authors of the publication according to these criteria;
4. Potential conflicts of interest have been disclosed to a) granting bodies, b) the editor or publisher of journals or other publications, and c) the head of the responsible academic unit; and
5. The original data will be held for at least five years from the date indicated below and is stored at the following **location(s)**:

Name(s) of Co-Author(s)	Contribution (%)	Nature of Contribution	Signature	Date
Jujiao Kuang	5%	Optimization of experiment protocol, data colection	[redacted]	12/04/2021
David Bishop	3%	Editing and approval final manuscript.		12/04/2021
Xu Yan Andrew Garnham	5% 2%	Data collection & manuscript editing.		12/04/2021
Javier Alvarez-Romeiro	5%	Data collection & manuscript editing.		12/04/2021
Fiona Munson Ioannis Papadimitriou	5% 5%	Data collection & manuscript editing.		12/04/2021
Sarah Voisin Nir Eynon	5% 5%	Editing and approval final manuscript.		12/04/2021

Updated: September 2019

PO Box 14428, Melbourne,
Vic 8001, Australia
+61 3 9919 6100

4.1 Introduction

The mitochondrion is a membrane-enclosed organelle found in eukaryotic cells. With its five specialized complexes, it produces adenosine triphosphate (ATP) and thus constitutes the powerhouse of the cell[95]. ATP is produced during oxidative phosphorylation (OXPHOS) via the tricarboxylic acid (TCA) cycle inside the mitochondrial matrix, and via the electron transport system (ETS) along the inner mitochondrial membrane[166]. Mitochondrial respiration measured by maximal oxygen consumption in skeletal muscle fibres, is currently the primary way of assessing mitochondrial capacity[18,19]. The most common method in human skeletal muscle uses fibres permeabilized by saponin[167,168]. Using a combination of different substrates, this technique is able to mimic the processes (i.e., TCA cycle & ETS) occurring within the mitochondrion.

Regular exercise has a beneficial effect on mitochondrial function[169]. Endurance exercise training improves mitochondrial respiration[12–17], with high-intensity exercise training leading to larger improvements (up to 40%)[12,14–16] than moderate continuous exercise training (up to 20%)[170,171]. However, changes in mitochondrial respiration using permeabilized muscle fibres following exercise training have been assessed in relatively small sample sizes, typically within a range of n = 8-15[157], and without assessing respiration changes in a control group[40]. Mitochondrial respiration values, as well as improvements in mitochondrial respiration following exercise training are also quite variable between studies[12,19,157,170,172,173]. For example, Irving *et al*.[172] observed a ~1.5 fold change in mitochondrial respiration after moderate intensity endurance training (n=34), while Robach *et al*.[174] did not observe any change after a similar intervention (n=17). Mitochondrial respiration capacity in human exercise intervention studies is not exclusive to skeletal muscle samples. Although such investigations are less common, different studies have measured respiration capacity in adipose tissue[175,176], and blood cells (including lymphocytes and platelet)[177–180].

Tests run in duplicates (or more) enable researchers to capture technical variability, and/or biological day-to-day variability within participants[40]. The typical error of measurement (TE_M), also called "within-subject standard deviation", provides an estimate of such variability[40]. In the only study to date investigating the reliability of mitochondrial respiration measurement[19], the TE_M between two fibre-bundles from the

same biopsy was 10.5 $pmol.s^{-1}.mg^{-1}$ in the maximal oxidative phosphorylation (OXPHOS) and the coefficient of variations (CVs) were worryingly high (15.2% between two fibre bundles, 23.9% between legs, and 33.1% at different time points)[19]. More studies are required to confirm whether such variability is consistently high, but, more importantly, this technical variability needs to be put into perspective with typical mitochondrial respiration changes following interventions (e.g. exercise training interventions). The qualifiers "high" and "low" variability only make sense when compared with the magnitude of the intervention-induced changes, which will determine how likely we are to detect those changes.

Therefore, the aim of the present study was to investigate the reliability of mitochondrial respiration measurements in human *vastus lateralis* muscle using large number of duplicate fibre bundles (160 pairs) collected from a range of participants at different time points. We correlated mitochondrial respiration values between two chambers containing bundles of same muscle sample for complexes CI_P, $CI+CII_P$ and $CI+CII_E$, and calculated the TE_M and the CV between experiments. Using the estimated TE_M and CV, we performed simulations to determine the minimum number of participants required to detect meaningful mitochondrial respiration changes of various effect sizes following a hypothetical intervention, at 80% power.

4.2 Materials and methods

4.2.1. Participants

Participants were from the Genes and Skeletal Muscle Adaptive Response to Training (Gene SMART) cohort. The full study methodology has been previously described elsewhere[136]. 68 apparently healthy, Caucasian men (age = 31.4 ± 8.2 years old; BMI = 25.2 ± 3.2 kg/m^2) participated in the study and signed a written informed consent. Participants were excluded if they had any pre-existing heart condition, health issues and/or pre-existing injury that could potentially impair exercise capacity. The study was approved by the Human Ethics Research Committee at Victoria University (HRE13-223).

4.2.2. Muscle biopsies

A controlled diet for 48 h prior to the muscle biopsies was provided to the participants, according to the guidelines of the Australian National Health & Medical

Research Council (NHMRC). Muscle biopsies were taken by an experienced medical doctor from the *vastus lateralis* muscle of the participants' dominant leg, following local anaesthesia (2mL, 1% Lidocaine (Lignocaine)). The needle was inserted in the participant leg and manual suction was applied for muscle collection. Care was taken not to contaminate the muscle samples with local anaesthetic during the biopsy. 2-6 mg of muscle was then immediately placed in ice-cold BIOPS for determination of mitochondrial respiration in two individual chambers (duplicates).

4.2.3. Mitochondrial respiration

Immediately after each biopsy (within max 30 minutes of collection), 2-6 mg of muscle fibres were mechanically separated using pointed forceps under a binocular microscope in 2mL ice-cold biopsy preservation solution on ice (BIOPS, 2.77 mM CaK_2EGTA, 7.23 mM K_2EGTA, 5.77 mM Na_2ATP, 6.56 mM $MgCl_2 \bullet 6H_2O$, 20 mM Taurine, 15 mM Na_2Phosphocreatine, 20 mM Imidazole, 0.5 mM Dithiothreitol, and 50 mM K^+-MES at pH 7.1)[168]. Permeabilization of the plasma membrane occurred in the same solution with 50 μg/ml of saponin (Sigma-Aldrich, St Louis, USA) for 30 minutes rotating on ice. This was followed by rinsing the muscle fibres for 3 × 7 minutes in mitochondrial respiration medium (MiR05, 0.5 mM EGTA, 3 mM $MgCl_2 \bullet 6H_2O$, 60 mM K-lactobionate, 20 mM Taurine, 10 mM KH_2PO_4, 20 mM Hepes, 110 mM sucrose, and 1 g/L bovine serum albumin at pH 7.1)[168] on ice. Mitochondrial respiration was measured in duplicates in washed muscle fibers (between 1 to 3 mg wet weight of muscle fibers/chamber) in MiR05 at 37°C using the high-resolution Oxygraph-2k (Oroboros, Innsbruck,Austria), with additional substrates. Oxygen concentration (mM) and flux (pmol × s^{-1} × mg^{-1}) were recorded using DatLab software. Reoxygenation by direct syringe injection of O_2 was necessary to maintain O_2 levels between 275 and 450 mM and to avoid potential oxygen diffusion limitation. A substrate-uncoupler-inhibition tritation sequence was used. The following substrates were added (final concentration): malate (2 mM) and pyruvate (5 mM) were added to measure the LEAK respiration (L) through Complex I (CI) (CI_L), followed by $MgCl_2$ (3mM) and ADP (5 mM) to measure oxidative phosphorylation (OXPHOS) capacity (P) through CI (CI_P), followed by succinate (10 mM) to measure P through CI + Complex II (CII) linked respiration ($CI+CII_P$)[181]. This respiration state represents the maximal respiratory capacity in the respirometer chamber[181]. Cytochrome *c* (10 μM) was used to test the integrity of the outer mitochondrial membrane, in this step if the respiration increased >10% when cytochrome

c was added, values from that chamber were removed due to a damaged membrane. A series of steps (steps of 0.5 µM) p-trifluoromethoxyphenylhydrazone (FCCP) titrations followed for measurement of electron transport system (ETS) capacity (E) through CI+CII ($CI+CII_E$). Antimycin (3.75 µM) was added to block the activity of complex III and to measure the residual oxygen consumption (ROX) indicative of non-mitochondrial oxygen consumption. Substrate and coupling control ratios were calculated from the different titration steps obtained from the protocol used[169]. A background calibration for the Oroboros machine was performed every three months, and air calibrations were performed before each experiment. The results were pasted into the excel spreadsheet supplied by the manufacturer (Oroboros). If air calibrations presented more than 5% deviation in the results, membranes were changed, and new background calibration was done. Instrument backgrounds were performed in MiR05, and oxygen levels were at 450 nmol/ml. Highly variable graphs indicative of poor quality, as shown on the O2K software, were removed.

4.2.4. Citrate synthase activity

Intrinsic changes in the mitochondria can be determined by quantitative measurements of specific markers. Such measurements can estimate the content of the mitochondria and are commonly used to normalise global measurements of mitochondrial function. The most commonly used measurement is the citrate synthase (CS) enzyme activity[137]. Thus, we have normalized our results by CS activity.

Complete enzyme extractions, from small pieces of frozen tissues, were performed in an ice-cold buffer (KH_2PO_4 & K_2HPO_4) using a TissueLyser II (Qiagen, Hilden, Germany). Protein concentration was assessed using the bicinchoninic acid assay. Total citrate synthase (CS) activity was measured (30 °C, pH 7.5) using standard spectrophotometric assays. CS activity is presented in international units (IU).

4.2.5. Statistical analyses

We calculated three different metrics to show the reliability of mitochondrial respiration measurements. First, we calculated the correlation between duplicates from the two chambers, for each complex, using non-parametric Spearman's test to down-weight the influence of outliers, and a stringent p-value<0.005 for significance. Then, we calculated the within-subject standard deviation, also called typical (or technical) error of measurement (TE_M) [40]:

$$TE_M = \sqrt{\frac{\sum_1^n (x_{i1} - x_{i2})^2}{2n}}$$

where n is the number of pairs of duplicates and x is the respiration measurement. We calculated the coefficient of variability (CV) estimated by: $CV = 100 * \frac{TE_M}{\mu}$, where μ is the mean respiration across all duplicates and all samples. While TE_M is expressed in the units of mitochondrial respiration (pmol · s^{-1} · mg^{-1}), CV is a percentage.

Lastly, we performed simulations based on the TE_M for the $CI+CII_P$ and $CI+CII_E$ respiration values. We simulated increases of 1-50% in mitochondrial respiration in each participant after a hypothetical intervention and estimated the sample size (number of participants) required to detect this change at 80% power. All analyses were performed using the R software.

4.3 Results

4.3.1. Large technical error in mitochondrial respiration measurement.

Two fibre bundles from the same muscle biopsy were run simultaneously in two chambers totalling 160 duplicate pairs after removal of results that indicated damaged membrane (i.e., cyt-c increased > 10%). Respiration measurements were correlated between chambers ($R \geq 0.71$-0.75, $p<0.005$ for all – **Figure 4.1**). Yet when compared with correlations obtained for gene expression data (generally $R>0.9$)[182,183], the correlation values obtained here are rather low.

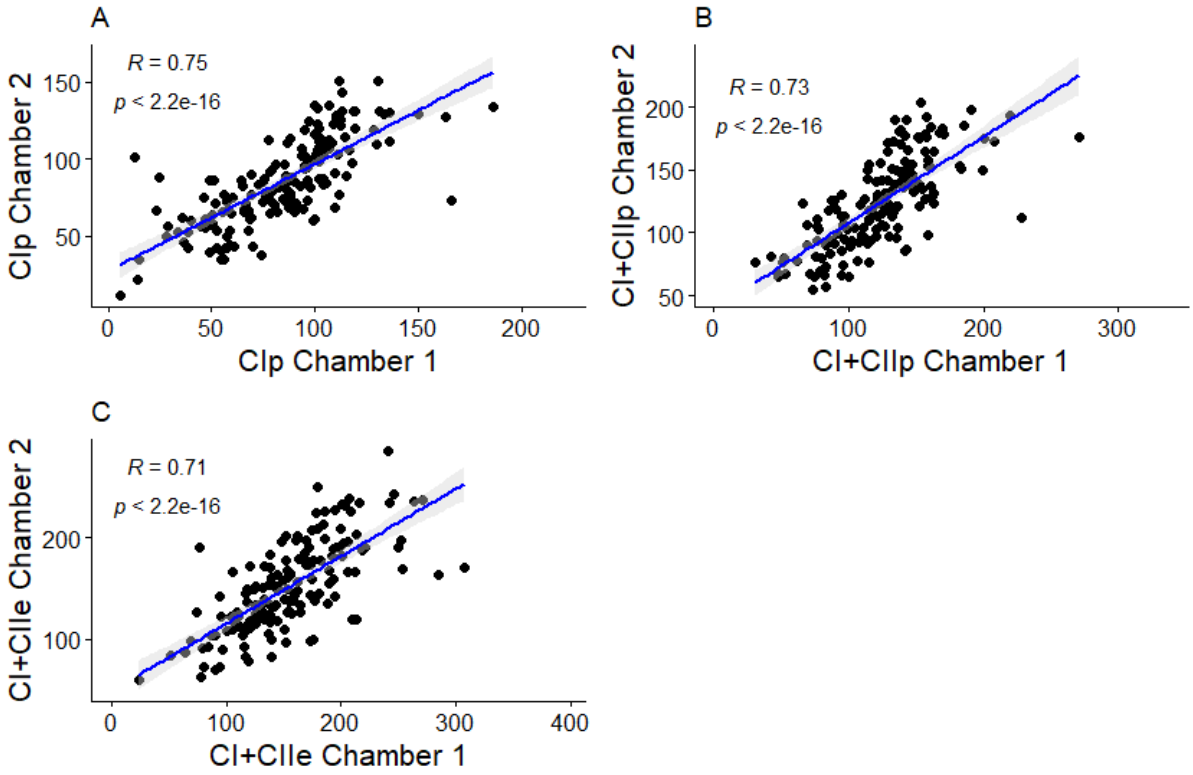


Figure 4.1. Spearman's correlation between chambers after the addition of (A) oxidative phosphorylation (OXPHOS) capacity ($_P$) through Complex I (CI_P), (B), measure P through CI+Complex II (CII) linked respiration ($CI+CII_P$), (C) electron transport system (ETS) capacity (E) through CI+CII ($CI+CII_E$).

All values are in pmol s^{-1} mg^{-1}

The poor correlation between chambers was consistent with high TE_M and CV estimates for all complexes (**Table 4.1**), as all complexes showed a CV $\geq$ 15%. When reporting the Flux Control Ratios (FCRs), to account for lab-to-lab variability[169], the TE_M and CV estimates were also significantly elevated, with some reaching more than 100%.

	Mean ± SD			
	Chamber 1	Chamber 2	TE_M	CV (%)
CI_P (pmol · s^{-1} · mg^{-1})	82.9 ± 30.6	85.1 ± 28.7	14.9	17.5 %
$CI+CII_P$ (pmol · s^{-1} · mg^{-1})	123.1 ± 39.0	123.0± 36.0	19.0	15.3 %
$CI+II_E$ (pmol · s^{-1} · mg^{-1})	154.9 ± 48.8	150.6 ± 44.1	24.4	15.9 %
LCR ($CI_L/CI+II_E$)	0.08 ± 0.08	0.09 ± 0.07	0.04	50.8%
PCR ($CI+II_P/CI+II_E$)	0.80 ± 0.12	0.82 ± 0.11	0.07	9.0%
RCR ($CI+II_P/CI_L$)	11.1 ± 31.3	11.6 ± 16.7	22.8	193.7%
Inv_RCR ($CI_L/CI+II_P$)	0.11 ± 0.09	0.11 ± 0.08	0.05	48.6%
SCR ($CI_P/CI+II_P$)	0.67 ± 0.11	0.69 ± 0.08	0.07	10.8%

Table 4.1. Chamber-specific respiration values and FCRs, typical error of measurement and coefficient of variation for each substrate.

*CI, Complex I; CI+CII, Complex I & II; L, leak respiration; P, oxphos capacity; E, ETS capacity; LCR, leak control ratio ($CI_L/CI+II_E$); PCR, phosphorylation control ratio ($CI+II_P/CI+II_E$); RCR, respiratory control ratio ($CI+II_P/CI_L$); Inv-RCR, inverse of respiratory control ratio ($CI_L/CI+II_P$); SCR, substrate control ratio at constant P ($CI_P/CI+II_P$); CI, electron input through CI; CI+II, convergent electron input through CI and CII. FCR were calculated from mass-specific mitochondrial respiration measurements in permeabilized muscle fibres (vastus lateralis).

The poor correlation along with high TE_M and CV (> 15%) estimates for all complexes, was still observed when we normalized mitochondrial respiration with CS activity, (**Table 4.2**).

	Mean ± SD			
	Chamber 1	Chamber 2	TE_M	CV (%)
CI_P*/CS**	5.38 ± 1.92	5.60 ± 1.79	1.02	18.5 %
$CI+CII_P$*/CS**	8.13 ± 2.73	8.24± 2.52	1.25	15.2 %
$CI+II_E$*/CS**	10.35 ± 3.65	10.22 ± 3.32	1.57	15.3%

Table 4.2. Chamber-specific respiration values normalized by CS activity, typical error of measurement and coefficient of variation for each substrate.

Results based on 128 muscle samples * (pmol · s^{-1} · mg^{-1}) ** (mIU × mg $protein^{-1}$)

4.3.2. Simulations to estimate the sample size required to detect changes in mitochondrial respiration at 80% power.

We performed simulations for both the CI+CII$_P$ and CI+II$_E$ respiration values since they are the most commonly reported respiration measurements in the literature, as well as the PCR and SCR ratios. We estimated the sample size required to detect true changes in mitochondrial respiration at 80% power. Since TE$_M$ and CV values for mitochondrial respiration and mitochondrial respiration/CS were similar we have not conducted simulations for the latter. However, we have attached the code for this calculation in the supplementary file of our published paper[124].

For the coupled and uncoupled respiration, the minimum sample size required to observe a percentage increase at 80% power is shown on **Figure 4.2**. An intervention that increases mitochondrial respiration by 10% at the group level requires a minimum of 23 participants to detect changes for CI+CII$_P$ and CI+CII$_E$ at 80% power. Our results suggest that with the typical sample size in exercise training studies (n = 12), only changes of >15% in mitochondrial respiration following training would be detectable at 80% power.

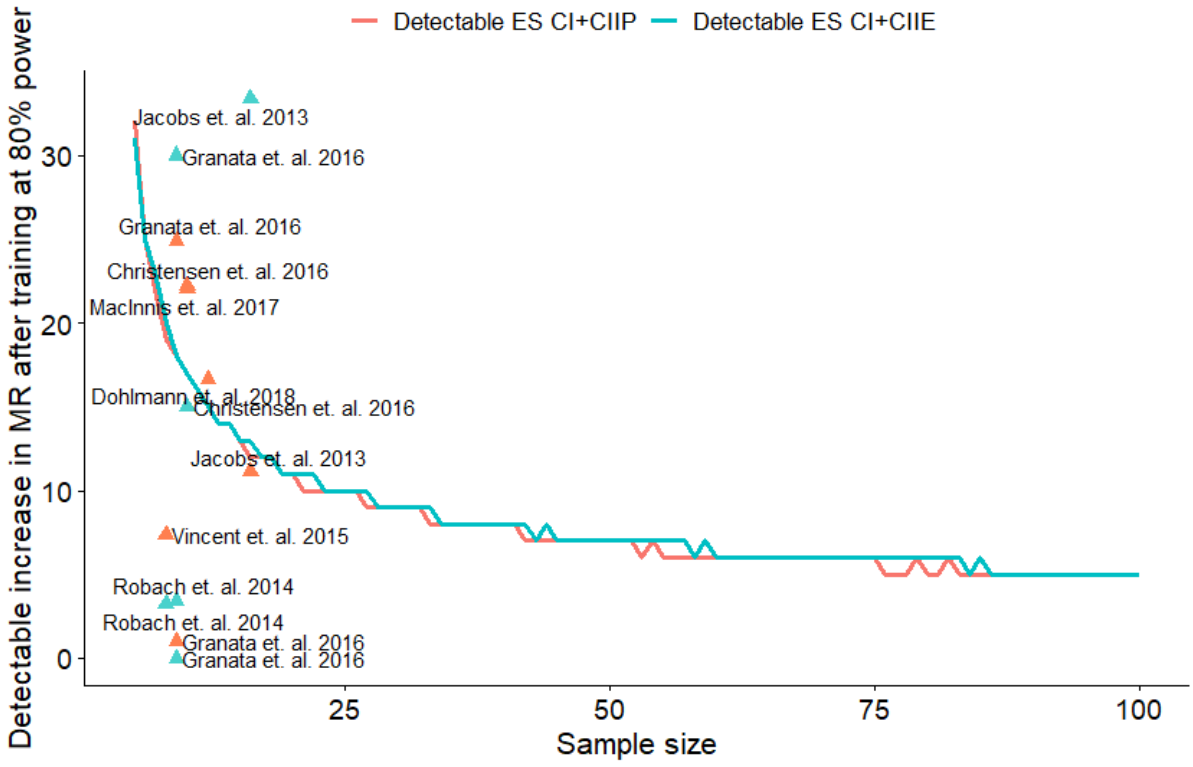


Figure 4.2. Minimum sample size required to detect increases in mitochondrial respiration (MR) after training at 80% power.

A minimum of ~65 (CI+CII$_P$) and ~75 (CI+CII$_E$) pairs of duplicate samples are necessary to detect an increase of 6% in mitochondrial respiration at the group-level, at 80% power. An intervention with ~25 samples/individuals

would require a change of at least 11% in mitochondrial respiration to achieve 80% power for both CI+CII$_P$ and CI+CII$_E$ respiration. Experiments with less than 20 individuals would require a change of at least 15-20% to achieve 80% power. The triangles represent real effect sizes and sample sizes reported in different studies: 1- MacInnis et al. 2017, 2- Granata et al. 2016, 3- Granata et al. 2016, 4- Dohlmann et al. 2018, 5- Robach et al. 2014, 6- Robach et al. 2014, 7- Jacobs et al. 2013, 8- Vincent et al. 2015, 9- Christensen et al. 2016 (changes from respective studies are reported as %)[12,14–16,170,174,184].

For the PCR and SCR respiration ratios, the minimum sample size required to observe a percentage increase at 80% power is shown on **Figure 4.3**. An intervention that increases mitochondrial respiration by 10%, at the group level, requires a minimum of 11 participants to detect changes for PCR ratio and 22 participants for the SCR ratio at 80% power.

We have also simulated percentage increases after hypothetical exercise training intervention in a cohort of 20 individuals (**Figure 4.4A**). We observed that an increase of ~11% or more in mitochondrial respiration is necessary for changes to be detected at 80% statistical power if each participant had duplicate respiration measurements. While for ratios (**Figure 4.4B**), a minimum of ~6% increase for PCR phosphorylation control ratio (CI+II$_P$/CI+II$_E$) and ~7% increase for SCR substrate control ratio at constant P (CI$_P$/CI+II$_P$) is required to be detected at 80% power, if each participant had duplicate respiration measurements.

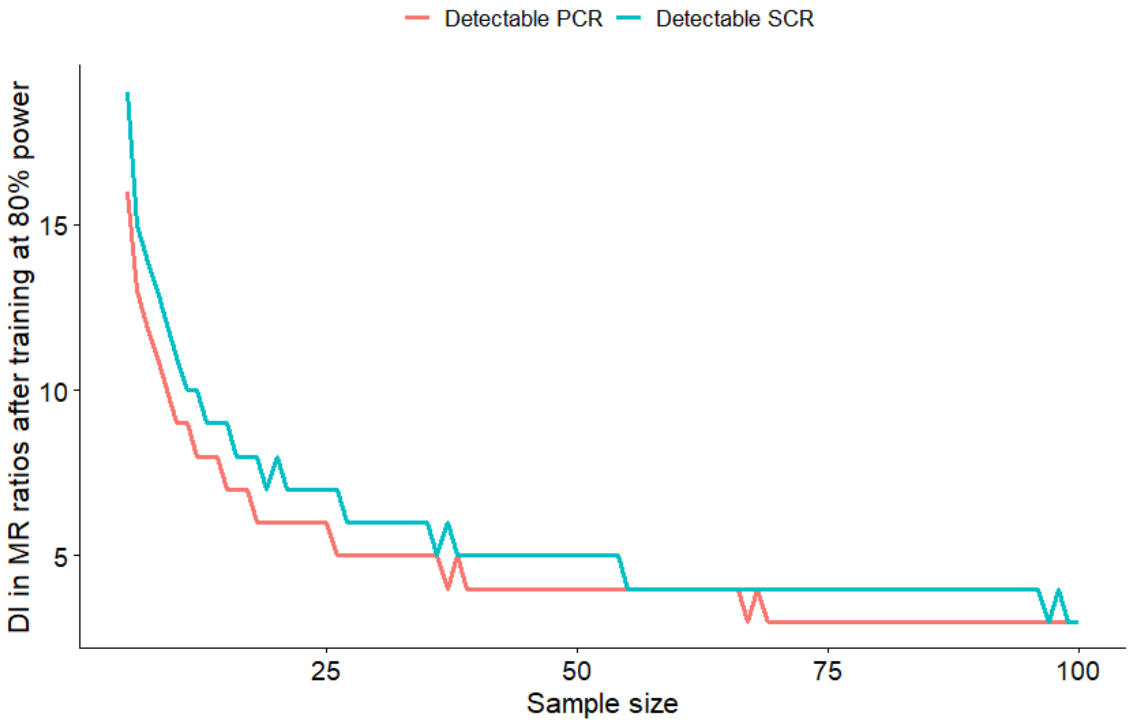


Figure 4.3. Minimum sample size required to detect increases (DI) in mitochondrial respiration (MR) ratios after training at 80% power.

A minimum of ~26 (PCR) and ~38 (SCR) pairs of duplicate samples are necessary to detect an increase of 5% in mitochondrial respiration at the group level, at 80% power. An intervention with ~20 samples/individuals would require a change of at least 6% and 8% in mitochondrial respiration to achieve 80% power for PCR and SCR ratios, respectively. Experiments with less than 20 individuals would require a change of at least 7%-10% to achieve 80% power. Due to inconsistencies in the literature in which ratios each study calculates we have not included data from published studies in our ratios graph.

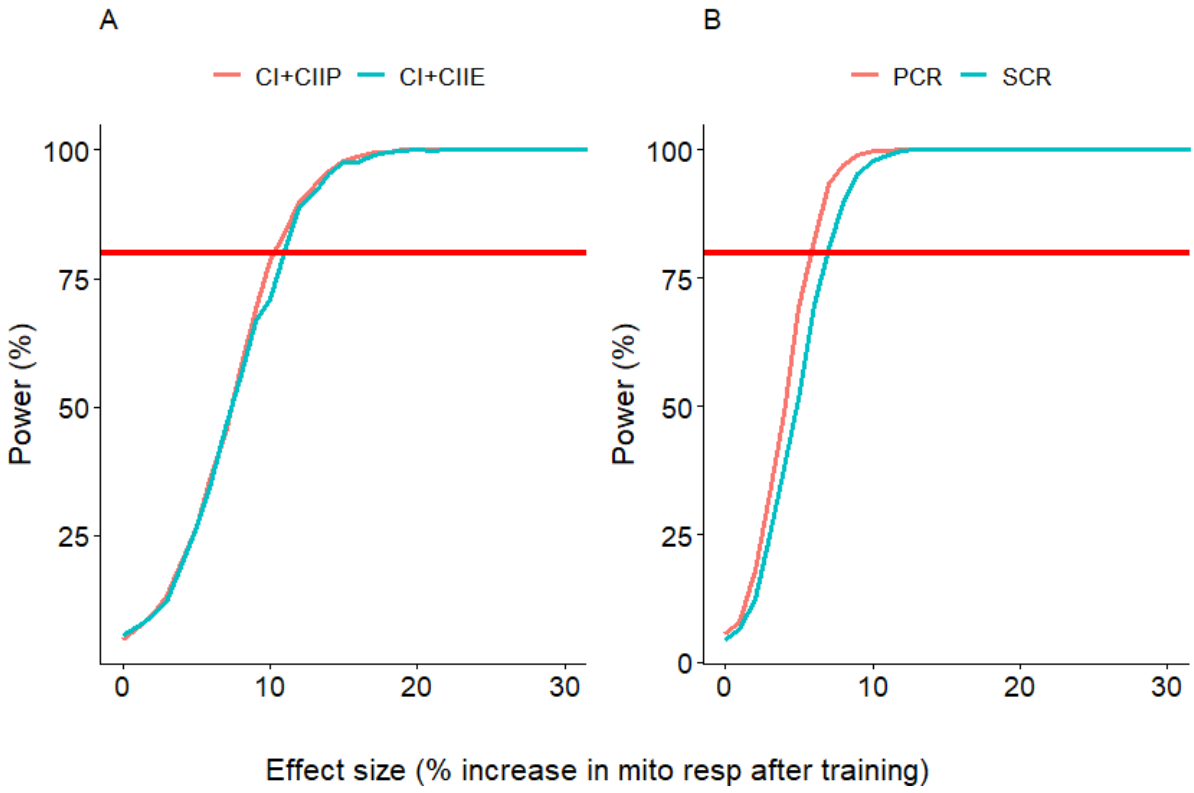


Figure 4.4. A: Power to detect percentage change in mitochondrial respiration (effect size) after a training intervention with n = 20 participants.
A minimum of ~11% increase in mitochondrial respiration is needed to be detected at 80% power for both the CI+CII$_P$ and CI+CII$_E$, if each participant had duplicate respiration measurements.

Figure 4.4 B: Power to detect percentage change in mitochondrial respiration ratios (effect size) after a training intervention with n = 20 participants.
A minimum of ~6% increase for PCR phosphorylation control ratio (CI+II$_P$/CI+II$_E$) and ~7% increase for SCR substrate control ratio at constant P (CI$_P$/CI+II$_P$) is required to be detected at 80% power, if each participant had duplicate respiration measurements.

4.4 Discussion

In the present study, we reported the TEM and CV for measurements of mitochondrial respiration for CIp, CI+CIIP, and CI+CIIE, using the OROBOROS equipment, and in a large sample (n = 160 pairs of duplicate respiration measurements). We also performed statistical simulations to uncover the minimum number of participants required to detect an intervention-induced change in mitochondrial respiration at 80% power. We found a very large variability in all measurements (CV >15%), suggesting that this measurement may only be appropriate in studies using large sample sizes (n≥55) or

that detect large effect sizes (>15%). To account for between-lab (lab-by-lab) variability, we have also computed the TEM and CV for mitochondrial respiration ratios including: LCR (L/E), PCR (P/E), RCR (P/L), Inv RCR (L/P) and SCR (constant P). Not surprisingly, the TEM and CV remained >9%, and the statistical simulations suggest a sample size of ≥26 is required to achieve 80% power. Mitochondrial respirations values were also normalized by CS activity, but no significant changes in TEM or CVs were observed. In other words, the type of training should be carefully selected to achieve effect sizes in mitochondrial respiration experiments if sample size is a limitation. For example, participants who did sprint interval training (SIT) (n=9) presented > 19% change in mitochondrial respiration after 4 weeks of training, while participants who did only moderate intensity (n=9) or high intensity interval training (n=11) did not show any changes in mitochondrial respiration after 4 weeks of training12. Higher numbers of technical replicates (i.e. number of chambers used for the same muscle - here we used two) could potentially lower the TEM, in which case the required sample size would be lower to detect a given effect size.

TEM includes variability due to machine calibration and human error, and is potentially specific to each research facility40. However, Cardinale et al.[19] recently reported a similarly high CV (15.2%), suggesting that the high variability we observed occurs across research facilities and is intrinsic to the OROBOROS equipment. Permeabilization of muscle pieces involves taking a small piece of muscle (typically 2-6 mg) and placing it in a dish with BIOPS solution; then, a technician uses two pairs of sharp forceps to separate individual fibre bundles[185]. Since this process is complicated, it is recommended that the same person performs the procedure in a given study to avoid variability between technicians[186]. The degree of fibre separation determines the amount of mitochondria present after the permeabilization, thus affecting the respiration measurements[186]. It is plausible that technicians vary in their ability to efficiently separate fibres, and this could lead to higher respiration values and potentially higher variability as well. Thus, different technical staff/researchers conducting the experiment can explain why experiments are variable. The largest variability in measurements, recently reported by Cardinale et al.[19] was in experiments conducted by two different technicians working on the same piece of muscle (mean ± SD = 31.3 ± 7.1 vs 26.3 ± 8.1 pmol · s-1 · mg-1, P = 0.12). In the present study, some of the experiments were conducted by one technician, and some by another technician (i.e. the two technicians never handled the same piece of

muscle), which might explain some of the variability we reported. Unfortunately, we are unable to calculate the variability due to technicians in this study as they worked on different muscle samples. It should also be noted that it is common to use creatine in the respiration chambers when working with permeabilized muscle. However, several papers have not used creatine in their experiments and have reported valid and replicable results[12,14,15,169].

To the best of our knowledge, the smallest meaningful change in mitochondrial respiration after training or other lifestyle interventions has never been reported, since this is dependent on the overall aim of each study[12]. In the present study, we calculated the minimum number of participants required to detect a change in mitochondrial respiration at 80% power. Our results suggest that with the typical sample size in exercise training studies (n = 12), only changes of >18% in mitochondrial respiration following training would be detectable at 80% power. This means that it would be a challenge to observe true changes in mitochondrial capacity using the OROBOROS technology, since most studies do not report such large increases following exercise training (-9% to 20%)[172,173,184,185,187–190]. We acknowledge that some of the studies have observed significant changes in mitochondrial respiration without reaching the effect sizes we presented here. This implies that although such studies were significant, the sample sizes were too low to detect the magnitude of changes they reported at 80% power. The simulations presented in this paper provide important information for planning experiments investigating mitochondrial capacity. Future studies can use this data and the code we provided (see Supplementary File 1) as a guide to determine the number of participants required to detect changes in mitochondrial respiration in their study. Alternatively, if the number of participants is a limitation, then a careful consideration of the exercise intervention duration is recommended, to trigger changes that are large enough to achieve 80% power.

In conclusion, we found very large variability in mitochondrial respiration measurements, reflected by TEM and CV, and calculated the required sample size necessary for studies aimed at detecting changes in mitochondrial respiration. We recommend that future studies utilising this method in skeletal muscle would follow the guidelines we provided here to detect significant changes in mitochondrial capacity, following lifestyle interventions. Finally, it should be noted that mitochondrial respiration is only one measure of mitochondrial function. The use of integrative approaches[95,157,185],

such as mitochondrial protein expression, mitochondrial content quantification, mitochondrial DNA sequencing, and appropriate statistical methods[40] may allow discoveries in the complex and integrative nature of exercise adaptations[157,191] as well as strengthen results from mitochondrial respiration measurements.

Chapter 5. DNA methylation patterns are associated with baseline fitness levels and change following high-intensity interval training

5.1 Introduction

Epigenetics has recently been suggested as underlying physiological adaptations to exercise training[192]. However, the field of exercise epigenetics is still in its infancy, and only a few studies have assessed genome-wide DNA methylation changes after short-term exercise training[23–28]. Reports from such studies are quite heterogeneous, since they used different exercise modalities (i.e. resistance or endurance) and length[25–27,123]. Although results were heterogeneous (i.e. some presented more hypomethylation and others more hypermethylation after exercise), the vast majority of studies did find changes in DNA methylation spread across multiple genes. Furthermore, a key study focused on candidate genes have also demonstrated that the promoter regions of key genes involved in exercise response are strongly hypomethylated immediately after strenuous exercise and become re-methylated at 3 hours post exercise[22]. However, no previous study linked DNA methylation profiles with baseline fitness or exercise training-induced fitness improvements.

The aim of this study was therefore to determine whether DNA methylation profiles are associated with physical fitness measures at baseline, and whether 4 weeks of high-intensity interval training (HIIT) is able to shift in the DNA methylation profiles consistent with higher fitness profiles. We conducted an epigenome-wide association study (EWAS) in 46 healthy young male participants from the Gene SMART study who underwent 4 weeks of HIIT. We intersected the fitness-related and exercise-induced DNA methylation sites to establish whether exercise-induced DNA methylation changes were consistent with DNA methylation patterns typically seen in highly trained individuals.

5.2 Methods

5.2.1. Participants

The study design has been described in detail in Chapter 3. To explore associations between DNA methylation patterns and measures of physical fitness or exercise training, we analysed all 46 males from the Gene SMART study who were profiled for DNA methylation patterns before and after the first 4 weeks of HIIT.

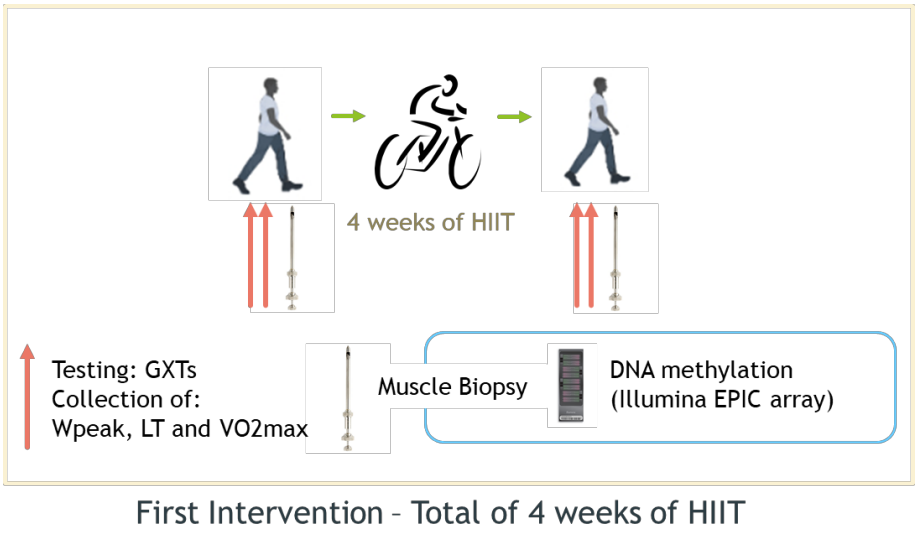


Figure 5.1. Study design

5.2.2. Muscle biopsies

Participants were provided a control diet for 48 h prior to the muscle biopsies, according to the guidelines of the Australian National Health & Medical Research Council (NHMRC). Muscle biopsies were taken by an experienced medical doctor from the vastus lateralis muscle of the participants' dominant leg, following local anaesthesia (2mL 1% Lidocaine (Lignocaine)). The needle was inserted in the participant leg and manual suction was applied for muscle collection. Excess blood from the biopsy was removed using an absorbent sheet, and muscle was then immediately frozen in liquid nitrogen and stored at -80 degrees until further analysis. Muscle biopsies were collected at 2 timepoints (before and after 4 weeks of HIIT) for DNA methylation profiling (**Figure 5.1**).

5.2.3. DNA extraction and DNA methylation analyses

DNA was extracted using the Qiagen All prep DNA/RNA kit (Cat/ID: 80204), according to manufacturer guidelines. In brief, ~15mg of muscle samples were

homogenised and separated by a column into genomic DNA and RNA, and then eluted separately. RNA was stored at -80 degrees for future analysis while dsDNA concentrations were estimated with the Invitrogen Qubit Fluorometer. DNA methylation analysis was conducted with the Illumina Infinium Methylation EPIC array (https://www.illumina.com/products/by-type/microarray-kits/infinium-methylation-epic.html) according to manufacturer protocols. In brief, DNA input amount was 500 ng for bisulfite conversion. The QC of bisulfite conversion was carried by MPS (Methylation specific PCR) on specific regions. Once QC was evaluated as good, whole genome amplification was conducted followed by Array hybridization and single base extension; finally, the array was scanned.

5.2.4 Pre-processing

DNA methylation data was pre-processed using the ChAMP analysis pipeline[193] in the R statistical software version 4.0.2. After loading the raw IDAT files into R, probes located on the sex chromosomes were removed. While the current study only focuses on males, both males and females were analysed and pre-processed together. Then, we removed probes with detection p-value > 0.01, with < 3 beads in at least 5% of samples, with missing β-values, aligning to multiple locations, and non-CpG probes. Probes mapping to single-nucleotide polymorphisms (SNPs) or located close to SNPs showing a high frequency in the Caucasian population ("EUR" population probes described in Zhou *et al.*[194]) were also removed. β-values were then obtained as follows:

$$\beta - value = \frac{intensity\ of\ the\ methylated\ allele}{intensity\ of\ the\ unmethylated\ allele + intensity\ of\ the\ methylated\ allele + 100}$$

Then, we applied β-mixture quantile normalisation (BMIQ) to normalize the distributions of Type I and Type II probes. We then performed singular value decomposition (SVD) to identify major sources of variability in the dataset. This data exploration step revealed that batch and position on the batch were significant sources of variation in the data. Therefore, data was converted to M-values according to the following formula:

$$M - value = log2(\beta/(1 - \beta))$$

and the ComBat function of the *sva* package was used to remove those technical artefacts. Finally, distributions of DNA methylation profiles and hierarchical clustering

of samples were performed as a last quality control step to ensure all unwanted sources of variability were accounted for.

5.2.5 Statistical analyses

As previously stated, we restricted this analysis to all males who were profiled for DNA methylation patterns before and after 4 weeks of HIIT (n = 46). We first converted β-values to M-values, as the latter are more homoscedastic and therefore more appropriate for differential analysis of methylation levels[195]. We used linear models and moderated Bayesian statistics as implemented in *limma*[196]. DNA methylation levels at each individual CpG were regressed against baseline fitness and other covariates. Baseline fitness was a z-score that combines W_{peak}, LT, and VO_{2max} into a single value to achieve greater power and provide a comprehensive view of whole-body changes associated with DNA methylation changes. We calculated the z-score for each fitness measure relative to body weight, and then we averaged those to obtain the final z-score. We included timepoint (PRE or POST training), as well as Age and Batch (batch #1 or batch #2) as covariates:

$$DNA\ methylation \sim Baseline\ fitness + Timepoint + Age + Batch$$

Note that here, batch does not refer to the Illumina Array, as this was adjusted in the pre-processing, but to the two groups of participants whose muscle methylomes were profiled a few years apart in different labs (i.e batch #1 or batch #2). We used the *duplicateCorrelation* function to account for the paired design (i.e. repeated measures on the same individuals before and after HIIT). CpGs associated with $Baseline\ fitness$ and $Timepoint$ at a false discovery rate (FDR) < 0.005 were considered differentially methylated positions (DMPs). If DMPs were identified, we then proceeded to identify differentially methylated regions (DMRs), i.e. contiguous clusters of DMPs showing consistent changes in DNA methylation. DMRs were identified using the *DMRcate* package[197].

To evaluate whether the shifts in DNA methylation profiles after 4 weeks of HIIT were consistent with DNA methylation patterns indicative of higher fitness, we overlapped the fitness-related DMPs with timepoint-related DMPs.

We then proceeded to identify pathways potentially altered as a result of the differential DNA methylation. We performed a gene set enrichment analysis (GSEA) to

identify enriched pathways, using the gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. We used the GOmeth method. This methodology takes into account the unequal number of CpGs mapped to different genes, but it performs a hypergeometric test on the DMPs (or CpGs in DMRs) to identify enriched pathways[198]. All pathways at FDR < 0.005 were considered significant. We used the *ggplot2*[148], *ggpubr*[147], *complexHeatmaps*[199], and *FactorMiner*[200] packages for data visualisation.

5.3 Results

5.3.1. Individuals with high aerobic fitness show a clear DNA methylation signature in skeletal muscle, related to muscle structure and function

To identify fitness-related DNA methylation patterns, we studied n = 46 males from the Gene SMART study who underwent 4 weeks of HIIT and for whom we had baseline aerobic fitness measures (W_{peak}, LT and VO_{2max}). To achieve greater statistical power, we combined all fitness measures into one comprehensive measurement (z-score – see Methods section). We found 12,170 DMPs associated with baseline fitness (FDR < 0.005), 81.8% of which were hypermethylated with higher fitness levels (**Figure 5.2**), while 1,268 DMRs were associated with baseline fitness, 85.8% of which were hypermethylated with higher fitness levels (**Table 5.1)**.

We then focused on the DMRs to remove spatial redundancy. CpG sites within ~500bp from each other are usually highly correlated[201], and therefore provide more robust and functionally important information than the DMPs[202,203]. DMRs' distribution in chromatin states was different from that of all tested CpGs ($\chi 2$ -test p-value < 2.2×10^{-16}, **Figure 5.3**). We observed a clear under-representation of DMRs at quiescent (i.e. silent) and Polycomb-repressed regions, and an over-representation at enhancers and in regions flanking active TSS. Hyper-DMRs were strongly depleted in regions of active transcription. Both hypo- and hyper-DMRs were under-represented in CpG islands, but only hyper-DMRs were over-represented outside of CpG islands ($\chi 2$ -test p-value < 2.2 x 10-16, **Figure 5.3**).

Finally, we used a comprehensive annotation of Illumina HumanMethylation arrays[194] with chromatin states from the Roadmap Epigenomics project[204] and the latest GeneHencer[205] information to map DMRs to genes (**Table 5.1)**. 1,000 unique genes

harboured at least one DMR. Of these genes, many are known to be associated with muscle function, such as the HOX family and MYOG. The differentially methylated genes were enriched for 11 gene ontologies (FDR <0.005) (**Table 5.2**) that were related to muscle structure development and muscle contraction, transmembrane receptor protein tyrosine kinase signalling, regulation of cell migration, supramolecular fiber organisation and regulation of cell adhesion. However, no enrichment for any KEGG term was identified (FDR <0.005).

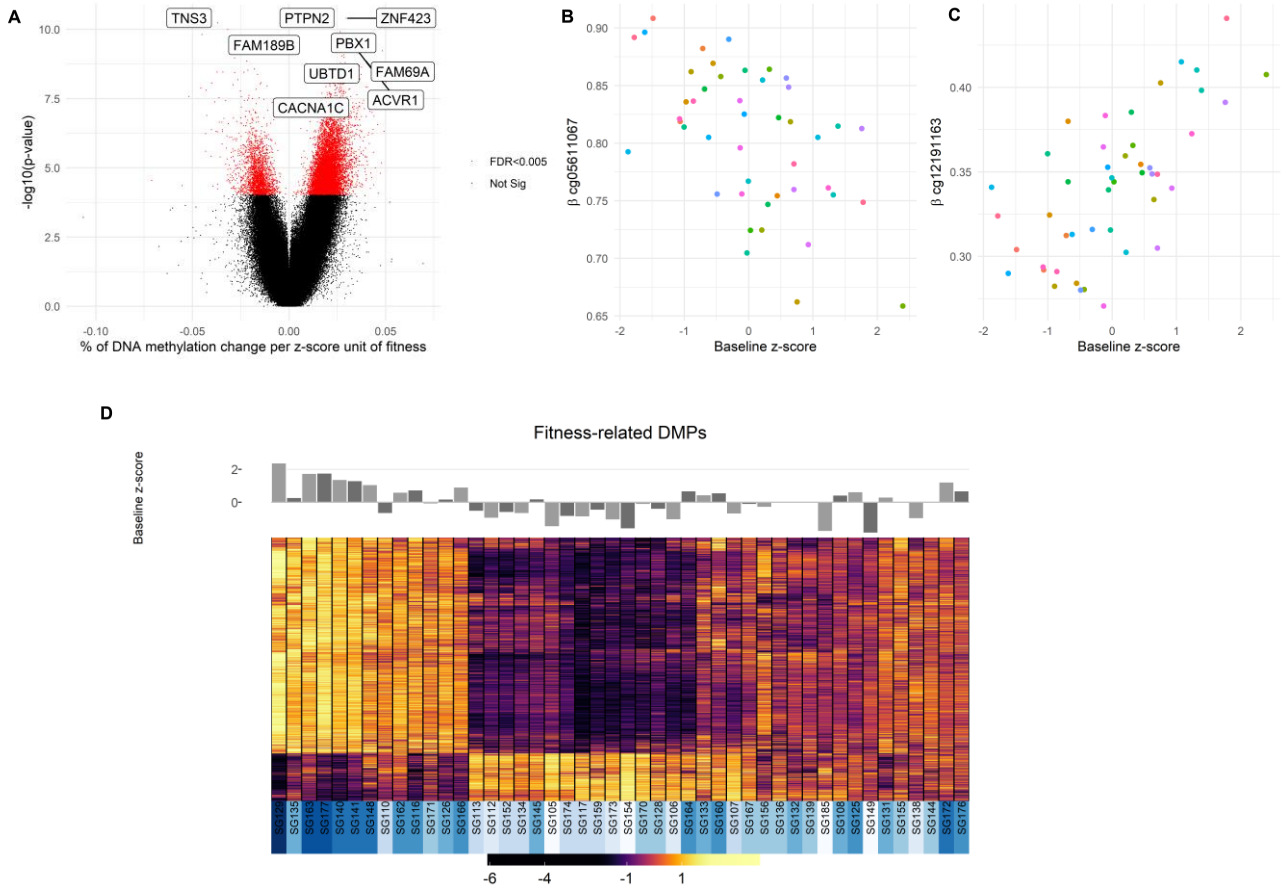


Figure 5.2. A: Volcano plot of fitness-related differential methylation. Each point represents a different CpG. Differentially methylated positions (DMPs) at false discovery rate < 0.005 appear in red. B: Top hypomethylated DMP. C: Top hypermethylated DMP. In plot B & C each colour represents a different individual. D: Unsupervised hierarchical clustering of individuals at fitness related DMPs.

Colour scale: purple represents hypomethylation and yellow hypermethylation; the units of the colour scale are arbitrary and do not represent % DNA methylation change.

Chromosome	Position start (hg38)	Position end (hg38)	Length of DMR (base pairs)	Number of CpGs in DMR	CpG island position	Chromatin state in male skeletal muscle	Annotated gene(s)	Maximum effect size in DMR	Mean effect size in DMR	Stouffer score	Harmonic mean of the individual component FDRs	Fisher multiple comparison statistic
chr13	35474754	35479270	4517	25	Shore; Island	Bivalent/Poised TSS; Flanking Active TSS; Active TSS; Weak transcription; Quiescent/Low	NBEA; MAB21L1	0.025787	0.016829	7.17E-42	0.000913	1.34E-35
chr7	27166924	27172121	5198	30	Shore; Island	Flanking Active TSS; Flanking Bivalent TSS/Enh; Bivalent/Poised TSS; Active TSS	HOXA10-HOXA9; HOXA9; HOXA10-AS; MIR196B; HOXA10	0.021663	0.012398	1.18E-29	0.004148	5.55E-25
chr3	1.28E+08	1.28E+08	2274	13	Island	NA; Bivalent Enhancer; Flanking Bivalent TSS/Enh	GATA2-AS1; GATA2; DNAJB8	0.018094	0.014172	3.22E-22	0.001243	3.55E-19
chr16	2210188	2213059	2872	19	Shelf; Shore	Genic enhancers; Flanking Active TSS	BRICD5; RP11-304L19.8; PGP	0.018623	0.012979	3.12E-21	0.004808	1.51E-18
chr2	33133991	33134620	630	11	Open sea	Flanking Active TSS; Active TSS	LTBP1	0.024924	0.016132	2.12E-20	0.001232	9.73E-18
chr17	5499562	5501827	2266	17	Shore; Island	Repressed PolyComb; Bivalent Enhancer; Flanking Bivalent TSS/Enh;	NLRP1	0.02533	0.013874	3.30E-14	0.000744	1.21E-17

						Bivalent/Poised TSS						
chr11	1838279	1839004	726	10	Open sea	Enhancers	TNNI2; LSP1, TNNI2	0.036443	0.022645	1.88E-19	0.000527	1.51E-17
chr21	45454328	45456470	2143	12	Shore; Island	Repressed PolyComb; Bivalent Enhancer; Flanking Bivalent TSS/Enh; Bivalent/Poised TSS	COL18A1	0.021817	0.013878	8.69E-18	0.000785	2.10E-17
chr5	66958090	66959943	1854	10	Open sea	Quiescent/Low; Active TSS	MAST4	0.024713	0.020398	5.78E-20	0.000823	2.34E-17
chr11	1867947	1871657	3711	18	Shelf; Shore; Island	Bivalent Enhancer; Flanking Bivalent TSS/Enh; Bivalent/Poised TSS	LSP1	0.024014	0.013143	2.73E-18	0.002501	1.03E-16
chr11	1.29E+08	1.29E+08	2280	16	Shelf; Shore; Island	Repressed PolyComb; Flanking Bivalent TSS/Enh; Bivalent Enhancer	RP11-744N12.3; FLI1	0.02051	0.012709	5.60E-16	0.002075	1.66E-16
chr21	29130409	29131153	745	8	Open sea	Flanking Active TSS	MAP3K7CL	0.024936	0.02034	1.32E-17	0.000586	1.97E-15
chr7	1708367	1709129	763	9	Open sea	Weak Repressed PolyComb	ELFN1	0.025187	0.018568	1.03E-17	0.001213	3.01E-15

chr3	1.88E+08	1.88E+08	2144	12	Shelf; Shore	Enhancers; Flanking Active TSS	BCL6	0.022107	0.015633	5.22E-18	0.003409	3.52E-15
chr11	72012928	72014543	1616	11	Open sea	Transcr. at gene 5' and 3'	RP11-849H4.4; NUMA1	0.024144	0.015696	9.20E-18	0.002014	3.90E-15
chr12	3200097	3201954	1858	9	Island; Shore	Flanking Active TSS; Active TSS; Flanking Bivalent TSS/Enh	TSPAN9	0.024198	0.014576	5.72E-17	0.002326	1.94E-14
chr5	1.41E+08	1.41E+08	1137	9	Shore; Island	Flanking Active TSS	PCDHGB5; PCDHGA6; PCDHGA12; PCDHGB6; PCDHGA7; PCDHGB7; PCDHGA8; PCDHGA9; PCDHGA1; PCDHGB1; PCDHGA2; PCDHGB2; PCDHGA3; PCDHGB3; PCDHGA4; PCDHGA10; PCDHGC3; PCDHGB4; PCDHGA5; PCDHGA11; RN7SL68P; AC008781.1	0.021786	0.017112	1.24E-16	0.001831	3.43E-14
chr16	85286252	85287275	1024	9	Shore; Island	Flanking Active TSS		0.024262	0.015722	1.64E-16	0.002115	4.34E-14
chr11	1918315	1921244	2930	17	Open sea	Flanking Active TSS	TNNT3	0.034986	0.015077	1.76E-12	0.00214	1.71E-13
chr19	13013158	13015173	2016	14	Island; Shore	Flanking Active TSS; Active TSS	NFIX; CTC-239J10.1	0.023715	0.013146	1.15E-08	0.000673	2.35E-13
chr6	30752029	30752706	678	6	Open sea	Flanking Active TSS; Enhancers	HLA-K, HLA-J, TRIM39, ABCF1, C6orf136, HCG20, VARS2, GTF2H4; PPP1R10,	0.020038	0.017981	4.67E-15	0.000495	2.73E-13

							DHX16, NRM, MDC1, IER3, LINC00243					
chr11	57540389	57543602	3214	13	Open sea	Enhancers; Flanking Active TSS; Active TSS; Genic enhancers; Transcr. at gene 5' and 3'	SMTNL1	0.043745	0.018461	6.42E-14	0.003631	4.68E-13
chr1	2.03E+08	2.03E+08	1783	10	Open sea	Transcr. at gene 5' and 3'; Active TSS; Flanking Active TSS; Enhancers	MYOG	0.024818	0.014643	2.48E-14	0.001961	5.53E-13
chr19	3369479	3370245	767	6	Island; Shore	Active TSS; Flanking Active TSS	NFIC; AC005514.2	0.021098	0.017537	1.45E-14	0.000649	7.99E-13
chr1	1.57E+08	1.57E+08	1113	7	Shore; Island	Repressed PolyComb	BCAN; RP11-284F21.10; RP11-284F21.7	0.017835	0.014972	2.06E-14	0.001096	1.01E-12
chr6	1605677	1608741	3065	14	Shore; Island	Repressed PolyComb; Bivalent Enhancer	FOXCUT	0.021209	0.01408	2.41E-14	0.004521	1.02E-12
chr7	47304006	47305151	1146	6	Open sea	Transcr. at gene 5' and 3'; Flanking Active TSS	TNS3	-0.04505	-0.02037	5.49E-14	0.000103	1.35E-12
chr16	28877425	28879164	1740	7	Shelf; Shore	Flanking Active TSS; Enhancers; Transcr. at gene 5' and 3'	ATP2A1; ATP2A1-AS1	0.029094	0.019395	2.80E-14	0.001081	1.72E-12
chr11	1.11E+08	1.11E+08	652	11	Open sea	Bivalent Enhancer	POU2AF1	-0.01998	-0.01366	1.58E-14	0.004084	1.85E-12

chr7	1.36E+08	1.36E+08	1126	10	Open sea	Enhancers; Flanking Active TSS; Weak transcription	FAM180A	0.025654	0.016217	6.52E-11	0.001484	2.51E-12
chr20	23086306	23087581	1276	12	Open sea	Flanking Active TSS; Enhancers	CD93; THBD, CD93	-0.02161	-0.01486	8.33E-15	0.004886	2.60E-12
chr13	49401246	49402336	1091	6	Open sea	Active TSS; Flanking Active TSS; Enhancers	CAB39L	0.022226	0.018262	2.08E-12	0.000163	2.61E-12
chr20	31818778	31819584	807	11	Shelf	Flanking Active TSS; Transcr. at gene 5' and 3'	MYLK2	0.025372	0.015012	1.62E-12	0.003796	4.13E-12
chr16	75266574	75268903	2330	13	Shore; Shelf	Flanking Active TSS; Enhancers; Weak transcription	BCAR1	0.022414	0.013189	1.17E-10	0.002404	4.65E-12
chr7	5495089	5496302	1214	13	Island; Shore	Flanking Active TSS	FBXL18; MIR589	0.020602	0.013678	8.03E-13	0.003912	4.90E-12
chr13	44158302	44160796	2495	8	Open sea	Flanking Active TSS; Enhancers	SMIM2-IT1; SMIM2; SMIM2-AS1	0.034547	0.022596	8.21E-14	0.003539	1.02E-11
chr16	30373962	30374828	867	5	Shelf	Enhancers; Flanking Active TSS	ZNF48, MYLPF; MYLPF; NPIPB12, GDPD3, SMG1P5	0.024435	0.02282	6.32E-13	0.00059	1.70E-11
chr11	2149181	2150463	1283	10	Open sea	Bivalent Enhancer	IGF2	0.023364	0.015798	4.34E-13	0.003877	1.95E-11
chr11	64555417	64555983	567	8	Open sea	Enhancers; Weak Repressed PolyComb	SLC22A11	0.024281	0.016176	2.20E-13	0.003144	2.28E-11

chr7	5428565	5429903	1339	6	Island; Shore	Flanking Active TSS	RP11-1275H24.1; RP11-1275H24.3	0.021589	0.019088	6.84E-13	0.001092	2.78E-11
chr14	1.03E+08	1.03E+08	1883	11	Shore; Island	Flanking Active TSS; Transcr. at gene 5' and 3'	TNFAIP2	0.018285	0.012554	1.95E-12	0.002478	3.11E-11
chr7	47582088	47583870	1783	13	Island; Shore	Active TSS; Flanking Active TSS; Enhancers	TNS3	0.02618	0.01196	2.45E-07	0.000754	4.50E-11
chr12	1.09E+08	1.09E+08	732	5	Open sea	Enhancers	CORO1C	0.02764	0.021309	4.55E-12	0.000229	4.74E-11
chr10	92833510	92834736	1227	6	Open sea	Enhancers; Active TSS	EXOC6	0.027764	0.019731	1.82E-09	0.000587	1.43E-10
chr11	1.22E+08	1.22E+08	2865	16	Open sea	Active TSS; Flanking Active TSS; Enhancers	RP11-166D19.1; MIR125B1; MIR100HG	0.025619	0.012445	1.64E-11	0.004334	1.44E-10
chr20	45826733	45827844	1112	7	Shelf	Flanking Active TSS	TNNC2	0.029903	0.023207	5.27E-12	0.002245	1.93E-10
chr4	94995818	94996334	517	5	Open sea	Enhancers; Active TSS	BMPR1B	0.022911	0.019972	8.94E-12	0.000956	2.03E-10
chr7	1.12E+08	1.12E+08	1488	10	Open sea	Flanking Active TSS; Active TSS	LSMEM1; IFRD1	0.024757	0.017756	2.78E-12	0.004524	2.56E-10
chr11	1298458	1300723	2266	11	Shelf; Open sea	Flanking Active TSS; Enhancers	TOLLIP; BRSK2	0.026169	0.016487	2.11E-09	0.001343	2.57E-10
chr14	80209930	80211503	1574	7	Open sea	Quiescent/Low; Active TSS	DIO2; DIO2-AS1	0.02876	0.01881	3.29E-11	0.000388	3.20E-10

chr2	1.05E+08	1.05E+08	814	8	Open sea	Flanking Active TSS; Enhancers	FHL2	0.032406	0.015972	2.07E-11	0.001308	3.63E-10
chr15	63595185	63597698	2514	12	Open sea; Shelf	Enhancers; Flanking Active TSS	USP3-AS1; USP3; FBXL22	0.027824	0.012432	8.87E-10	0.003961	4.52E-10
chr2	1.74E+08	1.74E+08	1612	9	Shore; Island	Flanking Active TSS; Enhancers	RP11-394I13.1; SP3, ENSG00000273258	0.018963	0.014382	9.30E-11	0.001195	5.65E-10
chr10	329307	329889	583	5	Shelf	Enhancers; Weak transcription	DIP2C	0.03367	0.022913	5.84E-11	0.000642	8.72E-10
chr6	89561619	89562772	1154	12	Open sea	Quiescent/Low; Active TSS; Flanking Active TSS	RP11-16C18.3; ANKRD6	0.01865	0.011411	3.06E-09	0.004806	1.09E-09
chr12	1.22E+08	1.22E+08	729	5	Open sea	Enhancers	HPD	0.022447	0.017866	1.42E-10	0.000521	1.14E-09
chr17	77883112	77885149	2038	7	Open sea; Shelf	Bivalent Enhancer; Weak Repressed PolyComb; Repressed PolyComb	FLJ45079	0.025695	0.014237	3.82E-10	0.001701	1.19E-09
chr18	26865169	26865823	655	6	Shore; Shelf	Active TSS	AQP4; AQP4-AS1	0.023752	0.019678	3.55E-11	0.003197	1.26E-09
chr16	58500776	58501651	876	8	Shore; Island	Bivalent Enhancer; Flanking Bivalent TSS/Enh	NDRG4	0.019632	0.012243	5.07E-09	0.001934	1.43E-09

chr6	46921997	46922774	778	6	Open sea	Weak transcription	GPR116	-0.01901	-0.01581	7.88E-11	0.000724	1.64E-09
chr13	98483288	98484325	1038	9	Open sea	Flanking Active TSS	STK24	0.017562	0.011806	8.54E-11	0.004082	1.91E-09
chr8	8381263	8383150	1888	9	Open sea; Shelf	Strong transcription; Weak transcription; Enhancers	SGK223	-0.025	-0.01139	1.55E-08	0.002238	2.28E-09
chr17	43842024	43843150	1127	6	Open sea	Enhancers; Weak transcription	MPP3	0.024127	0.01721	2.17E-10	0.001008	2.45E-09
chr22	30207118	30207470	353	5	Open sea	Bivalent Enhancer	MTMR3, CCDC157; RP3-438O4.4	0.023248	0.017267	3.10E-10	0.000647	2.52E-09
chr5	1.43E+08	1.43E+08	1301	13	Open sea	Flanking Active TSS; Enhancers	FGF1	0.023074	0.010244	3.81E-08	0.001923	2.53E-09
chr6	1.68E+08	1.68E+08	2731	11	Open sea; Shelf	Weak Repressed PolyComb; Repressed PolyComb	KIF25-AS1; KIF25	0.039523	0.017372	2.91E-10	0.00436	2.60E-09
chr15	81133405	81134478	1074	8	Shore; Island	Weak Repressed PolyComb; Bivalent/Poised TSS; Flanking Bivalent TSS/Enh; Repressed PolyComb	C15orf26	0.018462	0.012974	2.03E-09	0.000552	2.66E-09
chr9	1.22E+08	1.22E+08	1344	7	Shore; Island	Repressed PolyComb; Bivalent/Poised	LHX6	0.019848	0.011721	1.76E-08	0.001275	2.85E-09

						TSS; Bivalent Enhancer						
chr2	2.42E+08	2.42E+08	2005	9	Shelf; Shore	Bivalent Enhancer; Flanking Bivalent TSS/Enh	LOC285095; LINC01237; AC131097.4	0.020195	0.014497	1.22E-10	0.004258	2.89E-09
chr17	39193531	39195502	1972	8	Shelf; Shore	Transcr. at gene 5' and 3'; Flanking Active TSS	CACNB1	0.034895	0.016698	4.93E-10	0.00346	2.89E-09
chr4	1300994	1301289	296	6	Open sea	Enhancers; Flanking Active TSS	MAEA	0.023965	0.019764	8.41E-11	0.003921	2.90E-09
chr1	20684305	20685792	1488	7	Open sea	Active TSS; Flanking Active TSS	KIF17	-0.0188	-0.0146	1.38E-10	0.003322	2.91E-09
chr7	1.01E+08	1.01E+08	275	5	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh; Repressed PolyComb	RP11-44M6.1	0.020306	0.017709	1.45E-10	0.001912	2.92E-09
chr11	69012084	69012415	332	5	Shore	Bivalent/Poised TSS	MRGPRF; MRGPRF-AS1	0.018861	0.015548	1.77E-10	0.00113	2.95E-09
chr2	2.39E+08	2.39E+08	590	5	Open sea	Flanking Active TSS	HDAC4	0.0178	0.016493	1.65E-10	0.001695	2.99E-09
chr2	2.39E+08	2.39E+08	1694	7	Shore; Island	Enhancers; Flanking Active TSS	HDAC4	0.019292	0.012691	5.73E-08	0.00155	3.01E-09

chr2	23524055	23526216	2162	9	Shelf; Shore; Island	Flanking Active TSS; Enhancers	KLHL29; AC011239.1	0.032138	0.020429	1.20E-10	0.004138	3.04E-09
chr8	51809096	51810189	1094	10	Open sea	Flanking Active TSS; Enhancers	PXDNL	0.025342	0.012633	3.13E-09	0.002869	3.05E-09
chr1	9069427	9070035	609	5	Open sea	Active TSS; Flanking Active TSS	SLC2A5	0.028466	0.017839	7.73E-10	0.000361	3.08E-09
chr3	39192366	39193874	1509	8	Open sea	Transcr. at gene 5' and 3'; Active TSS; Enhancers; Flanking Active TSS	XIRP1	0.020253	0.014244	3.36E-09	0.001801	3.14E-09
chr6	1.39E+08	1.39E+08	465	5	Open sea	Weak transcription; Enhancers	RP11-445F6.2; TXLNB; RP1-225E12.3	0.026002	0.023432	1.65E-10	0.001838	3.15E-09
chr6	1.3E+08	1.3E+08	915	10	Open sea	Active TSS; Flanking Active TSS; Quiescent/Low	TMEM244	0.026003	0.015677	3.91E-08	0.001823	3.17E-09
chr21	45409684	45411368	1685	5	Shelf; Open sea	Flanking Bivalent TSS/Enh; Enhancers	COL18A1-AS2; COL18A1	0.024583	0.018869	2.37E-10	0.000934	3.28E-09
chr3	63967700	63968306	607	5	Open sea	Enhancers; Flanking Active TSS	PSMD6-AS2; ATXN7	0.018183	0.014844	2.50E-10	0.001105	3.31E-09
chr7	1.38E+08	1.38E+08	1014	4	Open sea	Weak transcription; Enhancers	CREB3L2; AC022173.2	0.032713	0.025703	4.39E-10	0.00036	3.67E-09

chr1	1.48E+08	1.48E+08	957	6	Open sea	Enhancers; Genic enhancers; Transcr. at gene 5' and 3'; Flanking Active TSS	BCL9	0.028073	0.021149	1.35E-10	0.00242	3.68E-09
chr13	30364272	30365833	1562	6	Open sea	Enhancers; Weak transcription; Quiescent/Low	LINC00426	-0.01764	-0.01417	2.44E-10	0.001685	3.83E-09
chr1	54152981	54154029	1049	9	Open sea	Quiescent/Low; Enhancers; Flanking Active TSS	RP11-446E24.4; CDCP2	0.02683	0.014622	1.50E-08	0.002656	3.94E-09
chr8	53945134	53945331	198	3	Open sea	Active TSS; Flanking Active TSS	RGS20	0.031202	0.027186	7.98E-10	9.17E-05	4.27E-09
chr13	74289308	74290644	1337	5	Open sea	Enhancers; Active TSS	RNY1P5	0.028429	0.021192	5.72E-10	0.000333	4.50E-09
chr5	1.76E+08	1.76E+08	1265	8	Open sea	Enhancers; Flanking Active TSS	HRH2	0.023644	0.016304	1.15E-10	0.004183	4.51E-09
chr9	1.27E+08	1.27E+08	847	5	Open sea	Enhancers	RALGPS1	0.039559	0.021049	2.09E-09	0.000661	5.63E-09
chr3	1.3E+08	1.3E+08	981	6	Open sea	Active TSS	TMCC1	0.025104	0.018245	2.82E-10	0.002597	6.00E-09
chr16	72989827	72990302	476	3	Open sea	Flanking Active TSS	ZFHX3	0.02619	0.022072	1.00E-09	0.000225	6.09E-09
chr14	34131132	34131700	569	6	Open sea	Flanking Active TSS; Enhancers	EGLN3	0.023578	0.017365	5.27E-10	0.001863	6.21E-09

chr5	34042870	34045233	2364	11	Open sea	Flanking Active TSS; Enhancers; Weak transcription	C1QTNF3; C1QTNF3-AMACR; AMACR	0.027418	0.01395	6.01E-10	0.000566	6.63E-09
chr1	1.11E+08	1.11E+08	474	6	Open sea	Weak transcription	C1orf162	-0.02328	-0.02008	3.07E-10	0.002646	6.84E-09
chr13	98307515	98308365	851	5	Open sea	Quiescent/Low; Enhancers	FARP1	-0.02144	-0.01515	8.77E-10	0.000565	6.88E-09
chr12	55719035	55720484	1450	6	Shelf; Open sea	Genic enhancers; Flanking Active TSS	RDH5, BLOC1S1; RDH5; BLOC1S1; RP11-644F5.10	0.023062	0.016086	4.47E-10	0.002611	7.21E-09
chr17	969521	970211	691	5	Open sea	Enhancers; Flanking Active TSS; Active TSS	NXN	0.025154	0.018329	5.21E-10	0.000982	7.32E-09
chr8	10350381	10351089	709	5	Open sea	Active TSS; Flanking Active TSS; NA	MSRA	0.020856	0.018303	7.99E-10	0.001303	8.52E-09
chr1	1.85E+08	1.85E+08	1567	6	Open sea	Genic enhancers; Enhancers	FAM129A	0.039404	0.027174	5.23E-10	0.002242	8.67E-09
chr19	49155290	49156041	752	6	Shelf; Shore	Transcr. at gene 5' and 3'; Active TSS; Flanking Active TSS	HRC	0.01845	0.012918	4.48E-10	0.001794	8.69E-09
chr15	90100533	90101445	913	7	Shore	Transcr. at gene 5' and 3'	IDH2; CTD-2315E11.1	0.022588	0.017625	5.79E-10	0.004163	9.55E-09
chr12	1.32E+08	1.32E+08	1206	5	Open sea	Enhancers; Flanking Active TSS	EP400	0.020608	0.015921	7.56E-10	0.001723	9.96E-09

chr21	42395575	42396976	1402	9	Open sea	Weak Repressed PolyComb	TMPRSS3	0.033839	0.017027	1.20E-09	0.00397	1.00E-08
chr16	1334624	1336288	1665	12	Island; Shore	Repressed PolyComb; Bivalent Enhancer	BAIAP3	0.020077	0.009918	6.03E-09	0.004779	1.04E-08
chr5	1.42E+08	1.42E+08	1256	5	Shore; Island	Repressed PolyComb		0.028428	0.014423	6.08E-09	0.000204	1.05E-08
chr10	29634990	29635923	934	7	Open sea	Active TSS; Flanking Active TSS	SVIL	0.021611	0.014466	1.90E-09	0.001777	1.16E-08
chr13	99312556	99312627	72	3	Open sea	Enhancers	UBAC2; DOCK9, GPR18, GPR183	0.029412	0.027	2.05E-09	0.000316	1.24E-08
chr16	85004689	85005768	1080	4	Open sea	Enhancers; Flanking Active TSS	ZDHHC7	0.026561	0.022979	1.45E-09	0.000743	1.28E-08
chr11	334250	334832	583	6	Shelf	Bivalent Enhancer; Weak Repressed PolyComb		0.023445	0.016994	1.51E-09	0.001262	1.47E-08
chr13	1.13E+08	1.13E+08	1192	9	Shore; Shelf	Bivalent Enhancer; Repressed PolyComb; Weak Repressed PolyComb	MCF2L	-0.01522	-0.01102	2.38E-08	0.003482	1.53E-08
chr17	7838807	7840091	1285	7	Shelf; Shore	Flanking Active TSS; Enhancers	KDM6B; POLR2A, EIF4A1, WRAP53, CHD3, CYB5D1, KDM6B; ZBTB4, CTC1, BORCS6, VAMP2, PER1	0.021087	0.014921	6.78E-10	0.003764	1.55E-08

chr9	91197388	91197869	482	3	Shelf	Enhancers	NFIL3, ENSG00000273381	0.024022	0.021982	3.06E-09	0.000307	1.76E-08
chr4	1.24E+08	1.24E+08	686	5	Open sea	Flanking Active TSS; Active TSS	LINC01091	0.021543	0.018237	1.25E-09	0.001914	1.76E-08
chr6	46325278	46326400	1123	8	Open sea	Enhancers	RCAN2	0.023523	0.013964	1.08E-08	0.000488	1.77E-08
chr5	1.25E+08	1.25E+08	834	6	Open sea	Active TSS; Flanking Active TSS	RP11-436H11.5; ZNF608	0.022003	0.013545	1.22E-07	0.001082	1.98E-08
chr1	2.26E+08	2.26E+08	230	4	Open sea	Active TSS	RP11-145A3.1	0.023279	0.021161	2.71E-09	0.000714	1.99E-08
chr12	52192001	52193025	1025	8	Open sea	Flanking Active TSS; Enhancers	KRT80; KRT7	0.026347	0.013773	5.88E-07	0.000494	2.18E-08
chr4	85827620	85827886	267	7	Open sea	Active TSS	ARHGAP24	0.017476	0.01278	9.25E-10	0.004197	2.29E-08
chr12	1.21E+08	1.21E+08	428	5	Open sea	Flanking Active TSS	RP11-173P15.7	0.020466	0.015781	6.43E-08	0.000842	2.31E-08
chr11	1923931	1924737	807	5	Shelf; Shore	Genic enhancers	TNNT3	0.024867	0.02363	1.46E-09	0.003235	2.51E-08
chr11	20022424	20023092	669	6	Open sea	Weak transcription; Enhancers	NAV2	0.02263	0.015253	3.73E-08	0.001396	2.70E-08
chr12	1.22E+08	1.22E+08	1309	12	Shore	Flanking Active TSS; Enhancers	RP13-941N14.1; KDM2B	0.016787	0.002081	5.12E-08	0.003376	2.72E-08

chr3	71542904	71544153	1250	6	Open sea	Flanking Active TSS; Weak transcription	FOXP1; MIR1284	0.021369	0.015015	6.11E-08	0.001377	2.74E-08
chr2	2.02E+08	2.02E+08	913	4	Open sea	Enhancers	AC007358.1	0.041744	0.026993	5.35E-09	0.000571	2.78E-08
chr6	1.68E+08	1.68E+08	1196	10	Shore; Island	Weak Repressed PolyComb; Flanking Active TSS	MLLT4-AS1; MLLT4	0.017644	0.01092	8.94E-06	0.002306	2.86E-08
chr2	47040667	47041312	646	3	Open sea	Enhancers; Flanking Active TSS	AC093732.1; TTC7A	0.0365	0.028456	5.04E-09	0.000395	2.88E-08
chr20	32031070	32032042	973	10	Island; Shore	Enhancers	RP1-310O13.7; CCM2L	0.016892	0.008712	2.46E-07	0.004151	2.98E-08
chr10	1.3E+08	1.3E+08	225	3	Shelf	Enhancers	EBF3	0.027627	0.024257	5.48E-09	0.000394	3.13E-08
chr11	1.11E+08	1.11E+08	578	4	Open sea	Quiescent/Low; Weak transcription	POU2AF1	0.029775	0.024109	3.19E-09	0.001521	3.23E-08
chr1	44617031	44618195	1165	7	Shore; Island	Bivalent Enhancer; Bivalent/Poised TSS; Weak Repressed PolyComb	RNF220	0.018887	0.013237	4.06E-08	0.00468	3.40E-08
chr5	72179283	72179638	356	4	Open sea	Weak Repressed PolyComb; Flanking Bivalent TSS/Enh	MAP1B	0.024015	0.021536	4.29E-09	0.00095	3.73E-08

chr5	1.49E+08	1.49E+08	721	3	Open sea	Genic enhancers		0.043958	0.033539	7.94E-09	0.000335	4.02E-08
chr13	1.1E+08	1.1E+08	573	4	Shore; Island	Flanking Bivalent TSS/Enh; Enhancers	COL4A2	-0.02457	-0.02013	5.92E-09	0.000835	4.10E-08
chr11	1890316	1891264	949	5	Open sea	Genic enhancers; Transcr. at gene 5' and 3'; Flanking Active TSS	PRR33; LSP1	0.02083	0.01318	2.95E-07	0.00136	4.20E-08
chr8	63469933	63471021	1089	7	Open sea	Enhancers; Weak transcription	RP11-45K10.2; CTD-3046C4.1	0.023902	0.01843	5.86E-09	0.00399	4.42E-08
chr7	2614016	2614485	470	5	Open sea	Flanking Active TSS	IQCE	0.021312	0.015054	4.43E-09	0.001488	4.51E-08
chr1	63621778	63623365	1588	7	Open sea	Enhancers; Flanking Active TSS	PGM1	0.023244	0.017697	1.51E-08	0.004724	5.00E-08
chr1	1.57E+08	1.57E+08	1365	6	Shelf; Open sea	Flanking Active TSS	RP11-284F21.8	0.022488	0.015465	8.13E-09	0.004041	5.05E-08
chr1	1.11E+08	1.11E+08	1757	7	Open sea	Enhancers; Quiescent/Low	CHI3L2; DENND2D	0.026265	0.016186	1.73E-07	0.000534	5.07E-08
chr7	1.43E+08	1.43E+08	1628	12	Shore; Island	Weak transcription; Enhancers; Flanking Active TSS; Active TSS	TMEM139; AC073342.12; CASP2	-0.015	-0.00718	1.21E-05	0.004445	5.17E-08

chr3	1.29E+08	1.29E+08	1132	5	Shore; Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer	KIAA1257; EFCC1	-0.02226	-0.01707	2.02E-08	0.000241	5.32E-08
chr13	35470722	35472439	1718	9	Island; Shore	Bivalent/Poised TSS; Active TSS	NBEA	0.030293	0.014774	4.40E-08	0.002582	5.33E-08
chr15	1.01E+08	1.01E+08	930	4	Open sea	Weak Repressed PolyComb	RP11-424I19.1	0.023537	0.018564	1.12E-08	0.00038	5.33E-08
chr1	1.55E+08	1.55E+08	1488	6	Open sea	Flanking Active TSS; Enhancers	KCNN3; ADAR, KCNN3	0.021505	0.015573	1.04E-08	0.003935	5.37E-08
chr1	1.52E+08	1.52E+08	835	6	Open sea	Flanking Active TSS; Enhancers	RORC	-0.01926	0.003135	1.24E-08	0.001412	5.45E-08
chr19	5953394	5954033	640	3	Open sea	Enhancers	FUT5, NDUFA11	0.030937	0.025341	1.02E-08	0.000443	5.46E-08
chr11	1.31E+08	1.31E+08	1202	4	Open sea	Enhancers; Flanking Active TSS	C11orf44	0.031503	0.023258	5.81E-09	0.00185	5.81E-08
chr6	1.56E+08	1.56E+08	1141	6	Shore; Island	Weak Repressed PolyComb		0.021439	0.013578	4.18E-09	0.002101	5.98E-08
chr3	1.26E+08	1.26E+08	323	3	Open sea	Enhancers		0.026658	0.023689	1.37E-08	0.000215	6.03E-08
chr7	1545722	1546419	698	8	Shore	Weak transcription	TMEM184A	-0.01276	-0.00926	1.63E-08	0.004318	6.16E-08
chr9	1.37E+08	1.37E+08	1528	4	Shelf	Enhancers; Genic enhancers	NOTCH1; INPP5E, SEC16A, NOTCH1; NALT1	-0.01725	-0.014	9.40E-09	0.001216	7.14E-08

chr14	55412327	55413018	692	4	Shore	Flanking Active TSS; Quiescent/Low	ATG14	0.030716	0.020759	1.36E-07	0.000511	7.23E-08
chr12	51820334	51821257	924	5	Shore; Island	Bivalent Enhancer; Flanking Bivalent TSS/Enh; Bivalent/Poised TSS	FIGNL2	0.018158	0.015669	6.12E-09	0.003163	8.33E-08
chr17	40551466	40552556	1091	6	Open sea	Flanking Active TSS; Enhancers		0.024586	0.014668	3.94E-08	0.001482	8.44E-08
chr6	1.13E+08	1.13E+08	635	5	Open sea	Quiescent/Low	RP1-124C6.1	0.024356	0.015801	1.04E-06	0.000515	8.55E-08
chr7	28279723	28280056	334	5	Open sea	Weak Repressed PolyComb	AC005017.2	0.027292	0.024278	6.54E-09	0.003642	9.20E-08
chr11	665385	665813	429	5	Open sea	Enhancers; Flanking Active TSS	DEAF1; RP11-754B17.1	0.022234	0.018605	6.54E-09	0.003618	9.23E-08
chr2	28664656	28665638	983	4	Open sea	Enhancers; Flanking Active TSS	ENSG00000230730; AC074011.2	0.028256	0.018721	1.06E-08	0.001791	9.34E-08
chr3	1.23E+08	1.23E+08	695	6	Open sea	Bivalent Enhancer		0.018845	0.013018	7.79E-09	0.003593	9.78E-08
chr17	35063716	35063834	119	3	Open sea	Active TSS; Flanking Active TSS	RFFL; RAD51L3-RFFL	0.019143	0.017185	1.92E-08	0.000589	1.03E-07
chr17	75557488	75557801	314	4	Shore	Enhancers	TSEN54, LLGL2	0.02225	0.018377	2.29E-08	0.000477	1.15E-07

chr12	1.29E+08	1.29E+08	749	4	Open sea	Flanking Active TSS	SLC15A4	0.022248	0.018952	1.20E-08	0.002364	1.18E-07
chr2	96895406	96895991	586	4	Open sea	Enhancers	FAM178B	0.028361	0.022314	1.33E-08	0.001967	1.22E-07
chr3	1.84E+08	1.84E+08	1579	12	Shelf; Open sea	Flanking Active TSS; Transcr. at gene 5′ and 3′	EIF4G1; EIF2B5	0.020179	0.008822	5.99E-08	0.004628	1.22E-07
chr2	1.05E+08	1.05E+08	1181	6	Shore	Flanking Active TSS; Active TSS	FHL2	0.030968	0.020349	2.66E-06	0.00114	1.22E-07
chr1	2.35E+08	2.35E+08	266	3	Open sea	Enhancers		0.024502	0.023826	2.36E-08	0.00063	1.24E-07
chr8	8461263	8462044	782	5	Open sea	Weak Repressed PolyComb	CTA-398F10.2	0.020854	0.015162	2.76E-07	0.001152	1.24E-07
chr7	1.52E+08	1.52E+08	617	4	Open sea	Enhancers; Flanking Active TSS	PRKAG2	0.021315	0.019008	1.28E-08	0.002439	1.26E-07
chr4	95524678	95525025	348	4	Open sea	Quiescent/Low	UNC5C	0.01975	0.018075	1.89E-08	0.001582	1.30E-07
chr4	7046331	7047198	868	5	Shelf	Flanking Active TSS; Transcr. at gene 5′ and 3′	RP11-367J11.2; TADA2B	0.025427	0.018274	8.72E-09	0.00409	1.31E-07
chr1	2.08E+08	2.08E+08	873	4	Open sea	Transcr. at gene 5′ and 3′; Active TSS	C1orf132	0.022623	0.016638	2.12E-08	0.000897	1.32E-07
chr7	194718	196129	1412	9	Shore; Island	Flanking Active TSS; Transcr. at gene 5′ and 3′	AC093627.12; FAM20C	0.022522	0.013848	7.51E-07	0.003087	1.33E-07

chr1	1.55E+08	1.55E+08	699	6	Open sea; Shelf	Flanking Active TSS; Enhancers	PBXIP1; PYGO2	0.025817	0.016337	9.51E-08	0.001036	1.34E-07
chr11	10899262	10899638	377	3	Open sea	Flanking Active TSS	CTD-2003C8.2; ZBED5-AS1	0.02595	0.019151	3.01E-08	0.000418	1.34E-07
chr3	15634421	15635130	710	2	Open sea	Enhancers	HACL1; BTD	0.031467	0.027603	5.12E-08	8.30E-05	1.39E-07
chr14	1.05E+08	1.05E+08	646	5	Shore	Flanking Bivalent TSS/Enh; Bivalent Enhancer	CRIP2; ENSG00000257270	0.019302	0.015321	1.60E-08	0.002838	1.46E-07
chr6	1.66E+08	1.66E+08	505	5	Open sea	Repressed PolyComb	PDE10A	0.020972	0.014239	3.31E-08	0.00158	1.48E-07
chr3	1E+08	1E+08	1022	5	Open sea	Active TSS; Flanking Active TSS	CMSS1; FILIP1L	0.021103	0.020133	1.06E-08	0.004872	1.57E-07
chr22	29062548	29062734	187	4	Open sea	Enhancers		0.021639	0.019545	2.93E-08	0.001524	1.68E-07
chr9	1.31E+08	1.31E+08	378	2	Open sea	Enhancers	FAM78A	0.022912	0.021301	6.44E-08	7.99E-05	1.70E-07
chr14	23407920	23408985	1066	4	Open sea	Enhancers	MYH6	-0.0184	-0.01457	1.84E-08	0.002429	1.73E-07
chr6	1.59E+08	1.59E+08	810	5	Open sea	Enhancers; Flanking Active TSS; Active TSS	SYTL3	0.02273	0.018502	2.35E-08	0.002784	1.73E-07
chr2	1.87E+08	1.87E+08	726	9	Shore; Island	Quiescent/Low; Active TSS	ZSWIM2	0.020667	0.010316	4.08E-07	0.000458	1.74E-07

chr19	3387812	3388322	511	4	Shore; Island	Enhancers; Flanking Active TSS	NFIC	0.021405	0.016725	1.86E-08	0.002497	1.75E-07
chr4	94584095	94584194	100	3	Open sea	Weak transcription	PDLIM5	0.025859	0.024602	3.26E-08	0.000815	1.75E-07
chr14	21034713	21035361	649	4	Open sea	Weak transcription	RNASE13; NDRG2; TPPP2	0.032294	0.027887	2.30E-08	0.001731	1.80E-07
chr19	15264471	15264638	168	8	Open sea	Genic enhancers		0.019817	0.014169	2.02E-08	0.004243	1.86E-07
chr5	91314459	91314604	146	3	Open sea	Enhancers; Quiescent/Low	LUCAT1; RP11-213H15.4	0.028254	0.020299	4.72E-08	0.000473	2.14E-07
chr17	57605040	57606327	1288	4	Open sea	Flanking Active TSS	MSI2; RP11-118E18.4	0.023586	0.017819	3.14E-08	0.001031	2.17E-07
chr4	15233582	15234506	925	4	Open sea	Enhancers; Weak transcription	RP11-665G4.1	0.021611	0.01726	3.36E-08	0.001512	2.18E-07
chr2	2.25E+08	2.25E+08	598	4	Open sea	Enhancers	CUL3	0.024566	0.017238	4.75E-08	0.000626	2.26E-07
chr3	11136299	11137111	813	8	Open sea	Weak Repressed PolyComb; Bivalent Enhancer; Flanking Active TSS	HRH1	0.019672	0.011111	2.61E-06	0.000764	2.34E-07
chr4	15478782	15479644	863	4	Open sea	Weak transcription; Flanking Active TSS	CC2D2A	0.021547	0.013818	5.95E-08	0.000481	2.37E-07

chr22	35685653	35685869	217	4	Open sea	Flanking Active TSS	RP1-41P2.7	0.047308	0.031278	2.85E-08	0.002708	2.58E-07
chr10	69416095	69417657	1563	10	Open sea	Repressed PolyComb; Weak Repressed PolyComb	TACR2	0.023227	0.011386	1.53E-07	0.004636	2.65E-07
chr15	29676891	29677515	625	5	Shore; Shelf	Repressed PolyComb	RP11-680F8.1	0.019716	0.014897	1.02E-07	0.001078	2.69E-07
chr8	1.44E+08	1.44E+08	850	4	Shore; Island	Genic enhancers; Transcr. at gene 5' and 3'	ZC3H3, EEF1D, PLEC; PLEC	0.035193	0.020176	9.65E-08	0.000409	2.69E-07
chr3	1.43E+08	1.43E+08	928	5	Open sea	Quiescent/Low	SLC9A9; RP13-635I23.3	0.022195	0.018117	6.33E-08	0.00138	2.69E-07
chr22	42695616	42695746	131	4	Shore; Shelf	Flanking Active TSS	A4GALT	0.017962	0.01738	2.99E-08	0.00289	2.75E-07
chr3	50303132	50303861	730	6	Shelf; Open sea	Flanking Active TSS	HYAL1	-0.04032	-0.02488	1.74E-08	0.004545	2.75E-07
chr13	77740659	77742014	1356	8	Open sea	Quiescent/Low	SLAIN1	0.026495	0.016627	6.27E-06	0.004961	3.01E-07
chr8	54469633	54470340	708	6	Shore; Island	Repressed PolyComb; Bivalent Enhancer; Flanking Bivalent TSS/Enh	RP11-53M11.5	0.018145	0.012093	2.14E-07	0.002101	3.32E-07
chr1	14929350	14929984	635	4	Open sea	Repressed PolyComb	KAZN	0.021979	0.018836	4.65E-08	0.001721	3.39E-07

chr22	41176919	41177171	253	5	Open sea	Genic enhancers		0.020016	0.018316	2.69E-08	0.004771	3.42E-07
chr7	1.01E+08	1.01E+08	1598	11	Shore; Island	Flanking Active TSS; Active TSS; Flanking Bivalent TSS/Enh	SLC12A9; TRIP6; MIR6875	0.01596	0.008716	0.000145	0.001913	3.53E-07
chr8	1745054	1745849	796	4	Open sea	Weak transcription; Enhancers	CLN8	0.02672	0.019397	6.08E-08	0.001503	3.67E-07
chr16	1043822	1044812	991	4	Open sea	Repressed PolyComb; Bivalent Enhancer		0.022128	0.015528	7.16E-08	0.000861	3.87E-07
chr2	1816396	1817328	933	4	Open sea	Enhancers; Flanking Active TSS	MYT1L	0.020398	0.016508	4.89E-08	0.002362	4.01E-07
chr1	2.04E+08	2.04E+08	77	2	Shelf	Enhancers		0.031517	0.028322	1.54E-07	0.00013	4.02E-07
chr11	10454694	10455428	735	5	Shelf; Open sea	Flanking Active TSS; Enhancers	AMPD3; AMPD3, CTR9	0.019396	0.016759	4.15E-08	0.004277	4.11E-07
chr8	60853950	60854509	560	3	Open sea	Enhancers	CHD7	0.025646	0.020543	7.92E-08	0.001162	4.15E-07
chr12	15984267	15984533	267	3	Open sea	Weak transcription	DERA	-0.02426	-0.01824	9.85E-08	0.000599	4.20E-07
chr1	77888095	77889980	1886	13	Shore; Island	Active TSS; Flanking Active TSS	NEXN-AS1; NEXN	0.024558	0.007658	9.28E-06	0.004009	4.43E-07

chr13	95311122	95311712	591	2	Open sea	Quiescent/Low	RNY3P8	0.030658	0.028887	2.02E-07	6.03E-05	4.53E-07
chr7	6015885	6016686	802	3	Open sea	Genic enhancers; Transcr. at gene 5' and 3'	AIMP2; SNORA42	0.021023	0.018669	9.74E-08	0.000854	4.63E-07
chr11	61879486	61880659	1174	5	Open sea	Strong transcription; Enhancers	FADS3; MIR6746	0.018674	0.011765	3.55E-07	0.001312	4.68E-07
chr11	33912476	33912833	358	3	Open sea	Enhancers	LMO2	0.023377	0.017759	1.39E-07	0.000381	4.69E-07
chr10	29369729	29370183	455	4	Open sea	Bivalent Enhancer		0.024368	0.018998	7.37E-08	0.001612	4.77E-07
chr4	40334237	40335425	1189	8	Open sea	Enhancers; Quiescent/Low	CHRNA9	0.028189	0.013665	1.06E-06	0.000557	4.78E-07
chr6	6320090	6321194	1105	9	Open sea	Bivalent/Poised TSS; Repressed PolyComb	F13A1	0.024668	0.011421	3.21E-07	0.002071	4.93E-07
chr5	39219132	39220046	915	4	Open sea	Active TSS; Enhancers; Weak transcription	FYB	0.026676	0.016661	3.47E-06	0.000447	4.98E-07
chr2	1.79E+08	1.79E+08	212	3	Open sea	Quiescent/Low	AC093911.1	0.020111	0.019447	9.68E-08	0.001228	5.01E-07
chr16	2766723	2767978	1256	8	Island; Shore	Strong transcription; Genic enhancers	SRRM2	0.022218	0.013206	7.32E-06	0.004294	5.20E-07
chr2	1.59E+08	1.59E+08	461	5	Open sea	Weak Repressed PolyComb	DAPL1	0.017613	0.01333	1.96E-06	0.00113	5.36E-07

chr12	1.32E+08	1.32E+08	911	5	Shore; Island	Repressed PolyComb	RP13-977J11.9	0.016758	0.01221	6.99E-08	0.003724	5.37E-07
chr15	81225199	81225416	218	5	Open sea	Flanking Active TSS; Active TSS	IL16	0.018484	0.014863	1.57E-07	0.001882	5.39E-07
chr2	2.31E+08	2.31E+08	1266	3	Open sea	Enhancers		0.023412	0.01974	1.14E-07	0.000933	5.40E-07
chr6	1.1E+08	1.1E+08	730	5	Open sea	Active TSS; Flanking Active TSS; Enhancers	DDO	0.029658	0.015225	3.97E-05	0.00093	5.50E-07
chr2	46844978	46845767	790	5	Open sea	Quiescent/Low; Enhancers	LINC01119	-0.02665	-0.01401	8.66E-08	0.003722	5.51E-07
chr7	1.43E+08	1.43E+08	718	4	Open sea	Weak Repressed PolyComb	C7orf34	0.02015	0.015715	6.98E-08	0.002859	5.55E-07
chr3	52830677	52831900	1224	4	Open sea	Genic enhancers; Flanking Active TSS	ITIH4; RP5-966M1.6	0.021344	0.017451	1.07E-07	0.000952	5.60E-07
chr5	41794070	41795039	970	6	Open sea	Quiescent/Low; Enhancers	OXCT1	0.025817	0.013493	3.37E-06	0.000726	5.63E-07
chr17	18951103	18951416	314	4	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh	SLC5A10	0.024188	0.019943	6.68E-08	0.003397	5.65E-07
chr8	1.25E+08	1.25E+08	903	3	Open sea	Enhancers; Flanking Active TSS	RP11-1082L8.4	0.019339	0.017563	1.29E-07	0.000694	5.76E-07

chr7	7535911	7537160	1250	5	Open sea	Flanking Active TSS; Quiescent/Low	COL28A1	0.027426	0.012752	1.11E-06	0.000972	5.83E-07
chr17	50101874	50102094	221	4	Open sea; Shelf	Flanking Active TSS	PDK2; RP5-875H18.4	0.017652	0.015939	9.83E-08	0.002348	5.90E-07
chr13	1.13E+08	1.13E+08	1537	9	Open sea; Shelf	Weak transcription; Enhancers	ATP11A; ATP11A-AS1	0.024919	0.011009	1.93E-05	0.001674	6.26E-07
chr3	31703702	31705097	1396	8	Open sea	Weak transcription; Enhancers	OSBPL10; OSBPL10-AS1	0.017505	0.011528	2.22E-06	0.004556	6.27E-07
chr11	12743761	12744890	1130	4	Open sea	Enhancers	TEAD1	0.022357	0.018823	8.05E-08	0.003115	6.48E-07
chr9	14779578	14780006	429	4	Open sea	Flanking Active TSS	FREM1	0.02313	0.0213	7.95E-08	0.003146	6.49E-07
chr17	15264590	15265859	1270	5	Shore; Shelf	Flanking Active TSS; Weak transcription	RP11-849N15.1; PMP22	0.022143	0.014737	2.45E-07	0.001235	6.76E-07
chr7	1.5E+08	1.5E+08	365	3	Open sea	Flanking Bivalent TSS/Enh; Bivalent Enhancer	GIMAP8; GIMAP6	-0.01908	-0.01658	1.46E-07	0.00097	6.80E-07
chr10	95273414	95273913	500	3	Open sea	Flanking Active TSS	PDLIM1	-0.03075	-0.0251	1.45E-07	0.001019	6.81E-07
chr14	1.03E+08	1.03E+08	932	5	Open sea	Transcr. at gene 5' and 3'	CDC42BPB	0.019954	0.016386	6.86E-08	0.004299	6.84E-07

chr1	1033661	1034444	784	5	Island	Bivalent/Poised TSS; Bivalent Enhancer; Flanking Bivalent TSS/Enh	AGRN	0.019881	0.01534	1.58E-07	0.002574	7.01E-07
chr1	2.02E+08	2.02E+08	1066	3	Open sea	Enhancers; Flanking Active TSS	SHISA4; IPO9	0.024708	0.02027	1.68E-07	0.00098	7.31E-07
chr15	55916651	55917307	657	3	Open sea	Active TSS	NEDD4	0.021256	0.019092	1.69E-07	0.00087	7.49E-07
chr11	78063290	78063997	708	4	Open sea	Enhancers; Flanking Active TSS	KCTD14; NDUFC2-KCTD14; THRSP	0.025716	0.019709	8.41E-08	0.004306	7.58E-07
chr5	37668652	37668707	56	2	Open sea	Enhancers	WDR70	0.026885	0.026534	2.99E-07	0.000206	7.74E-07
chr3	54358471	54359479	1009	4	Open sea	Enhancers		0.022844	0.02124	1.23E-07	0.001411	7.80E-07
chr18	58255889	58257655	1767	4	Island; Shore	Active TSS	NEDD4L	0.025109	0.020563	1.24E-07	0.002022	8.50E-07
chr11	69466908	69467528	621	4	Open sea; Shelf	Weak Repressed PolyComb; Enhancers	AP000439.5	-0.01836	-0.01098	3.68E-07	0.00177	8.52E-07
chr21	42467221	42467579	359	3	Open sea	Flanking Active TSS		0.021717	0.017821	1.96E-07	0.000796	8.55E-07
chr12	6376206	6378510	2305	14	Open sea	Enhancers; Flanking Active TSS	LTBR; SCNN1A	0.028352	0.012513	0.002726	0.002998	9.25E-07

chr22	35895682	35895702	21	3	Open sea	Flanking Active TSS	RBFOX2	0.026519	0.02378	1.93E-07	0.001273	9.32E-07
chr8	23573310	23573395	86	3	Shore	Enhancers		0.017244	0.016304	2.18E-07	0.001175	9.75E-07
chr15	99438780	99439378	599	5	Open sea	Enhancers	ENSG00000259341	-0.02217	-0.01838	1.33E-07	0.003619	9.81E-07
chr1	2.23E+08	2.23E+08	700	4	Open sea	Enhancers		0.024242	0.017863	1.37E-07	0.003096	9.83E-07
chr8	98399462	98400034	573	3	Open sea	Repressed PolyComb; Bivalent Enhancer		0.022184	0.018845	2.23E-07	0.001146	1.03E-06
chr3	1.22E+08	1.22E+08	601	6	Open sea	Active TSS	FBXO40	0.017046	0.012351	2.32E-07	0.002413	1.05E-06
chr8	1.21E+08	1.21E+08	600	4	Shore	Weak transcription	RP11-713M15.2; SNTB1	0.037523	0.027047	1.75E-07	0.00235	1.07E-06
chr17	29138560	29139755	1196	3	Open sea	Transcr. at gene 5' and 3'; Flanking Active TSS	MYO18A; RP11-321A17.4	0.018911	0.016496	2.44E-07	0.001256	1.11E-06
chr2	1.28E+08	1.28E+08	332	3	Open sea	Enhancers	MYO7B	-0.02445	-0.01958	3.14E-07	0.000561	1.14E-06
chr12	1.24E+08	1.24E+08	259	4	Shore	Enhancers; Flanking Active TSS	NCOR2	-0.01745	-0.01638	1.49E-07	0.003361	1.15E-06
chr18	22176620	22177506	887	5	Shore; Island	Repressed PolyComb	GATA6; RP11-627G18.2	0.020722	0.012699	6.64E-07	0.003891	1.16E-06
chr15	67915470	67915812	343	3	Open sea	Enhancers	CALML4	0.022232	0.017915	3.35E-07	0.000406	1.16E-06

chr7	2760561	2761613	1053	5	Shelf; Shore	Flanking Active TSS; Enhancers	GNA12; AMZ1	0.017095	0.014209	1.47E-07	0.003329	1.18E-06
chr1	2256322	2257587	1266	5	Open sea	Enhancers; Flanking Active TSS	SKI	0.022168	0.007271	5.43E-05	0.002734	1.19E-06
chr11	33892540	33893098	559	3	Open sea	Weak transcription	LMO2	0.036737	0.028273	2.47E-07	0.001537	1.20E-06
chr18	49726418	49726587	170	2	Open sea	Weak transcription		0.036265	0.034169	4.77E-07	0.000241	1.21E-06
chr22	27124391	27124826	436	2	Open sea	Repressed PolyComb		0.018801	0.018111	4.91E-07	0.000244	1.24E-06
chr17	78224526	78224873	348	5	Open sea	Bivalent Enhancer	BIRC5	0.019798	0.017211	1.34E-07	0.004853	1.25E-06
chr17	82871151	82871727	577	4	Shore; Island	Genic enhancers	ZNF750; TBCD	-0.01591	-0.01495	1.87E-07	0.002622	1.25E-06
chr18	62610374	62611214	841	4	Open sea	Enhancers; Flanking Active TSS; Flanking Bivalent TSS/Enh		0.015102	0.012873	7.25E-07	0.00085	1.26E-06
chr10	1.19E+08	1.19E+08	1346	6	Shore	Enhancers	NANOS1	0.028397	0.018353	4.80E-07	0.004219	1.27E-06
chr5	5658422	5658528	107	2	Open sea	Flanking Active TSS; Enhancers		0.025857	0.024096	5.40E-07	0.00017	1.29E-06
chr1	1.6E+08	1.6E+08	468	5	Open sea	Repressed PolyComb	SLAMF9	0.028498	0.019286	1.60E-06	0.001287	1.29E-06
chr2	2.41E+08	2.41E+08	191	3	Shore	Flanking Active TSS	SNED1	0.022027	0.018981	3.01E-07	0.001304	1.32E-06

chr7	1567509	1568150	642	6	Shelf; Shore	Genic enhancers	PSMG3; PSMG3-AS1	0.020499	0.01648	2.48E-07	0.004881	1.33E-06
chr14	64806680	64806902	223	4	Open sea	Enhancers	SPTB	0.023799	0.014943	3.51E-06	0.000855	1.35E-06
chr17	47847521	47848237	717	4	Island	Bivalent Enhancer; Repressed PolyComb	SP6	0.015796	0.012944	2.20E-07	0.001832	1.36E-06
chr8	686370	687467	1098	4	Shore; Island	Weak Repressed PolyComb; Bivalent Enhancer; Flanking Bivalent TSS/Enh	ERICH1; ERICH1, ENSG00000254207	0.026244	0.020934	3.57E-07	0.002484	1.39E-06
chr16	67346330	67347295	966	3	Open sea	Quiescent/Low	LRRC36	-0.01799	-0.01187	3.48E-07	0.001147	1.40E-06
chr17	77834635	77834717	83	2	Open sea	Flanking Active TSS		0.024124	0.022215	5.58E-07	0.000287	1.43E-06
chr16	20408899	20409393	495	4	Open sea	Quiescent/Low	ACSM5	0.019346	0.017391	2.28E-07	0.003166	1.52E-06
chr2	23563717	23564799	1083	3	Shore; Shelf	Enhancers; Flanking Active TSS	KLHL29	0.028465	0.021853	4.94E-07	0.000499	1.58E-06
chr2	96898763	96898944	182	4	Open sea	Enhancers	FAM178B	0.021259	0.017904	2.28E-07	0.003292	1.60E-06
chr1	44642882	44643009	128	3	Open sea	Genic enhancers; Transcr. at gene 5' and 3'	TMEM53; RNF220	0.029615	0.022977	3.30E-07	0.001836	1.60E-06

chr11	34346323	34346845	523	3	Open sea	Enhancers; Flanking Active TSS	ABTB2	0.021959	0.020122	4.34E-07	0.000838	1.64E-06
chr16	4052291	4053531	1241	10	Open sea	Enhancers; Flanking Active TSS	ADCY9	0.026359	0.013676	1.07E-05	0.00385	1.64E-06
chr2	2.02E+08	2.02E+08	298	5	Open sea	Weak Repressed PolyComb		-0.01772	-0.01276	6.02E-07	0.003674	1.65E-06
chr22	42770132	42770340	209	3	Island; Shore	Flanking Bivalent TSS/Enh		0.01453	0.013696	3.58E-07	0.001648	1.67E-06
chr3	12350680	12351521	842	4	Open sea	Quiescent/Low	PPARG	0.025086	0.016606	0.000376	0.000531	1.70E-06
chr11	35083330	35084102	773	3	Open sea	Enhancers; Flanking Active TSS	CD44	0.031978	0.02346	5.50E-07	0.00072	1.72E-06
chr1	2.23E+08	2.23E+08	792	7	Shore	Flanking Active TSS; Enhancers	TLR5; RP11-239E10.2	0.02647	0.012686	1.07E-05	0.00186	1.77E-06
chr17	12549740	12549962	223	4	Open sea	Quiescent/Low	LINC00670	0.021089	0.017384	2.55E-07	0.004102	1.87E-06
chr12	26523845	26523915	71	2	Open sea	Active TSS	ITPR2	0.024618	0.023873	7.57E-07	0.000334	1.92E-06
chr21	27423296	27423301	6	2	Open sea	Weak transcription	AP001604.3	0.021515	0.021256	7.59E-07	0.000331	1.92E-06
chr1	1.46E+08	1.46E+08	750	5	Shelf	Active TSS; Weak transcription	HFE2	0.024006	0.018165	2.44E-07	0.002993	1.93E-06

chr10	78248477	78248879	403	3	Open sea	Active TSS	LINC00856	0.019978	0.017714	4.52E-07	0.001406	1.96E-06
chr9	35673720	35674002	283	4	Shore	Enhancers; NA	CA9; ARHGEF39	0.018627	0.015955	2.64E-07	0.00449	2.02E-06
chr6	43016552	43016956	405	4	Shelf	Flanking Active TSS	KLHDC3	0.014919	0.013598	3.07E-07	0.003033	2.04E-06
chr11	6362750	6363069	320	4	Open sea	Enhancers; Flanking Active TSS	SMPD1; RP11-304C12.3	0.019957	0.017346	4.37E-07	0.002215	2.04E-06
chr17	83081449	83081943	495	4	Island; Shore	Flanking Bivalent TSS/Enh	METRNL	0.017415	0.013872	7.66E-07	0.002021	2.06E-06
chr7	1.51E+08	1.51E+08	897	4	Shore; Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer	NOS3; ATG9B; KCNH2, ENSG00000244151, ATG9B	0.016214	0.012767	3.33E-07	0.003607	2.08E-06
chr7	93043169	93043700	532	4	Shore; Island	Weak Repressed PolyComb; Active TSS; Quiescent/Low		0.021365	0.014335	1.31E-05	0.001509	2.13E-06
chr16	65071677	65072381	705	4	Open sea	Flanking Active TSS; Enhancers	CDH11	0.020719	0.018591	2.81E-07	0.004806	2.13E-06
chr7	4714870	4715709	840	6	Shelf	Flanking Active TSS	FOXK1	0.02479	0.013652	0.001188	0.00125	2.13E-06
chr17	80858842	80859683	842	4	Shore	Genic enhancers; Enhancers; Flanking Active TSS	RPTOR	0.019699	0.014869	5.16E-07	0.001711	2.13E-06
chr16	48393926	48394104	179	3	Open sea	Flanking Active TSS	SIAH1	0.020604	0.020153	4.45E-07	0.002184	2.16E-06

chr1	93614126	93614812	687	6	Open sea	Active TSS; Flanking Active TSS	BCAR3	0.0195	0.012997	7.52E-06	0.003354	2.18E-06
chr19	45216940	45217817	878	5	Shore; Island	Repressed PolyComb; Bivalent Enhancer	EXOC3L2; MARK4	0.019755	0.013261	7.63E-06	0.002279	2.20E-06
chr7	1.4E+08	1.4E+08	576	3	Open sea	Flanking Active TSS; Quiescent/Low	SLC37A3	0.019735	0.018981	7.52E-07	0.000834	2.21E-06
chr17	81036657	81037741	1085	5	Shore	Enhancers; Flanking Active TSS	BAIAP2	0.024491	0.014721	4.74E-07	0.003735	2.21E-06
chr2	55133707	55134173	467	3	Open sea	Flanking Active TSS		0.019507	0.016929	5.53E-07	0.001315	2.22E-06
chr18	10122233	10122237	5	2	Open sea	Flanking Active TSS		0.027992	0.026219	8.82E-07	0.000364	2.23E-06
chr14	75453464	75453494	31	2	Open sea	Flanking Active TSS	JDP2	0.024402	0.023152	9.02E-07	0.000329	2.24E-06
chr9	1.3E+08	1.3E+08	733	4	Island; Shore	Active TSS; Flanking Active TSS	C9orf78; USP20	0.020304	0.01277	0.000111	0.000938	2.27E-06
chr2	1.02E+08	1.02E+08	835	5	Open sea	Flanking Active TSS; Weak transcription	AC007271.3	0.022305	0.014935	1.23E-05	0.002375	2.28E-06
chr9	1.12E+08	1.12E+08	74	2	Open sea	Enhancers	RNU6-710P	0.020678	0.019227	9.19E-07	0.000338	2.29E-06

chr16	2036844	2037930	1087	4	Island; Shore	Transcr. at gene 5' and 3'	SLC9A3R2	-0.0202	-0.01334	4.12E-07	0.003194	2.36E-06
chr11	69545633	69546198	566	4	Open sea	Repressed PolyComb		0.018685	0.015475	5.01E-07	0.003229	2.36E-06
chr12	49912331	49913102	772	5	Open sea	Weak Repressed PolyComb	RP11-70F11.8; RP11-70F11.11	0.023123	0.016042	5.22E-06	0.003514	2.37E-06
chr2	1.05E+08	1.05E+08	582	5	Open sea	Flanking Active TSS	AC012360.6; FHL2	0.028596	0.015709	3.57E-07	0.004809	2.37E-06
chr17	77477261	77477987	727	5	Open sea	Enhancers; Flanking Active TSS	Sep-09	0.022495	0.015019	2.82E-06	0.003054	2.38E-06
chr5	4772860	4773383	524	4	Open sea	Quiescent/Low	CTD-2161F6.3; RP11-445O3.2	0.027664	0.020705	3.36E-07	0.004496	2.38E-06
chr12	1.31E+08	1.31E+08	76	2	Open sea	Weak Repressed PolyComb		0.020686	0.01837	9.62E-07	0.000365	2.41E-06
chr6	1.7E+08	1.7E+08	521	4	Open sea	Flanking Bivalent TSS/Enh; Bivalent Enhancer		0.01573	0.014281	4.18E-07	0.002811	2.43E-06
chr13	24223994	24224101	108	3	Open sea	Enhancers	SPATA13; RP11-307N16.6	-0.0188	-0.01576	6.93E-07	0.000992	2.47E-06
chr1	2.46E+08	2.46E+08	255	3	Open sea	Weak Repressed PolyComb	KIF26B	0.021592	0.014265	6.81E-07	0.000762	2.48E-06
chr8	60865205	60865728	524	4	Open sea	Flanking Active TSS; Enhancers	CHD7	0.018599	0.016769	3.64E-07	0.004862	2.55E-06
chr14	1.04E+08	1.04E+08	206	3	Open sea	Repressed PolyComb		0.020467	0.016253	6.34E-07	0.001408	2.55E-06

chr2	2.23E+08	2.23E+08	130	2	Open sea	Enhancers	ACSL3	0.023488	0.022827	1.02E-06	0.000382	2.56E-06
chr10	1.24E+08	1.24E+08	922	2	Shelf; Shore	Enhancers; Flanking Active TSS	OAT	0.033197	0.033012	1.08E-06	0.000295	2.60E-06
chr2	3459786	3460395	610	4	Shore; Shelf	Flanking Active TSS	TRAPPC12	0.027158	0.022657	6.53E-07	0.00307	2.62E-06
chr14	63273520	63274303	784	3	Open sea	Enhancers; Quiescent/Low	RHOJ	0.022725	0.01774	7.62E-07	0.00065	2.63E-06
chr15	90047250	90048122	873	3	Open sea	Enhancers	ZNF710	0.01864	0.017192	5.46E-07	0.002386	2.64E-06
chr14	59354509	59355009	501	3	Open sea	Enhancers	DAAM1	0.04032	0.029479	6.27E-07	0.001697	2.64E-06
chr6	41053229	41053421	193	3	Open sea	Active TSS	APOBEC2; OARD1	0.018957	0.016548	5.70E-07	0.002079	2.67E-06
chr17	49013675	49014158	484	4	Island	Repressed PolyComb	IGF2BP1; RP11-501C14.6	0.01736	0.01565	4.87E-07	0.003443	2.71E-06
chr8	1.44E+08	1.44E+08	1201	4	Open sea	Transcr. at gene 5' and 3'; Genic enhancers	DGAT1; GS1-393G12.12	0.015675	0.013909	4.86E-07	0.003476	2.72E-06
chr5	59124371	59124582	212	2	Open sea	Enhancers	PDE4D; ENSG00000247345	0.037877	0.03477	1.46E-06	0.000105	2.74E-06
chr2	98726221	98727530	1310	5	Open sea; Shelf	Weak transcription; Enhancers	MGAT4A	-0.01332	-0.01104	5.72E-07	0.002663	2.85E-06

chr10	1.02E+08	1.02E+08	6	2	Open sea	Weak transcription	C10orf76	-0.01759	-0.01354	1.15E-06	0.000393	2.85E-06
chr2	2.39E+08	2.39E+08	198	3	Open sea	Flanking Active TSS	HDAC4	0.027311	0.017657	8.32E-07	0.000965	2.88E-06
chr17	78862165	78862341	177	3	Open sea	Enhancers		0.021932	0.016829	9.56E-07	0.000985	2.89E-06
chr17	1405321	1405380	60	3	Open sea	Flanking Active TSS		0.022539	0.02062	6.12E-07	0.002336	2.90E-06
chr6	57313796	57314239	444	5	Open sea	Enhancers; Weak transcription	PRIM2	0.023502	0.012283	4.46E-06	0.002041	2.94E-06
chr17	48627655	48628663	1009	6	Shore	Repressed PolyComb	HOXB-AS4; HOXB7; HOXB9	0.020269	0.013967	1.23E-06	0.002564	2.95E-06
chr17	67520629	67520660	32	2	Open sea	Flanking Active TSS	PITPNC1	0.027944	0.023013	1.19E-06	0.000391	2.96E-06
chr2	98564920	98565315	396	3	Open sea	Genic enhancers	INPP4A	-0.01943	-0.01607	9.38E-07	0.001158	3.02E-06
chr1	1.15E+08	1.15E+08	196	4	Open sea	Active TSS; Flanking Active TSS	RP4-666F24.3	0.022204	0.012877	8.81E-07	0.00167	3.05E-06
chr8	38937699	38938627	929	3	Open sea	Flanking Active TSS	PLEKHA2	0.016899	0.013959	6.54E-07	0.002437	3.10E-06
chr3	46612162	46612430	269	3	Open sea	Weak Repressed PolyComb; Enhancers	TDGF1	0.027523	0.017877	0.000173	0.000212	3.14E-06
chr1	59359972	59360381	410	4	Open sea	Enhancers		0.02651	0.015651	1.50E-06	0.001833	3.15E-06

chr4	1.85E+08	1.85E+08	307	4	Open sea	Flanking Active TSS	ACSL1	0.021081	0.015705	5.68E-07	0.002516	3.18E-06
chr2	2.2E+08	2.2E+08	179	3	Open sea	Enhancers		0.020776	0.019265	8.14E-07	0.001277	3.27E-06
chr3	1.22E+08	1.22E+08	453	4	Open sea	Quiescent/Low; Enhancers	SLC15A2	0.024025	0.017459	1.21E-06	0.002912	3.28E-06
chr6	1.34E+08	1.34E+08	16	2	Open sea	Enhancers	SGK1	0.025887	0.02332	1.41E-06	0.00031	3.32E-06
chr22	20476214	20476367	154	3	Open sea	Weak transcription	RNY1P9; KLHL22	0.022787	0.019217	7.90E-07	0.001884	3.37E-06
chr2	17880051	17880304	254	3	Shore	Flanking Active TSS	KCNS3	0.032798	0.026551	9.51E-07	0.001456	3.49E-06
chr10	95288894	95289852	959	3	Shore	Active TSS; Flanking Active TSS	PDLIM1	-0.02892	-0.02625	8.08E-07	0.002026	3.55E-06
chr1	2.07E+08	2.07E+08	1202	6	Open sea	Genic enhancers	PFKFB2; C4BPB	-0.03422	-0.0117	0.000192	0.000261	3.56E-06
chr16	1537608	1537808	201	3	Shore; Island	Transcr. at gene 5' and 3'	TMEM204; IFT140	-0.0186	-0.01596	8.52E-07	0.001696	3.60E-06
chr6	1.12E+08	1.12E+08	189	3	Open sea	Flanking Active TSS		0.022871	0.020124	8.70E-07	0.001961	3.66E-06
chr10	6204983	6205137	155	2	Shore	Flanking Active TSS	PFKFB3	0.043525	0.042521	1.48E-06	0.00046	3.67E-06
chr9	1.29E+08	1.29E+08	330	2	Open sea	Flanking Active TSS	CCBL1	0.031277	0.03101	1.49E-06	0.000468	3.70E-06

chr17	56834495	56835107	613	3	Island	Flanking Active TSS	C17orf67; DGKE	-0.02494	-0.01766	8.72E-07	0.001981	3.72E-06
chr10	73888767	73889098	332	2	Open sea	Flanking Active TSS		0.027964	0.022587	1.70E-06	0.000257	3.79E-06
chr7	229224	229599	376	4	Shelf; Shore	Flanking Active TSS	AC145676.2; FAM20C	0.017009	0.015267	6.16E-07	0.00419	3.82E-06
chr1	54390361	54390837	477	4	Shore; Island	Flanking Active TSS	SSBP3	0.02706	0.017894	1.20E-06	0.002816	3.86E-06
chr3	1.7E+08	1.7E+08	272	3	Shore	Repressed PolyComb		0.022748	0.015299	1.83E-06	0.000685	3.86E-06
chr12	1.32E+08	1.32E+08	218	3	Open sea	Strong transcription	RP13-820C6.2; EP400	-0.01701	-0.01298	9.29E-07	0.00181	3.89E-06
chr5	1.5E+08	1.5E+08	217	2	Open sea	Enhancers; Genic enhancers	ARHGEF37	0.024837	0.024712	1.97E-06	0.000166	3.92E-06
chr12	1.25E+08	1.25E+08	750	2	Open sea	Enhancers		0.023976	0.020736	2.07E-06	0.00015	3.99E-06
chr10	1.3E+08	1.3E+08	1165	4	Open sea	Bivalent Enhancer		0.019793	0.017204	7.83E-07	0.003057	4.01E-06
chr3	1.95E+08	1.95E+08	417	6	Open sea	Active TSS; Flanking Active TSS	XXYLT1; XXYLT1-AS2; RN7SL36P	-0.02478	-0.01528	8.22E-07	0.00299	4.07E-06
chr8	1.03E+08	1.03E+08	565	4	Shore	Enhancers; Flanking Active TSS	GASAL1; KB-1732A1.1	0.028632	0.017761	2.05E-05	0.000913	4.10E-06
chr16	85309674	85310043	370	4	Open sea	Flanking Active TSS		0.020688	0.017039	6.60E-07	0.004529	4.21E-06

chr10	3500435	3500793	359	3	Open sea	Flanking Active TSS	RP11-482E14.1	0.023329	0.019978	1.43E-06	0.00111	4.24E-06
chr4	1.48E+08	1.48E+08	173	2	Open sea	Enhancers		0.030684	0.028112	1.92E-06	0.0003	4.33E-06
chr3	1.95E+08	1.95E+08	185	2	Open sea	Weak transcription	XXYLT1	0.035066	0.029393	1.77E-06	0.000492	4.36E-06
chr18	77058445	77058858	414	4	Island; Shore	Enhancers	MBP	0.016454	0.014621	6.87E-07	0.003695	4.37E-06
chr19	13002640	13003078	439	3	Shore	Active TSS; Flanking Active TSS	NFIX	0.016656	0.014448	9.53E-07	0.002645	4.38E-06
chr2	10493768	10494047	280	3	Open sea	Weak Repressed PolyComb		0.015042	0.012417	1.02E-06	0.002391	4.53E-06
chr17	1783332	1784053	722	4	Open sea	Flanking Active TSS; Enhancers	SMYD4	0.026398	0.015891	1.03E-05	0.001942	4.62E-06
chr6	1.59E+08	1.59E+08	233	3	Open sea	Weak Repressed PolyComb	RP1-111C20.3	0.026509	0.019878	3.29E-06	0.00048	4.63E-06
chr17	596390	596691	302	2	Open sea	Enhancers	VPS53	-0.02067	-0.02048	1.88E-06	0.000532	4.64E-06
chr3	1.6E+08	1.6E+08	119	3	Open sea	Quiescent/Low	IL12A-AS1	0.025295	0.019932	1.73E-06	0.000522	4.66E-06
chr10	78543724	78544084	361	2	Open sea	Enhancers		0.020624	0.018129	1.89E-06	0.000535	4.66E-06
chr6	1.11E+08	1.11E+08	807	4	Shelf	Weak transcription; Enhancers	RNU6-960P; SLC16A10	0.028425	0.019911	3.23E-06	0.002073	4.66E-06

chr5	1.07E+08	1.07E+08	352	3	Open sea	Enhancers; Quiescent/Low	CTC-254B4.1	0.022932	0.019942	1.36E-06	0.000959	4.69E-06
chr12	1.06E+08	1.06E+08	7	2	Open sea	Enhancers	ENSG00000257890	0.022351	0.021217	1.93E-06	0.000524	4.73E-06
chr1	2.02E+08	2.02E+08	2054	10	Island; Shore	Bivalent/Poised TSS; Flanking Bivalent TSS/Enh	NAV1	0.015605	0.009327	2.27E-05	0.004347	4.75E-06
chr17	55732959	55733451	493	3	Open sea	Enhancers; Flanking Active TSS	TMEM100	0.022205	0.02083	1.09E-06	0.002219	4.76E-06
chr17	4902635	4903611	977	8	Shore	Enhancers; Flanking Active TSS	CHRNE; C17orf107	0.022768	0.010547	1.92E-06	0.004383	4.76E-06
chr16	15607603	15608562	960	7	Open sea	Genic enhancers; Enhancers	C16orf45; CTB-193M12.1; KIAA0430	0.022195	0.016187	1.39E-06	0.002331	4.81E-06
chr7	29019484	29019892	409	2	Open sea	Enhancers	CPVL	0.037389	0.030298	2.91E-06	0.00011	4.85E-06
chr14	51368570	51368591	22	2	Open sea	Enhancers	RP11-255G12.2	0.021406	0.020906	2.01E-06	0.000512	4.90E-06
chr14	1.05E+08	1.05E+08	900	9	Shore; Island	Flanking Active TSS	PACS2; BRF1	0.022159	0.010082	0.000512	0.003207	4.93E-06
chr1	67653059	67653215	157	2	Open sea	Enhancers		0.028729	0.022935	2.16E-06	0.000373	4.98E-06
chr1	8590958	8591171	214	2	Open sea	Quiescent/Low	RERE	0.023858	0.021279	2.31E-06	0.000277	5.00E-06

chr2	33069855	33070070	216	2	Open sea	Enhancers	LTBP1	0.025291	0.022274	2.80E-06	0.000144	5.06E-06
chr1	10787483	10788085	603	3	Open sea	Enhancers	CASZ1	0.027383	0.021575	1.23E-06	0.002218	5.08E-06
chr5	1.73E+08	1.73E+08	534	2	Open sea	Enhancers; Flanking Active TSS	ERGIC1	0.019524	0.018339	2.27E-06	0.000327	5.10E-06
chr6	1.51E+08	1.51E+08	53	3	Open sea	Enhancers	MTHFD1L	0.021967	0.018256	1.15E-06	0.002665	5.18E-06
chr4	1.56E+08	1.56E+08	208	3	Open sea	Quiescent/Low	TDO2	0.023788	0.020111	1.28E-06	0.002049	5.20E-06
chr17	63838472	63839369	898	5	Shelf	Flanking Active TSS	SMARCD2	0.01862	0.012162	2.48E-05	0.001389	5.24E-06
chr16	67184680	67185690	1011	8	Island; Shore	Flanking Active TSS	EXOC3L1; KIAA0895L	0.017374	0.008168	0.001664	0.001446	5.31E-06
chr8	23450435	23450652	218	2	Open sea	Enhancers		0.024802	0.024133	2.23E-06	0.000487	5.34E-06
chr12	1.09E+08	1.09E+08	116	5	Open sea	Enhancers	SELPLG, SSH1, CORO1C	0.038545	0.022287	1.86E-06	0.003687	5.35E-06
chr1	1.51E+08	1.51E+08	459	5	Open sea	Transcr. at gene 5' and 3'; Genic enhancers	TMOD4; VPS72	0.023729	0.014835	1.75E-06	0.00389	5.42E-06
chr10	1.29E+08	1.29E+08	51	3	Open sea	Weak Repressed PolyComb		-0.01522	-0.01377	1.17E-06	0.003105	5.43E-06
chr21	32494806	32495240	435	4	Open sea	Weak transcription	EVA1C	0.02246	0.014179	2.23E-06	0.002511	5.44E-06

chr10	1.03E+08	1.03E+08	583	2	Open sea	Enhancers	ARL3; NFKB2, SFXN2, WBP1L, BORCS7	0.030881	0.026389	2.70E-06	0.000219	5.45E-06
chr11	1930583	1931447	865	3	Shore; Shelf	Enhancers; Flanking Active TSS	TNNT3	0.029477	0.02561	2.22E-06	0.000552	5.45E-06
chr16	58115434	58116112	679	3	Open sea	Genic enhancers; Strong transcription	CTB-134F13.1; CFAP20	-0.01925	-0.01664	1.40E-06	0.001778	5.52E-06
chr11	64313685	64314401	717	5	Shelf	Flanking Active TSS; Transcr. at gene 5’ and 3’	ESRRA; AP001453.1	0.020208	0.014563	5.94E-06	0.004	5.52E-06
chr17	81052248	81052511	264	4	Island	Flanking Active TSS	BAIAP2	-0.01666	-0.01285	1.24E-06	0.004423	5.55E-06
chr2	2.37E+08	2.37E+08	318	2	Open sea	Bivalent Enhancer		0.020787	0.020779	2.31E-06	0.000521	5.57E-06
chr18	76528076	76528624	549	4	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh	LINC00908	0.020691	0.01455	1.48E-06	0.004123	5.63E-06
chr13	20702093	20703222	1130	7	Shore; Island	Active TSS; Flanking Active TSS	RP11-172H24.4; IL17D	0.023675	0.011161	0.003242	0.00194	5.64E-06
chr2	84807215	84807641	427	3	Open sea	Weak Repressed PolyComb	DNAH6	0.02064	0.016524	2.48E-06	0.000927	5.78E-06
chr3	1.53E+08	1.53E+08	127	2	Open sea	Quiescent/Low		0.020564	0.01987	2.43E-06	0.000527	5.83E-06

chr3	64067137	64067712	576	3	Open sea	Quiescent/Low; Enhancers	PRICKLE2-AS1	0.020327	0.015606	2.14E-06	0.000787	5.87E-06
chr4	7250595	7250859	265	3	Open sea	Enhancers; Flanking Active TSS	SORCS2	0.01984	0.014653	1.31E-06	0.002939	5.92E-06
chr21	29327046	29327340	295	3	Open sea	Genic enhancers	BACH1	0.024719	0.022445	1.32E-06	0.00287	5.94E-06
chr1	1.65E+08	1.65E+08	202	2	Open sea	Flanking Active TSS	PBX1	0.022416	0.021293	2.45E-06	0.000616	6.01E-06
chr2	1.13E+08	1.13E+08	303	6	Open sea	Quiescent/Low	IL1A	0.020404	0.012268	4.18E-06	0.004886	6.01E-06
chr5	1.43E+08	1.43E+08	91	3	Open sea	Enhancers	ARHGAP26	0.02573	0.023174	1.46E-06	0.002343	6.16E-06
chr7	50595721	50596006	286	2	Open sea	Weak transcription	GRB10	0.030811	0.026889	2.76E-06	0.000421	6.30E-06
chr15	72752611	72752685	75	2	Open sea	Weak transcription; Strong transcription	ADPGK	0.022582	0.020065	2.71E-06	0.00049	6.37E-06
chr13	50222708	50222988	281	2	Open sea	Flanking Active TSS	DLEU1	0.02107	0.018983	2.83E-06	0.000453	6.53E-06
chr7	77281890	77282211	322	2	Open sea	Enhancers		0.020568	0.020259	2.72E-06	0.000594	6.57E-06
chr2	2.16E+08	2.16E+08	9	2	Open sea	Enhancers	LINC00607	0.02323	0.023042	2.70E-06	0.000629	6.57E-06

chr16	2028822	2029656	835	6	Shore; Shelf	Flanking Active TSS	SLC9A3R2	-0.01611	-0.00988	1.58E-05	0.003849	6.59E-06
chr13	20142498	20143457	960	6	Island; Shore	Bivalent Enhancer; Repressed PolyComb	GJA3	-0.02078	-0.01271	6.30E-06	0.001829	6.76E-06
chr2	9741275	9741879	605	3	Open sea	ZNF genes & repeats; Weak transcription	RNU4-73P	0.023226	0.015883	5.94E-05	0.000382	6.78E-06
chr17	8868629	8869101	473	4	Open sea	Weak Repressed PolyComb	PIK3R6	0.023176	0.013678	7.57E-06	0.002574	6.79E-06
chr15	40926066	40926153	88	3	Island	Flanking Bivalent TSS/Enh		0.014581	0.012489	1.62E-06	0.00256	6.84E-06
chr1	9438284	9439274	991	3	Open sea	Enhancers; Flanking Active TSS	RNA5SP40	0.025918	0.023643	3.07E-06	0.000899	6.88E-06
chr1	12540198	12540717	520	3	Shore; Island	Flanking Active TSS		0.016874	0.014278	1.64E-06	0.002825	7.09E-06
chr11	315907	317045	1139	4	Island; Shore	Bivalent/Poised TSS; Active TSS		-0.01992	-0.01601	1.39E-06	0.004998	7.12E-06
chr12	1.19E+08	1.19E+08	623	3	Open sea	Flanking Active TSS; Transcr. at gene 5' and 3'; Active TSS	RP11-64B16.4; HSPB8	0.025933	0.02213	1.65E-06	0.003002	7.29E-06
chr1	1.72E+08	1.72E+08	130	2	Open sea	Quiescent/Low	DNM3	0.021688	0.020512	3.46E-06	0.000325	7.31E-06

chr21	46055904	46056481	578	3	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh	COL6A1, MCM3AP-AS1; AP001476.3; DSTNP1	0.021297	0.02035	2.30E-06	0.001507	7.32E-06
chr16	88194650	88195173	524	3	Open sea	Flanking Active TSS	RP11-863P13.2; LA16c-444G7.2	0.021235	0.019355	1.65E-06	0.003595	7.57E-06
chr16	85187140	85187269	130	2	Open sea	Enhancers		0.023894	0.023067	3.21E-06	0.000632	7.69E-06
chr5	1.35E+08	1.35E+08	517	3	Open sea	Strong transcription	C5orf66; H2AFY	0.02453	0.019273	1.81E-06	0.002741	7.73E-06
chr10	1.03E+08	1.03E+08	739	2	Open sea	Enhancers; Flanking Active TSS		0.039957	0.037477	3.49E-06	0.000427	7.77E-06
chr3	1.88E+08	1.88E+08	615	3	Shore	Active TSS	BCL6	0.019479	0.017242	2.33E-06	0.001481	7.85E-06
chr4	1211570	1212361	792	5	Shore; Island	Genic enhancers	CTBP1; CTBP1-AS	0.022136	0.014977	9.75E-06	0.002254	7.98E-06
chr1	9539007	9540661	1655	9	Shore; Island	Enhancers; Flanking Active TSS; Active TSS	SLC25A33	-0.01892	-0.00709	3.11E-05	0.003335	8.01E-06
chr1	2.07E+08	2.07E+08	652	5	Shore	Enhancers	RASSF5; EIF2D	0.015951	0.012568	2.18E-05	0.003614	8.04E-06
chr3	52518669	52519416	748	5	Open sea	Flanking Active TSS; Active TSS; Weak transcription	STAB1	0.023354	0.01501	2.89E-05	0.003973	8.09E-06
chr8	18886935	18887210	276	3	Open sea	Bivalent/Poised TSS; Repressed PolyComb	PSD3	0.016557	0.014662	1.81E-06	0.003337	8.09E-06

chr13	1.06E+08	1.06E+08	423	4	Shore	Strong transcription	EFNB2	-0.02303	-0.01637	3.26E-06	0.000549	8.11E-06
chr7	2149730	2149852	123	3	Shelf	Genic enhancers		0.021615	0.019281	1.79E-06	0.003556	8.12E-06
chr5	31737827	31738229	403	3	Open sea	Quiescent/Low	RP11-5N11.3; PDZD2	0.021416	0.020908	1.79E-06	0.003622	8.16E-06
chr3	10468830	10469268	439	2	Open sea	Flanking Active TSS	ATP2B2	0.02307	0.019408	3.52E-06	0.000562	8.21E-06
chr20	60062860	60062913	54	2	Open sea	Flanking Active TSS	C20orf197	0.022999	0.020121	4.12E-06	0.000282	8.22E-06
chr14	1.05E+08	1.05E+08	481	4	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh	C14orf180; TMEM179	0.017605	0.012826	2.76E-06	0.002779	8.45E-06
chr8	37520757	37521021	265	4	Open sea	Flanking Active TSS; Enhancers	RP11-150O12.1	0.026698	0.019135	2.18E-06	0.003639	8.50E-06
chr1	1.09E+08	1.09E+08	497	2	Open sea	Flanking Active TSS	SORT1	0.021092	0.020542	3.87E-06	0.000436	8.54E-06
chr11	628301	629522	1222	5	Shore	Flanking Active TSS	SCT	0.027466	0.012123	0.00172	0.000839	8.66E-06
chr14	1.01E+08	1.01E+08	1069	4	Shore; Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer; Bivalent/Poised TSS; Repressed PolyComb		0.017629	0.012655	2.58E-05	0.002314	8.69E-06
chr16	89443286	89443351	66	2	Open sea	Enhancers	RNU6-430P; ANKRD11	0.020555	0.019835	3.65E-06	0.000725	8.80E-06

chr4	1.84E+08	1.84E+08	780	6	Open sea	Repressed PolyComb; Weak Repressed PolyComb	ENPP6	0.020661	0.012347	1.12E-05	0.003041	8.84E-06
chr11	71478338	71478970	633	6	Open sea	Genic enhancers; Strong transcription	NADSYN1	-0.01777	-0.00929	0.000774	0.002056	8.84E-06
chr10	1.19E+08	1.19E+08	636	3	Open sea	Bivalent Enhancer		0.027876	0.019825	2.32E-06	0.001963	8.88E-06
chr8	1.02E+08	1.02E+08	51	2	Open sea	Quiescent/Low	NCALD	0.023445	0.022166	3.79E-06	0.000624	8.90E-06
chr3	1.78E+08	1.78E+08	526	4	Open sea	Enhancers		-0.04319	-0.0214	0.000234	0.0002	9.08E-06
chr7	98245946	98246189	244	4	Open sea	Genic enhancers; Weak transcription	TECPR1	-0.01377	-0.01197	1.83E-06	0.002633	9.13E-06
chr19	2627323	2628033	711	4	Open sea	Enhancers; Flanking Active TSS	TLE2, GNG7; GNG7; DOT1L, SPPL2B, GADD45B	0.018918	0.014978	1.50E-06	0.004831	9.20E-06
chr3	50111873	50112726	854	3	Open sea	Genic enhancers		0.023789	0.021126	2.09E-06	0.003684	9.36E-06
chr2	2.38E+08	2.38E+08	426	4	Open sea	Enhancers; Flanking Active TSS	LRRFIP1	0.017845	0.015387	3.61E-06	0.001625	9.43E-06
chr17	848046	849257	1212	3	Open sea	Enhancers	NXN	-0.01709	-0.01412	2.30E-06	0.002934	9.47E-06

chr3	3665168	3665183	16	2	Open sea	Quiescent/Low		0.027771	0.025792	4.51E-06	0.000385	9.49E-06
chr15	72228008	72228817	810	4	Shore	Flanking Active TSS; Enhancers	PKM	0.025478	0.0149	3.54E-06	0.000654	9.57E-06
chr6	1.34E+08	1.34E+08	276	3	Open sea	Enhancers; Flanking Active TSS	SGK1	0.023785	0.020811	3.30E-06	0.001949	1.00E-05
chr4	61933366	61933804	439	3	Open sea	Quiescent/Low	LPHN3	0.023672	0.01815	5.67E-06	0.00061	1.01E-05
chr5	76607571	76607874	304	6	Open sea	Quiescent/Low	CTD-2236F14.1; IQGAP2	0.018677	0.010979	7.02E-06	0.002931	1.02E-05
chr14	1.05E+08	1.05E+08	776	4	Shelf	Flanking Active TSS	PACS2	0.02303	0.016841	1.68E-06	0.004896	1.02E-05
chr12	57868799	57869085	287	3	Shelf	Enhancers; Flanking Active TSS	AVIL, CTDSP2	0.021339	0.020365	2.33E-06	0.003774	1.03E-05
chr19	8334234	8335318	1085	3	Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer; Bivalent/Poised TSS	KANK3; ELAVL1	0.023431	0.018933	6.20E-06	0.001181	1.05E-05
chr11	61627650	61628719	1070	3	Open sea	Flanking Active TSS; Enhancers	RPLP0P2; DAGLA	-0.03729	-0.02054	6.23E-05	0.000156	1.05E-05
chr2	2.38E+08	2.38E+08	334	2	Open sea	Active TSS	RAB17	0.016684	0.016682	4.57E-06	0.000628	1.05E-05
chr17	2212502	2212925	424	2	Open sea	Flanking Active TSS	SMG6; AC090617.1	0.021076	0.020215	4.37E-06	0.000832	1.05E-05

chr22	17165198	17165372	175	4	Open sea	Weak transcription	CECR5-AS1; CECR5	0.019087	0.014597	1.03E-05	0.002822	1.06E-05
chr1	9443989	9444369	381	2	Open sea	Enhancers; Weak transcription		0.042945	0.036176	5.19E-06	0.000387	1.07E-05
chr5	1.49E+08	1.49E+08	159	2	Open sea	Active TSS; Flanking Active TSS	MIR143HG; IL17B	-0.03278	-0.02565	5.72E-06	0.000272	1.07E-05
chr15	62843130	62843841	712	5	Open sea	Weak transcription	TLN2; RP11-1069G10.1	0.023225	0.014913	9.60E-05	0.003684	1.08E-05
chr20	603047	603360	314	2	Open sea	Enhancers; Flanking Active TSS		-0.01706	-0.01631	4.49E-06	0.000844	1.08E-05
chr12	47837341	47837353	13	2	Open sea	Enhancers		0.029472	0.027204	4.58E-06	0.00076	1.08E-05
chr14	89206064	89206587	524	3	Open sea	ZNF genes & repeats	FOXN3	0.031506	0.020872	0.001603	0.000396	1.09E-05
chr2	37318412	37318524	113	4	Open sea	Enhancers		0.026375	0.020325	3.41E-06	0.003009	1.10E-05
chr1	1.84E+08	1.84E+08	687	4	Open sea	Enhancers		-0.01418	-0.01054	9.32E-06	0.003108	1.10E-05
chr11	1.12E+08	1.12E+08	356	4	Open sea	Flanking Active TSS	ALG9; RP11-108O10.8	0.022227	0.019106	2.11E-06	0.003906	1.11E-05
chr19	3383484	3383727	244	3	Shore	Transcr. at gene 5' and 3'	NFIC	0.019738	0.015963	3.68E-06	0.001981	1.11E-05

chr11	73213962	73214806	845	3	Open sea; Shelf	Enhancers; Flanking Active TSS		0.023645	0.019201	2.54E-06	0.003949	1.12E-05
chr6	1.18E+08	1.18E+08	491	2	Shore; Island	Flanking Active TSS	GOPC; DCBLD1	0.021095	0.017572	4.67E-06	0.000862	1.13E-05
chr4	1E+08	1E+08	508	2	Open sea	Enhancers		0.031721	0.026706	8.94E-06	0.000105	1.14E-05
chr1	55443725	55443788	64	2	Open sea	Enhancers	RNU6-830P	0.019354	0.018452	5.41E-06	0.000448	1.15E-05
chr16	49663057	49663226	170	2	Open sea	Flanking Active TSS	ZNF423	0.029613	0.020869	1.73E-05	3.65E-05	1.16E-05
chr4	1502753	1502926	174	3	Shore	Bivalent Enhancer		-0.01779	-0.01418	2.82E-06	0.003145	1.16E-05
chr3	42066806	42067646	841	3	Open sea	Flanking Active TSS; Enhancers	TRAK1; ULK4	0.012568	0.01193	2.59E-06	0.00418	1.16E-05
chr11	66547767	66548398	632	3	Shore; Shelf	Flanking Active TSS; Enhancers	ACTN3	0.023331	0.01761	3.53E-06	0.002361	1.16E-05
chr7	1.01E+08	1.01E+08	689	2	Open sea	Weak Repressed PolyComb	RP11-44M6.1	0.021244	0.020231	4.93E-06	0.000861	1.18E-05
chr17	78412255	78413111	857	3	Open sea	Flanking Active TSS	PGS1	0.031426	0.021095	4.04E-06	0.001708	1.19E-05
chr2	2.37E+08	2.37E+08	75	3	Open sea	Enhancers		0.022844	0.018726	5.51E-06	0.001559	1.19E-05
chr21	32932847	32933045	199	3	Open sea	Weak Repressed PolyComb	AP000282.2	0.016493	0.014882	4.60E-06	0.000993	1.20E-05

chr16	11781949	11782588	640	6	Open sea	Enhancers	ZC3H7A	0.0151	0.010301	2.07E-05	0.004072	1.21E-05
chr8	1.3E+08	1.3E+08	227	2	Open sea	Weak Repressed PolyComb		0.018665	0.01811	5.12E-06	0.000829	1.21E-05
chr11	2827079	2827261	183	2	Open sea	Flanking Active TSS	KCNQ1	-0.01508	-0.01476	5.14E-06	0.000816	1.22E-05
chr17	44087064	44087131	68	3	Open sea	Genic enhancers	ASB16; ASB16-AS1, HDAC5	0.018997	0.01691	3.14E-06	0.002626	1.22E-05
chr7	29146884	29147402	519	3	Open sea	Flanking Active TSS	CPVL; CHN2	0.01928	0.018961	2.79E-06	0.004314	1.25E-05
chr2	1.62E+08	1.62E+08	10	2	Open sea	Quiescent/Low	DPP4	0.025136	0.024213	5.63E-06	0.000632	1.26E-05
chr2	2.24E+08	2.24E+08	641	2	Open sea	Quiescent/Low	SERPINE2	0.022818	0.0214	6.93E-06	0.000289	1.27E-05
chr22	50304118	50304990	873	3	Shelf; Shore	Enhancers; Flanking Bivalent TSS/Enh	PLXNB2; DENND6B, PLXNB2	0.020234	0.018562	2.92E-06	0.004179	1.29E-05
chr2	1.97E+08	1.97E+08	74	3	Open sea	Enhancers	CCDC150	0.019739	0.016946	5.16E-06	0.001129	1.29E-05
chr3	51962780	51964034	1255	9	Open sea; Shelf	Flanking Active TSS	RP11-155D18.12; PCBP4	0.01684	0.008703	0.001111	0.004824	1.31E-05
chr17	7026434	7027027	594	4	Shelf	Enhancers	BCL6B	-0.01749	-0.01243	6.96E-06	0.003215	1.31E-05
chr5	1.8E+08	1.8E+08	590	3	Open sea	Genic enhancers	LOC101928445	0.034457	0.021568	1.60E-05	0.000675	1.34E-05

chr15	67199884	67200202	319	2	Open sea	Enhancers	IQCH; AAGAB	0.0278	0.02486	5.72E-06	0.000921	1.36E-05
chr17	35868129	35869018	890	3	Open sea	Quiescent/Low	AC015849.2; HEATR9	0.014717	0.013407	3.31E-06	0.003731	1.37E-05
chr7	1.56E+08	1.56E+08	410	4	Open sea	Weak Repressed PolyComb		0.018775	0.015379	9.87E-06	0.003672	1.37E-05
chr14	99809399	99810133	735	3	Open sea	Enhancers	EML1	0.025217	0.02283	4.77E-06	0.001211	1.37E-05
chr4	3500653	3500847	195	3	Island	Strong transcription; Genic enhancers	DOK7	-0.01713	-0.01426	3.49E-06	0.003394	1.38E-05
chr17	15463271	15464312	1042	3	Open sea	Flanking Active TSS; Enhancers	TVP23C-CDRT4; CDRT4	0.017251	0.014818	3.31E-06	0.003652	1.39E-05
chr20	23091858	23092241	384	2	Open sea	Enhancers		0.023796	0.021722	5.91E-06	0.000913	1.40E-05
chr17	47852032	47852666	635	7	Shore	Bivalent Enhancer; Repressed PolyComb	SP6	0.015635	0.01017	1.96E-05	0.002303	1.41E-05
chr10	1.27E+08	1.27E+08	345	3	Shore	Repressed PolyComb; Bivalent Enhancer	FAM196A; DOCK1	0.026421	0.018368	3.86E-06	0.002434	1.41E-05
chr3	1.52E+08	1.52E+08	181	2	Open sea	Active TSS; Flanking Active TSS	MBNL1	0.02564	0.022659	6.85E-06	0.000492	1.43E-05
chr7	1E+08	1E+08	629	12	Open sea	Genic enhancers		0.020416	0.00944	1.19E-05	0.004356	1.43E-05

chr3	1.88E+08	1.88E+08	476	2	Open sea	Flanking Active TSS	LPP	0.022478	0.020633	6.42E-06	0.000674	1.43E-05
chr1	1.54E+08	1.54E+08	582	2	Open sea	Flanking Active TSS; Active TSS	S100A16	0.023647	0.021897	6.01E-06	0.000978	1.43E-05
chr3	45139601	45139881	281	3	Open sea	Enhancers	RNU5B-3P, CDCP1	0.032431	0.02995	3.82E-06	0.003191	1.44E-05
chr17	42324705	42325247	543	3	Open sea	Genic enhancers		0.025455	0.017466	7.75E-06	0.000929	1.45E-05
chr1	45913184	45913506	323	3	Open sea	Flanking Active TSS; Active TSS	MAST2	0.023305	0.018696	3.47E-06	0.00384	1.46E-05
chr12	80714210	80714254	45	3	Shelf	Active TSS		0.01679	0.013214	7.10E-06	0.000888	1.48E-05
chr10	3107567	3107659	93	3	Shore	Flanking Active TSS	PFKP	0.01847	0.015837	3.48E-06	0.004276	1.50E-05
chr6	42820288	42821314	1027	7	Open sea	Enhancers; Flanking Active TSS	GLTSCR1L	0.022045	0.014492	6.86E-05	0.003883	1.50E-05
chr10	71597897	71598664	768	3	Open sea	Enhancers	CDH23	0.020177	0.017501	8.31E-06	0.000691	1.50E-05
chr7	77840107	77840259	153	2	Open sea	Flanking Active TSS	PHTF2	0.032344	0.029005	6.57E-06	0.00079	1.50E-05
chr16	88956980	88957224	245	3	Open sea	Bivalent Enhancer	CBFA2T3	0.017719	0.015468	3.45E-06	0.00444	1.51E-05
chr16	66926447	66927152	706	3	Shore	Flanking Active TSS; Enhancers	RRAD; PLEKHG4	0.025006	0.015625	7.87E-06	0.000663	1.52E-05

chr2	12314004	12314258	255	3	Open sea	Enhancers	AC096559.1	-0.02111	-0.01726	3.81E-06	0.003532	1.53E-05
chr12	1.18E+08	1.18E+08	507	3	Open sea	Active TSS; Flanking Active TSS	TAOK3	0.017518	0.01528	5.77E-06	0.000918	1.53E-05
chr13	1.06E+08	1.06E+08	284	3	Open sea	Weak Repressed PolyComb; Flanking Active TSS; Quiescent/Low		0.01677	0.014464	3.74E-06	0.003632	1.54E-05
chr13	1.12E+08	1.12E+08	153	3	Open sea	Weak Repressed PolyComb; Repressed PolyComb		-0.01557	-0.01434	3.76E-06	0.003942	1.55E-05
chr15	31223546	31223923	378	4	Open sea	Flanking Active TSS; Flanking Bivalent TSS/Enh	RP11-16E12.1; RP11-16E12.2	-0.03101	-0.02029	0.000103	0.002226	1.55E-05
chr6	1.56E+08	1.56E+08	6	2	Open sea	Quiescent/Low		0.024936	0.024748	6.58E-06	0.001013	1.56E-05
chr8	41770718	41771033	316	2	Shelf	Enhancers	ANK1	0.0202	0.018986	6.90E-06	0.000789	1.57E-05
chr1	6084493	6085070	578	2	Open sea	Bivalent Enhancer; Repressed PolyComb	KCNAB2	0.025667	0.021491	7.58E-06	0.000526	1.58E-05
chr12	26287716	26288353	638	3	Open sea	Flanking Active TSS	SSPN; RP11-283G6.4; RP11-283G6.5	0.019093	0.015836	3.79E-06	0.003864	1.58E-05
chr2	1.09E+08	1.09E+08	511	4	Island; Shore	Flanking Active TSS	SH3RF3-AS1; SH3RF3	-0.03775	-0.02346	0.000667	0.001298	1.60E-05

chr7	1.49E+08	1.49E+08	771	3	Open sea	Genic enhancers; Transcr. at gene 5' and 3'	CUL1	0.023634	0.021156	4.12E-06	0.003631	1.62E-05
chr11	2839758	2839973	216	2	Open sea	Weak transcription	KCNQ1	-0.01717	-0.01466	6.91E-06	0.000985	1.63E-05
chr19	34594568	34594771	204	3	Open sea	Flanking Active TSS	SCGB2B2	0.019299	0.018094	3.89E-06	0.004168	1.63E-05
chr1	12618348	12619299	952	5	Island; Shore	Active TSS; Flanking Active TSS	DHRS3; RP11-474O21.5	-0.02256	-0.01547	4.57E-06	0.003729	1.64E-05
chr14	1E+08	1E+08	742	3	Open sea	Enhancers; Flanking Active TSS; Transcr. at gene 5' and 3'	SLC25A29	0.01832	0.014702	7.93E-06	0.001229	1.68E-05
chr6	1.7E+08	1.7E+08	629	3	Open sea	Bivalent Enhancer	LOC102724511	0.019736	0.018763	4.81E-06	0.002993	1.68E-05
chr18	11857270	11857784	515	2	Open sea	Active TSS; Flanking Active TSS	RP11-78A19.4; GNAL	0.023049	0.019037	7.74E-06	0.00067	1.69E-05
chr17	81867804	81869021	1218	4	Shore; Island	Genic enhancers		0.016326	0.011871	4.80E-06	0.004638	1.72E-05
chr19	3384855	3385006	152	2	Shelf	Enhancers		0.019172	0.01694	7.60E-06	0.000833	1.72E-05
chr19	33507464	33507691	228	2	Open sea	Weak transcription	PEPD	-0.02707	-0.02131	8.43E-06	0.00053	1.73E-05
chr1	1.81E+08	1.81E+08	663	3	Open sea	Enhancers; Flanking Active TSS		0.022441	0.017444	4.77E-06	0.003323	1.75E-05

chr19	30450950	30451188	239	3	Island	Bivalent/Poised TSS	ZNF536	0.024406	0.018066	5.05E-06	0.002118	1.75E-05
chr4	1.85E+08	1.85E+08	37	2	Open sea	Genic enhancers	ACSL1	0.024695	0.023933	7.40E-06	0.001096	1.76E-05
chr12	1.01E+08	1.01E+08	672	3	Island	Repressed PolyComb; Bivalent/Poised TSS	ANO4	-0.02281	-0.01692	5.42E-06	0.002684	1.77E-05
chr17	45291362	45291381	20	2	Open sea	Flanking Active TSS	MAP3K14	0.026482	0.023065	8.57E-06	0.000556	1.77E-05
chr20	49604596	49605305	710	3	Open sea	Weak Repressed PolyComb		0.017074	0.015141	4.09E-06	0.004936	1.79E-05
chr12	95611884	95611999	116	2	Open sea	Quiescent/Low	PGAM1P5	-0.01692	-0.01477	1.04E-05	0.000305	1.79E-05
chr12	1918638	1918989	352	3	Open sea	Enhancers		0.02559	0.01928	8.62E-06	0.001268	1.80E-05
chr1	11176208	11176589	382	3	Open sea	Flanking Active TSS	MTOR	0.020615	0.018889	4.74E-06	0.003405	1.83E-05
chr4	1.41E+08	1.41E+08	367	3	Shore	Quiescent/Low	RNF150	0.018286	0.017314	4.48E-06	0.004168	1.84E-05
chr15	37015183	37015311	129	3	Open sea	Quiescent/Low	MEIS2	0.021087	0.020535	4.28E-06	0.004756	1.84E-05
chr18	57754714	57754775	62	3	Open sea	Weak transcription; Enhancers	ATP8B1; RNU6-742P	0.024668	0.023108	4.41E-06	0.004644	1.87E-05
chr14	1.05E+08	1.05E+08	245	2	Shelf	Enhancers		0.023974	0.022742	8.10E-06	0.001062	1.90E-05

chr10	7552064	7552501	438	2	Open sea	Bivalent Enhancer; Enhancers	ITIH5	0.017175	0.016425	8.53E-06	0.000849	1.91E-05
chr2	1.09E+08	1.09E+08	133	3	Open sea	Flanking Active TSS	SH3RF3	0.017641	0.015927	4.84E-06	0.003915	1.96E-05
chr15	96234445	96234507	63	2	Open sea	Quiescent/Low	RP11-759A24.3; RP11-327J17.1; NR2F2-AS1	0.027328	0.024096	9.99E-06	0.000512	1.98E-05
chr11	1.3E+08	1.3E+08	934	3	Open sea	Enhancers	LOC646383	0.021089	0.005658	1.42E-05	0.001342	1.99E-05
chr6	1.51E+08	1.51E+08	724	2	Open sea	Weak Repressed PolyComb	PLEKHG1	0.024256	0.004876	8.78E-06	0.000916	1.99E-05
chr11	57293252	57293438	187	2	Open sea	Enhancers	APLNR, SSRP1, TNKS1BP1	0.025254	0.021719	8.58E-06	0.001108	2.01E-05
chr16	31129361	31130331	971	3	Open sea	Transcr. at gene 5' and 3'; Strong transcription	KAT8; RP11-388M20.2	0.022611	0.017372	1.61E-05	0.00068	2.04E-05
chr5	3225741	3226351	611	3	Open sea	Weak Repressed PolyComb		0.018505	0.015588	4.90E-06	0.004906	2.08E-05
chr16	22915788	22916263	476	2	Shore	Weak Repressed PolyComb	HS3ST2	0.026329	0.020987	9.34E-06	0.000943	2.11E-05
chr11	73263014	73263109	96	3	Shore	Weak Repressed PolyComb	P2RY6	0.021936	0.019376	5.52E-06	0.003671	2.13E-05
chr12	54463247	54463636	390	2	Open sea	Quiescent/Low; Enhancers	RP11-753H16.3; GTSF1; RP11-753H16.5	0.029624	0.022938	1.05E-05	0.000591	2.14E-05

chr1	2.05E+08	2.05E+08	202	2	Open sea	Weak transcription; Enhancers	DSTYK; LOC101929459, DSTYK	0.0186	0.018355	9.27E-06	0.001077	2.14E-05
chr4	6886310	6886423	114	2	Open sea	Flanking Active TSS		0.017349	0.015922	9.16E-06	0.00122	2.16E-05
chr4	83548409	83548716	308	2	Open sea	Flanking Active TSS	AGPAT9	0.022416	0.021172	9.47E-06	0.001036	2.17E-05
chr19	15116407	15116879	473	2	Shelf	Genic enhancers	ILVBL	-0.01099	-0.00944	1.12E-05	0.000504	2.18E-05
chr8	85350373	85351032	660	3	Open sea	Flanking Active TSS	CA1	0.018358	0.017645	5.22E-06	0.004822	2.18E-05
chr1	2.13E+08	2.13E+08	263	2	Open sea	Repressed PolyComb; Weak Repressed PolyComb		0.017963	0.016577	9.72E-06	0.001004	2.21E-05
chr17	50117135	50118330	1196	6	Shore; Island	Flanking Active TSS; Enhancers	SAMD14	0.022049	0.01121	4.94E-05	0.003809	2.22E-05
chr8	1.23E+08	1.23E+08	281	3	Open sea	Weak transcription	RP11-557C18.4	0.022252	0.021616	5.75E-06	0.003568	2.22E-05
chr10	60911377	60911469	93	2	Open sea	Genic enhancers	RHOBTB1	0.032409	0.030369	9.58E-06	0.001147	2.23E-05
chr8	48557214	48557907	694	2	Shore	Bivalent Enhancer; Repressed PolyComb	SNAI2; RP11-770E5.1; RP11-567J20.2	0.022714	0.019682	1.04E-05	0.000779	2.25E-05
chr20	52494415	52494553	139	2	Open sea	Quiescent/Low	RP5-1022J11.2; LINC01524	0.02199	0.021449	9.61E-06	0.001214	2.25E-05

chr3	42090817	42091498	682	7	Open sea	Genic enhancers; Transcr. at gene 5' and 3'; Flanking Active TSS	TRAK1	0.025089	0.01355	1.89E-05	0.003542	2.29E-05
chr2	1.52E+08	1.52E+08	55	2	Open sea	Enhancers	NEB	0.023903	0.023812	9.97E-06	0.001102	2.29E-05
chr17	78278661	78278774	114	2	Open sea	Enhancers	RP11-219G17.4	0.01974	0.017367	9.80E-06	0.001234	2.30E-05
chr3	58627495	58627760	266	3	Open sea	Bivalent Enhancer	FAM107A	0.016218	0.014022	5.75E-06	0.004491	2.30E-05
chr8	48789744	48789782	39	2	Open sea	Quiescent/Low		-0.02008	-0.01521	9.82E-06	0.00124	2.30E-05
chr16	80174131	80174704	574	3	Open sea	Quiescent/Low	RP11-525K10.3	0.021784	0.019156	1.12E-05	0.001873	2.31E-05
chr7	35716028	35716664	637	3	Open sea	Quiescent/Low; Active TSS; Flanking Active TSS	AC018647.3	0.020299	0.016701	7.71E-06	0.00176	2.31E-05
chr1	2.05E+08	2.05E+08	109	2	Open sea	Weak Repressed PolyComb		0.02634	0.024875	9.83E-06	0.001264	2.31E-05
chr17	57899131	57899160	30	2	Open sea	Flanking Active TSS	CUEDC1	0.018557	0.016401	1.06E-05	0.000883	2.32E-05
chr17	20086408	20086988	581	4	Open sea	Enhancers; Weak transcription	SPECC1	-0.02045	-0.01266	0.000112	0.002347	2.38E-05
chr14	68088932	68089272	341	2	Open sea	Enhancers		0.0245	0.02078	1.07E-05	0.000999	2.40E-05

chr12	1.19E+08	1.19E+08	642	2	Open sea	Enhancers; Flanking Active TSS	RP11-64B16.3	0.028348	0.020547	1.55E-05	0.000276	2.40E-05
chr16	27245447	27246008	562	2	Open sea	Enhancers	ENSG00000259940, NSMCE1; KDM8	0.021589	0.019062	1.04E-05	0.001183	2.41E-05
chr7	30225892	30226620	729	2	Open sea	Flanking Active TSS		0.02251	0.020132	1.03E-05	0.001303	2.43E-05
chr22	50013985	50014000	16	2	Shore	Repressed PolyComb	IL17REL	0.019629	0.016165	1.26E-05	0.000548	2.44E-05
chr16	1794925	1795458	534	5	Shelf	Weak transcription	IGFALS; SPSB3	0.020148	0.010084	1.69E-05	0.004347	2.44E-05
chr3	1.96E+08	1.96E+08	313	3	Open sea	Flanking Active TSS		0.02136	0.015012	8.71E-06	0.002434	2.45E-05
chr6	1.39E+08	1.39E+08	142	3	Open sea	Genic enhancers	TXLNB; RP1-225E12.3	0.027918	0.020981	6.10E-06	0.004475	2.47E-05
chr14	97977813	97978669	857	5	Open sea	Enhancers; Flanking Active TSS	LINC01550	0.01697	0.011544	0.001078	0.003865	2.48E-05
chr3	62179360	62179590	231	2	Open sea	Quiescent/Low; Enhancers	PTPRG	0.037252	0.032409	1.07E-05	0.001204	2.48E-05
chr3	1.7E+08	1.7E+08	219	2	Open sea	Quiescent/Low	LRRC31	0.023574	0.022551	1.10E-05	0.00108	2.49E-05
chr21	43732301	43732452	152	2	Shelf	Flanking Active TSS	PDXK	0.022127	0.020972	1.09E-05	0.001218	2.52E-05
chr2	2.33E+08	2.33E+08	1187	5	Open sea	Enhancers	ATG16L1, DGKD	0.030465	0.002569	6.74E-06	0.002287	2.52E-05

chr12	2999058	2999448	391	3	Open sea	Enhancers; Flanking Active TSS	TEAD4	0.01876	0.013486	6.49E-06	0.004643	2.57E-05
chr10	44380540	44380713	174	2	Shelf	Enhancers	CXCL12	0.026044	0.022479	1.13E-05	0.001131	2.57E-05
chr11	58575523	58576317	795	3	Shelf; Shore	Flanking Active TSS; Enhancers	LPXN	0.022497	0.013163	0.004854	0.000457	2.57E-05
chr5	1.42E+08	1.42E+08	129	2	Open sea	Bivalent Enhancer		0.016304	0.015674	1.10E-05	0.001325	2.58E-05
chr11	1907884	1908260	377	6	Open sea	Enhancers; Weak transcription	LINC01150	-0.01681	-0.01021	4.95E-05	0.003336	2.58E-05
chr13	1.12E+08	1.12E+08	20	3	Open sea	Weak Repressed PolyComb		0.018361	0.014954	1.08E-05	0.001022	2.60E-05
chr21	45051091	45051539	449	3	Open sea	Enhancers		0.024152	0.015499	7.08E-06	0.003827	2.61E-05
chr7	55109488	55109929	442	2	Open sea	Enhancers	EGFR	0.027704	0.025131	1.28E-05	0.0007	2.61E-05
chr16	84643251	84643744	494	2	Open sea	Enhancers	KLHL36	0.018448	0.016745	1.14E-05	0.001183	2.62E-05
chr6	1.7E+08	1.7E+08	666	3	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh; Bivalent/Poised TSS		-0.02167	-0.01616	1.31E-05	0.001449	2.63E-05
chr10	1.03E+08	1.03E+08	103	2	Shelf; Open sea	Transcr. at gene 5' and 3'; Flanking Active TSS	TRIM8	0.024889	0.020537	1.21E-05	0.000981	2.66E-05

chr11	1.26E+08	1.26E+08	23	2	Open sea	Enhancers	RP11-680F20.4; RP11-680F20.5	-0.01157	-0.00912	1.18E-05	0.001119	2.66E-05
chr8	22927942	22928860	919	4	Open sea	Flanking Active TSS	PEBP4	0.019605	0.015145	1.57E-05	0.003816	2.68E-05
chr12	47755416	47756079	664	3	Shelf	Enhancers; Flanking Active TSS	ENDOU, RAPGEF3; RAPGEF3; SLC48A1	-0.01642	-0.01396	6.97E-06	0.004093	2.70E-05
chr13	28322019	28323283	1265	5	Open sea	Quiescent/Low; Weak transcription	FLT1	-0.01517	-0.01144	2.96E-05	0.003732	2.71E-05
chr14	51360592	51360699	108	3	Open sea	Quiescent/Low	RP11-255G12.2; LINC00640	-0.01297	-0.01042	8.21E-06	0.002771	2.75E-05
chr3	30631966	30632457	492	2	Open sea	Enhancers		0.035573	0.032767	1.19E-05	0.001353	2.77E-05
chr10	90921156	90921324	169	2	Open sea	Enhancers	ANKRD1	0.024517	0.022864	1.29E-05	0.000962	2.81E-05
chr11	1.32E+08	1.32E+08	413	2	Open sea	Enhancers; Active TSS	NTM	0.023658	0.019164	1.73E-05	0.000354	2.81E-05
chr7	24477794	24477868	75	2	Open sea	Enhancers	MPP6	0.020145	0.013941	1.20E-05	0.001406	2.81E-05
chr7	2638298	2638613	316	2	Open sea	Flanking Active TSS	TTYH3	0.018287	0.01475	1.21E-05	0.001403	2.82E-05
chr1	1.66E+08	1.66E+08	357	2	Open sea	Flanking Active TSS; Enhancers	RP11-280O1.2	0.021391	0.020767	1.21E-05	0.001403	2.83E-05
chr3	58571725	58572052	328	3	Open sea	Weak transcription	RP11-475O23.2; FAM107A	-0.0153	-0.00472	1.58E-05	0.001625	2.83E-05

chr9	1.34E+08	1.34E+08	285	3	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh	TMEM8C	0.015758	0.014126	7.56E-06	0.004391	2.83E-05
chr14	1.01E+08	1.01E+08	417	4	Open sea	Bivalent Enhancer		0.017331	0.013643	1.18E-05	0.00357	2.86E-05
chr4	11625011	11625526	516	3	Open sea	Quiescent/Low	RP11-281P23.2	-0.01908	-0.0171	8.54E-06	0.003736	2.86E-05
chr2	1.58E+08	1.58E+08	114	2	Open sea	Flanking Active TSS	ACVR1	0.026238	0.020688	1.44E-05	0.000682	2.87E-05
chr11	12066598	12066928	331	4	Open sea	Enhancers	ENSG00000254991	0.022397	0.014407	6.07E-05	0.003925	2.88E-05
chr5	56409541	56409768	228	2	Open sea	Quiescent/Low; Enhancers		0.021992	0.020698	1.24E-05	0.001427	2.89E-05
chr9	1.22E+08	1.22E+08	748	4	Shore; Island	Flanking Active TSS; Active TSS	DAB2IP	0.01416	0.012982	6.54E-06	0.004605	2.90E-05
chr14	72476752	72476831	80	2	Open sea	Flanking Active TSS	RGS6	0.021503	0.02106	1.25E-05	0.001396	2.91E-05
chr16	716395	716402	8	2	Shore	Flanking Bivalent TSS/Enh; Bivalent Enhancer	MSLN; METRN	0.015187	0.014817	1.25E-05	0.001433	2.91E-05
chr15	72785635	72785851	217	2	Shore	Enhancers	ADPGK	0.029715	0.023757	1.58E-05	0.000531	2.93E-05
chr4	1.06E+08	1.06E+08	70	2	Open sea	Active TSS	INTS12; RP11-45L9.1; GSTCD	0.01884	0.01732	1.27E-05	0.001442	2.97E-05
chr6	87725593	87725796	204	2	Open sea	Enhancers	AKIRIN2	0.027757	0.024903	1.28E-05	0.00144	2.98E-05

chr6	1.09E+08	1.09E+08	105	2	Open sea	Enhancers	ARMC2	0.021723	0.019354	1.32E-05	0.001222	2.99E-05
chr16	85616767	85616915	149	3	Shore	Flanking Active TSS	GSE1	0.018514	0.016139	1.07E-05	0.002983	3.00E-05
chr5	1.8E+08	1.8E+08	339	3	Open sea	Strong transcription; Weak transcription	RP11-1379J22.2; RUFY1	-0.01798	-0.01083	1.46E-05	0.002202	3.08E-05
chr5	54455662	54456302	641	3	Open sea	Active TSS	HSPB3	0.02543	0.022897	1.02E-05	0.001993	3.10E-05
chr3	13480760	13481239	480	4	Shore	Flanking Active TSS	HDAC11-AS1; HDAC11	0.019894	0.014302	0.000116	0.003965	3.10E-05
chr14	75409675	75409975	301	2	Open sea	Enhancers		0.030388	0.026845	1.47E-05	0.000939	3.12E-05
chr10	72089058	72089867	810	6	Shore	Flanking Bivalent TSS/Enh; Repressed PolyComb	SPOCK2	0.017598	0.007632	1.17E-05	0.002854	3.14E-05
chr4	37908629	37908651	23	2	Open sea	Flanking Active TSS	TBC1D1	0.028111	0.025629	1.38E-05	0.001342	3.16E-05
chr1	8880001	8880400	400	2	Shore	Enhancers	LOC102724552	0.024917	0.020417	3.10E-05	0.000146	3.18E-05
chr4	23877869	23878341	473	2	Open sea	Enhancers		0.023609	0.022174	1.45E-05	0.00109	3.18E-05
chr1	6637972	6638396	425	2	Open sea	Genic enhancers	DNAJC11	0.020181	0.017547	1.40E-05	0.001294	3.18E-05

chr7	73893873	73894525	653	2	Open sea	Enhancers		0.017191	0.016643	1.38E-05	0.001494	3.21E-05
chr17	62895427	62895789	363	3	Open sea	Weak Repressed PolyComb		0.018377	0.017409	8.43E-06	0.004911	3.24E-05
chr9	34255095	34255150	56	2	Open sea	Flanking Active TSS	KIF24	0.017594	0.017565	1.43E-05	0.001413	3.28E-05
chr3	1.29E+08	1.29E+08	269	2	Open sea	Enhancers	IFT122; RPL32P3	0.032713	0.027528	1.44E-05	0.00151	3.33E-05
chr10	45181549	45181592	44	2	Open sea	Quiescent/Low	RP11-445N18.5	0.025806	0.019719	1.50E-05	0.001244	3.34E-05
chr8	2043703	2045041	1339	13	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh; Active TSS; Flanking Active TSS	MYOM2	0.019741	0.007793	0.003304	0.003638	3.36E-05
chr1	2.37E+08	2.37E+08	16	2	Open sea	Quiescent/Low	RYR2	0.021602	0.017881	1.57E-05	0.001004	3.36E-05
chr18	3526389	3526903	515	2	Open sea	Flanking Active TSS	DLGAP1	0.024882	0.020802	1.60E-05	0.000925	3.36E-05
chr17	78173130	78173521	392	3	Shelf; Open sea	Flanking Active TSS	SYNGR2	0.021147	0.017197	9.24E-06	0.00459	3.39E-05
chr1	1.54E+08	1.54E+08	992	5	Open sea	Flanking Active TSS	S100A2	0.027334	0.014544	0.000185	0.003018	3.40E-05
chr1	94544120	94544513	394	2	Shelf	Weak Repressed PolyComb		0.018566	0.018287	1.47E-05	0.001512	3.40E-05

chr1	2.31E+08	2.31E+08	122	2	Open sea	Weak Repressed PolyComb		0.021577	0.019974	1.52E-05	0.001326	3.43E-05
chr13	45207042	45207467	426	2	Open sea	Flanking Active TSS; Enhancers	GTF2F2	0.024305	0.022259	1.52E-05	0.001353	3.43E-05
chr17	2214514	2214852	339	2	Open sea	Flanking Active TSS	SMG6	0.024451	0.021969	1.55E-05	0.001212	3.44E-05
chr22	30292348	30292404	57	2	Shelf	Genic enhancers	CASTOR1	-0.01285	-0.0121	1.57E-05	0.001145	3.44E-05
chr19	30373959	30374578	620	2	Shore	Weak Repressed PolyComb	ZNF536	-0.02224	-0.02116	1.53E-05	0.001339	3.46E-05
chr17	8707631	8708293	663	2	Open sea	Weak Repressed PolyComb		0.018961	0.015847	1.51E-05	0.001564	3.49E-05
chr15	82805973	82806459	487	3	Shelf	Active TSS; Weak transcription	FSD2	0.020475	0.018821	1.07E-05	0.003896	3.49E-05
chr1	61606589	61606795	207	2	Open sea	Enhancers		0.024919	0.019568	1.73E-05	0.000831	3.51E-05
chr12	1.31E+08	1.31E+08	233	2	Open sea	Repressed PolyComb		0.01964	0.016838	1.52E-05	0.001549	3.51E-05
chr11	1841078	1841246	169	2	Open sea	Transcr. at gene 5’ and 3’	TNNI2	0.029506	0.026257	1.55E-05	0.001456	3.54E-05
chr11	8778722	8779080	359	2	Open sea	Flanking Active TSS; Enhancers	ST5; RP11-318C2.1	0.02114	0.018823	1.63E-05	0.001209	3.58E-05
chr12	50102422	50103253	832	5	Open sea	Enhancers		0.022703	0.013998	3.09E-05	0.004795	3.58E-05

chr13	50387291	50387534	244	2	Open sea	Active TSS	DLEU1	0.025647	0.021842	1.82E-05	0.000797	3.63E-05
chr13	1.11E+08	1.11E+08	570	3	Shelf	Flanking Active TSS; Enhancers	CARS2	0.01824	0.015731	1.03E-05	0.004529	3.64E-05
chr2	2.38E+08	2.38E+08	117	2	Open sea	Flanking Active TSS	TRAF3IP1	0.024916	0.022429	1.58E-05	0.001606	3.65E-05
chr20	63208639	63209099	461	5	Open sea	Genic enhancers		0.020458	0.012518	7.85E-05	0.003444	3.66E-05
chr17	74430329	74430700	372	3	Shore; Island	Flanking Active TSS	GPRC5C	0.016146	0.01489	1.02E-05	0.004074	3.68E-05
chr1	59056471	59057478	1008	3	Open sea	Enhancers		0.019787	0.016312	3.92E-05	0.001578	3.69E-05
chr2	47193314	47193727	414	2	Open sea	Enhancers		0.033051	0.029665	1.62E-05	0.001528	3.70E-05
chr1	1.85E+08	1.85E+08	541	2	Open sea	Weak transcription; Enhancers	FAM129A	0.041251	0.024185	1.89E-05	0.000766	3.70E-05
chr6	22147852	22148508	657	3	Open sea	Flanking Active TSS; Enhancers	NBAT1; CASC15	0.027012	0.013817	0.003505	0.000691	3.70E-05
chr11	65559061	65559684	624	4	Shore	Enhancers	LTBP3; POLA2	0.025801	0.013332	0.000988	0.001762	3.70E-05
chr17	59428129	59428235	107	2	Open sea	Enhancers	YPEL2	0.050097	0.033719	3.18E-05	0.000208	3.72E-05
chr6	3848008	3849583	1576	23	Shore; Island	Weak transcription; Enhancers;	RP11-420L9.4; FAM50B	0.017902	0.007307	0.00257	0.003753	3.72E-05

						Flanking Active TSS; Active TSS						
chr5	1.73E+08	1.73E+08	79	2	Open sea	Weak transcription		0.025492	0.022519	1.61E-05	0.001634	3.72E-05
chr1	63592508	63593377	870	11	Shore; Island	Flanking Active TSS; Active TSS	ITGB3BP; PGM1	0.028591	0.00855	0.00334	0.002429	3.76E-05
chr8	76680521	76680822	302	3	Shore	Active TSS	ZFHX4-AS1; ZFHX4	0.027454	0.015472	0.000224	0.000521	3.77E-05
chr12	1.05E+08	1.05E+08	32	2	Open sea	Enhancers		0.023672	0.022912	1.76E-05	0.001112	3.77E-05
chr19	19501496	19502355	860	6	Island; Shore	Strong transcription	GATAD2A	-0.01373	-0.00779	0.001057	0.001467	3.79E-05
chr5	1.46E+08	1.46E+08	202	2	Open sea	Quiescent/Low	PRELID2	0.025223	0.024126	1.65E-05	0.001631	3.81E-05
chr3	1.31E+08	1.31E+08	15	2	Open sea	Enhancers		0.032328	0.02542	1.65E-05	0.001645	3.82E-05
chr2	2.01E+08	2.01E+08	109	2	Open sea	Weak transcription	CFLAR-AS1; RNU7-45P; CFLAR	0.032773	0.03063	1.67E-05	0.001547	3.82E-05
chr12	1.11E+08	1.11E+08	404	2	Shelf	Flanking Active TSS	PPTC7	0.022027	0.021178	1.70E-05	0.001506	3.87E-05
chr9	1.28E+08	1.28E+08	549	2	Open sea	Transcr. at gene 5' and 3'	ENG	-0.01623	-0.01501	1.71E-05	0.001569	3.90E-05
chr6	1524258	1524382	125	2	Island; Shore	Flanking Bivalent TSS/Enh; Bivalent/Poised TSS		-0.02545	-0.0251	1.80E-05	0.001229	3.91E-05

chr2	1.76E+08	1.76E+08	845	3	Shore	Enhancers; Weak transcription		0.023619	0.015918	9.77E-05	0.000641	3.91E-05
chr8	13514622	13514981	360	4	Open sea	Active TSS; Quiescent/Low	DLC1	0.017918	0.013391	1.61E-05	0.004209	3.91E-05
chr14	22547337	22547384	48	3	Open sea	Enhancers	C14orf93, PRMT5, RBM23, DAD1; MMP14, ABHD4, TRAC, LOC105370399	0.017045	0.014453	1.16E-05	0.004313	3.94E-05
chr22	49039838	49039884	47	2	Open sea	Weak Repressed PolyComb		0.016387	0.015198	1.72E-05	0.001642	3.95E-05
chr1	15158849	15159370	522	3	Shelf; Open sea	Enhancers	TMEM51	-0.01423	-0.01259	1.37E-05	0.003324	3.97E-05
chr1	15326739	15326950	212	3	Open sea	Repressed PolyComb	RP3-467K16.2; FHAD1	0.0227	0.015579	1.44E-05	0.00181	3.97E-05
chr15	67131113	67131376	264	2	Open sea	Enhancers; Flanking Active TSS	SMAD3	0.019201	0.015117	1.77E-05	0.00158	4.02E-05
chr5	60487207	60488380	1174	5	Open sea	Quiescent/Low	PART1_2; PDE4D; PART1; PART1_1	0.018257	0.010535	0.000764	0.00339	4.05E-05
chr4	24245162	24245362	201	2	Open sea	Enhancers; Flanking Active TSS		0.023537	0.021147	2.00E-05	0.000934	4.06E-05
chr17	45092282	45092974	693	2	Open sea	Genic enhancers; Enhancers	NMT1	0.02313	0.022574	2.05E-05	0.000893	4.11E-05
chr8	2512607	2512628	22	2	Open sea	Weak Repressed PolyComb	AC133633.2	0.019536	0.019503	1.79E-05	0.001685	4.12E-05

chr2	1.28E+08	1.28E+08	533	4	Open sea	Weak Repressed PolyComb	RNA5SP103	0.027586	0.017314	0.000885	0.00178	4.18E-05
chr22	49658645	49658758	114	3	Open sea	Bivalent Enhancer	C22orf34	-0.01875	-0.0158	2.21E-05	0.001663	4.22E-05
chr19	12997340	12997402	63	2	Shore	Transcr. at gene 5' and 3'	NFIX	0.019893	0.01875	1.89E-05	0.001514	4.25E-05
chr11	3614550	3614870	321	2	Open sea	Flanking Active TSS; Enhancers	TRPC2	0.021838	0.019089	1.88E-05	0.001597	4.26E-05
chr9	1.14E+08	1.14E+08	287	2	Open sea	Flanking Active TSS	COL27A1	0.019255	0.019096	1.88E-05	0.001661	4.29E-05
chr15	22952898	22953235	338	3	Open sea	Enhancers		0.022506	0.017511	1.28E-05	0.004022	4.33E-05
chr3	1.95E+08	1.95E+08	264	3	Open sea	Enhancers	FAM43A, XXYLT1-AS2; XXYLT1	-0.01747	-0.01314	2.26E-05	0.002087	4.34E-05
chr8	1.24E+08	1.24E+08	47	3	Open sea	Quiescent/Low	ANXA13	-0.01574	-0.01481	1.65E-05	0.001706	4.35E-05
chr10	69494692	69494943	252	3	Open sea	Weak transcription	TSPAN15	0.029042	0.022021	2.04E-05	0.001739	4.35E-05
chr2	47063248	47063834	587	2	Open sea	Enhancers	TTC7A	0.028499	0.02381	2.23E-05	0.000871	4.37E-05
chr3	50230610	50230826	217	3	Shelf	Quiescent/Low	GNAI2	0.020998	0.014761	1.26E-05	0.004052	4.38E-05
chr13	1.13E+08	1.13E+08	41	3	Shelf	Bivalent Enhancer	MCF2L	-0.01255	-0.01114	1.36E-05	0.00365	4.40E-05
chr14	21036930	21037338	409	2	Open sea	Enhancers		0.024509	0.02409	2.37E-05	0.000783	4.49E-05

chr8	29315089	29315480	392	4	Open sea	Flanking Active TSS; Active TSS	AC084262.2	-0.03203	-0.01785	3.99E-05	0.002969	4.50E-05
chr3	1.41E+08	1.41E+08	76	2	Open sea	Enhancers	PXYLP1	0.022715	0.021957	2.01E-05	0.001559	4.51E-05
chr2	2.07E+08	2.07E+08	361	3	Open sea	Enhancers	ENSG00000240440; AC007879.7	0.025892	0.017451	0.000147	0.001133	4.52E-05
chr11	1.12E+08	1.12E+08	808	2	Shore	Enhancers	C11orf52	0.028583	0.026329	2.00E-05	0.00164	4.53E-05
chr15	67148563	67148728	166	2	Open sea	Enhancers	SMAD3	0.025854	0.021989	2.01E-05	0.001634	4.54E-05
chr7	25359490	25360419	930	5	Open sea	Quiescent/Low	RP5-978I12.1	0.026227	0.019069	1.70E-05	0.004658	4.61E-05
chr2	82007178	82007321	144	2	Open sea	Quiescent/Low	AC079896.1	0.024073	0.021434	2.11E-05	0.001419	4.62E-05
chr19	18002775	18002794	20	2	Shore	Repressed PolyComb; Weak Repressed PolyComb	ARRDC2	-0.01483	-0.01466	2.07E-05	0.001585	4.63E-05
chr4	75034904	75034927	24	2	Open sea	Quiescent/Low	PARM1; RP11-44F21.2	0.018843	0.016601	2.13E-05	0.001423	4.66E-05
chr6	32054692	32054864	173	3	Open sea	Weak transcription	TNXB	0.019908	0.016947	1.47E-05	0.003104	4.69E-05
chr17	10549533	10549943	411	4	Open sea	Active TSS; Weak transcription	MYHAS; MYH2	0.025832	0.016958	0.000104	0.002231	4.71E-05
chr6	2743696	2743829	134	2	Open sea	Flanking Active TSS	MYLK4	0.027562	0.025791	2.06E-05	0.001866	4.72E-05

chr7	1.51E+08	1.51E+08	625	5	Shore; Island	Weak transcription	AGAP3	0.016308	-0.00202	0.000113	0.004272	4.82E-05
chr20	62242208	62242376	169	2	Shelf	Genic enhancers	OSBPL2	-0.01869	-0.01792	2.14E-05	0.001684	4.82E-05
chr8	1.3E+08	1.3E+08	149	2	Open sea	Enhancers; Flanking Active TSS		0.017945	0.012192	2.13E-05	0.001796	4.84E-05
chr2	1.89E+08	1.89E+08	57	2	Open sea	Enhancers	COL5A2	0.022376	0.020786	2.18E-05	0.001612	4.86E-05
chr1	26790569	26791216	648	2	Shelf	Enhancers	RPS6KA1, ARID1A, ZDHHC18, PIGV	-0.02055	-0.01849	2.36E-05	0.001139	4.87E-05
chr10	3825439	3826143	705	3	Open sea	Flanking Active TSS		0.021408	0.014916	1.69E-05	0.003639	4.87E-05
chr15	40103274	40103711	438	2	Open sea	Flanking Active TSS	BMF	0.018679	0.016641	2.15E-05	0.00179	4.89E-05
chr14	1.02E+08	1.02E+08	97	2	Shore	Enhancers	WDR20	0.023108	0.020548	2.13E-05	0.001899	4.89E-05
chr2	1.73E+08	1.73E+08	105	3	Shore	Repressed PolyComb	RAPGEF4; RAPGEF4-AS1	0.021705	0.018089	1.42E-05	0.004726	4.91E-05
chr2	1.48E+08	1.48E+08	57	2	Open sea	Weak transcription	ACVR2A	0.035506	0.030201	2.16E-05	0.001828	4.93E-05
chr8	66559958	66560257	300	2	Open sea	Enhancers		0.021895	0.021818	2.15E-05	0.001909	4.93E-05
chr1	1.62E+08	1.62E+08	304	2	Open sea	Flanking Active TSS; Enhancers	OLFML2B	0.018581	0.017279	2.77E-05	0.0007	4.96E-05

chr22	29074220	29075111	892	5	Island; Shore	Flanking Active TSS; Enhancers	KREMEN1; Chr22-38_28785274-29006793.1	0.023072	0.012304	4.62E-05	0.00411	4.97E-05
chr17	40511176	40511579	404	2	Open sea	Enhancers; Flanking Active TSS		0.025145	0.024284	2.18E-05	0.001852	4.97E-05
chr9	1.34E+08	1.34E+08	328	2	Shelf	Enhancers		0.021834	0.018692	2.34E-05	0.00133	4.98E-05
chr17	79837112	79837883	772	3	Shore	Flanking Bivalent TSS/Enh	CBX4	0.021135	0.015368	6.32E-05	0.001766	4.99E-05
chr4	1.86E+08	1.86E+08	267	2	Open sea	Enhancers		0.01976	0.019597	2.21E-05	0.001768	5.00E-05
chr5	75539060	75539066	7	2	Open sea	Weak transcription	RNU7-175P; POLK	0.022877	0.021815	2.20E-05	0.001843	5.00E-05
chr15	60650349	60650467	119	2	Open sea	Enhancers		0.025672	0.021639	2.23E-05	0.001681	5.00E-05
chr16	67993864	67994036	173	3	Shore	Flanking Active TSS	DUS2; DPEP2	-0.01785	-0.01458	2.59E-05	0.002871	5.05E-05
chr12	4025162	4025373	212	2	Open sea	Weak Repressed PolyComb	RP11-320N7.2	0.01977	0.018908	2.21E-05	0.001923	5.07E-05
chr17	75635379	75635704	326	3	Shelf	Flanking Active TSS	RECQL5; SMIM5	0.017739	0.012641	1.82E-05	0.003943	5.08E-05
chr19	13016574	13017058	485	3	Shelf	Enhancers; Flanking Active TSS	NFIX	0.019927	0.017661	1.41E-05	0.004994	5.10E-05
chr5	1.43E+08	1.43E+08	359	2	Open sea	Enhancers	ARHGAP26; ARHGAP26-AS1	0.023599	0.020934	2.56E-05	0.001063	5.14E-05

chr22	42688972	42688978	7	2	Shelf	Flanking Active TSS		0.018448	0.017531	2.25E-05	0.001955	5.16E-05
chr10	34628593	34628640	48	2	Open sea	Enhancers		0.017932	0.01604	2.29E-05	0.001901	5.22E-05
chr5	1.43E+08	1.43E+08	366	2	Open sea	Enhancers		0.024614	0.024597	2.54E-05	0.001252	5.29E-05
chr15	81374329	81374505	177	4	Open sea	Weak Repressed PolyComb	TMC3	0.020539	0.014001	0.000734	0.00126	5.29E-05
chr9	23672395	23672726	332	4	Open sea	Quiescent/Low	RP11-315I14.2	0.025542	0.016385	0.000142	0.002831	5.31E-05
chr10	1.3E+08	1.3E+08	49	3	Shelf	Weak Repressed PolyComb		0.020712	0.018412	1.61E-05	0.004599	5.38E-05
chr6	1.68E+08	1.68E+08	607	4	Open sea	Flanking Active TSS	RP11-351J23.2	0.015255	0.011912	2.24E-05	0.003672	5.42E-05
chr20	57714607	57714640	34	2	Shelf	Flanking Active TSS		0.021611	0.020484	2.41E-05	0.001944	5.48E-05
chr16	57635813	57636460	648	2	Open sea	Bivalent Enhancer; Flanking Bivalent TSS/Enh	GPR56	-0.01672	-0.0154	2.41E-05	0.00201	5.49E-05
chr3	32003665	32004153	489	2	Open sea	Quiescent/Low	OSBPL10	0.017943	0.017663	2.77E-05	0.001109	5.54E-05
chr14	68583691	68583840	150	2	Open sea	Flanking Active TSS	RAD51B	0.017625	0.01696	2.58E-05	0.001479	5.55E-05
chr6	52552057	52552239	183	3	Open sea	Flanking Active TSS	TRAM2	0.017262	0.014121	1.96E-05	0.004233	5.58E-05

chr20	33423411	33423726	316	2	Open sea	Flanking Active TSS	SNTA1	0.019004	0.0168	3.42E-05	0.000588	5.59E-05
chr2	2.1E+08	2.1E+08	54	2	Open sea	Flanking Active TSS	LANCL1-AS1	0.029019	0.025457	2.53E-05	0.00178	5.63E-05
chr13	31982662	31982669	8	2	Open sea	Quiescent/Low		0.021677	0.020001	3.32E-05	0.000653	5.63E-05
chr14	92600465	92600676	212	2	Open sea	Enhancers; Weak Repressed PolyComb	RIN3	0.021285	0.019159	2.48E-05	0.002044	5.66E-05
chr2	2.18E+08	2.18E+08	298	3	Island	Repressed PolyComb	RUFY4	-0.02587	-0.01742	1.81E-05	0.004556	5.75E-05
chr16	11105285	11105334	50	2	Open sea	Flanking Active TSS	CLEC16A	0.016706	0.016424	2.54E-05	0.002003	5.76E-05
chr5	35853501	35854446	946	3	Open sea	Quiescent/Low	IL7R	0.019806	0.008096	2.85E-05	0.001529	5.76E-05
chr4	60865354	60865465	112	2	Open sea	Quiescent/Low		0.023798	0.020307	2.65E-05	0.001684	5.81E-05
chr5	1.13E+08	1.13E+08	285	2	Open sea	Enhancers		0.026709	0.020261	2.90E-05	0.001148	5.82E-05
chr9	87010519	87010831	313	2	Shore	Weak Repressed PolyComb	CDC20P1; RP11-276H19.2	-0.02103	-0.01755	3.07E-05	0.000952	5.84E-05
chr14	91244402	91244820	419	3	Open sea	Flanking Bivalent TSS/Enh; Bivalent Enhancer	CTD-2547L24.3; GPR68	0.013814	0.013144	2.02E-05	0.004255	5.87E-05
chr9	77731753	77732188	436	3	Open sea	Enhancers; Weak transcription	GNAQ	0.025548	0.019082	1.92E-05	0.003731	5.89E-05

chr10	1.04E+08	1.04E+08	555	2	Open sea	Flanking Active TSS	SH3PXD2A	0.018493	0.01721	2.60E-05	0.00204	5.90E-05
chr16	12265402	12265439	38	2	Shelf	Enhancers	SNX29	0.023859	0.023816	2.64E-05	0.001914	5.92E-05
chr14	64118175	64118264	90	2	Open sea	Active TSS	ESR2; SYNE2	0.022604	0.021243	3.35E-05	0.000774	5.94E-05
chr2	1.06E+08	1.06E+08	571	2	Open sea	Enhancers	ENSG00000235522	0.018801	0.017942	2.62E-05	0.002072	5.96E-05
chr7	22812277	22812715	439	2	Open sea	Active TSS	TOMM7	0.021281	0.021148	2.62E-05	0.002117	5.97E-05
chr10	33559129	33559316	188	2	Open sea	Weak Repressed PolyComb		0.024873	0.019465	3.75E-05	0.000578	5.98E-05
chr13	1.11E+08	1.11E+08	906	6	Open sea	Flanking Active TSS; Enhancers	ARHGEF7	0.022615	0.008968	0.00396	0.001955	6.00E-05
chr17	13544347	13544535	189	2	Open sea	Quiescent/Low	MIR548H3; HS3ST3A1	0.023148	0.020437	2.67E-05	0.001998	6.01E-05
chr7	55056808	55057148	341	2	Open sea	Enhancers	EGFR	0.030079	0.022846	3.20E-05	0.000964	6.05E-05
chr1	22652487	22653245	759	7	Open sea	Repressed PolyComb	C1QB	0.025111	0.010105	0.00024	0.003297	6.07E-05
chr1	1.54E+08	1.54E+08	53	2	Shore	Flanking Active TSS	RP11-350G8.5; IL6R	0.017028	0.015441	2.73E-05	0.001945	6.10E-05
chr11	69089348	69089482	135	4	Open sea	Genic enhancers	TPCN2	0.017952	0.012681	0.000217	0.000517	6.14E-05

chr12	92466271	92466311	41	2	Open sea	Flanking Active TSS	RP11-693J15.4; RP11-693J15.5	0.020996	0.020995	2.87E-05	0.00156	6.14E-05
chr7	1.17E+08	1.17E+08	30	2	Open sea	Weak transcription	MET	0.037115	0.035565	2.74E-05	0.002039	6.18E-05
chr7	51034325	51034431	107	2	Open sea	Enhancers		0.016891	0.016539	2.73E-05	0.002164	6.22E-05
chr3	99938421	99938749	329	3	Open sea	Enhancers	FILIP1L	0.025421	0.020091	2.47E-05	0.003339	6.24E-05
chr2	2.17E+08	2.17E+08	370	2	Open sea	Flanking Active TSS		0.014681	0.0142	2.85E-05	0.001785	6.24E-05
chr2	1.29E+08	1.29E+08	1008	3	Open sea	Flanking Active TSS; Enhancers	AC079586.1	0.025557	0.018636	2.10E-05	0.002943	6.27E-05
chr10	72637518	72637577	60	2	Open sea	Flanking Active TSS	HMGN2P34	0.017284	0.016271	2.80E-05	0.002079	6.31E-05
chr9	1.28E+08	1.28E+08	71	2	Shore	Weak transcription	PTRH1	0.0268	0.02513	2.81E-05	0.002014	6.31E-05
chr13	79988753	79988892	140	2	Open sea	Quiescent/Low		0.021838	0.019719	2.77E-05	0.002171	6.31E-05
chr20	50009899	50010083	185	2	Island	Bivalent/Poised TSS		0.016851	0.015373	2.92E-05	0.001783	6.38E-05
chr1	99852264	99852664	401	2	Shore; Shelf	Active TSS	AGL	0.023463	0.022208	2.82E-05	0.002101	6.38E-05
chr2	85433373	85434439	1067	6	Open sea; Shelf	Enhancers; Flanking Active TSS	TGOLN2, CAPG; SH2D6; Y_RNA	0.021666	0.01231	0.000309	0.003906	6.38E-05

chr12	1.11E+08	1.11E+08	442	2	Open sea	Enhancers		0.027356	0.023821	2.91E-05	0.001807	6.39E-05
chr19	13001547	13001634	88	2	Island	Transcr. at gene 5' and 3'; Flanking Active TSS	NFIX	0.01809	0.016819	2.88E-05	0.00195	6.40E-05
chr15	84752380	84753168	789	4	Shelf; Open sea	Enhancers; Flanking Active TSS; NA	RP11-7M10.2; ZNF592	0.021206	0.014004	5.37E-05	0.002256	6.42E-05
chr13	48595650	48596083	434	2	Open sea	Quiescent/Low		0.01885	0.015279	4.20E-05	0.000559	6.46E-05
chr6	35061134	35061371	238	2	Open sea	Genic enhancers	ANKS1A	-0.01611	-0.01338	2.88E-05	0.002088	6.47E-05
chr22	35615357	35616173	817	4	Open sea	Transcr. at gene 5' and 3'	MB	0.024049	0.01606	3.30E-05	0.004594	6.51E-05
chr7	503809	503871	63	2	Open sea	Flanking Active TSS	PDGFA	0.022045	0.021235	3.15E-05	0.001424	6.52E-05
chr17	79080009	79080275	267	2	Open sea	Genic enhancers	ENGASE	-0.0186	-0.01811	2.95E-05	0.001908	6.52E-05
chr22	49963114	49963616	503	3	Island; Shore	Transcr. at gene 5' and 3'	PIM3	-0.02618	-0.01926	2.81E-05	0.002125	6.58E-05
chr17	81656987	81657433	447	5	Open sea	Enhancers; Weak transcription	PDE6G	-0.01906	-0.01061	4.68E-05	0.004001	6.58E-05
chr7	45978411	45978831	421	3	Open sea	Enhancers	AC073115.6	0.022616	0.012479	3.15E-05	0.001252	6.60E-05
chr16	81431820	81432262	443	2	Open sea	Enhancers; Quiescent/Low	CMIP	0.02211	0.018576	3.25E-05	0.001377	6.65E-05

chr19	35405335	35405575	241	3	Open sea	Weak Repressed PolyComb	LINC01531	0.015232	0.010858	0.00012	0.001727	6.73E-05
chr12	1.02E+08	1.02E+08	56	2	Shore	Flanking Active TSS; Active TSS	RP11-285E23.2; CHPT1	0.019549	0.017261	3.01E-05	0.002194	6.78E-05
chr16	2031192	2031444	253	2	Shelf	Genic enhancers	SLC9A3R2	-0.02248	-0.02023	3.00E-05	0.002247	6.80E-05
chr17	3879609	3880053	445	2	Open sea	Enhancers	CAMKK1	0.028139	0.021969	3.00E-05	0.002274	6.82E-05
chr5	3325580	3326529	950	4	Shore; Island	Weak Repressed PolyComb; Bivalent/Poised TSS		0.019962	0.012754	0.000633	0.004187	6.82E-05
chr15	40063493	40064228	736	3	Open sea	Enhancers	SRP14-AS1; SRP14	0.017629	0.013495	4.96E-05	0.001484	6.85E-05
chr20	57374619	57374900	282	3	Open sea	Genic enhancers	RBM38	-0.01555	-0.01171	0.000106	0.001902	6.91E-05
chr3	11633232	11633850	619	3	Open sea	Flanking Active TSS	VGLL4	0.025156	0.002984	2.31E-05	0.003168	6.92E-05
chr22	41862283	41862533	251	2	Open sea	Enhancers	ENSG00000184068, CENPM; MEI1, SREBF2	0.023058	0.022126	3.11E-05	0.002073	6.92E-05
chr1	54273095	54273502	408	2	Open sea	Flanking Active TSS	SSBP3	0.017797	0.015953	3.08E-05	0.002263	6.95E-05
chr17	80773379	80773426	48	2	Open sea	Flanking Active TSS	RPTOR	0.024857	0.018778	4.26E-05	0.000687	6.96E-05
chr13	1.13E+08	1.13E+08	198	2	Shelf	Weak Repressed PolyComb	ATP11AUN	-0.01722	-0.01475	4.15E-05	0.000747	6.99E-05

chr5	1.22E+08	1.22E+08	230	2	Open sea	Quiescent/Low	CTC-441N14.4; ZNF474	0.019521	0.017884	3.11E-05	0.002306	7.04E-05
chr6	43890847	43891003	157	3	Open sea	Weak Repressed PolyComb		-0.01404	-0.00947	2.07E-05	0.004898	7.05E-05
chr6	1.33E+08	1.33E+08	230	5	Open sea	Quiescent/Low	VNN1	0.022188	0.015993	0.000529	0.004455	7.08E-05
chr7	30920093	30920802	710	5	Open sea	Enhancers; Flanking Active TSS	RP5-877J2.1; AQP1	-0.01925	-0.01103	9.82E-05	0.001205	7.10E-05
chr5	1.43E+08	1.43E+08	18	2	Open sea	Flanking Active TSS	NR3C1	0.022831	0.020963	3.74E-05	0.00109	7.10E-05
chr2	43269073	43269503	431	3	Open sea	Flanking Active TSS	THADA	0.027911	0.014823	0.000367	0.001278	7.14E-05
chr6	1.5E+08	1.5E+08	16	2	Shore	Flanking Active TSS	RAET1E-AS1; LRP11	0.019918	0.017011	3.78E-05	0.001091	7.16E-05
chr18	9824696	9824959	264	2	Open sea	Enhancers	PPP4R1; RAB31, VAPA	0.029654	0.026436	3.24E-05	0.002039	7.16E-05
chr17	83087618	83087904	287	3	Shelf; Shore	Bivalent Enhancer	METRNL	0.018618	0.014938	3.17E-05	0.003418	7.19E-05
chr1	43270618	43270719	102	2	Shelf	Repressed PolyComb	EBNA1BP2; TMEM125	0.014753	0.014522	3.19E-05	0.002307	7.21E-05
chr3	1.68E+08	1.68E+08	390	3	Open sea	Flanking Active TSS; Active TSS	RP11-298O21.6; RP11-298O21.7	-0.01386	-0.01307	2.79E-05	0.003148	7.22E-05
chr7	46969296	46969667	372	4	Open sea	Quiescent/Low	AC004901.1; AC004870.4	0.023329	0.015855	4.24E-05	0.003929	7.22E-05

chr10	73859661	73859741	81	2	Open sea	Genic enhancers		0.019524	0.016226	3.72E-05	0.001241	7.28E-05
chr6	90286386	90286395	10	2	Open sea	Enhancers	BACH2	0.028044	0.024005	5.10E-05	0.000513	7.29E-05
chr11	73215917	73216786	870	5	Shelf; Shore	Enhancers; Flanking Active TSS	ENSG00000215841; RP11-800A3.2	0.023525	0.015164	0.000338	0.004345	7.29E-05
chr2	1.73E+08	1.73E+08	533	3	Shore	Weak Repressed PolyComb	CDCA7; AC092573.2	0.025412	0.014139	0.000101	0.00201	7.33E-05
chr16	46649731	46649814	84	2	Open sea	Flanking Active TSS	RP11-93O14.1	0.016401	0.013862	3.50E-05	0.001647	7.35E-05
chr8	1.25E+08	1.25E+08	224	3	Open sea	Quiescent/Low	RP11-1082L8.2; LINC00964	0.015734	0.013383	3.99E-05	0.001042	7.42E-05
chr8	1E+08	1E+08	47	2	Open sea	Enhancers		0.018577	0.018259	3.60E-05	0.001569	7.45E-05
chr5	74316056	74316377	322	2	Open sea	Enhancers		0.029139	0.024722	4.05E-05	0.001023	7.46E-05
chr5	1.77E+08	1.77E+08	1283	9	Island; Shore	Genic enhancers; Transcr. at gene 5' and 3'	PDLIM7; RP11-1334A24.6	0.026653	0.008738	0.000231	0.002357	7.46E-05
chr2	1.27E+08	1.27E+08	465	4	Shore	Enhancers; Flanking Active TSS	GYPC	-0.01879	-0.01328	2.01E-05	0.004116	7.48E-05
chr2	2.16E+08	2.16E+08	57	2	Open sea	Enhancers	LINC00607	0.016372	0.015411	3.37E-05	0.00216	7.48E-05

chr11	62555743	62556309	567	2	Open sea	Flanking Active TSS	AHNAK	0.022897	0.01855	5.14E-05	0.000546	7.49E-05
chr22	23149811	23150041	231	2	Open sea	Bivalent Enhancer	RAB36	-0.01995	-0.01664	3.71E-05	0.001477	7.55E-05
chr7	1.3E+08	1.3E+08	716	2	Open sea	Weak transcription	CEP41	0.022555	0.020324	3.50E-05	0.001906	7.56E-05
chr2	1.72E+08	1.72E+08	71	2	Open sea	Quiescent/Low	AC104088.1	0.018307	0.018218	3.47E-05	0.001985	7.57E-05
chr1	41724648	41724863	216	2	Open sea	Enhancers		-0.01366	-0.01107	3.48E-05	0.00199	7.57E-05
chr20	48828894	48829047	154	2	Shore	Flanking Active TSS	PREX1; RP5-906C1.1	-0.01359	-0.01046	3.72E-05	0.00158	7.69E-05
chr21	38156969	38157033	65	2	Open sea	Weak Repressed PolyComb	KCNJ15; DSCR8	0.020116	0.019854	3.95E-05	0.001262	7.69E-05
chr8	1.03E+08	1.03E+08	296	2	Open sea	Quiescent/Low	BAALC; BAALC-AS1	0.022235	0.016928	4.78E-05	0.000755	7.84E-05
chr18	58998847	58999006	160	2	Open sea	Flanking Active TSS	OACYLP	0.018092	0.01757	4.37E-05	0.000998	7.88E-05
chr20	38211883	38211942	60	2	Open sea	Enhancers		0.020451	0.019674	3.49E-05	0.00246	7.88E-05
chr11	2600042	2600134	93	2	Open sea	Quiescent/Low	KCNQ1	-0.02269	-0.02126	4.10E-05	0.001255	7.92E-05
chr12	5480277	5480526	250	2	Open sea	Weak Repressed PolyComb	NTF3	0.022981	0.019765	3.77E-05	0.001802	7.98E-05

chr13	1.13E+08	1.13E+08	135	2	Open sea	Strong transcription	PCID2	-0.01293	-0.01222	4.52E-05	0.000958	8.01E-05
chr17	48972527	48972593	67	2	Open sea	Bivalent Enhancer		0.014209	0.013292	3.59E-05	0.002373	8.04E-05
chr1	36320683	36321754	1072	5	Shore; Island	Flanking Bivalent TSS/Enh	SH3D21	0.014538	0.008685	0.004973	0.003909	8.09E-05
chr10	71757002	71757199	198	2	Open sea	Enhancers; Flanking Active TSS	C10orf54; CDH23	0.024064	0.022925	3.59E-05	0.002481	8.09E-05
chr12	1.14E+08	1.14E+08	39	2	Open sea	Repressed PolyComb	LINC01234	0.020543	0.018564	3.70E-05	0.002171	8.11E-05
chr19	2546878	2547068	191	3	Shore	Flanking Active TSS	GNG7	-0.01725	-0.00918	8.12E-05	0.002424	8.16E-05
chr5	91182898	91183125	228	2	Open sea	Enhancers		0.026888	0.021309	5.04E-05	0.000775	8.23E-05
chr2	2E+08	2E+08	113	2	Shelf	Enhancers	SPATS2L	0.030207	0.023955	3.66E-05	0.002505	8.24E-05
chr13	1.09E+08	1.09E+08	235	2	Open sea	Active TSS	LINC00370	-0.02773	-0.02519	3.77E-05	0.002165	8.25E-05
chr2	1.28E+08	1.28E+08	421	2	Shore; Shelf	Flanking Bivalent TSS/Enh		0.015224	0.014413	4.33E-05	0.001233	8.25E-05
chr1	1.57E+08	1.57E+08	381	2	Shelf; Shore	Flanking Active TSS	ARHGEF11	0.016465	0.015812	4.07E-05	0.001568	8.28E-05
chr10	11757636	11757667	32	2	Open sea	Enhancers		0.017634	0.016789	3.79E-05	0.002168	8.30E-05

chr7	898067	898312	246	2	Shore	Genic enhancers	ADAP1	-0.01721	-0.01597	3.80E-05	0.002187	8.32E-05
chr17	75695607	75695911	305	2	Open sea	Genic enhancers	SAP30BP	0.028292	0.021893	3.76E-05	0.002328	8.34E-05
chr21	39984634	39985036	403	2	Open sea	Weak Repressed PolyComb		0.020309	0.020108	5.67E-05	0.000625	8.44E-05
chr22	42887707	42887713	7	2	Open sea	Strong transcription	PACSIN2	-0.01502	-0.01172	3.83E-05	0.002308	8.45E-05
chr8	1.27E+08	1.27E+08	50	2	Open sea	Quiescent/Low	RP11-89K10.1	0.018125	0.016859	4.06E-05	0.00185	8.54E-05
chr1	59597955	59598025	71	2	Open sea	Enhancers		0.035698	0.031208	3.84E-05	0.002402	8.54E-05
chr18	1075646	1075732	87	2	Open sea	Quiescent/Low	RP11-78F17.1	0.021198	0.020141	4.71E-05	0.001076	8.54E-05
chr5	68292357	68292556	200	3	Shelf	Active TSS; Flanking Active TSS	PIK3R1	0.021527	0.015908	4.24E-05	0.002779	8.58E-05
chr1	1.55E+08	1.55E+08	523	5	Island	Flanking Active TSS	RP11-540D14.8; EFNA4; ADAM15	0.013145	0.009236	5.58E-05	0.00456	8.59E-05
chr10	1.02E+08	1.02E+08	30	2	Open sea	Transcr. at gene 5' and 3'	ACTR1A	0.026373	0.019455	4.20E-05	0.001661	8.60E-05
chr16	89370038	89370429	392	2	Open sea	Enhancers		0.021588	0.018976	3.85E-05	0.002526	8.62E-05
chr12	1.24E+08	1.24E+08	394	2	Open sea	Enhancers		-0.01567	-0.01483	3.92E-05	0.002332	8.66E-05

chr10	1.22E+08	1.22E+08	143	3	Open sea	Enhancers		0.018631	0.014883	0.000201	0.00191	8.70E-05
chr7	1.11E+08	1.11E+08	349	2	Open sea	Quiescent/Low	IMMP2L	0.023516	0.017199	4.88E-05	0.001035	8.71E-05
chr5	56298326	56298790	465	3	Open sea	Enhancers; Quiescent/Low	RNU6ATAC2P	0.016894	0.014159	5.56E-05	0.003262	8.79E-05
chr5	1.25E+08	1.25E+08	295	2	Open sea	Weak transcription; Active TSS		0.022313	0.019368	4.05E-05	0.002177	8.79E-05
chr21	25944348	25944452	105	2	Open sea	Weak transcription	APP; RNU6-123P	0.01817	0.016411	3.92E-05	0.002569	8.80E-05
chr10	69337570	69337978	409	5	Open sea	Enhancers	HK1	0.025599	0.012978	0.000236	0.004879	8.93E-05
chr2	75605957	75606369	413	2	Open sea	Repressed PolyComb		0.019254	0.017541	5.14E-05	0.000979	8.94E-05
chr5	1.32E+08	1.32E+08	386	2	Open sea	Enhancers; Weak transcription	AC034220.3	-0.01885	-0.01668	4.13E-05	0.002223	8.97E-05
chr2	2E+08	2E+08	66	2	Open sea	Active TSS; Flanking Active TSS	SPATS2L	0.014637	0.013556	4.02E-05	0.002645	9.02E-05
chr10	43878470	43878941	472	2	Open sea	Enhancers	LINC00840	-0.01846	-0.01777	4.02E-05	0.002643	9.04E-05
chr12	68374164	68374404	241	2	Open sea	Enhancers	RP11-81H14.2	0.02706	0.02602	4.30E-05	0.001945	9.06E-05
chr8	1.03E+08	1.03E+08	341	2	Shore	Flanking Active TSS	SLC25A32; DCAF13	0.01588	0.013837	4.08E-05	0.002657	9.15E-05

chr2	2.41E+08	2.41E+08	289	2	Shelf	Enhancers; Flanking Active TSS	AC104809.3	0.021773	0.01979	5.08E-05	0.001128	9.20E-05
chr2	48107460	48107477	18	2	Open sea	Flanking Active TSS	AC079807.4	-0.01893	-0.01829	4.11E-05	0.002646	9.21E-05
chr19	39935055	39935703	649	4	Open sea	Repressed PolyComb	FCGBP	0.018263	0.010258	0.001182	0.001126	9.25E-05
chr3	72995661	72995895	235	2	Shore	Enhancers	PPP4R2	0.022006	0.020977	4.22E-05	0.002397	9.27E-05
chr11	75572935	75573597	663	4	Open sea	Genic enhancers		0.024527	0.015711	5.87E-05	0.004891	9.29E-05
chr2	10427623	10427716	94	2	Shore	Strong transcription; Repressed PolyComb		0.02403	0.019281	4.26E-05	0.002372	9.33E-05
chr2	2.19E+08	2.19E+08	86	2	Open sea	Active TSS	PLCD4	0.02115	0.019933	4.23E-05	0.002489	9.35E-05
chr16	648071	648634	564	3	Shore	Flanking Active TSS	WDR90; MSLN; FAM195A; AL022341.3	0.013565	0.011548	3.41E-05	0.004997	9.36E-05
chr15	36601747	36601764	18	2	Open sea	Flanking Active TSS	C15orf41	0.02192	0.021884	4.21E-05	0.002592	9.37E-05
chr1	59574129	59574185	57	2	Open sea	Heterochromatin	FGGY	0.027978	0.023667	4.29E-05	0.002357	9.37E-05
chr8	1.1E+08	1.1E+08	39	2	Open sea	Quiescent/Low		0.019699	0.018361	4.35E-05	0.002216	9.38E-05
chr5	61312603	61312699	97	2	Open sea	Repressed PolyComb		0.019777	0.017593	4.29E-05	0.002448	9.44E-05

chr8	1.43E+08	1.43E+08	52	2	Shore	Flanking Active TSS	ZC3H3	0.022717	0.019843	4.62E-05	0.001798	9.47E-05
chr8	1.05E+08	1.05E+08	351	2	Open sea	Quiescent/Low	ZFPM2	0.021351	0.018408	5.85E-05	0.000839	9.48E-05
chr6	1.48E+08	1.48E+08	37	2	Open sea	Quiescent/Low	SASH1	0.026499	0.024511	4.41E-05	0.002203	9.49E-05
chr11	46710825	46711235	411	2	Open sea	Enhancers		0.014638	0.0128	4.83E-05	0.001576	9.57E-05
chr19	48630163	48630389	227	4	Island	Genic enhancers; Transcr. at gene 5' and 3'	SPACA4; SPHK2; DBP	0.022011	0.015595	0.000155	0.003348	9.65E-05
chr1	2.03E+08	2.03E+08	19	2	Open sea	Flanking Bivalent TSS/Enh	PRELP	0.01944	0.015959	4.78E-05	0.001789	9.76E-05
chr1	59198509	59198757	249	2	Open sea	Flanking Active TSS; Enhancers	RP11-470E16.2; HSD52	0.021062	0.019956	5.22E-05	0.001315	9.78E-05
chr12	2841197	2841221	25	2	Open sea	Weak transcription	ITFG2	0.023633	0.015866	5.36E-05	0.001209	9.79E-05
chr8	48583484	48583830	347	3	Open sea	Enhancers	LOC101929268	0.020788	0.016406	4.73E-05	0.003298	9.81E-05
chr2	96833004	96833164	161	2	Open sea	Transcr. at gene 5' and 3'	CNNM3; ANKRD23	0.017647	0.017361	4.45E-05	0.002695	9.90E-05
chr5	1.41E+08	1.41E+08	557	3	Shelf	Weak transcription	PCDHGC4; PCDHGB5; PCDHGA6; PCDHGA12; PCDHGC5; PCDHGB6; PCDHGA7; PCDHGB7; PCDHGA8; PCDHGA9; PCDHGA1; PCDHGB1;	0.023756	0.012921	0.000571	0.00053	0.0001

							PCDHGA2; PCDHGB2; PCDHGA3; PCDHGB3; PCDHGA4; PCDHGA10; PCDHGC3; PCDHGB4; PCDHGA5; PCDHGA11					
chr16	70692045	70692160	116	2	Open sea	Flanking Active TSS	VAC14	0.01699	0.015557	4.55E-05	0.002624	0.000101
chr11	1.19E+08	1.19E+08	800	3	Shore	Transcr. at gene 5' and 3'; Flanking Active TSS	SLC37A4	0.022867	0.016553	5.41E-05	0.002232	0.000101
chr10	69505882	69506153	272	2	Open sea	Flanking Active TSS	TSPAN15	0.027989	0.025873	4.62E-05	0.002502	0.000101
chr1	25686375	25686489	115	3	Open sea	Enhancers		0.021849	0.01644	6.05E-05	0.001919	0.000102
chr7	44635251	44635425	175	2	Open sea	Flanking Active TSS	OGDH	0.022839	0.019084	4.56E-05	0.002765	0.000102
chr17	78058139	78058199	61	2	Open sea	Enhancers	TNRC6C	0.025936	0.023925	4.68E-05	0.002423	0.000102
chr7	74643391	74643699	309	2	Open sea	Flanking Active TSS; Active TSS		0.017256	0.014	4.64E-05	0.002561	0.000102
chr8	76778263	76778345	83	2	Open sea	Flanking Active TSS	ZFHX4	0.024096	0.020793	4.63E-05	0.0028	0.000103
chr8	1.21E+08	1.21E+08	56	2	Open sea	Quiescent/Low	SNTB1	-0.01141	-0.01115	5.01E-05	0.001965	0.000103
chr10	78409107	78409114	8	2	Open sea	Flanking Active TSS	LINC00856	0.017391	0.015608	4.71E-05	0.002637	0.000104

chr2	2.42E+08	2.42E+08	117	2	Shelf	Genic enhancers		0.021877	0.019285	4.69E-05	0.002825	0.000104
chr17	28722538	28722637	100	2	Shelf	Genic enhancers	RAB34, ENSG00000264577; RPL23A, NEK8	0.019715	0.015685	7.64E-05	0.000607	0.000105
chr6	1.6E+08	1.6E+08	312	2	Open sea	Quiescent/Low		0.020052	0.018492	4.71E-05	0.002808	0.000105
chr10	12784787	12784834	48	2	Open sea	Quiescent/Low	CAMK1D	0.023874	0.022408	4.96E-05	0.002266	0.000105
chr6	16334043	16334815	773	2	Open sea	Enhancers		0.021654	0.020215	6.98E-05	0.000766	0.000106
chr10	1.25E+08	1.25E+08	336	2	Open sea	Flanking Active TSS	FAM53B; RP11-12J10.3	0.01983	0.015566	5.39E-05	0.00166	0.000106
chr6	1.67E+08	1.67E+08	367	3	Open sea	Weak Repressed PolyComb	TTLL2	0.020509	0.015104	8.78E-05	0.002638	0.000106
chr8	80965359	80965950	592	3	Open sea	Enhancers; Weak transcription	ZNF704	0.016778	0.012168	4.60E-05	0.00256	0.000108
chr1	51744831	51744916	86	2	Open sea	Enhancers		0.029147	0.02263	5.05E-05	0.002466	0.000109
chr8	76403811	76403980	170	2	Open sea	Flanking Active TSS; Active TSS	LINC01109; RP11-706J10.2	0.020568	0.02027	6.04E-05	0.001293	0.00011
chr9	1.32E+08	1.32E+08	369	3	Open sea	Enhancers	RAPGEF1; POMT1	0.021672	0.014589	0.000773	0.001062	0.00011
chr7	77467609	77467649	41	2	Open sea	Quiescent/Low		0.015806	0.014306	5.01E-05	0.002754	0.00011

chr3	1.24E+08	1.24E+08	137	2	Open sea	Enhancers		0.01843	0.016022	7.14E-05	0.000859	0.000112
chr4	1.4E+08	1.4E+08	200	2	Open sea	Flanking Active TSS; Enhancers	MAML3; LOC101927516	0.018921	0.018556	5.16E-05	0.002547	0.000112
chr21	27954824	27954849	26	2	Open sea	Quiescent/Low	AJ006995.3	0.022531	0.022063	5.01E-05	0.002969	0.000112
chr15	76995420	76995564	145	3	Open sea	Weak Repressed PolyComb	PSTPIP1	0.012568	0.010764	4.34E-05	0.004712	0.000113
chr11	1559606	1559870	265	2	Shore; Shelf	Transcr. at gene 5' and 3'	DUSP8	0.015712	0.014482	5.32E-05	0.002449	0.000114
chr4	1644222	1644451	230	3	Island	Weak transcription	FAM53A	0.023655	0.018041	7.43E-05	0.002326	0.000114
chr8	1E+08	1E+08	183	2	Open sea	Enhancers	KB-173C10.2	0.019876	0.019063	5.14E-05	0.002951	0.000114
chr12	94651698	94651969	272	2	Shore	Quiescent/Low	TMCC3	0.023061	0.016656	5.71E-05	0.001888	0.000115
chr10	50527562	50527579	18	2	Open sea	Weak transcription	SGMS1	0.024683	0.022423	5.22E-05	0.002849	0.000115
chr10	618778	619053	276	2	Open sea	Flanking Active TSS	DIP2C	0.020115	0.019931	5.31E-05	0.002735	0.000116
chr9	86983466	86983876	411	2	Open sea	Enhancers		0.020168	0.019612	5.45E-05	0.002461	0.000116
chr4	1.5E+08	1.5E+08	120	3	Open sea	Quiescent/Low	RNU6-1230P	0.019109	0.0138	0.000103	0.002237	0.000117

chr19	19232280	19232725	446	4	Open sea	Repressed PolyComb	NCAN; RNU6-1028P	0.022837	0.016441	4.69E-05	0.004457	0.000118
chr1	27567374	27567413	40	2	Shore	Flanking Active TSS	AHDC1	0.018898	0.016524	5.32E-05	0.003068	0.000118
chr20	25686679	25686830	152	2	Open sea	Enhancers		0.017827	0.017241	5.55E-05	0.002525	0.000119
chr13	30466986	30467083	98	2	Shore	Enhancers	HMGB1	0.019044	0.016688	5.54E-05	0.002583	0.000119
chr12	19205288	19205354	67	2	Open sea	Quiescent/Low	PLEKHA5	0.023393	0.020009	6.95E-05	0.001152	0.000119
chr17	41309077	41309460	384	4	Open sea	Flanking Bivalent TSS/Enh; Repressed PolyComb	KRTAP16-1	0.01705	0.011719	0.000102	0.002519	0.000119
chr5	1.4E+08	1.4E+08	295	2	Island; Shore	Flanking Active TSS		0.017091	0.01458	6.90E-05	0.001201	0.00012
chr14	31881117	31881221	105	2	Open sea	Enhancers		0.022491	0.019646	6.04E-05	0.00188	0.00012
chr10	49134035	49134099	65	2	Shore	Repressed PolyComb	FAM170B-AS1; FAM170B	0.019652	0.018967	5.45E-05	0.003106	0.000121
chr22	38260882	38261261	380	2	Open sea	Enhancers	TMEM184B	0.019831	0.017899	5.56E-05	0.002801	0.000121
chr9	1979163	1979254	92	2	Open sea	Weak transcription	RP11-443B9.1	0.021767	0.019242	5.69E-05	0.002588	0.000122
chr16	58551242	58551630	389	2	Open sea	Weak transcription; Genic enhancers	CNOT1	0.030257	0.026319	5.57E-05	0.002911	0.000122

chr20	35854367	35854474	108	2	Open sea	Weak transcription	Y_RNA; PHF20	0.034475	0.031477	5.51E-05	0.003104	0.000122
chr17	70661257	70661421	165	2	Open sea	Quiescent/Low		0.021675	0.018975	5.82E-05	0.002393	0.000122
chr19	13009740	13010172	433	2	Shore	Transcr. at gene 5' and 3'	NFIX	0.018761	0.016176	5.53E-05	0.00308	0.000122
chr16	17343062	17343185	124	3	Open sea	Weak transcription	XYLT1	0.026268	0.022092	4.86E-05	0.002846	0.000123
chr8	1.28E+08	1.28E+08	614	2	Open sea	Enhancers	PVT1; TMEM75	0.014957	0.014831	7.48E-05	0.001043	0.000123
chr20	4835440	4835551	112	2	Open sea	Enhancers		0.022907	0.016251	0.000115	0.000429	0.000123
chr15	47185637	47185713	77	2	Shore	Bivalent Enhancer	SEMA6D	0.019095	0.01792	5.59E-05	0.003144	0.000124
chr17	75468360	75468481	122	2	Open sea	Flanking Active TSS	KIAA0195	0.019129	0.01782	5.95E-05	0.002377	0.000125
chr17	1987438	1987572	135	2	Open sea	Enhancers		0.021962	0.020485	5.72E-05	0.002878	0.000125
chr13	49352172	49352177	6	2	Open sea	Quiescent/Low	CAB39L	0.021989	0.021647	6.06E-05	0.002297	0.000126
chr22	17563693	17564044	352	2	Open sea	Flanking Active TSS	SLC25A18	0.020419	0.01763	6.30E-05	0.001992	0.000126
chr17	933233	933776	544	4	Open sea	Enhancers; Weak transcription	NXN	0.028024	0.016585	0.000822	0.001604	0.000127

chr17	7994144	7994378	235	2	Shelf	Weak Repressed PolyComb		-0.0163	-0.01557	5.88E-05	0.002851	0.000128
chr17	45431051	45431061	11	2	Island	Flanking Bivalent TSS/Enh	ARHGAP27	0.016502	0.01594	5.77E-05	0.00314	0.000128
chr15	62076071	62076132	62	2	Open sea	Quiescent/Low		0.018993	0.017995	5.91E-05	0.002967	0.000129
chr2	1.45E+08	1.45E+08	982	4	Shore; Shelf	Active TSS	ZEB2; ZEB2-AS1; ZEB2_AS1_3; ZEB2_AS1_4	0.02155	0.012937	0.001539	0.00126	0.000129
chr1	2.3E+08	2.3E+08	46	2	Open sea	Enhancers	GALNT2	0.019108	0.018838	6.24E-05	0.002309	0.000129
chr3	72339292	72339460	169	2	Open sea	Active TSS; Flanking Active TSS		0.013986	0.013641	5.82E-05	0.003202	0.000129
chr1	2.05E+08	2.05E+08	538	2	Open sea; Shelf	Enhancers	RP11-383G10.5; TMCC2	0.022906	0.016242	7.41E-05	0.001304	0.00013
chr1	50176465	50176768	304	3	Open sea	Enhancers		0.030106	0.018599	0.000284	0.001059	0.000131
chr3	1.97E+08	1.97E+08	291	2	Open sea	Strong transcription	SENP5	0.026658	0.025084	6.37E-05	0.002349	0.000132
chr1	1.1E+08	1.1E+08	184	2	Open sea	ZNF genes & repeats		0.017929	0.017186	6.01E-05	0.003143	0.000132
chr22	30308828	30309078	251	2	Open sea	Enhancers	MTMR3, CCDC157	-0.02062	-0.016	0.000136	0.000389	0.000133

chr20	36828071	36828097	27	2	Open sea	Genic enhancers		0.019749	0.017935	6.07E-05	0.003178	0.000134
chr12	53600857	53601369	513	3	Open sea	Weak transcription; Enhancers	ATF7; RP11-793H13.10	0.03192	0.019317	0.000101	0.001538	0.000135
chr10	72086689	72087099	411	2	Shore; Island	Weak Repressed PolyComb; Bivalent/Poised TSS	SPOCK2	-0.01442	-0.01326	0.000312	0.000146	0.000136
chr17	44502060	44502283	224	2	Shore	Flanking Active TSS; Active TSS	GPATCH8	0.013605	0.013172	6.78E-05	0.002157	0.000136
chr13	94480981	94481084	104	2	Open sea	Quiescent/Low	DCT	0.016102	0.015262	6.38E-05	0.002875	0.000137
chr6	22297860	22297916	57	2	Open sea	Quiescent/Low	RP3-404K8.2; PRL	-0.01362	-0.01232	7.66E-05	0.001483	0.000138
chr19	50500724	50500872	149	2	Open sea	Active TSS		0.018531	0.017417	6.30E-05	0.003298	0.000139
chr5	1.78E+08	1.78E+08	151	2	Open sea	Flanking Active TSS	FAM193B	0.02497	0.020981	7.64E-05	0.001576	0.00014
chr16	4213891	4214353	463	2	Open sea	Flanking Active TSS; Enhancers	SRL	0.020572	0.015179	0.00013	0.000486	0.000141
chr1	6097998	6098630	633	3	Open sea	Weak transcription; Strong transcription	KCNAB2	0.017333	0.005104	0.000119	0.002621	0.000142
chr10	11242584	11242638	55	2	Open sea	Enhancers	CELF2	0.021264	0.020425	6.70E-05	0.002821	0.000142

chr9	97529896	97530008	113	2	Open sea	Enhancers	TMOD1; LOC105376168	0.018658	0.015989	6.57E-05	0.00311	0.000143
chr16	85586367	85586637	271	2	Island	Flanking Active TSS		0.01432	0.013689	6.47E-05	0.003391	0.000143
chr1	33874923	33874966	44	2	Open sea	Weak Repressed PolyComb	CSMD2; CSMD2-AS1	0.018669	0.018581	6.54E-05	0.003296	0.000143
chr11	66909096	66909162	67	2	Open sea	Enhancers	PC	-0.02867	-0.02247	6.57E-05	0.003264	0.000144
chr16	10832346	10832445	100	3	Open sea	Quiescent/Low		0.029275	0.018718	9.07E-05	0.001936	0.000144
chr3	51568104	51568189	86	2	Open sea	Enhancers		0.017874	0.016099	6.78E-05	0.002909	0.000145
chr9	86127383	86127496	114	2	Open sea	Enhancers	GOLM1	0.022328	0.019172	6.64E-05	0.003444	0.000147
chr1	15412235	15412334	100	2	Shore	Bivalent Enhancer	EFHD2	-0.0168	-0.01559	7.91E-05	0.001745	0.000148
chr10	1.03E+08	1.03E+08	16	2	Shore	Flanking Active TSS	NT5C2	0.025849	0.025274	7.23E-05	0.002496	0.000148
chr11	86151563	86151773	211	2	Open sea	Enhancers		0.017126	0.011104	9.70E-05	0.001027	0.000149
chr5	1.78E+08	1.78E+08	261	2	Shore	Flanking Active TSS; Enhancers	FAM193B; RP11-1277A3.3	0.025059	0.023428	6.95E-05	0.003117	0.00015
chr17	41187011	41187024	14	2	Open sea	Repressed PolyComb	AC006070.12	0.024909	0.023165	7.31E-05	0.002659	0.000152

chr6	1.49E+08	1.49E+08	151	3	Open sea	Enhancers; NA; Flanking Active TSS		0.022564	0.015572	7.18E-05	0.002165	0.000152
chr2	69200112	69200365	254	2	Open sea	Enhancers		0.024108	0.020825	7.28E-05	0.002835	0.000153
chr3	64443930	64444323	394	2	Shore	Repressed PolyComb	RP11-14D22.1; PRICKLE2	0.028674	0.02857	0.000101	0.001021	0.000154
chr11	15663998	15664004	7	2	Open sea	Enhancers		0.017608	0.016752	7.15E-05	0.003226	0.000155
chr1	1.51E+08	1.51E+08	46	2	Open sea	Transcr. at gene 5' and 3'	LYSMD1; SCNM1	0.017178	0.016758	7.27E-05	0.002974	0.000155
chr19	41500832	41501041	210	2	Open sea	Active TSS	PCAT19	-0.01315	-0.01277	9.30E-05	0.001335	0.000156
chr11	57641040	57641277	238	3	Open sea	Enhancers	UBE2L6; ENSG00000254602	0.018015	0.011854	0.000145	0.003381	0.000158
chr19	17468529	17468749	221	3	Shore	Flanking Active TSS	SLC27A1	0.016452	0.013327	9.32E-05	0.004427	0.000158
chr21	38498702	38499578	877	4	Open sea	Weak Repressed PolyComb	ERG	-0.0172	-0.01025	0.000462	0.002033	0.000158
chr8	23855036	23855248	213	2	Open sea	Weak Repressed PolyComb	STC1	0.019206	0.015834	0.000102	0.001098	0.000159
chr8	1.16E+08	1.16E+08	207	2	Shore	Active TSS	TRPS1	0.017395	0.016045	7.41E-05	0.003229	0.000159
chr10	72217736	72218429	694	2	Shore; Shelf	Enhancers	ASCC1; ANAPC16	0.022563	0.020284	0.000105	0.001053	0.00016

chr13	21672930	21673074	145	2	Shore	Flanking Bivalent TSS/Enh	FGF9	-0.02416	-0.0224	8.76E-05	0.00173	0.00016
chr17	1713340	1713807	468	2	Shelf; Shore	Transcr. at gene 5' and 3'	MIR22HG	0.015158	0.012694	7.62E-05	0.003128	0.000162
chr3	8593207	8593670	464	2	Open sea	Enhancers		0.022418	0.020501	8.33E-05	0.002266	0.000164
chr17	48555602	48555918	317	3	Shore	Repressed PolyComb	HOXB3; HOXB-AS3; HOXB-AS2	0.013766	0.011294	7.70E-05	0.004711	0.000164
chr11	67416074	67416144	71	2	Shore	Flanking Bivalent TSS/Enh	CARNS1; PPP1CA	0.015044	0.013192	7.52E-05	0.003582	0.000165
chr6	2750898	2751167	270	2	Open sea	Weak transcription	MYLK4	0.025479	0.022856	7.54E-05	0.003612	0.000165
chr10	1.2E+08	1.2E+08	59	2	Open sea	Flanking Active TSS	BAG3	0.016721	0.014418	8.68E-05	0.002051	0.000166
chr3	1.5E+08	1.5E+08	61	2	Open sea	Enhancers		0.019123	0.018827	7.65E-05	0.003506	0.000166
chr5	1.42E+08	1.42E+08	44	2	Open sea	Weak Repressed PolyComb		0.013558	0.011868	0.000116	0.000939	0.000167
chr13	66666112	66666351	240	2	Open sea	Quiescent/Low	PCDH9	0.024759	0.021838	9.28E-05	0.001715	0.000167
chr8	23922727	23922838	112	2	Open sea	Quiescent/Low		0.02267	0.020533	0.000101	0.001377	0.000169
chr8	1.43E+08	1.43E+08	27	2	Open sea	Repressed PolyComb		0.01824	0.017302	9.28E-05	0.001805	0.00017

chr1	8016612	8016745	134	2	Open sea	Enhancers	PARK7, ENSG00000238290	0.022874	0.01884	0.000113	0.001061	0.00017
chr15	90596636	90597015	380	2	Open sea	Enhancers	CTD-3065B20.2; CRTC3; IQGAP1, CRTC3	0.026257	0.023863	8.18E-05	0.002937	0.000171
chr3	1.16E+08	1.16E+08	107	2	Open sea	Quiescent/Low	LSAMP	0.020011	0.017241	8.26E-05	0.002855	0.000171
chr19	46466574	46466661	88	3	Open sea	Weak transcription	PNMAL1	0.018123	0.014923	8.47E-05	0.00367	0.000171
chr6	1.64E+08	1.64E+08	36	2	Open sea	Active TSS		0.022354	0.019022	8.49E-05	0.002579	0.000171
chr10	97540346	97540766	421	2	Open sea	Enhancers		0.027342	0.018438	0.001023	8.42E-05	0.000171
chr1	13800912	13801296	385	3	Open sea	Enhancers		0.034574	0.021583	0.000485	0.002376	0.000172
chr12	1.05E+08	1.05E+08	152	2	Open sea	Quiescent/Low	CHST11	-0.02362	-0.01983	0.000106	0.001311	0.000172
chr12	1.24E+08	1.24E+08	170	2	Shore	Enhancers		0.021087	0.020885	8.24E-05	0.003034	0.000173
chr4	20883458	20883918	461	4	Open sea	Quiescent/Low	KCNIP4	0.0254	0.014929	0.001281	0.002876	0.000174
chr20	51507390	51507594	205	2	Open sea	Enhancers		0.025458	0.019896	0.000117	0.001053	0.000174
chr6	1.7E+08	1.7E+08	33	2	Open sea	Flanking Active TSS		0.020192	0.019491	8.07E-05	0.003554	0.000175

chr14	55383991	55384082	92	2	Open sea	Enhancers	ATG14	0.022348	0.019541	8.02E-05	0.003706	0.000175
chr16	24819696	24819813	118	2	Open sea	Strong transcription	CTD-2515A14.1; TNRC6A	0.018096	0.017733	8.93E-05	0.002375	0.000176
chr3	66004072	66004226	155	3	Open sea	Weak transcription; Enhancers	MAGI1; Y_RNA	-0.01348	-0.01063	0.000334	0.001666	0.000176
chr16	67260796	67260995	200	2	Open sea	Enhancers		0.018791	0.016501	9.18E-05	0.002272	0.000178
chr22	37904593	37904743	151	2	Shore	Enhancers		0.020883	0.019977	8.17E-05	0.003821	0.000179
chr1	10541052	10541521	470	3	Open sea	Enhancers; Genic enhancers		-0.01613	-0.01315	9.98E-05	0.003188	0.00018
chr3	1.22E+08	1.22E+08	32	2	Open sea	Quiescent/Low	SLC15A2	0.030496	0.026718	8.32E-05	0.003596	0.00018
chr1	2.27E+08	2.27E+08	466	2	Open sea	Enhancers	ITPKB	-0.01994	-0.01773	8.49E-05	0.003344	0.00018
chr20	11687564	11687576	13	2	Shore	Enhancers	RP11-268G13.1	0.015132	0.013943	8.64E-05	0.003255	0.000182
chr19	10636465	10636651	187	3	Island	Strong transcription	SLC44A2	0.011834	0.008187	0.000188	0.002246	0.000183
chr1	1.81E+08	1.81E+08	288	3	Shore; Island	Flanking Active TSS	KIAA1614-AS1; RP11-46A10.5	0.01999	0.012424	0.002367	0.00086	0.000184
chr1	1.6E+08	1.6E+08	127	2	Shelf	Flanking Active TSS		0.016747	0.015898	8.45E-05	0.003813	0.000184

chr13	1.11E+08	1.11E+08	46	2	Open sea	Enhancers	ARHGEF7	-0.02319	-0.02077	8.45E-05	0.003824	0.000184
chr1	2.02E+08	2.02E+08	259	2	Open sea	Flanking Active TSS	LMOD1	0.0184	0.017828	8.46E-05	0.003837	0.000185
chr2	1540579	1540701	123	3	Island	Flanking Bivalent TSS/Enh	TPO	0.018363	0.013571	8.41E-05	0.004311	0.000185
chr19	11035027	11035343	317	3	Shore	Strong transcription; Genic enhancers	SMARCA4	0.022089	0.015692	0.000111	0.002716	0.000185
chr5	79561426	79561677	252	2	Open sea	Enhancers; Quiescent/Low	CMYA5	0.02461	0.020286	0.000112	0.00145	0.000185
chr17	9958394	9958829	436	2	Open sea	Repressed PolyComb	GAS7	-0.02074	-0.015	0.000148	0.000784	0.000187
chr14	60204305	60204322	18	2	Open sea	Flanking Active TSS		0.019867	0.01497	0.000101	0.002001	0.000187
chr3	1.85E+08	1.85E+08	226	2	Shore	Flanking Active TSS	MAGEF1	0.017336	0.015306	0.000105	0.001812	0.000187
chr4	7646812	7647327	516	4	Shore	Repressed PolyComb	SORCS2	-0.01627	-0.01146	0.000429	0.002321	0.000189
chr8	1760271	1760280	10	2	Shelf	Weak Repressed PolyComb	CLN8	0.020428	0.020186	8.68E-05	0.003942	0.00019
chr3	66406175	66406373	199	3	Open sea	NA; Transcr. at gene 5′ and 3′	LRIG1	0.019664	0.014779	0.000116	0.004963	0.000193
chr10	1.27E+08	1.27E+08	27	2	Open sea	Weak Repressed PolyComb	C10orf90	0.020469	0.017192	0.000107	0.001985	0.000196

chr12	1.23E+08	1.23E+08	223	2	Shore	Flanking Active TSS	PITPNM2	-0.01473	-0.01364	0.000105	0.002131	0.000196
chr10	33134012	33134213	202	2	Open sea	Weak transcription		0.021398	0.016506	0.00036	0.00025	0.000197
chr6	11851660	11851701	42	2	Open sea	Flanking Active TSS		0.021616	0.018543	0.000125	0.001359	0.000198
chr1	1.46E+08	1.46E+08	27	2	Shore	Flanking Active TSS	CH17-270A2.1; CH17-270A2.2; POLR3GL; ANKRD34A	0.014228	0.012518	9.80E-05	0.003236	0.000203
chr4	1.28E+08	1.28E+08	30	2	Open sea	Enhancers	LOC100507487	0.015245	0.011665	9.33E-05	0.004074	0.000203
chr17	1423098	1423552	455	3	Open sea	Enhancers	RP11-818O24.3; CRK	0.026927	0.014875	0.00171	0.000158	0.000203
chr16	49560570	49560791	222	2	Open sea	Flanking Active TSS	ZNF423	0.027669	0.02245	9.42E-05	0.003945	0.000203
chr8	28374596	28374658	63	2	Open sea	Weak transcription	FBXO16; ZNF395	0.025007	0.024664	9.41E-05	0.003984	0.000204
chr22	31223915	31224026	112	2	Open sea	Enhancers	PLA2G3; RNF185, LIMK2, SFI1	0.019191	0.018995	0.000141	0.001118	0.000205
chr11	66337330	66337676	347	2	Shelf	Genic enhancers	RIN1	0.027189	0.022004	0.000135	0.001249	0.000205
chr2	1.73E+08	1.73E+08	130	2	Shore	Enhancers	MAP3K20	0.018136	0.017639	9.46E-05	0.004105	0.000206
chr2	1.5E+08	1.5E+08	291	3	Open sea	Quiescent/Low	RNU6-601P; AC144449.1	0.021298	0.016252	0.000278	0.002911	0.000206

chr2	1.56E+08	1.56E+08	201	2	Shore	Flanking Active TSS	GPD2	0.022299	0.020381	0.000103	0.002956	0.000207
chr1	11484395	11484431	37	2	Shelf	Bivalent Enhancer	PTCHD2	0.017447	0.016086	0.000102	0.003415	0.000211
chr7	55150870	55151397	528	3	Open sea	Enhancers; Genic enhancers		0.022271	0.021231	0.000113	0.003937	0.000212
chr16	1543212	1543555	344	3	Shore	Enhancers	IFT140; TELO2, TMEM204, JPT2	-0.02017	-0.01322	0.000114	0.002248	0.000213
chr16	84933874	84933978	105	2	Open sea	Flanking Active TSS	RP11-254F19.3	0.014093	0.013996	9.94E-05	0.00401	0.000214
chr1	87154587	87154799	213	2	Shelf	Quiescent/Low	LINC01140; RP5-1052I5.2	0.019193	0.01594	0.000111	0.002531	0.000214
chr2	2.06E+08	2.06E+08	144	2	Shore	Repressed PolyComb	NRP2	-0.01308	-0.01264	0.000108	0.00285	0.000214
chr14	53367879	53368295	417	3	Open sea	Quiescent/Low	AL163953.3	0.019864	0.015682	9.73E-05	0.004958	0.000215
chr4	76987273	76987816	544	3	Open sea	Quiescent/Low	Sep-11	0.030908	0.008876	0.00061	0.000842	0.000216
chr21	42684839	42685363	525	3	Shore; Island	Flanking Bivalent TSS/Enh; Repressed PolyComb; Weak Repressed PolyComb	PDE9A	0.021025	0.016814	0.000441	0.002935	0.000217
chr16	82995451	82995809	359	3	Open sea	Quiescent/Low	CDH13	-0.01649	-0.01324	0.000185	0.004222	0.000217

chr10	1.03E+08	1.03E+08	130	2	Open sea	Flanking Active TSS; Enhancers	SUFU	0.027516	0.023028	0.000126	0.001898	0.00022
chr13	97429927	97430098	172	2	Shelf	Active TSS		0.022458	0.022091	0.000102	0.004297	0.000222
chr1	11839579	11839885	307	3	Shore	Strong transcription	CLCN6; NPPA-AS1	-0.01739	-0.00907	0.001201	0.001903	0.000222
chr8	1863593	1863608	16	2	Open sea	Bivalent Enhancer	ARHGEF10	0.019291	0.01615	0.00014	0.001499	0.000223
chr17	39192393	39192419	27	2	Open sea	Transcr. at gene 5' and 3'	CACNB1	0.020388	0.019381	0.000105	0.003851	0.000223
chr17	81919437	81919630	194	3	Shore	Genic enhancers		0.019796	0.010415	0.001455	0.002598	0.000223
chr21	41142441	41142502	62	2	Open sea	Enhancers	LINC00323	0.017391	0.015038	0.00011	0.003324	0.000225
chr4	2067941	2068022	82	3	Shelf	Bivalent Enhancer		0.020101	0.012841	0.001934	0.002226	0.000227
chr14	22815308	22815349	42	2	Open sea	Flanking Bivalent TSS/Enh	SLC7A7	0.015447	0.015093	0.000109	0.003894	0.000231
chr4	71231225	71231255	31	2	Open sea	Flanking Active TSS	SLC4A4	0.021372	0.018053	0.000113	0.003437	0.000233
chr2	2.16E+08	2.16E+08	467	2	Shore	Weak Repressed PolyComb	XRCC5	0.017195	0.015678	0.000108	0.004422	0.000233
chr8	1.3E+08	1.3E+08	845	6	Open sea	Quiescent/Low	GSDMC	0.022594	0.005942	0.000351	0.002639	0.000234
chr19	37931977	37932328	352	3	Open sea	Flanking Active TSS	SIPA1L3	0.020698	0.01296	0.000339	0.00315	0.000236

chr20	17962759	17963050	292	2	Open sea	Enhancers	MGME1	0.020359	0.019883	0.000288	0.000481	0.000239
chr4	1.39E+08	1.39E+08	24	2	Shore	Active TSS	NDUFC1; NAA15	0.012918	0.009542	0.000221	0.000716	0.000239
chr10	1.22E+08	1.22E+08	44	2	Shore	Enhancers		0.021466	0.02146	0.000113	0.004115	0.000241
chr3	1.88E+08	1.88E+08	361	4	Open sea	Weak transcription	LPP	0.026523	0.011211	0.000888	0.000212	0.000242
chr19	1254066	1254435	370	2	Island	Transcr. at gene 5' and 3'	MIDN	-0.01491	-0.00988	0.000993	0.000146	0.000244
chr5	1.07E+08	1.07E+08	343	2	Open sea	Enhancers		0.017651	0.013797	0.000344	0.000409	0.000247
chr11	1.29E+08	1.29E+08	815	5	Island; Shore	Flanking Bivalent TSS/Enh; Repressed PolyComb; Weak Repressed PolyComb		0.022744	0.009548	0.000478	0.004085	0.000254
chr8	1.28E+08	1.28E+08	252	2	Open sea	Enhancers		0.019235	0.016443	0.000154	0.00183	0.000255
chr18	76487228	76487276	49	2	Shelf	Enhancers	ZNF516	0.024938	0.02327	0.000123	0.003953	0.000256
chr1	2.08E+08	2.08E+08	107	2	Shore	Enhancers; Flanking Active TSS	CD46	-0.02229	-0.02042	0.00023	0.000797	0.000257
chr2	2.39E+08	2.39E+08	357	2	Open sea	Flanking Active TSS	HDAC4	0.018421	0.017248	0.000132	0.003382	0.000264
chr2	85578497	85578507	11	2	Open sea	Flanking Active TSS	VAMP8	0.020488	0.019527	0.000128	0.004211	0.000268

chr14	90674934	90675488	555	2	Open sea	Flanking Active TSS; Enhancers	TTC7B; RP11-661G16.1	0.020786	0.018365	0.000132	0.003733	0.000269
chr17	62706422	62707000	579	3	Shore; Shelf	Bivalent Enhancer	MARCH10; RP11-156L14.1	-0.01613	-0.01043	0.001537	0.002771	0.00027
chr19	35449271	35449959	689	5	Open sea	Bivalent Enhancer; Repressed PolyComb	FFAR2	-0.01512	-0.0094	0.000573	0.004174	0.00027
chr9	1.35E+08	1.35E+08	26	2	Open sea	Bivalent Enhancer		0.021244	0.019835	0.000126	0.004764	0.000271
chr2	39505104	39505310	207	2	Open sea	Flanking Active TSS; Enhancers	AC007246.3	-0.02216	-0.01908	0.000185	0.001411	0.000271
chr5	5572436	5572514	79	2	Open sea	Bivalent Enhancer		0.015479	0.012389	0.00022	0.000991	0.000271
chr2	67260597	67260660	64	2	Open sea	Enhancers	AC023115.2	0.015868	0.015205	0.000136	0.003573	0.000273
chr5	1.39E+08	1.39E+08	579	2	Open sea	Flanking Active TSS; Enhancers	SIL1	0.019961	0.018746	0.000136	0.003582	0.000273
chr2	2.07E+08	2.07E+08	542	4	Open sea	Enhancers; Quiescent/Low		-0.01844	-0.01016	0.000424	0.004055	0.000279
chr17	10698421	10698716	296	3	Shore	Active TSS; Flanking Active TSS	SCO1; ADPRM	0.024608	0.016646	0.001177	0.002238	0.000279
chr1	2.07E+08	2.07E+08	160	2	Shore	Active TSS	YOD1; PFKFB2; C4BPB	-0.02242	-0.02145	0.000131	0.004721	0.000279

chr2	44944200	44944443	244	2	Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer	SIX3; SIX3-AS1	-0.01316	-0.01295	0.00013	0.004863	0.00028
chr16	11024846	11025543	698	3	Open sea	Genic enhancers; Strong transcription	CLEC16A; RPL7P46	-0.01663	-0.01018	0.002072	0.001625	0.000281
chr6	1.59E+08	1.59E+08	31	2	Shore	Enhancers		-0.01304	-0.01236	0.000141	0.003598	0.000282
chr10	4514848	4514921	74	2	Open sea	Enhancers	RNU6-163P	-0.01605	-0.01547	0.000138	0.004043	0.000284
chr4	7872246	7872568	323	3	Open sea	Flanking Active TSS; Enhancers	AFAP1	0.019528	0.014581	0.000245	0.004895	0.000285
chr1	2.04E+08	2.04E+08	10	2	Shore	Bivalent Enhancer	ETNK2	0.019342	0.017776	0.000133	0.004937	0.000285
chr11	1.19E+08	1.19E+08	523	3	Shelf	Flanking Active TSS; Enhancers	USP2; RNF26	0.017523	0.012842	0.00025	0.001094	0.000285
chr3	1.28E+08	1.28E+08	96	2	Open sea	Genic enhancers		-0.01548	-0.01436	0.000136	0.004944	0.000291
chr11	19201969	19202087	119	2	Open sea	Active TSS	CSRP3; RP11-428C19.4	0.019651	0.017893	0.000143	0.003932	0.000291
chr15	65612375	65612754	380	3	Shore	Enhancers	SLC24A1; VWA9; DENND4A, INTS14	0.021705	0.013921	0.000825	0.002065	0.000292
chr6	90135884	90136132	249	2	Open sea	Enhancers	BACH2	0.018485	0.018073	0.000249	0.000974	0.000294
chr2	1.22E+08	1.22E+08	144	2	Open sea	Enhancers		0.026726	0.020079	0.000515	0.00036	0.000295

chr12	51024884	51025380	497	2	Shore	Active TSS	SLC11A2	0.028071	0.017552	0.000518	0.00036	0.000296
chr2	1.36E+08	1.36E+08	53	2	Open sea	Weak Repressed PolyComb		0.019733	0.01285	0.000278	0.000827	0.000297
chr6	1.46E+08	1.46E+08	85	2	Shore	Quiescent/Low	RP3-466P17.2; EPM2A; RP3-466P17.1	0.020424	0.019708	0.000142	0.004581	0.000298
chr17	77467777	77467901	125	3	Open sea	Enhancers	Sep-09	-0.01733	-0.01039	0.000186	0.003412	0.000299
chr8	1.42E+08	1.42E+08	257	3	Open sea	Repressed PolyComb		0.020403	0.015915	0.000141	0.004281	0.0003
chr5	1.61E+08	1.61E+08	177	2	Open sea	Repressed PolyComb		0.012583	0.012453	0.000142	0.004989	0.000304
chr9	1.09E+08	1.09E+08	330	3	Open sea	Flanking Active TSS; Transcr. at gene 5’ and 3’	PTPN3	-0.02188	-0.01433	0.001033	0.002962	0.000304
chr1	2.11E+08	2.11E+08	396	4	Open sea	Repressed PolyComb	RD3	0.018246	0.013633	0.000365	0.003958	0.000304
chr5	1.73E+08	1.73E+08	10	2	Open sea	Genic enhancers		-0.01612	-0.01511	0.000145	0.004693	0.000305
chr2	3040964	3041558	595	4	Open sea	Repressed PolyComb; Bivalent Enhancer	LINC01250	0.016939	0.010502	0.002367	0.002929	0.000305
chr3	1.78E+08	1.78E+08	512	4	Open sea	Enhancers	LINC00578	0.018594	0.011614	0.000316	0.003	0.000305
chr16	52258420	52258458	39	2	Open sea	Quiescent/Low	RP11-142G1.1; CASC22	0.019761	0.018578	0.000151	0.004892	0.000318

chr3	1.02E+08	1.02E+08	219	2	Shore	Flanking Active TSS	NXPE3	0.021188	0.013336	0.001654	0.000158	0.000321
chr6	1.05E+08	1.05E+08	230	2	Open sea	Active TSS	BVES-AS1; POPDC3	0.021714	0.020814	0.000159	0.004186	0.000323
chr2	2.32E+08	2.32E+08	70	2	Open sea	Enhancers; Weak transcription	AC105461.1; DIS3L2	0.019606	0.018964	0.000166	0.003659	0.000325
chr2	1.09E+08	1.09E+08	7	2	Open sea	Enhancers	SH3RF3	-0.01231	-0.01099	0.000157	0.004638	0.000325
chr5	80713769	80713848	80	3	Open sea	Weak transcription	MSH3	-0.01278	-0.01041	0.000299	0.004643	0.000327
chr13	41463164	41463296	133	2	Open sea	Weak Repressed PolyComb	RGCC	-0.01536	-0.01404	0.000193	0.002364	0.000329
chr10	43859152	43859397	246	2	Open sea	Weak transcription	LINC00840	-0.01722	-0.01176	0.000291	0.001008	0.000333
chr1	2.35E+08	2.35E+08	364	3	Shelf	Flanking Active TSS		0.022351	0.008789	0.000697	0.000405	0.000335
chr3	1.51E+08	1.51E+08	320	2	Open sea	Quiescent/Low	IGSF10	0.019222	0.016132	0.000198	0.002449	0.000338
chr15	65101694	65101724	31	2	Shore	Strong transcription	UBAP1L	0.011079	0.010124	0.000178	0.003537	0.000342
chr10	84244638	84244903	266	3	Open sea	Weak Repressed PolyComb; Bivalent Enhancer; Flanking Bivalent TSS/Enh	RGR	0.016583	0.011288	0.00025	0.003217	0.000346

chr19	6721004	6721015	12	2	Open sea	Weak Repressed PolyComb	C3	0.016124	0.016067	0.000171	0.004838	0.000354
chr14	1.04E+08	1.04E+08	152	2	Open sea	Strong transcription	RP11-73M18.6; KLC1	-0.01226	-0.01209	0.000247	0.001788	0.000365
chr1	7051067	7051255	189	4	Open sea	Repressed PolyComb	CAMTA1	0.01942	0.011213	0.000909	0.004828	0.000366
chr6	1.39E+08	1.39E+08	49	2	Open sea	Flanking Active TSS	NHSL1	0.020996	0.019225	0.000215	0.002659	0.00037
chr9	1.23E+08	1.23E+08	145	2	Open sea	Weak transcription	MIR600HG; STRBP	0.026226	0.021862	0.000223	0.002638	0.00038
chr4	1.19E+08	1.19E+08	47	2	Open sea	Enhancers		0.02273	0.020643	0.000227	0.002662	0.000386
chr14	50233397	50233577	181	2	Shore	Enhancers		0.022479	0.020679	0.000197	0.00424	0.000387
chr9	1.37E+08	1.37E+08	53	2	Island	Bivalent Enhancer	SEC16A, NOTCH1	-0.01731	-0.01327	0.000352	0.001096	0.000393
chr6	1.26E+08	1.26E+08	282	2	Shore	Flanking Active TSS; Active TSS	RP11-624M8.1; HEY2	0.01851	0.015115	0.000212	0.003614	0.000396
chr18	37486041	37486418	378	2	Shore	Bivalent Enhancer	CELF4	0.0152	0.011784	0.000421	0.000941	0.000416
chr8	37625939	37625978	40	2	Open sea	Flanking Active TSS	RP11-150O12.4	0.020589	0.013188	0.000352	0.001287	0.000419
chr10	30116214	30116398	185	2	Open sea	Weak Repressed PolyComb	KIAA1462	0.019875	0.017784	0.000273	0.002227	0.000423
chr5	68683789	68683964	176	2	Open sea	Quiescent/Low		0.024682	0.017727	0.00069	0.000532	0.00043

chr17	41027222	41027507	286	3	Open sea	Repressed PolyComb	KRTAP1-5	0.021964	0.013617	0.000289	0.002834	0.000436
chr11	1.18E+08	1.18E+08	440	2	Open sea	Weak transcription; ZNF genes & repeats	TMPRSS4-AS1; TMPRSS4	0.027203	0.023761	0.000334	0.00162	0.000441
chr5	1464125	1464224	100	3	Shore	Strong transcription	LPCAT1	0.016897	0.011526	0.000408	0.004965	0.000441
chr1	1.78E+08	1.78E+08	255	2	Open sea	Active TSS	RP4-798P15.3; SEC16B	0.026658	0.017025	0.001085	0.00036	0.000443
chr5	1.76E+08	1.76E+08	360	2	Open sea	Weak Repressed PolyComb; Bivalent Enhancer	HRH2	0.025528	0.016718	0.000434	0.001053	0.000446
chr8	42378397	42378736	340	3	Open sea	Weak Repressed PolyComb	DKK4	0.029352	0.018989	0.001206	0.004331	0.000449
chr5	1.46E+08	1.46E+08	356	2	Shore	Quiescent/Low; Active TSS	SH3RF2	0.0219	0.016539	0.000802	0.000527	0.000467
chr3	1.94E+08	1.94E+08	999	8	Shelf; Shore	Flanking Bivalent TSS/Enh; Bivalent Enhancer	LINC00887	0.018419	0.008438	0.003976	0.003311	0.000468
chr7	1.35E+08	1.35E+08	56	2	Open sea	Quiescent/Low	CALD1	-0.01845	-0.01789	0.000245	0.004406	0.000468
chr18	77101857	77102083	227	2	Open sea	Flanking Active TSS	MBP	0.026622	0.022371	0.000265	0.003437	0.000469
chr3	51955352	51955675	324	2	Shore	Flanking Active TSS	GPR62; RP11-155D18.13	0.017409	0.013792	0.000364	0.001724	0.00048

chr20	63454275	63454497	223	2	Island	Bivalent Enhancer	KCNQ2; ENSG00000226390, RTEL1	0.008223	0.005735	0.000286	0.003037	0.000481
chr8	1.23E+08	1.23E+08	123	2	Open sea	Flanking Active TSS; Enhancers	DERL1; RP11-557C18.3	0.020877	0.01717	0.000249	0.00477	0.000484
chr19	49832849	49833525	677	2	Open sea	Genic enhancers; Flanking Active TSS	MED25	0.018402	0.016162	0.000556	0.000898	0.000488
chr2	2.38E+08	2.38E+08	66	2	Shore	Flanking Active TSS	PER2	0.022318	0.018989	0.000284	0.003594	0.000501
chr8	1.33E+08	1.33E+08	51	2	Open sea	Quiescent/Low	TG	0.021552	0.017854	0.00033	0.002524	0.000509
chr5	1.39E+08	1.39E+08	26	2	Open sea	Enhancers	SLC23A1, PROB1	0.021791	0.017563	0.00034	0.002466	0.000516
chr14	1E+08	1E+08	337	3	Open sea	Genic enhancers	RP11-638I2.2; YY1	0.020312	0.013014	0.001079	0.00132	0.000517
chr2	29064056	29064238	183	2	Open sea	Flanking Active TSS; Enhancers	C2orf71	0.024348	0.015954	0.001493	0.00036	0.000521
chr10	1.3E+08	1.3E+08	259	4	Open sea; Shelf	Weak transcription	EBF3	0.020317	0.012165	0.001093	0.00262	0.000527
chr19	40283949	40283958	10	2	Shore	Flanking Active TSS	AKT2; MIR641	0.014035	0.012834	0.000295	0.00395	0.000531
chr2	23030200	23030682	483	3	Open sea	Weak Repressed PolyComb	AC016768.1	-0.01447	-0.0085	0.000512	0.001595	0.000534

chr1	2.27E+08	2.27E+08	36	2	Open sea	Genic enhancers	PSEN2	-0.01151	-0.01111	0.000424	0.001722	0.000535
chr10	11012060	11012279	220	2	Open sea	Enhancers	CELF2; RP1-251M9.3	0.023676	0.022377	0.000323	0.003172	0.000536
chr2	1.05E+08	1.05E+08	314	3	Open sea	Weak transcription	AC012360.4; TGFBRAP1	-0.01136	-0.00596	0.003197	0.000237	0.000565
chr2	2.19E+08	2.19E+08	97	3	Open sea	Flanking Active TSS; Enhancers	DNPEP; AC053503.4	0.01962	0.015348	0.000303	0.004299	0.000588
chr20	50111526	50111575	50	2	Shore	Flanking Active TSS	UBE2V1; TMEM189-UBE2V1	0.015768	0.009815	0.000767	0.00091	0.000602
chr16	85912744	85912823	80	2	Open sea	Repressed PolyComb	IRF8	0.020922	0.01727	0.000814	0.000876	0.000613
chr15	51738321	51738548	228	4	Shore	Flanking Active TSS	LYSMD2	0.026375	0.015335	0.000536	0.003354	0.000651
chr15	71547190	71547307	118	2	Open sea	Enhancers		0.018233	0.014127	0.00044	0.002791	0.000653
chr3	1.2E+08	1.2E+08	311	3	Open sea	Active TSS	POPDC2; COX17	0.019282	0.014693	0.000421	0.004199	0.00068
chr4	1173620	1173925	306	3	Shore	Enhancers	SPON2; ENSG00000273179	0.018421	0.010933	0.004762	0.004735	0.000696
chr3	45996449	45996483	35	2	Shore	Flanking Active TSS	FYCO1	0.023385	0.020509	0.000497	0.002646	0.000702
chr3	1.08E+08	1.08E+08	114	4	Shore	Active TSS	CD47	-0.01874	-0.01195	0.000463	0.002775	0.000726
chr16	88392209	88392260	52	2	Shore	Enhancers		-0.02217	-0.01987	0.000427	0.004244	0.000727

chr14	1.03E+08	1.03E+08	237	2	Shelf	Genic enhancers		0.020918	0.015667	0.000585	0.00213	0.00073
chr2	1.75E+08	1.75E+08	44	2	Open sea	Enhancers	AC018890.6; CHRNA1	0.024482	0.018511	0.000545	0.002517	0.000739
chr1	1.6E+08	1.6E+08	119	2	Shore	Flanking Active TSS	RP11-536C5.7; AL121987.1; PEA15	0.018025	0.014788	0.000469	0.003618	0.000746
chr18	48942675	48942843	169	2	Open sea	Flanking Active TSS	SMAD7	0.033653	0.022023	0.000706	0.001643	0.000748
chr1	33306430	33306545	116	2	Shore; Island	Bivalent Enhancer	RP11-415J8.3	-0.01838	-0.01301	0.000709	0.001657	0.000753
chr16	977356	977382	27	2	Shelf	Flanking Bivalent TSS/Enh	RP11-161M6.2; LMF1	0.014703	0.01431	0.00045	0.004843	0.000786
chr1	2E+08	2E+08	76	2	Shore	Repressed PolyComb	NR5A2	0.016318	0.012305	0.001397	0.000776	0.000787
chr1	9872766	9872967	202	2	Open sea	Enhancers	CTNNBIP1	0.018832	0.016921	0.000474	0.004827	0.000818
chr1	1.55E+08	1.55E+08	86	2	Shore	Bivalent/Poised TSS	TDRD10; SHE	-0.01532	-0.013	0.000564	0.003362	0.000838
chr5	1.4E+08	1.4E+08	513	2	Shelf	Genic enhancers	CXXC5, PSD2	0.018047	0.013587	0.000595	0.003245	0.000861
chr10	48288107	48288495	389	2	Open sea	Enhancers; Quiescent/Low		0.017796	0.012654	0.002113	0.000753	0.000966
chr4	16627950	16628209	260	2	Open sea	Quiescent/Low	LDB2	0.016645	0.016638	0.000943	0.002187	0.001027
chr6	1.12E+08	1.12E+08	175	2	Open sea	Active TSS		0.019519	0.01471	0.001601	0.001204	0.001077

chr7	4689321	4689474	154	3	Open sea	Enhancers		0.027302	0.017589	0.001998	0.001701	0.001094
chr17	917640	917698	59	2	Open sea	Enhancers	NXN	-0.02115	-0.0159	0.001555	0.001383	0.001139
chr4	1.15E+08	1.15E+08	349	5	Open sea	Quiescent/Low	NDST4	0.020431	0.011942	0.001364	0.002126	0.001184
chr17	39165636	39165774	139	2	Island	Flanking Active TSS; Enhancers	RP5-906A24.1; ARL5C	0.018311	0.013291	0.00152	0.001579	0.001201
chr11	69001410	69001545	136	2	Shelf	Bivalent Enhancer	MRPL21, MRGPRD, ENSG00000261625; IGHMBP2	0.014935	0.013284	0.000787	0.004926	0.001222
chr4	1.4E+08	1.4E+08	138	2	Shelf	Enhancers	MAML3	-0.02133	-0.01695	0.000913	0.004304	0.001305
chr17	82239483	82239880	398	3	Shore	Genic enhancers	CSNK1D; SLC16A3	0.012359	0.011632	0.001497	0.003899	0.001522
chr11	72812839	72812982	144	2	Shore	Flanking Active TSS	ATG16L2	0.018715	0.012728	0.003672	0.001077	0.001579
chr1	12745799	12746078	280	3	Open sea	Quiescent/Low	C1orf158	0.017687	0.010918	0.002796	0.003357	0.001675
chr8	9615197	9615273	77	2	Open sea	Enhancers		0.018408	0.012658	0.00288	0.001525	0.001703
chr9	35909377	35909468	92	3	Open sea	Flanking Active TSS	LINC00961	-0.01915	-0.01266	0.001698	0.003125	0.00172
chr10	45453573	45454163	591	2	Open sea	Weak transcription; Enhancers	RP11-67C2.2	0.020565	0.014886	0.002933	0.001554	0.001738

chr10	1.33E+08	1.33E+08	302	2	Shore	Weak Repressed PolyComb	CFAP46	0.021565	0.017096	0.001929	0.003626	0.002058
chr5	1.39E+08	1.39E+08	98	2	Open sea	Enhancers		0.018799	0.01656	0.003237	0.001994	0.002104
chr9	1.35E+08	1.35E+08	62	2	Open sea	Repressed PolyComb	FCN2	-0.01473	-0.01061	0.001843	0.004128	0.00211
chr5	1.39E+08	1.39E+08	493	2	Open sea	Enhancers		0.020266	0.014972	0.004555	0.002069	0.002584

Table 5.1. Significant DMRs associated with baseline z-score.

GO term ID	Ontology	Name	Number of genes in GO term	Number of genes differentially methylated in GO term	P-value	FDR
GO:0030029	BP	actin filament-based process	771	290	3.36E-11	3.79E-07
GO:0043292	CC	contractile fiber	232	103	2.67E-11	3.79E-07
GO:0003012	BP	muscle system process	452	163	4.80E-10	2.71E-06
GO:0030016	CC	myofibril	221	97	4.40E-10	2.71E-06
GO:0030036	BP	actin cytoskeleton organization	675	253	2.79E-09	1.26E-05
GO:0030017	CC	sarcomere	201	87	8.82E-09	3.32E-05
GO:0006936	BP	muscle contraction	350	125	8.07E-08	0.000228
GO:0007015	BP	actin filament organization	412	160	7.48E-08	0.000228
GO:0003779	MF	actin binding	414	165	2.39E-07	0.0006
GO:0015629	CC	actin cytoskeleton	492	180	5.28E-07	0.001193
GO:0006941	BP	striated muscle contraction	168	67	1.72E-06	0.003538

Table 5.2. Gene Set Enrichment Analyses (GSEA) of significant CpGs associated with baseline z-score fitness using the Gene Ontology (GO) data set.

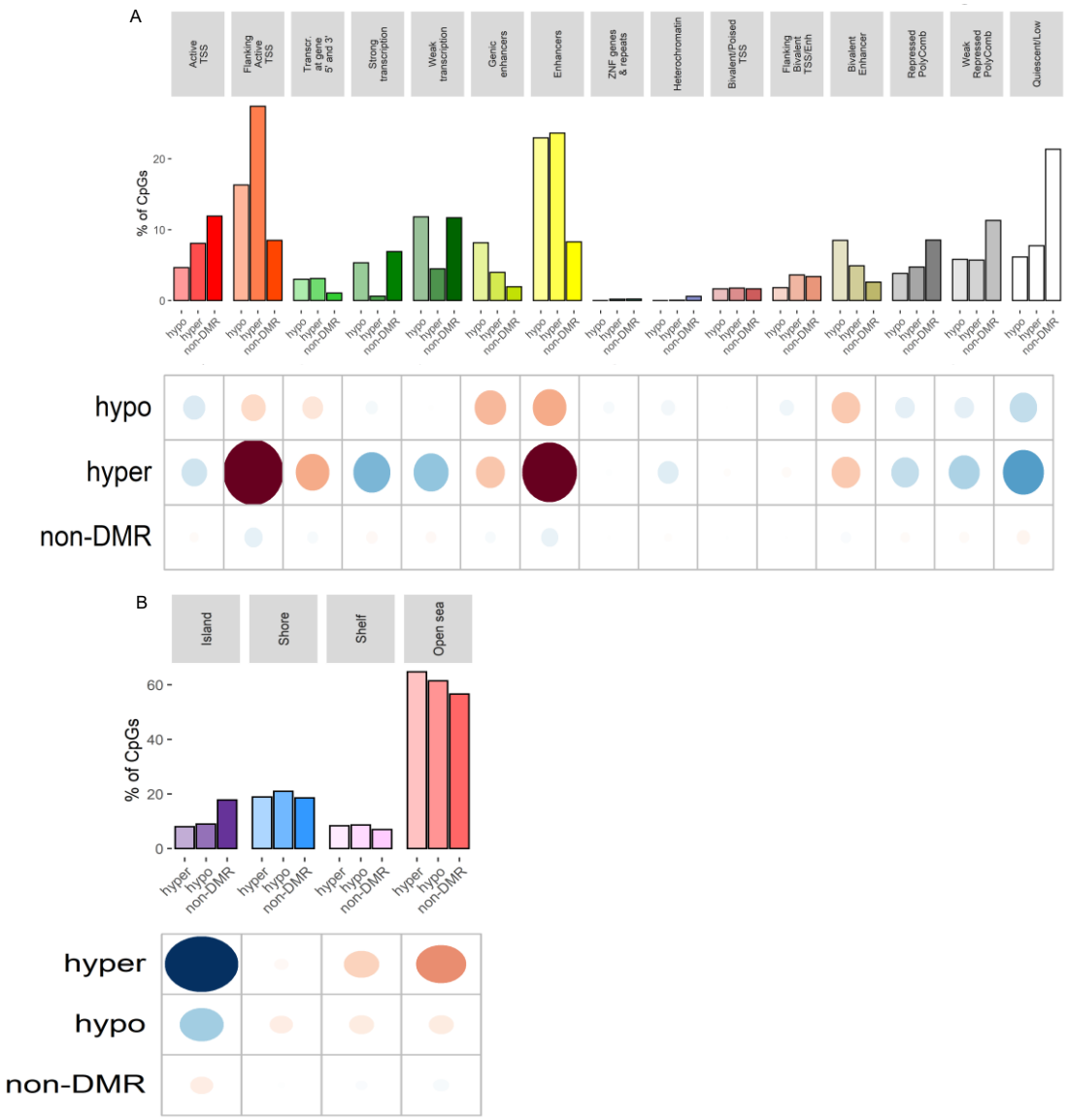


Figure 5.3. Distribution of fitness-related differentially methylated regions (DMRs) and non-DMRs in functional regions of the genome.

A: Distribution in chromatin states from male skeletal muscle from the Roadmap Epigenomics Project[206] B: distribution with respect to CpG islands (b), shore = ± 2kb from the CpG island, shelf = ± 2-4 kb from the CpG island, open sea = > 4kb from a CpG island. The grids under chromatin state distribution and next CpG island distribution represent the residuals from the χ2-test, with the size of the circles being proportional to the cell's contribution; red indicates an enrichment of the DMR category in the functional region, while blue indicates a depletion of the DMR category in the functional region.

5.3.2 Few DNA methylation changes following 4 weeks of HIIT

We then investigated the effect of HIIT on the muscle methylome. After 4 weeks of HIIT we found 568 DMPs (FDR < 0.005), 69.9% of which were hypomethylated (**Figure 5.4**), and 17 DMRs, all of which were hypomethylated (**Table 5.3)**.

The distribution of DMRs in chromatin states was different from that of all tested CpGs ($\chi 2$ -test p-value < 2.2 x 10^{-16}, **Figure 5.5**). There was a clear under-representation of DMRs around quiescent (i.e. silent) regions, at enhancers and in bodies of actively transcribed genes, and an over-representation at active TSS, bivalent TSS and regions actively repressed by PolyComb proteins. DMRs were over-represented at CpG island and in open sea, while under-represented in CpG island shores ($\chi 2$ -test p-value < 2.2 x 10-16, **Figure 5.5**).

We found 25 unique genes that showed at least one DMR after 4 weeks of HIIT (**Table 5.3)**. However, no enrichment for any GO or KEGG term was identified (FDR <0.05).

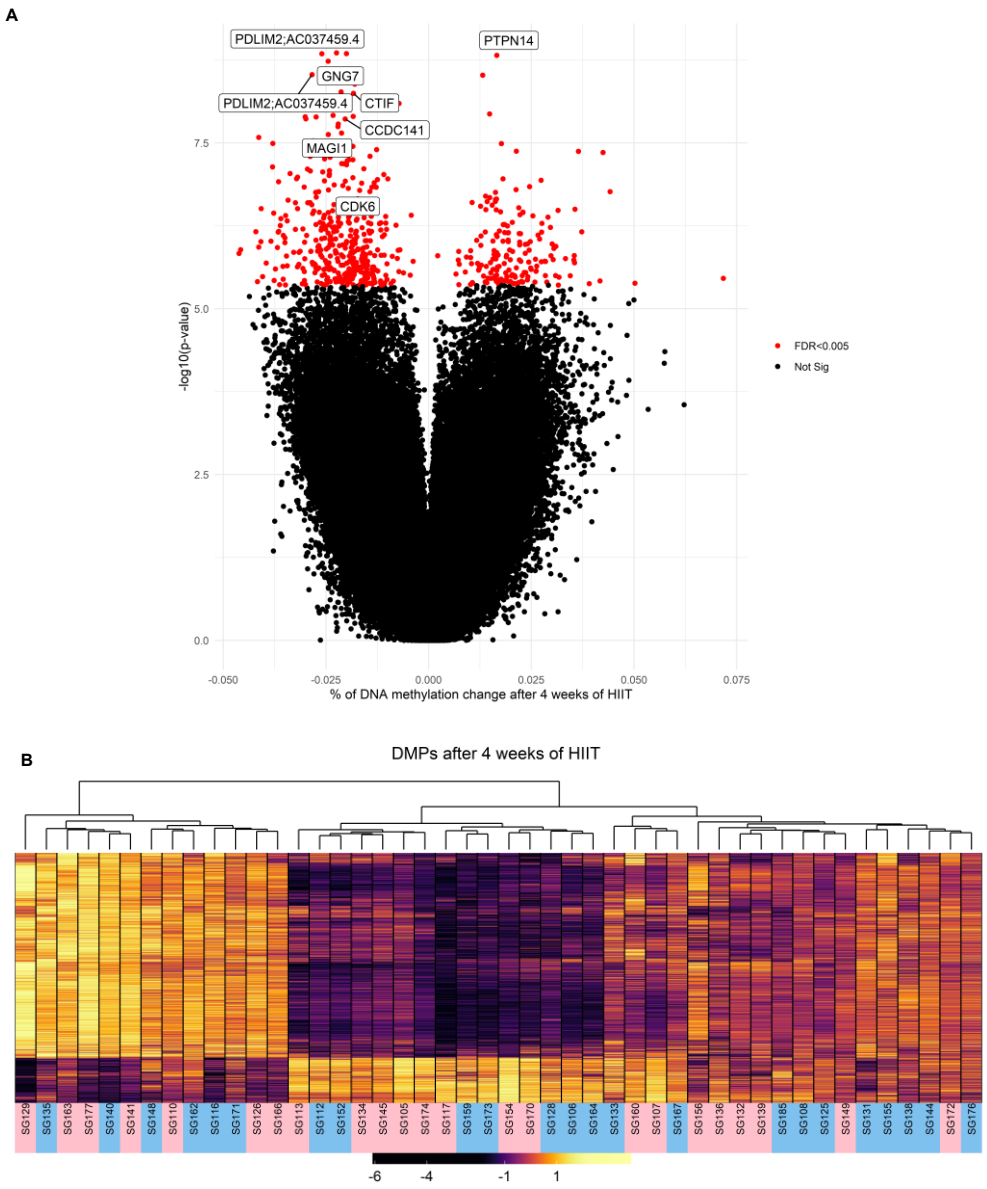


Figure 5.4. A: Volcano plot showing significant CpGs (false discovery rate <0.005) associated 4 weeks of HIIT. CpGs in red are significant (FDS <0.005). Each point represents a different CpG. B: Heatmap composed of significant CpGs associated with 4 weeks of HIIT. The purple colours represent higher levels of hypomethylation and yellow represents higher levels of hypermethylation.

The colours pink and blue highlighted in each participant represent timepoint with PRE = Pink and 4WP = Blue.

Chromosome	Position start (hg38)	Position end (hg38)	Length of DMR (base pairs)	No. of CpGs in DMR	CpG island position	Chromatin state in male skeletal muscle	Annotated gene(s)	Maximum effect size in DMR	Mean effect size in DMR	Stouffer score	Harmonic mean of the individual component FDRs	Fisher multiple comparison statistic
chr19	1070688	1071208	521	7	Shore; Island	Repressed PolyComb; Bivalent Enhancer	HMHA1	-0.03654	-0.02915	2.40E-12	0.001773	1.10E-10
chr1	1.72E+08	1.72E+08	914	9	Open sea	Active TSS	DNM3; DNM3OS; MIR199A2	-0.02281	-0.01333	6.26E-08	0.004938	1.36E-08
chr8	22588970	22589667	698	4	Open sea	Flanking Active TSS; Active TSS	PDLIM2; AC037459.4	-0.02838	-0.02374	5.81E-09	0.000504	3.28E-08
chr6	15504612	15505228	617	5	Open sea	Flanking Active TSS	JARID2	-0.02886	-0.02103	2.12E-09	0.003421	3.55E-08
chr19	2540908	2541105	198	3	Island	Flanking Active TSS	GNG7	-0.02559	-0.02483	1.39E-08	0.000469	7.32E-08
chr6	10555448	10556289	842	8	Open sea	Enhancers; Flanking Active TSS; Active TSS	GCNT2	-0.024	-0.01279	5.42E-08	0.00467	3.62E-07

chr15	42457137	42457686	550	6	Open sea	Active TSS; Flanking Active TSS	ZNF106	-0.01853	-0.01552	4.38E-07	0.004049	1.77E-06
chr1	39633108	39633697	590	6	Open sea	Enhancers; Flanking Active TSS	ENSG0000 0225903; RP1-144F13.3; HEYL	-0.02578	-0.01993	2.54E-05	0.003982	2.27E-06
chr17	7019612	7020206	595	5	Shelf; Shore	Active TSS; Flanking Active TSS	RP11-589P10.7; MIR497HG	-0.0295	-0.01747	4.55E-07	0.0043	3.69E-06
chr16	17972550	17972582	33	3	Open sea	Enhancers		-0.02882	-0.01981	1.00E-06	0.001874	4.19E-06
chr1	54781466	54781734	269	3	Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer	TTC22	-0.04137	-0.03322	3.45E-06	0.001634	1.14E-05
chr11	64759767	64760373	607	8	Open sea	Transcr. at gene 5’ and 3’; Flanking Active TSS	PYGM	-0.02988	-0.01704	8.26E-06	0.003068	1.74E-05
chr7	1537339	1537379	41	3	Shore	Active TSS; Flanking Active TSS	MAFK	-0.03612	-0.03288	4.76E-06	0.004227	1.97E-05

chr19	39307840	39307922	83	2	Island	Repressed PolyComb	LRFN1	-0.03026	-0.02683	4.35E-05	0.002536	9.60E-05
chr12	57736071	57736399	329	3	Island	Flanking Bivalent TSS/Enh; Bivalent Enhancer	AGAP2; NEMP1, AGAP2	-0.03032	-0.01898	0.000114	0.002309	0.000133
chr16	75238746	75238805	60	2	Shelf	Flanking Active TSS	BCAR1	-0.01856	-0.01707	6.73E-05	0.00339	0.000148
chr5	1.39E+08	1.39E+08	104	2	Island	Active TSS; Flanking Active TSS	PROB1	-0.04145	-0.03959	7.84E-05	0.002964	0.000165

Table 5.3. DMRs after 4 weeks of HIIT.

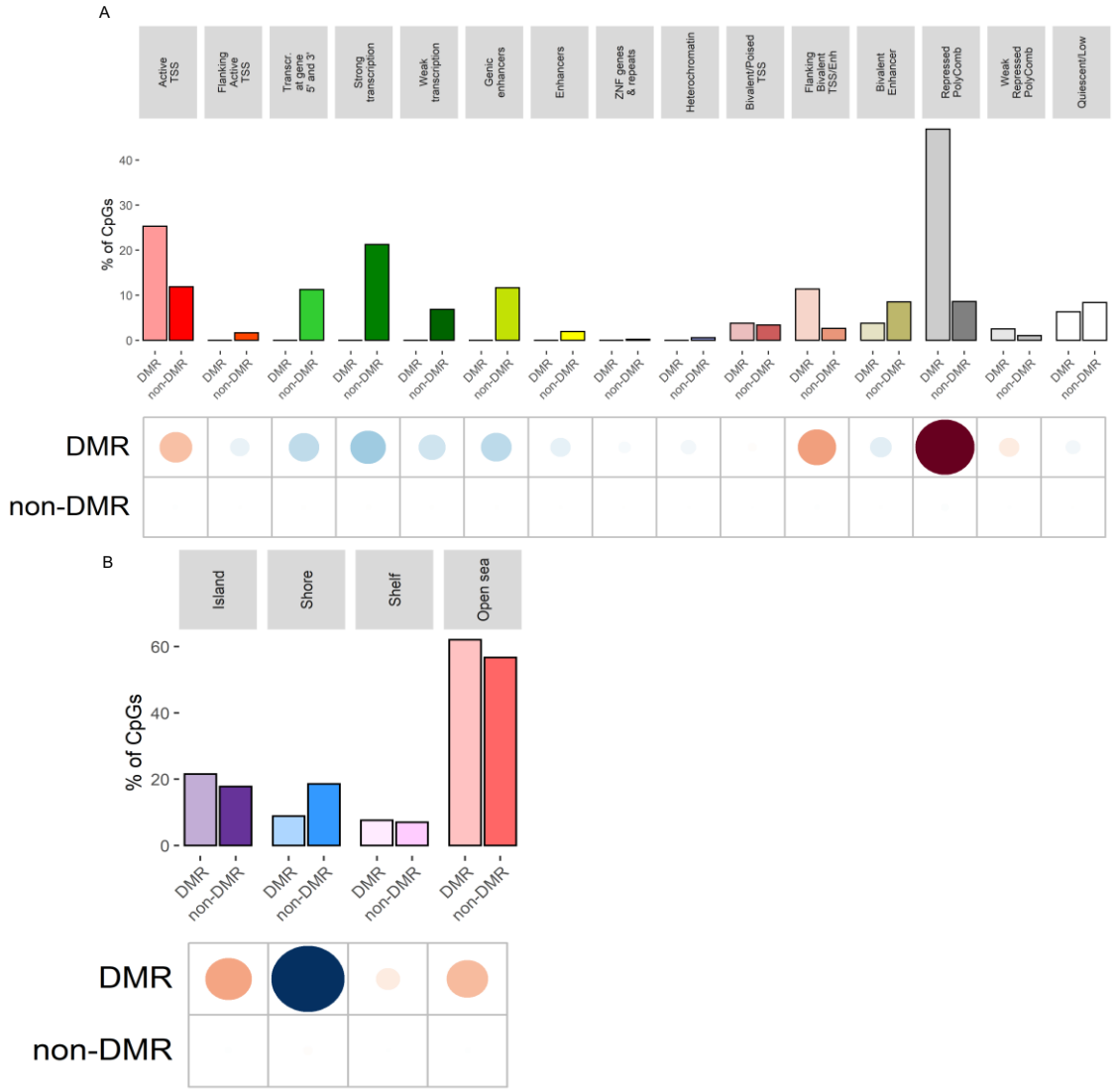


Figure 5.5. Distribution of hypomethylated, differentially methylated regions (DMRs) and non-DMRs in functional regions of the genome.

A: Distribution in chromatin states from male skeletal muscle from the Roadmap Epigenomics Project19 B: distribution with respect to CpG islands (b), shore = ± 2kb from the CpG island, shelf = ± 2-4 kb from the CpG island, open sea = > 4kb from a CpG island. The grids under chromatin state distribution and next CpG island distribution represent the residuals from the $\chi 2$-test, with the size of the circles being proportional to the cell's contribution; red indicates an enrichment of the DMR category in the functional region, while blue indicates a depletion of the DMR category in the functional region.

5.3.3 Significant DMPs at baseline and after 4 weeks of HIIT

We then overlapped fitness-related DNA methylation at baseline, and exercise-induced DNA methylation. We hypothesised that if exercise-induced DNA methylation changes underpin physiological adaptations to exercise training, DNA methylation at fitness-related DMPs would shift in a direction consistent with higher fitness levels. For instance, if CpG_x shows hypermethylation in individuals with higher fitness levels, DNA methylation at this CpG should *increase* following four weeks of HIIT. We found only five overlapping DMPs between baseline fitness and exercise training (cg14936543 – *AGBL1*, cg01466031 – *BCAR3*, cg09869357 - No annotated gene, cg25874123 – *MYOM1* and cg01383128 – *FZD5*). Surprisingly, DNA methylation levels at these CpGs decreased after 4 weeks of training, yet they were all hypermethylated with higher baseline fitness levels (**Figure 5.6**).

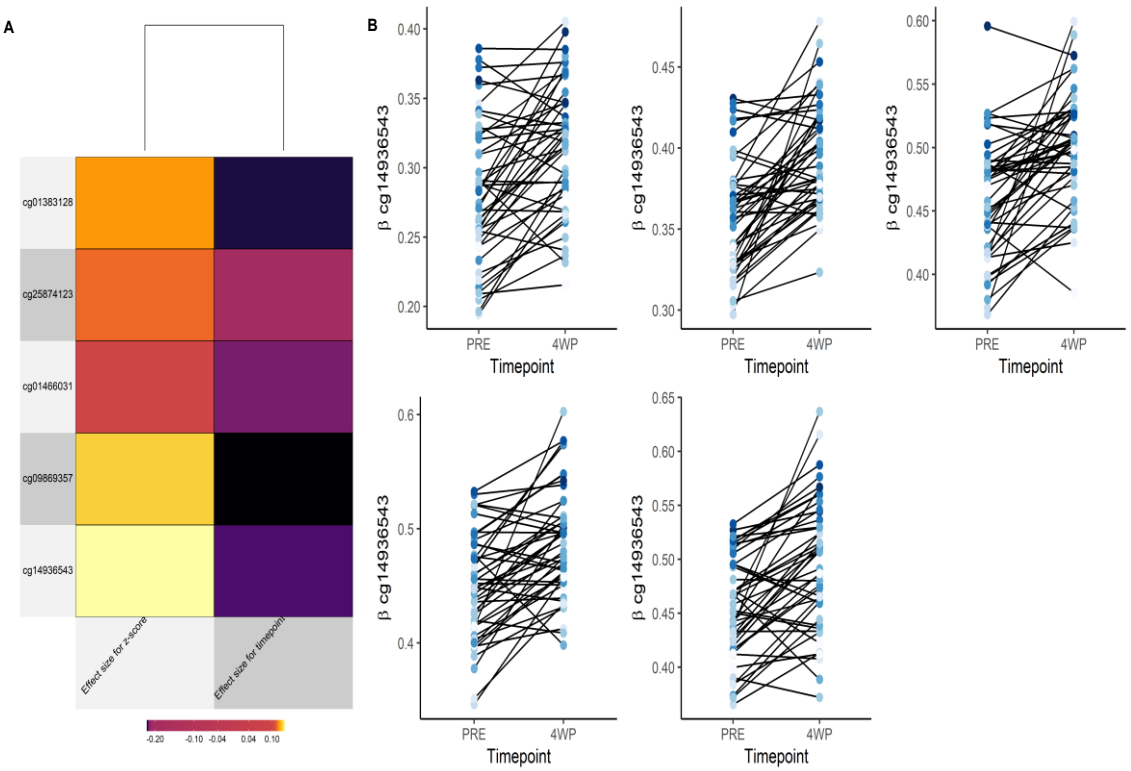


Figure 5.6. A: Heatmap of effect sizes for DMPs at baseline fitness and after 4 weeks of HIIT. The purple colours represent higher levels of hypomethylation and yellow represents higher levels of hypermethylation. B: Dot plot of the five intersected DMPs.

Each point represents a different participant, the darker the colour of the points the higher fitness z-score is.

5.4 Discussion

We conducted an EWAS of aerobic fitness and exercise training in human skeletal muscle in 46 healthy males who underwent 4 weeks of HIIT. We uncovered distinct DNA methylation signatures of high aerobic fitness at baseline, which target genes related to skeletal muscle structure and function. Subsequently, we investigated the effect of 4 weeks of HIIT on the methylome but found only few changes, with no clear affected pathway. Finally, we overlapped CpGs both associated with baseline fitness and showing changes following exercise training. We found surprisingly little overlap, and the few CpGs in common (n=5) presented exercise-induced shifts inconsistent with higher fitness levels.

A meta-analysis of 12 studies totalling n = 3,880 individuals assessed global DNA methylation levels in blood in relation to physical activity levels, and reported that higher fitness levels tend to be associated with higher DNA methylation levels[207]. In line with what has been observed in blood, we found that higher levels of aerobic fitness are associated with higher levels of methylation in human skeletal muscle. We have also identified a large number of genes showing altered DNA methylation with baseline fitness. One genomic region of no less than 12 DMPs over a distance of ~2kb (chr3:187735074-187737217) was hypermethylated near BCL6 Transcription Repressor (*BCL6*). Interestingly, higher mRNA expression of this gene in muscle was previously associated with low VO_{2max} levels (Spearman's = -0.42, p-value=0.008)[208]. Another interesting region located near *CD93* (chr20:23086306-23087581, 12 CpGs, 1276 bp) was hypomethylated, suggesting higher expression of *CD93*. Depletion of *CD93* plasma in mice is associated with poor metabolic control[209], suggesting a positive effect of higher fitness on metabolism.

While baseline fitness was mostly associated with increased DNA methylation levels, we only found decreased DNA methylation levels following 4 weeks of HIIT. This is surprising, as following exercise training, we expected a shift of the muscle methylome towards a profile that is typical of fitter individuals. Furthermore, we found very little overlap between fitness-related and exercise-induced differentially methylated genes. None of the four genes in common (*BCAR3*, *FZD5*, *MYOM1* and *AGBL1*) presented a clear link to exercise. However, other studies have described potential pathways affected by these genes that might regulate skeletal muscle function. For example, the BCAR3

gene has been previously described to have a functional role in the signalling pathway of insulin and IGF-1[210]. FZD5 encoded receptors for the Wnt5A ligand, and was linked to muscle mass regulation via the mTOR pathway[211]. Recent study suggest that the FZD5 gene is associated with sarcopenia-related hypermethylation in CpG islands[212]. MYOM1 encodes the structural myomesin-1 protein, which is expressed in muscle cells and is stabilizing the three-dimensional conformation of thick filament[213]. A decrease MYOM1 gene expression was associated with diabetic skeletal muscle atrophy[214], and therefore exercise is a potential form of prevention of this condition, although this hypothesis remains to be tested. Finally, AGBL1 is a metallocarboxypeptidase that mediates deglutamylation of targeted proteins and has been previously associated with corneal dystrophy and endothelial dystrophy, and its role in skeletal muscle is completely unknown[215]. Further studies utilizing mendelian randomization and epigenome editing are warrant to investigate the causality between DNA methylation and exercise responses.

The length of the exercise intervention (4 weeks) might have been too short to generate a profound change in the DNA methylation status of genes important for exercise training. Another potential explanation for such a low overlap has to do with unmeasured confounders: DNA methylation signatures of higher fitness levels may actually be reflecting other lifestyle factors or anthropometric measures that typically associate with higher fitness levels (e.g. a healthier diet). Furthermore, a limitation of the present study was the inability to include a control group in the analyses of DNA methylation, and therefore any changes in the methylation cannot be attributed to an effect of exercise per se in the absence of a no-exercise comparison. Finally, it should be noted that we only assessed one type of exercise training (HIIT) and other exercise modalities may show more consistent associations with higher aerobic fitness.

In summary, we have confirmed that baseline fitness is associated with distinct skeletal muscle DNA methylation profiles, but these signatures were surprisingly inconsistent with the DNA methylation changes induced by exercise training. Future studies should focus on applying longer exercise interventions, with larger sample sizes via multi-site collaborations and consortia, to establish the effects of exercise in the methylome.

Chapter 6. Individual DNA methylation response to exercise training

6.1 Introduction

There is growing evidence that exercise shapes the muscle methylome. A seminal study in 2012 demonstrated that the promoter regions of key genes involved in exercise response are strongly hypomethylated immediately after strenuous exercise and become re-methylated at 3 hours post exercise[22]. Further studies have shown that exercise training induces changes in the methylation status of pivotal genes involved in muscle function, thus possibly modelling a long-lasting advantageous expression pattern for increased trainability[56,216–219]. However, all these studies have been focused on *group* level changes and *individual* DNA methylation responses have not been investigated. Yet, it is essential to uncover how each individual uniquely responds to exercise training to develop personalised regimes and to understand individual differences in physiological adaptations to training. To do so, within-subject variability in response to exercise must be accounted for, but it requires specific study designs that are rarely implemented. In a recent publication by Bajpeyi et al.[122], participants performed a single bout of exercise and were divided into responders and non-responders based on their levels of methylation at an important regulatory region in *PGC-1α*. High-responders showed nucleosome repositioning after the exercise bout, along with a significant decrease in intra-myocellular lipid content.

Thus, the aim of the current study was to investigate individual responses to HIIT, using the unique design of the Gene SMART study. We took advantage of the repeated intervention, and repeated testing on a subset of individuals (n = 19 for the repeated intervention and n = 16 for the longer intervention with repeated testing) to see whether the DNA methylation response to an exercise stimulus could be individual-specific (i.e. trainability).

6.2 Methods

6.2.1. Participants

The study design has been described in detail in Chapter 3. Out of a sub-set of 20 participants from the 4-week Gene SMART (Skeletal Muscle Adaptive Responses to Training) intervention[136], 16 completed a repeated intervention of 12 weeks of HIIT. From the 20 initially recruited participants, **19 completed 4 weeks of HIIT** (1 dropout)**, of these 18 completed 8 weeks** (1 dropout)**, and 16 completed the full 12-weeks of HIIT (Repeated testing)** (1 dropout and 1 exclusion due to inconsistent results (i.e. duplicate tests provided more than 10% difference)).

Participants were apparently healthy, moderately trained men (VO_{2max} 35–60 $mL \cdot min^{-1} \cdot kg^{-1}$), aged 18 to 45 years old. The study was approved by the Victoria University Human Ethics Committee (HRE13-223) and written informed consent was obtained from each participant. Participants were excluded from the study if they had a past history of definite or possible coronary heart disease, significant chronic or recurrent respiratory condition, significant neuromuscular, major musculoskeletal problems interfering with ability to cycle, uncontrolled endocrine and metabolic disorders, or diabetes requiring insulin and other therapies[136].

6.2.2. Study design

Participants from the Gene SMART study[136] completed 4 weeks of HIIT, followed by a washout period of ≥12 months; participants that agreed to return for a follow up repeated the 4-week HIIT intervention. Two graded Exercise Tests (GXTs) were conducted at each time point to determine peak power output (W_{peak}), the lactate threshold (LT) and maximal oxygen consumption (VO_{2max}) **(Figure 6.1**).

HIIT – first intervention (4 weeks)

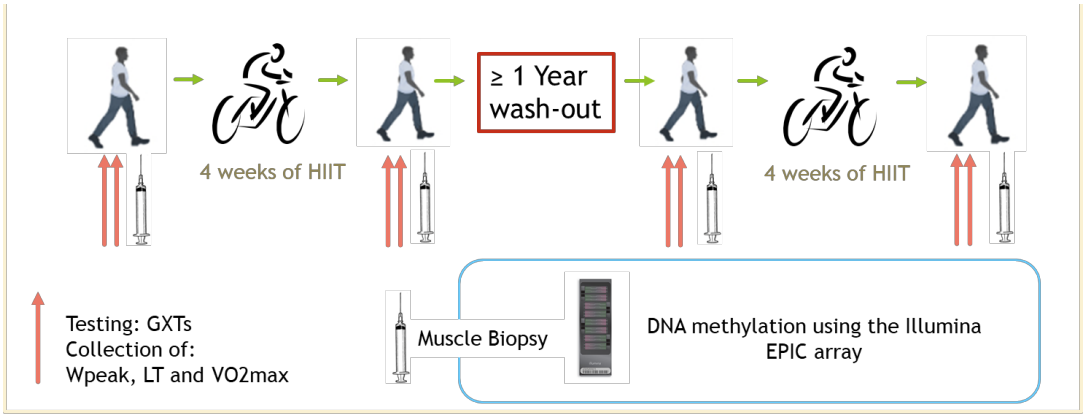
Participants trained 3 times/week under supervision. All training sessions were completed on an electronically braked cycle ergometer (Velotron, Racer Mate Inc, Seattle, USA) and were preceded by a 5-min warm up at 50 W. Each session consisted of six to twelve 2-min intervals performed at different intensities 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).

HIIT – second intervention (4 weeks)

After a washout of at least 1 year, participants repeated the HIIT described above. A total of 16 participants completed the repeated intervention and were used for the analyses.

HIIT – repeated testing (12 weeks)

Participants were tested at baseline and after 4 weeks, 8 weeks, and 12 weeks of HIIT. To ensure progression, training intensity was re-adjusted every 4 weeks based on the newly determined Peak Power output (W_{peak}) and Lactate Threshold (LT) from the Graded Exercise Test (GXTs). These tests also allowed monitoring individual progress of participants for the longitudinal analysis of training adaptations. To increase accuracy in measurement and to reduce biological day-to-day variability in participants' performance, physiological measures of fitness (W_{peak}, LT, and VO_{2max}) were assessed from two GXTs conducted at each time point **(Figure 5.1)**.



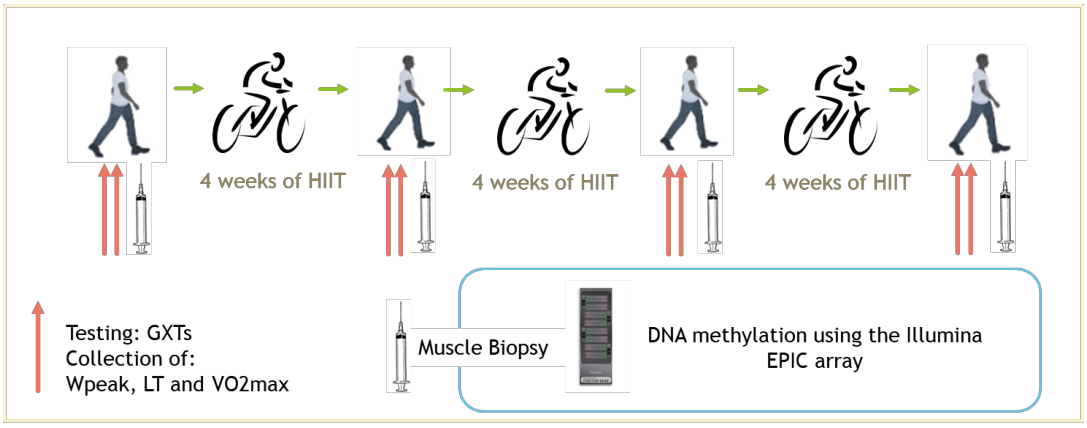


Figure 6.1. Study design

6.2.3. Muscle biopsies, DNA extraction and DNA methylation analyses and pre-processing

A detailed report of how muscle biopsies, DNA extraction and DNA methylation analyses and how DNA methylation data was pre-processed are detailed in Chapter 5.

6.2.4. Statistical analyses

We estimated individual DNA methylation response to exercise training using two study designs: in the first one, we focused on the repeated intervention (**Figure 6.1 First vs Second Intervention**). In this design, each individual was profiled for DNA methylation patterns before and after four weeks of HIIT twice (i.e. repeated intervention). This allowed us to estimate how consistent individual response to four weeks of HIIT was (i.e. whether individuals responded similarly to HIIT after the first and the second intervention at the DNA methylation level). The *limma* package that is traditionally used for differential DNA methylation analysis does not allow extracting random effects, which are the measures of interest in this study, since we focus on individual response. Therefore, we used linear mixed models using the *lmerTest* package [48]; DNA methylation at each CpG was regressed against timepoint (before or after 4 weeks of HIIT), intervention (1st or 2nd intervention), with the addition of age and batch as covariates.

$$DNA\ methylation = Timepoint + Intervention + Age + Batch + random\ intercept + random\ slope$$

The $Timepoint$ coefficient represents DNA methylation changes that are shared between all participants after 4 weeks of HIIT; the $Intervention$ coefficient accounts for baseline DNA methylation differences that may be present between the 1st and 2nd intervention; the $random\ intercept$ accounts for differences in baseline DNA methylation between individuals, and the $random\ slope$ represents the subject-by-training interaction, that is, the individual response to four weeks of HIIT at the DNA methylation level.

In the second study design, we focused on the repeated testing over 12 weeks of HIIT (**Figure 6.1 Second Intervention**). In this design, each participant was profiled for DNA methylation patterns in skeletal muscle at regular intervals over the course of 12

weeks of HIIT (at baseline, after 4 weeks of HIIT, after 8 weeks, and after 12 weeks). This unique design is another way to investigate individual response to HIIT at the DNA methylation level since it is possible to build individual DNA methylation progress curves and estimate whether some individuals show exceptionally high (or low) improvements. We used linear mixed models using the *lmerTest* package[48]. DNA methylation at each CpG was regressed against timepoint (baseline, after 4 weeks, after 8 weeks or after 12 weeks), with the addition of age as covariate. Batch was not included as a covariate in this model as all DNA methylation samples were profiled on batch #2.

$$DNA\ methylation\ =\ Timepoint + Age\ +\ random\ intercept + \ random\ slope$$

The $Timepoint$ coefficient represents DNA methylation changes that are shared between all participants after 12 weeks of HIIT; the $random\ intercept$ accounts for differences in baseline DNA methylation between individuals, and the $random\ slope$ represents the subject-by-training interaction, that is, the individual response to 12 weeks of HIIT at the DNA methylation level. All CpGs associated with the random slope at FDR < 0.005 were considered DMPs. We used the *ggplot2*[148], *ggpubr*[147], *complexHeatmaps*[199], and *FactorMiner*[200] packages for data visualisation.

6.3 Results

6.3.1 No individual DNA methylation response after 4 weeks of HIIT

We identified only one CpG showing consistent, individual response after both interventions (cg11260483, p-value: 3.22000e-10, adj.p-value: 0.00022) (**Figure 6.2**). This DMP is located in the chr11:12010278-12010280 and annotated to the *DKK3* gene.

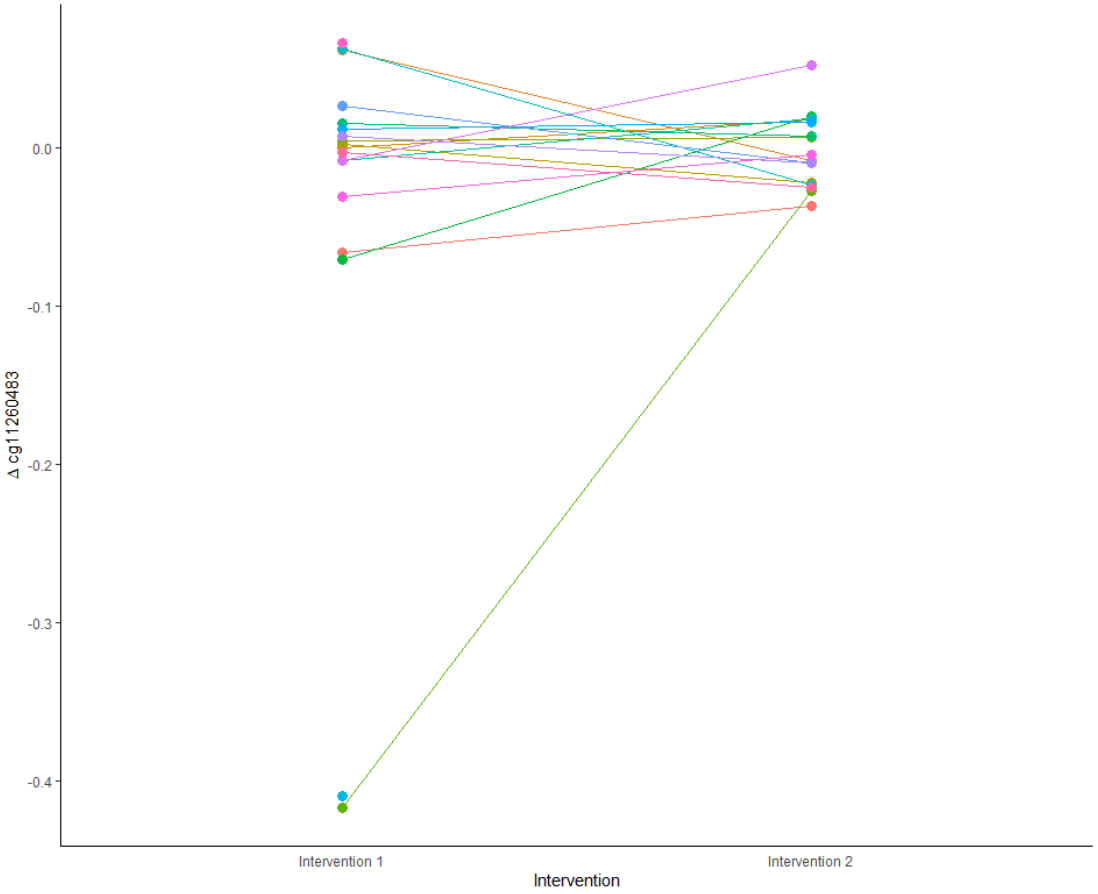


Figure 6.2. Significant CpG presenting consistent individual response after a repeated HIIT intervention of 4 weeks.

6.3.2 No individual DNA methylation response to 12 weeks of HIIT

We applied mixed modelling to identify *individual* DNA methylation response to 12 weeks of HIIT. As we profiled individual DNA methylation patterns at regular intervals (at baseline, after 4 weeks of HIIT, after 8 weeks and after 12 weeks), we could fit a random slope to the model to estimate the individual DNA methylation trajectory over the training program, which corresponds to trainability. We did not identify any CpG that showed individual response to training (adjusted p-value <0.005). To estimate whether this lack of results reflects a true lack of individual response to HIIT at the epigenetic level, we looked at the histogram and quantile-quantile (Q-Q) plot of all p-values for the random effects across all tested CpGs (**Figure 6.3**). The Q-Q plot is used to assess the number and magnitude of observed associations between CpGs and the trait under study (here, individual response to training, or trainability), compared with the association statistics expected under the null hypothesis of no association[220]. The Q-Q

plot for the random effect is highly unusual and suggests either heteroscedasticity (i.e. large residuals)[221], or that individual effects are smaller than expected by the model.

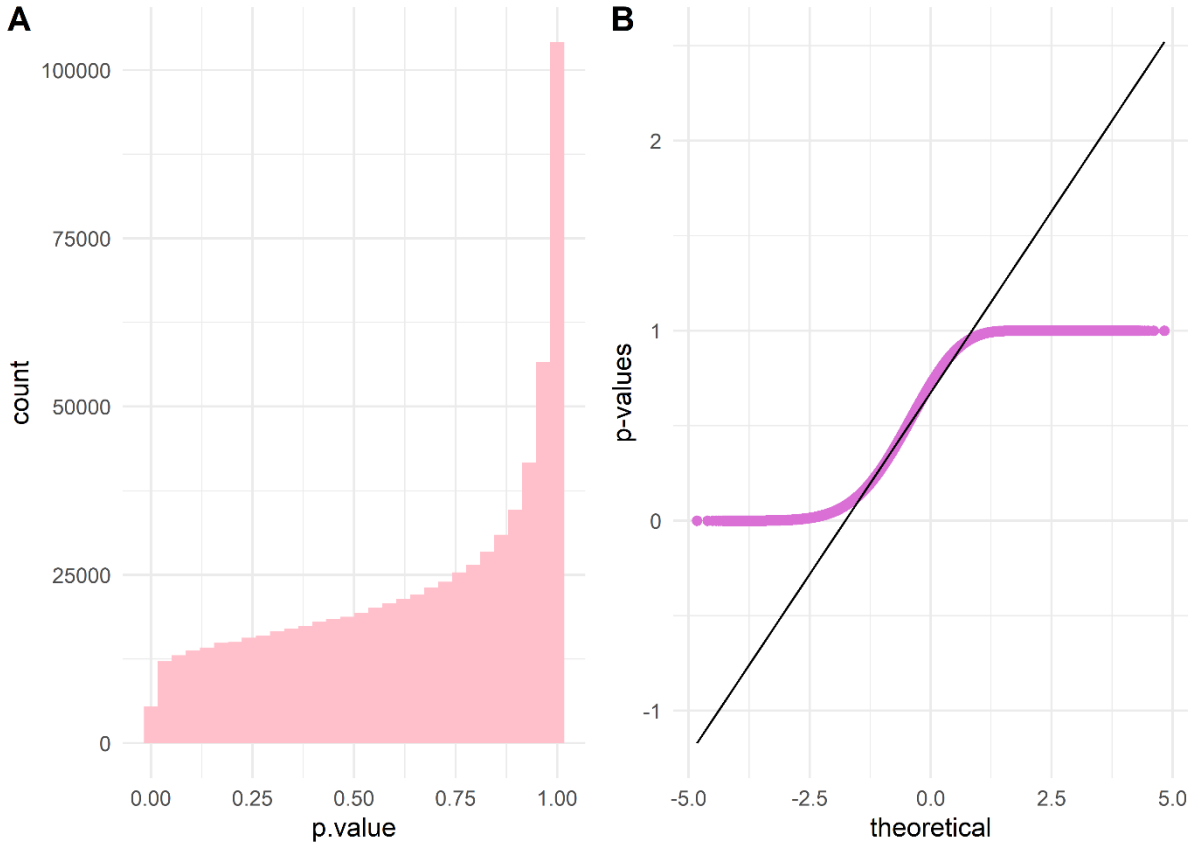


Figure 6.3. A: p-values for random effect (i.e. trainability, individual response). B: Quantile-Quantile plot of p-values for individual response.

6.4 Discussion

For the first time, we investigated DNA methylation changes at the individual level following exercise training, by combining two recommended approaches in the field of personalised training response (i.e. repeated intervention and repeated testing during the intervention)[5]. We found no evidence for a consistent, individual response to 4 or 12 weeks of HIIT at the DNA methylation level in healthy young males.

Only one CpG was associated with consistent individual response after both interventions (cg11260483, p-value: 3.22000e-10, adj.p-value: 0.00022). This DMP is located in the chr11:12010278-12010280 and is annotated to the DKK3 gene. DKK3 is a secreted glycoprotein belonging to the dickkopf (DKK) family[222]. Unlike other members of the DKK family, the pathway involving DKK3 remains to be elucidated. Given its increased level in circulating blood in aged population it was speculated that DKK3 play

a role in age related diseases[223].Furthermore, DKK3 has been suggested to influence myogenesis, and in mice increased expression of DKK3 is associated with muscle atrophy[223]. Although only one CpG located in this gene consistently changed after exercise interventions, this gene appears to be a great candidate for further investigation of causality between DNA methylation and exercise response. Further studies focusing on this gene are therefore warrant.

This is the first study to investigate epigenetics in the context of individual response to exercise. By using a repeated intervention (i.e. two HIIT interventions of 4 weeks interspaced by at least 1 year of wash out) and a repeated testing approach (i.e. tests at multiple timepoints during the course of 12 weeks), we aimed to separate between- from within-subject variability in training response, and to obtain a robust estimate of individual epigenetic response to HIIT. Although DNA methylation changes are highly dynamic[224], they can also accumulate overtime if the environmental stimulus is sustained[225]. For example, DNA methylation decreased immediately after exercise and re-methylation occurred a few hours later, but not fully back to baseline levels[22], supporting the idea that DNA methylation changes may be accumulated after repeated training sessions. To our surprise, we found no consistent changes in DNA methylation within the same individuals after HIIT.

Emerging evidence suggests that environmental stimuli such as exercise lead skeletal muscle to retain molecular information to be primed for future plasticity, following encounters with the same stimulus[21]. To date, only one study has investigated muscle memory in response to exercise using a resistance intervention approach[123]. Although this is an interesting topic of research, our study design was not aimed to investigate muscle memory. Trainability is the consistent response of an individual to training. In other words, it is an individual inherent capacity for response that does not depend on previous exposure to a stimulus. Muscle memory refers to how exposure to a stimulus (exercise, in utero programming, etc.) "primes" the muscle to responds later, it is not an inherent capacity to respond. For example, genetics would influence trainability, but genetics would not influence muscle memory as genetics is an innate characteristic, it is not an "exposure". The long wash out period, in the present study, was purposely chosen to avoid any carry over effects that could influence (i.e. muscle memory). Future studies with multiple timepoints should be conducted to investigate how long muscle exhibits an epigenetic memory in response to exercise.

Individual changes in the muscle methylome appeared much more variable within the same individual than we expected, and this may have hindered our ability to detect consistent, robust individual responses to HIIT. Larger cohorts and/or longer training programs with regular assessments would provide greater statistical power to detect these individual responses. We cannot dismiss the possibility that larger samples sizes might still not be sufficient to capture individual changes at the methylome level, as there are many unwanted sources of variability that introduce noise in the DNA methylation signal. We attempted to reduce any acute influence on the muscle methylome by sampling muscle biopsies in the fasted state at the same time of the day for all participants, following a 48h control diet, but we did not control sleep and other social stresses that may have influenced DNA methylation patterns. Finally, DNA methylation from muscle biopsies highly depends on the relative proportions of different cell types (i.e. type I and type II fibres, endothelial cells, leucocytes) that may vary between different pieces of muscle taken at adjacent sites on the leg. A deconvolution of the DNA methylation signal from the different cell types may help reduce noise in the data and build a more accurate profile of individual DNA methylation changes in muscle.

Chapter 7. Overall discussion, limitations, and future directions

7.1 Discussion

The benefits of exercise are well established[27,98,226–232], yet most exercise studies focus on responses at the group level, and little has been done to address the reality of individual response to exercise training[2,5,9,10,35,38,40,43]. For the first time, we have combined **robust statistical methods** with an **innovative and comprehensive exercise trial design** in order to discriminate between- and within-subject variability in exercise response at both the **physiological** and **molecular** (i.e. mitochondrial and epigenetic) level.

We first painted a comprehensive picture of all statistical methods that can be used to estimate individual response to an exercise intervention. Then, we implemented two of these methods to identify individual response to exercise training. We also showed that the mitochondrial respiration technique in permeabilised human muscle we had considered as a molecular outcome was too unreliable to be used in the present thesis and requires a larger sample size due to significant between-samples variability. We successfully quantified individual responses to 12 weeks of HIIT at the *physiological* level using repeated testing at regular intervals over the course of the intervention, with some participants showing greater fitness improvements than average and others showing lower improvements. However, we did not identify individual responses to 4 weeks of HIIT, as individuals showed highly inconsistent responses to the two interventions. In both designs, we failed to identify individual responses at the *molecular* level (i.e. mitochondrial and DNA methylation measures), as within-subject variability (i.e. the variability in response *within* each person after the same stimulus) was large. As a result, the signal-to-noise ratio (i.e. the relative magnitude of true individual response and within-subject variability) was very low, which hindered our ability to detect individual molecular response to HIIT. Finally, we found that baseline DNA methylation patterns were significantly associated with baseline aerobic fitness levels and exercise triggered changes in the methylome that were consistent with previous reports[22,219]. However, to our surprise such changes in DNA methylation after exercise occurred in the opposite

direction to those observed in fitter individuals, who presented more global hypermethylation, while changes after exercise were all hypomethylated. Next, we intersected the results and only 5 DMPs were the same for both baseline and intervention changes, which in part could explain why we saw different directions on DNA methylation at baseline and after 4 weeks of HIIT (i.e. DMPs are related to different pathways and mechanisms).

Reducing technical variability in the phenotype of interest is important to increase our ability to detect subtle changes, and therefore to increase power. In the early stages of this PhD, we wanted to ensure we would be able to detect potentially small differences in individual responses in molecular traits, so we quantified the technical variability of one of the molecular techniques we were planning to use. We were surprised to find that a popular method to measure mitochondrial function in skeletal muscle (i.e. mitochondrial respiration) showed very large technical variability. There was a high correlation between measurements from the two chambers ($R>0.7$ $p<0.001$) for all complexes, but the TEM was large (7.9 - 27 pmol·s-1·mg-1; complex dependent), and the CV was > 15% for all complexes. We performed statistical simulations of a range of effect sizes at 80% power and found that 75 participants (with duplicate measurements) are required to detect a 6% change in mitochondrial respiration after an intervention, while for interventions with 11% effect size, ~24 participants are sufficient. The high variability in respiration suggests that the typical sample sizes in exercise studies may not be sufficient to capture exercise-induced changes. While we could not pinpoint the exact reason for the high variability in this technique, we suspect that there is a high margin of error for technicians to properly separate the muscle fibre before permeabilization. For this reason we chose not to use this technique in this PhD project as our sample size was too small (n = 20) to overcome the technical variability of this method. Instead, we decided to use another approach where several mitochondrial markers are analysed and then combined into a comprehensive mitochondrial health index (MHI). This functional MHI has been recently proposed in blood by mathematically integrating biochemical enzymatic activities and mtCN into a single score, which may represent an optimized measure of mitochondrial functional capacity[135]. This method successfully captured a reduction in mitochondrial health in blood as a result of chronic psychological stress[135]. During the 12-week HIIT intervention, the mitochondrial enzyme maximal activity and therefore the MHI were highly variable, and no consistent changes were observed at either

the group or individual level. This was surprising given that mitochondrial content and function are upregulated by exercise[126–128]. To ensure that the variance was not due to technical variability, we removed any duplicate results that presented a variance >10%. However, it is known that enzyme activity is highly dynamic and the timeframe in which enzymes are fired may vary both within as well as between subjects[152]. In addition, we cannot rule out the potential involvement of other enzymes in similar pathways, or the fact that enzyme Km and not the maximal activity could be different between people[153]; however, these hypotheses remain to be tested. Furthermore, due to the nature of skeletal muscle (i.e. multi-nucleated) we could not account for cell number as suggested by Picard *et al*. The multi-nucleated nature of skeletal muscle promotes the possibility that each myonucleus differs in transcriptional rates and is independently regulated and distinctive from the others, to the extent that local differences in skeletal muscle (i.e. two pieces of same biopsy) might be present following exercise[9,154,155]; this would potentially explain part of the variability observed between and among measures.

Researchers usually focus on the *average* response to an intervention to determine its overall efficacy in the general population[233]. However, variability in response is commonly observed between individuals, and the term "non-responder" has sometimes been used[7,52,234] to refer to individuals who have failed to present positive changes in a specific outcome[235]. However, such terminology may promote the erroneous perception that exercise maybe not be collectively beneficial[234]. This perception has serious implications from a public health viewpoint, given that exercise is well-known to promote numerous health benefits[236]. Exercise affects virtually all tissues via myriads of different pathways (e.g. activation of *BDNF* in the brain, leading to greater cognitive function[237]), and likely provides health benefits even if improvements at the physiological level are not visible with exercise testing[236]. However, we propose that the hasty classification of individuals into "responders" and "non-responders" may also stem from the lack of consideration for how inconsistent a response may be *within* a given individual. Why label an individual a "non-responder" if we cannot provide a robust prediction as to how this individual will respond to a given exercise intervention[234,238]? The paucity of studies on individual response to training is a major hindrance to the development of exercise training programs that are tailored to the individual ("personalised medicine"). In addition, most studies ignore the existence of within-subject variability, which leads to an erroneous classification of individuals into responders or non-responders[234]. Within-

subject variability is well known and routinely accounted for in pharmacology[239] and dietetics[240]. It ensures that the individual response to a drug or to a certain food is robust and predictable within the same individual. In exercise physiology, within-subject variability is often ignored because it is tedious to measure repeating an exercise training program of a few weeks or a few months is both time- and resource-consuming. In addition, an exercise training program may remodel the muscle substantially to introduce a form of muscle memory (i.e. "carry-over effect") that would modulate the way participants respond to a future intervention. In this thesis, we took on this challenge by repeating a relatively short intervention (4 weeks of HIIT) after a long wash out of at least one year (to limit this carry-over effect), and we estimated how consistent the response to four weeks of HIIT was within a given individual. We also implemented another study design that could estimate within-subject variability at a lower cost and within a shorter time frame by repeatedly measuring the desired outcome at regular intervals during the exercise training program. This clever study design allows multiple sampling per participant and discriminates individual responses (i.e. the slope of the progress curve) from within-subject variability (i.e. the spread of individual measurements around the slope). Using this approach, we were able to isolate all sources of variability and identify consistent responses at the individual level for all physiological measures of aerobic fitness (W_{peak}, LT and VO_{2max}). With this approach, we accurately labelled individuals as "better" or "worse" responders than the average participant.

Finally, we investigated the roles of DNA methylation in response to exercise, as well as its influence on individual measures of fitness. DNA methylation changes were observed at the group level, but no consistent changes were found at the individual level. Several DMPs and DMRs were associated with W_{peak}, LT and VO_{2max}. Significant enriched pathways were also identified for both W_{peak} and LT, but not for VO_{2max}, indicating that our sample size might still be small for analysis on the relationship between DNA methylation and VO_{2max}. No consistent changes in DNA methylation were observed after a repeated intervention, and baseline methylation values did not influence physiological changes in response to exercise. This seems to indicate that cell memory of DNA methylation changes in response to exercise is not long-lasting and might be erased, and therefore one year of wash out period was too long to detect any epigenetic memory in skeletal muscle.

The repeated intervention approach is rather novel in exercise studies, and only one study to our knowledge has applied this methodology, albeit not aimed at exercise response[47]. Using the supplementary files of this study we were able to extract the data and compare responses for some variables analysis between first and second intervention. Surprisingly a poor correlation between interventions was observed. These findings matched our observations after a repeated intervention, whereby we did not observe a consistent response in the same participants. This indicates that aspects outside of the intervention's control (i.e. diet, training history, between-session recovery, sleep, etc.) might have a large influence at the individual level, and the same adaptative state is likely not to be the same as the initial intervention[234]. Thus, this type of approach may yield better results if applied to variables that do not present large variable aspects, such as genetics.

When we transitioned to the molecular level, our initial expectation was that trainability would be identified by the repeated testing approach, as we observed at the physiological level. However, at the molecular level (i.e. mitochondrial and fibre type measures) intra-individual variability was very large, and trainability could not be identified. As previously mentioned, exercise modifies many pathways and mechanisms, and we hypothesise that the variability observed may be due to compensatory mechanisms occurring in response to the intervention applied. Furthermore, our sample size was rather small, and due to the large variability observed it is likely that we might have been underpowered. Finally, it has been recently reported that intra-biopsy variability can also lead to large noise and thus contribute to the lack of significant results[9].

Sources of variability can also be observed at the experimental level. A recent study reported a large intra-biopsy variability was large and results from different portions of same muscle biopsy did not correlate. Furthermore, substantial intra-individual variability was noted at baseline for gene expression even after accounting for experimental variability and between visits[9]. Within our own experiments, we have also observed a large variability between duplicate samples of same muscle biopsy when performing mitochondrial respiration[124,241]. Such findings led us to perform a detailed analysis of the technique and calculate TE_M for the estimation of minimal effect size or sample size necessary to be confident in the results. Our study was the first to provide guidelines (i.e. sample size or effect size) to future studies aiming to use mitochondrial

respiration as a phenotype of interest. Studies such as this are important, so false positives or low confidence studies may highlight their limitations or reconsider their study design before experiments are completed.

DNA methylation has been shown to be modulated by exercise, and to be involved in the process of cell memory. However, only four studies to date have investigated DNA methylation genome-wide in skeletal muscle in response to exercise[25–27,123]. Furthermore, investigations were based only at the group level and no associations between DNA methylation and exercise-induced physiological markers were included. For the first time we have investigated DNA methylation at the individual level in association with physiological markers and as a mechanism of cell memory after a long wash-out period. However, based on our findings, we were underpowered for most analyses. We have shown change in the methylome in the present study. However, changes present very small effect sizes and thus it is necessary to acquire large cohorts to identify robust and replicable methylation changes in response to exercise at both the individual and the group level. In addition, we observed very clear DNA methylation patterns associated with physiological variables independently of time, indicating that such patterns are a result of years of training, and to detect shifts in DNA methylation in skeletal muscle we either need to have large cohorts or long interventions (i.e. >1 year). Our findings suggest that no DNA methylation patterns consistently observed after a second intervention, indicating that change in DNA methylation might have been lost after such a long wash-out period. These results strengthen the notion that exercise must be continuously applied throughout life to have long-lasting benefits, and when it is stopped, adaptations and health benefits are likely to be lost[121]. Finally, epigenetic modifications are not restricted to DNA methylation changes as highlighted in the introduction of this thesis, and histone modifications as well as microRNA changes should also be further investigated. In fact, these modifications are likely to occur in synchrony and affect each other, leading to a complex network of changes resulting in exercise adaptations.

7.2 Limitations and future directions

The main limitation of our study was the small number of participants that agreed to come back for the repeated and longer intervention. This caused us to be underpowered for most of our analyses, and after adjusting for multiple testing most of our results were

not significant. While we could detect signals in response to exercise, larger cohorts are needed to overcome noise-to-ratio and achieve significance.

Our cohort was composed of only male participants, and therefore sex differences have not been accounted for. Furthermore, our experiments at the molecular level were all conducted utilising different pieces of same biopsy. It has been shown that intra-biopsy variability is large, and this could potentially explain the lack of relationship between experiments. Futures studies should aim to include males and females to account for sex differences, and if possible, use the same muscle piece for all analyses to avoid additional sources of variability.

Based on our findings, we suggest future studies implementing the repeated testing approach should be considered the "gold standard" for identification of individual trainability at the physiological level. This has important implications for personalised training, as it shifts the focus from the group level and can confidently classify true response to exercise for each individual.

Exercise response involves many pathways and mechanisms, and thus future interventions should combine not only epigenetic markers, but also others such as proteomics and transcriptomics, and integrate findings to achieve a broader understanding of molecular changes in response to exercise. Furthermore, many compensatory mechanisms may occur at once in response to exercise stimulus. Thus, future studies should also aim to investigate molecular networks that are activated at the individual level in response to exercise.

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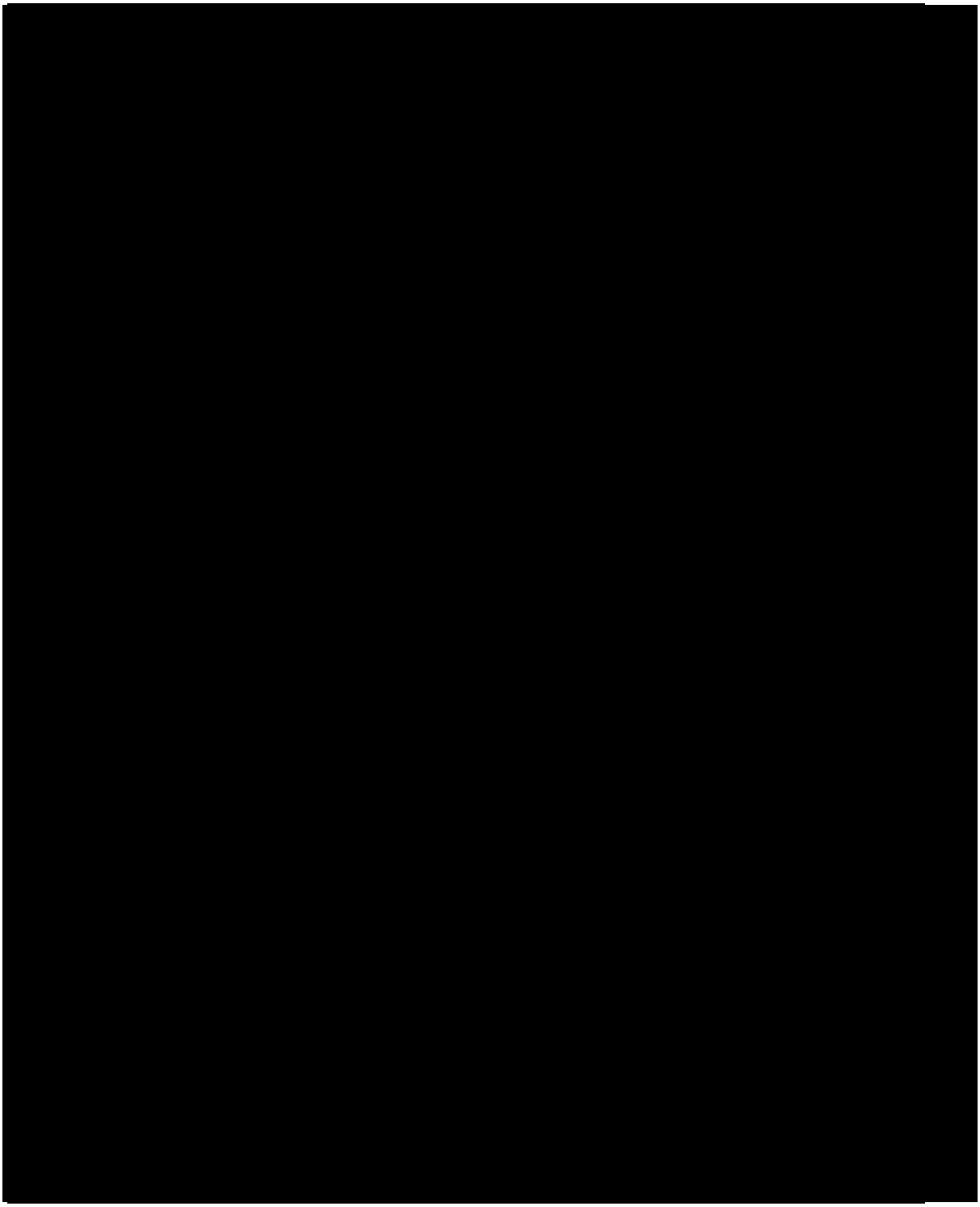
Appendix: Included Publications



Macsue Jacques, Danielle Hiam, Jeffrey Craig, Romain Barrès, Nir Eynon & Sarah Voisin (2019) Epigenetic changes in healthy human skeletal muscle following exercise– a systematic review, Epigenetics, 14:7, 633-648, DOI: 10.1080/15592294.2019.1614416

Voisin, Sarah1; Jacques, Macsue1; Lucia, Alejandro2,3; Bishop, David J.1,4; Eynon, Nir1,5 Statistical Considerations for Exercise Protocols Aimed at Measuring Trainability, Exercise and Sport Sciences Reviews: January 2019 - Volume 47 - Issue 1 - p 37-45 doi: 10.1249/JES.0000000000000176

Implementation of multiple statistical and exercise training methods to measure trainability

Macsue Jacques[1], Shanie Landen[1], Javier Alvarez Romero[1], Xu Yan[1], Danielle Hiam[1], Nir Eynon[1, 2*#], Sarah Voisin[1*]

*Co-senior authors

[1] Institute for Health and Sport (iHeS), Victoria University, Melbourne, Australia.

[2] Murdoch Children's Research Institute, Melbourne, Australia

Corresponding Author:
Associate Professor Nir Eynon
Institute for Health and Sport (iHeS),
Victoria University
PO Box 14428
Melbourne, VIC 8001, Australia.
Tel: (61-3) 9919 5615, Fax: (61-3) 9919 5532,
E-mail: Nir.Eynon@vu.edu.au

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Individual physiological and mitochondrial responses during 12 weeks of intensified exercise

Running title: Physiological and molecular adaptations in muscle

Macsue Jacques[1], Shanie Landen[1], Javier Alvarez Romero[1], Xu Yan[1], Andrew Garnham[1], Danielle Hiam[1], Mélina Siegwald[3], Emma Mercier[3], Anne Hecksteden[4], Sarah Voisin[1*], Nir Eynon[1, 2*#]

*Co-senior authors

[1] Institute for Health and Sport (iHeS), Victoria University, Melbourne, Australia.

[2] Murdoch Children's Research Institute, Melbourne, Australia

[3] Rennes Agrocampus Ouest, Rennes, France

[4] Institute of Sports and Preventive Medicine, Saarland University, Saarbrücken, Germany

Corresponding Author:

Associate Professor Nir Eynon

Institute for Health and Sport (iHeS),

Victoria University

PO Box 14428

Melbourne, VIC 8001, Australia.

Tel: (61-3) 9919 5615, Fax: (61-3) 9919 5532,

E-mail: Nir.Eynon@vu.edu.au

Abstract

Aim: Observed effects of exercise are highly variable between individuals, and subject-by-training interaction (i.e. individual response) is often not estimated. Here, we measured mitochondrial (citrate synthetase, cytochrome-c oxidase, succinate dehydrogenase and mitochondrial copy-number), performance markers (W_{peak}, LT and VO_{2peak}), and fibre type proportions/expression (Type I, Type IIa and Type IIx) in multiple time points during 12-week of High-Intensity Interval Training (HIIT) to investigate effects of exercise at the individual level.

Methods: 16 young (Age: 33.1±9.0 years), healthy men (VO_{2peak} 35–60 $mL \cdot min^{-1} \cdot kg^{-1}$ & BMI: 26.4±4.2) from the Gene SMART study completed 12-week of progressive HIIT. Performance markers and muscle biopsies were collected every 4-week. We used mixed-models and bivariate growth-models to quantify individual response and to estimate correlations between variables.

Results: All performance markers exhibited significant (W_{peak} 0.56±0.33 p=0.003, LT 0.37±0.35 p=0.007, VO_{2peak} 3.81±6.13 p=0.02) increases overtime, with subject-by-training interaction being present (CI: W_{peak} 0.09-0.24, LT 0.06-0.18, VO_{2peak} 0.27-2.32). All other measurements did not exhibit significant changes. Fibre type IIa proportions at baseline was significantly associated with all physiological variables ($p<0.05$), and citrate synthetase & cytochrome-c oxidase levels at baseline and overtime (i.e. intercept & slope) presented significant covariance ($p<0.05$). Finally, low-correlations between performance and mitochondrial markers were observed.

Conclusion: We identified a significant subject-by-training interaction for the performance markers. While for all other measures within-subject variability was too large and interindividual differences in training efficacy could not be verified. Changes in measurements in response to exercise were not correlated, and such disconnection should be further investigated by future studies.

Key words: VO_{2peak}, training variability, mitochondria, exercise

Introduction

Exercise training leads to many physiological adaptations, such as increased maximal oxygen uptake (VO_{2max}) as well as molecular adaptations, such as mitochondrial biogenesis (1). The magnitude of these adaptations depends on the duration, intensity, volume, and type of exercise training (2). Although the benefits of exercise are well described, large inter-individual variability in the observed responses to well standardized exercise training is consistently reported (3–7), for all exercise-related phenotypes (5), independently of the intervention duration (7). Furthermore, gross measures of variability in response to exercise interventions, commonly measured by pre-post approaches are not a conclusive representation of individual response (6). Individual response (also known as subject-by-training interaction) relies on the assumption that consistent training changes occur for each individual (6,8–10). However, we and others, have shown that measuring individual response for any given variable is more complex than previously assumed, and exercise studies often fail to robustly measure it (6–8,11–14).

The key to quantifying individual responses is to isolate sources of variation in exercise training responses by first quantifying the magnitude of variation in training response, given that if subject-by-training interaction is low assessing response of individuals is futile; and only then quantifying individual responses (15). In exercise studies, two stances are commonly observed as sources of variation: 1) day-to-day or biological variability (i.e. sleep, nutrition, etc), and 2) statistical variance such as random error. In order to isolate such sources of variation and obtain true effects of exercise training specific study designs and methods (8,11,13) have been proposed. With the reference standard being a replicated cross-over design, and repeated testing measuring gradual adaptations at consecutive timepoints being a relative substitute. To date, one study to our knowledge has implemented the repeated testing design solely for VO_{2max} measurement (11), and no molecular markers have been investigated thus far.

Among the many molecular changes that are led by exercise (i.e. fibre type switch, glucose uptake, etc.), the mitochondria is known to be heavily regulated by exercise training (16–19). The mitochondrion is responsible for energy production to the cells, and mitochondrial deficiency can lead to both physical and psychological disorders (20–23). Exercise studies often rely on isolated mitochondrial markers measures; however, one human cell contains multiple copies of mitochondria and consequently mitochondrial DNA (mtDNA). MtDNA

encodes critical components of the respiratory complexes and is necessary for ATP production. An increase in mtDNA copy number (mtCN) does not necessarily equate with an increase in mitochondrial capacity and could simply be a consequence of compensatory mechanisms (i.e. reduction in mitochondrial quality and elevated mitochondrial content) (24,25). Thus, mitochondrial markers measured in isolation do not provide the full picture of mitochondrial health (26). Combining measures of mitochondrial content and quality is essential to access mitochondrial health. A functional index of mitochondrial health in blood has been recently proposed (Mitochondrial Health Index), by mathematically integrating biochemical enzymatic activities and mtCN into a single score, that may represent an optimized measure of mitochondrial functional capacity (26). This method successfully captured a reduction in mitochondrial health in blood as a result of chronic psychological stress (26). However, this approach has not been explored in skeletal muscle, either in the basal state, or following a chronic physiological stimulus, such as exercise training. Furthermore, variability across mitochondrial measures has been overlooked, and no study to date has thoroughly investigated the efficacy of training on mitochondrial markers or estimated subject-by-training interaction by the mitochondria.

Therefore, our aim was to use a repeated testing approach to estimate individual response for performance and molecular measures and to investigate the relationship between measurements changes overtime as a response to a 12-week HIIT intervention. We hypothesised that measuring multiple physiological and molecular components at regular intervals would allow to account for sources of variability and identification of true individual responses to exercise for performance as well as molecular measures, and by using a bivariate growth model we will also find associations between different markers in response to training.

Results

Individual responses to 12 weeks of HIIT training are evident at the physiological level but not at the molecular level.

The exercise training triggered a positive physiological adaptation in a dose-response (i.e. overtime) manner ($p<0.05$ for all physiological variables, **Table 1, Figure 2 & fixed effects Supplementary Table 1**).

115

116 As we used a repeated testing approach, we were able to estimate within-subject variability
117 between multiple segments during the training period (**Supplementary Table 1** – random
118 effects). We separated trainability from within-subject variability (and random error) that
119 correspond to the error surrounding the segmental changes of the slope. We were able to
120 delineate individual response, meaning each participant responded differently to the
121 intervention, with some participants showing rapid and large increases in fitness, while others
122 showed slower improvements **(Figure 2)**. For example, in **Figure 3** the highest responder
123 presented a VO_{2peak} slope change of +3.34, while the lowest responder presented a slope of -
124 2.29.

125 We did not detect any change in MHI (**Figure 4**) or mitochondrial markers in isolation
126 (**Supplementary Figure 1**) at either the group or the individual level following 12 weeks of
127 HIIT **(Supplementary Table 2).** Of note, mtCN was strongly associated with age in all models
128 ($p<0.005$) (**Supplementary Table 2**), which is in accordance with the literature (40,41).

129 Next, we investigated whether the training led to changes in fibre type distributions and
130 whether those changes were associated with physiological or molecular changes
131 (**Supplementary table 2&3**). First, we tested whether fibre type proportion (as a percentage
132 of number of fibres that were counted) and expression of myosin heavy chains (MHC) were
133 correlated. Fibre type proportion and MHC expression presented small but significant
134 correlation (**Supplementary Figure 2**). We did not detect any shifts in fibre type percentage
135 distributions or MHC expression after 12 weeks of HIIT at either the group or individual
136 level (p-value >0.05) (**Figure 5, Supplementary Table 3**). However, the proportions of
137 types I and IIa, but not IIx, were associated with physiological markers (W_{peak}, LT and
138 VO_{2peak}, $p<0.05$) (**Supplementary Table 3**). Finally, fibre type proportion and expression

were not associated with any of the mitochondrial markers after adjusting p-values ($p>0.05$, data not shown).

Relationship between physiological and mitochondrial variables – bivariate latent growth models

To understand the relationship between changes in molecular variables and changes in physiological variables, we built a bivariate latent growth model. **Table 2** summarises the interaction between each bivariate model. As expected, W_{peak}, LT & VO_{2peak} were correlated at baseline, which means that participants with high W_{peak} at baseline also had high LT and VO_{2peak} at baseline ($p<0.05$). A similar correlation was observed between baseline CS and COX ($p=0.049$). W_{peak} and LT showed similar increases over time across participants ($p=0.05$), but VO_{2peak} did not present any correlations with the other performance measurements. However, no other associations were observed between physiological and mitochondrial measures. Finally, participants with higher baseline CS displayed smaller changes in COX following training ($p=0.042$), and vice versa, participants with higher baseline COX had smaller changes in CS following training ($p=0.026$) **(Table 2).**

Discussion

In the present study, we provided a comprehensive analysis of changes in performance, mitochondrial, and fibre type profiles of 16 young to middle aged men, at three time points throughout a 12-week HIIT intervention. We found that performance measurements improved more consistently than molecular (mitochondrial) measurements during the 12-week HIIT intervention. While there were clear changes in performance at the group level and we were able to establish individual response to exercise, we were unable to do so with the other markers as they were highly variable both within and between participants. Type I and IIa fibres were associated with physiological variables (W_{peak}, LT and VO_{2peak}). In our growth model no

163 significant associations were found between intercepts and slopes withing same parameters,
164 which means that baseline fitness did not influence magnitude of change for any physiological,
165 molecular or fibre type related measurement. Finally, changes at the physiological level were
166 not associated with changes at the molecular level.

167 Repeated measures during exercise interventions (i.e. testing participants at multiple
168 timepoints), constitute a cost-effective approach to estimate individual exercise responses
169 (6,8,11). Using this method, we could detect individual response for W_{peak}, LT and VO_{2peak},
170 and identified individuals who responded better (or poorer) to the training than the group
171 average. This methodology could therefore be the standard for studies aiming at uncovering
172 any exercise related phenotype/measure at the individual level, since it has successfully isolated
173 sources of variability providing a true trainability estimate. However, due to the lack of a
174 control group, separating within-subject variability and random error was not captured (6).

175 We have assessed, for the first time in skeletal muscle, a comprehensive mitochondrial health
176 "score", MHI, originally assessed in blood (26). Although biologically relevant, mitochondrial
177 markers measured in isolation are hard to interpret as compensatory mechanisms may be
178 occurring among variables. Thus, the novel MHI measurement integrating biochemical and
179 molecular mitochondrial measures, aims to obtain higher sensitivity to mitochondrial responses
180 as it accounts for any relationship between variables (26). During the 12-week HIIT
181 intervention, the mitochondrial enzyme maximal activity and therefore the MHI were highly
182 variable, and no consistent changes were observed at either the group or individual level. This
183 was surprising given that mitochondrial content and function are upregulated by exercise (16–
184 18). To ensure that the variance was not due to technical variability, we removed any duplicate
185 results that presented a variance >10%. However, it is known that enzyme activity is highly
186 dynamic and the timeframe in which enzymes are fired may vary both within as well as between
187 subjects (42). We cannot rule out the potential involvement of other enzymes in similar

188 pathways, or enzyme Km and not the maximal activity could be different between people (43),
189 but these hypothesis remains to be tested. Furthermore, due to the nature of skeletal muscle
190 (i.e. multi-nucleated) we could not account for cell number as suggested by Picard *et al*. The
191 multi-nucleated characteristic of skeletal muscle promotes the possibility that each myonuclear
192 differ in transcriptional rates and are independently regulated and distinctive from each other,
193 to the extent that local differences in skeletal muscle (i.e. two pieces of same biopsy) might be
194 present following exercise (44–46), and thus potentially explaining part of the variability
195 observed between and among measures.

196 Adaptive mechanisms that improve muscle function and enhance response to exercise are
197 initiated at the transcriptional level (46,47). However, apart from correlational observations
198 there is no direct evidence linking changes in mRNA expression to training induced adaptations
199 (46,48,49). Here, we investigated whether fibre type gene expression was associated with fibre
200 type composition. The proportions of fibre type have been previously reported to be associated
201 with different modalities of exercise (50–52). For example, a high proportion of type I fibres
202 are beneficial to endurance athletes as they are slow twitch, oxidative and relatively more
203 resistant to fatigue. We found significant associations between the proportions of type I and IIa
204 fibres with physiological markers. Fibre type I was positively associated with LT and VO_{2peak}
205 while fibre type IIa was negatively associated with all physiological measurements (W_{peak}, LT
206 and VO_{2peak}) ($p<0.05$). However, neither fibre type composition nor MHC mRNA expression
207 significantly changed with 12-weeks of HIIT at either the group or individual level. It is
208 recommended that for better accuracy a minimum of 150 fibres to be used for estimation of
209 fibre type proportions (35), and these guidelines were followed whenever possible (i.e. large
210 enough sample) in our study, and the absolutely minimum number of fibres considered was
211 100. However, even though we were careful to reduce variability within the data whenever
212 possible, it is possible that the large noise to ratio observed by these techniques might have

213 hindered any significant changes in our cohort (53). Further, we hypothesise that, the apparent
214 lack of association between composition and expression may be due to the confounding
215 influence of random error (i.e. technical error of measurement and/or biological variability)
216 (46). A recent study investigating repeatability of exercise-induced changes, has shown a large
217 intra-biopsy variation, most like to due to slight changes in the site for sampling, for fibre type
218 distribution as well as gene expression (46), this could potentially explain the poor correlation
219 observed between our results for fibre type composition and expression, and even the
220 mitochondrial enzyme analysis.

221 Growth models allowed testing as to whether an individual who started with higher baseline
222 values had lower improvements after training, without suffering from the statistical artefact of
223 regression to the mean that often plagues exercise training studies (7). To achieve this, slope
224 factors within each system were regressed on the corresponding intercepts to control for any
225 false associations (i.e. regression to the mean) (54). We found that baseline values did not affect
226 the rate of change of any of the physiological or molecular variables ($p>0.05$). Although we
227 have conducted a power analyses for our models and our sample size is sufficient to detect with
228 a confidence of 80% we could not derive the power of covariance between intercepts and slopes
229 and therefore we are unsure on the certainty of this measure. New studies with repeated testing
230 and larger sample sizes should further investigate this hypothesis. We also hypothesised that
231 changes at the physiological level are a consequence of changes at the molecular level, and
232 therefore physiological changes should be associated with changes molecular changes.
233 Surprisingly, only W_{peak} and LT changed similarly over time, meaning that changes in most
234 variables were independent from one another, and improvement in one variable did not
235 necessarily mean improvement in another variable. Finally, we found an interesting
236 relationship between CS and COX. CS and COX levels were correlated at baseline, and
237 baseline CS values were associated with changes in COX and vice versa. CS activity is closely

238 associated with mitochondrial content, while COX activity is strongly associated with
239 mitochondrial oxidative phosphorylation capacity (26,29). CS activity influences the oxidation
240 of substrates in some respirations protocols, and complex IV is part of the mitochondrial
241 substrate oxidation (29), which could potentially explain the relationship observed in our
242 results. COX/CS ratio has been previously reported to be a biochemical marker of
243 mitochondrial dysfunction related to obesity in blood (55). An increase of this ratio (i.e. energy-
244 coupled substrate oxidation) could potentially lead to increase of ATP synthesis which in turn
245 may be channelled toward lipid formation (56). Based on our results exercise might be acting
246 as a regulator of CS/COX ratio, which might represent an important mechanism regulating
247 adipocyte formation and reducing the risk of obesity. This is an important finding, that needs
248 to be further explored and validated.

249 In summary, the repeated testing approach applied here could detect subject-by-training
250 interaction for the performance markers. However, we could only estimate trainability for
251 physiological measures of fitness, while mitochondrial markers were highly variable both
252 between and within participants over time. We also reported a low correlation between
253 physiological and molecular markers of fitness. Further studies utilizing the repeated testing
254 approach in larger cohorts are needed in order to clarify the relationship between molecular
255 and physiological responses to training. Furthermore, future studies should also include a
256 control group for the same length in order to obtain a clear measure of random error, and then
257 use this to normalise the effects of training. Finally, variability between and within variables
258 might due to compensatory molecular mechanisms, and other associations might be occurring
259 such as the one reported here by CS/COX, however further studies in the field are necessary to
260 elucidate such networks.

261

Methods

Participants

A subset of 20 participants from the Gene SMART (Skeletal Muscle Adaptive Responses to Training) study 4-week training intervention (27) were recruited to complete a second intervention of 12 weeks. Sixteen of those 20 participants completed the full 12-weeks of HIIT (3 dropouts and 1 exclusion due to pre-intended criterion (i.e. duplicate tests provided more than 10% difference)). 19 completed 4 weeks (1 dropout), and 18 completed 8 weeks (1 dropout).

Participants were apparently healthy, moderately trained men (VO_{2peak} 35–60 $mL \cdot min^{-1} \cdot kg^{-1}$), aged 18 to 45 years old (**Supplementary table 1**). The study was approved by the Victoria University human ethics committee (HRE13-223) and written informed consent was obtained from each participant. Participants were excluded from the study if they had a past history of definite or possible coronary heart disease, significant chronic or recurrent respiratory condition, significant neuromuscular, major musculoskeletal problems interfering with ability to cycle, uncontrolled endocrine and metabolic disorders or diabetes requiring insulin and other therapies (27).

Study design

Participants were tested at baseline and after 4 weeks, 8 weeks, and 12 weeks of HIIT. To ensure progression, training intensity was re-adjusted every 4 weeks based on the newly determined peak power output (W_{peak}) and lactate threshold (LT) from the graded exhaustive exercise test (GXTs). These tests also allowed for the monitoring of individual participant progress for the longitudinal analysis of training adaptations. To increase accuracy in measurement and to reduce biological day-to-day variability in participants' performance, physiological measures of fitness (W_{peak}, LT, and VO_{2peak}) were assessed from two GXTs, at a maximum of 2 days apart, conducted at each time point **(Figure 1**). Muscle biopsies were taken

48h after performance tests from each cycle. LT was determined using the modified d-max method (28). More details on testing criteria and participants have been described elsewhere (27).

Muscle biopsies

A controlled diet for 48 h prior to the muscle biopsies was provided to the participants, according to the guidelines of the Australian National Health & Medical Research Council (NHMRC). Muscle biopsies were taken using a Bergstrom needle by an experienced medical doctor from the *vastus lateralis* muscle of the participants' following local anaesthesia (2mL, 1% Lidocaine (Lignocaine)). The needle was inserted in the participant leg and manual suction was applied for muscle collection. Care was taken not to contaminate the muscle samples with local anaesthetic during the biopsy. Excess blood, fat and fibrosed tissue was gently removed from biopsy if any. A piece of the muscle was immediately frozen in liquid nitrogen and stored in -80 degrees, and another piece was imbedded in optimum cutting temperature compound (O.C.T.), and then snap frozen and stored in -80 for immunology analyses. Muscle biopsies were collected at four timepoints (pre, 4W, 8W and 12W) for comprehensive analyses of mitochondrial markers, including: citrate synthase, succinate dehydrogenase, mitochondrial copy number, cytochrome oxidase and fibre type composition (**Figure 1**). Due to dropouts previously described, all statistical analyses are based on 16 participants.

Molecular analyses and immunohistochemistry

Succinate Dehydrogenase Activity (Complex II activity)

We utilised the succinate dehydrogenase (SDH) activity assay kit (Colorimetric) (#ab228560) to measure maximal enzyme activity. Muscle was lysed according to the kit's protocol and 15ul of muscle lysate was used in each reaction (well). Assay was performed in duplicates. The assay kit is able to detect less than 0.1mU SDH activity in muscle samples. Protocol was

311 followed according to kit user guide. SDH activities (mU/mg of tissue) were averaged and if
312 CV >10% for the duplicate results then values were removed.

313 *Cytochrome C Oxidase Activity (Complex IV activity)*

314 We utilised the assay kit (#ab239711) to measure maximal cytochrome C oxidase activity
315 (COX). Muscle was lysed using the SDH buffer from the kit described above and 10ul of lysate
316 was used for each reaction (well). The activity of the enzyme was determined colorimetrically
317 in triplicates according to the kit user guide. COX results were averaged and if CV >10% for
318 the triplicate then divergent results were removed. COX results are presented as mol/h/kg of
319 protein. Protein was quantified using the bicinchoninic acid assay (BCA).

320 *Citrate Synthase Activity*

321 The most commonly used measurement of mitochondrial content is the maximal citrate
322 synthase (CS) enzyme activity (14,29). Small pieces of tissue were lysed in an ice-cold buffer
323 (KH_2PO_4 & K_2HPO_4) using a TissueLyser II (Qiagen, Hilden, Germany). Protein concentration
324 was assessed using the BCA. Total CS activity (mol/h/kg of protein) was measured in
325 triplicates (30°C, pH 7.5) using standard spectrophotometric assays. Values that presented a
326 CV > 10% between triplicated samples were removed.

327 *Mitochondrial DNA Copy Number*

328 Mitochondrial DNA copy number also reflects the content of mtDNA, and it is usually
329 associated with mitochondrial gene stability and mitochondrial biogenesis (26). Mitochondrial
330 copy number (mtCN) was determined in quadruplicates using multiplex qPCR. This method
331 allows for simultaneous amplification of a mitochondrial (ND1) and a nuclear (RNAseP)
332 amplicon to verify their relative abundance (26,30). The sequences for the ND1 amplicon (IDT)
333 are as follows:

Forward primer (300nM), 5'CCCTAAAACCCGCCACATCT3'; Reverse primer (300nM): 5'GAGCGATGGTGAGAGCTAAGGT3'; and Probe (100nM): 5'FAMCCATCACCCTCTACATCACCGCCC-TAMRA3'. We utilised the RNAseP assay kit (Thermofisher Scientific #4403328). Taqman Universal Mastermix (Thermofisher #4304437) was used and the assay ran on a QuantStudio™ 7 Flex Real-Time PCR System. The average CV for mtCN Cts was 1.02%. Data was manually curated and in cases in which samples yielded a standard deviation > 0.3, the divergent sample was removed.

Mitochondrial Health Index (MHI)

We calculated MHI as was previously reported in blood (26), using the following equation:

$$MHI = \left[\frac{Energy\ production\ capacity}{Mitochondrial\ content}\right]$$

$$= \left[\frac{Compex\ II\ (SDH) + Complex\ IV\ (COX)}{CS + mtDNAcn}\right] * 100$$

Fibre Typing (immunohistochemistry), and Expression (RT-PCR)

Exercise is known to affect fibre type composition, area and expression (31,32). Shifts in fibre type composition might not be achieved so easily in human interventions, however changes in myosin heavy chain expression patterns might occur even after short interventions (33). Thus, in our study we have measured both parameters to see whether trainability is observed in either measurement.

Immunofluorescence analyses of muscle fibre types were performed on muscle tissue sections imbedded in optimum cutting temperature compound (O.C.T.). Primary and secondary antibodies information have been previously described (34). Briefly, muscle tissue was sectioned at ~8 µm, and sections were then incubated for blocking with 10% goat serum for one hour. Primary antibodies in 10% goat serum (ThermoFisher #50062Z) (1:25) were used to

incubate slides overnight in the dark at 4°C. Primary antibody was then removed, and slides washed 3x5min in ddH2O. Secondary antibodies in 10% goat serum (1:500) were then used to incubate slides for two hours in the dark. Slides were washed 3x5 min in ddH2O and then stained with wheat germ agglutin (WGA) for 10 min in the dark (10ug/ml). Slides were then washed once in ddH2O and mounted with PBS for imaging. Fibre type distribution was quantified using Fiji software and values are presented in percentage distribution. We have attempted where possible to keep our fibre counts above 150 as previously suggested (35), and a minimum of 100 fibres was considered the lowest threshold for analyses.

RT-PCR for MHCs was performed on muscle tissue that was flash frozen in liquid nitrogen. RNA was extracted using the AllPrep DNA/RNA FFPE Kit (#80234 Qiagen). 10 ng of RNA was then diluted into 50ul and reverse transcription was conducted using the iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad) with a thermomixer. Primers for myosin heavy chain I, IIa and IIx used for this experiment have been described elsewhere (33). RT-PCR was conducted using the QuantumStudio-7 (Bio-Rad). MRNA expression levels were quantified by real-time PCT using SYBR green fluorescence. Cycle threshold (Ct) values were normalized to a housekeeping gene, Cycl1. Samples were analysed in triplicates and data was manually curated. In cases where samples yielded a standard deviation > 0.4, the divergent sample was removed.

Statistical Analyses

Responses to training at the group level and individual level (subject-by-training interaction)

We utilised a linear mixed model (using the lmer package in R (36)) of the form:

$$\text{Outcome} = \text{timepoint} + \text{random intercept (ID)} + \text{random slope (ID x Timepoint)}$$

where outcome was either physiological measures of fitness (W_{peak} or LT or VO_{2peak}) or a mitochondrial marker (CS activity, mtCN, SDH, COX) or a fibre type (Type I, Type IIa or Type IIx), timepoint was a numeric variable (0, 4, 8 or 12 weeks). The fixed effect for "timepoint" estimates whether there were changes in outcome at the group level over time (i.e. mean slope). The random intercept accounts for baseline differences in outcome between individuals, and the random slope assesses whether there are significant differences in how individuals change in outcome with time (subject-by-training interaction, or trainability). The individual segmental changes between the different timepoints estimates within-subject variability.

Next, we used bivariate latent growth curve models (using the package Lavaan in R (37)) to test whether changes in physiological measurements were correlated with changes in molecular markers (i.e. correlation between slopes). A complete explanation of this method can be found in the supplementary files. Due to limited muscle there were missing data points therefore multiple imputations using the *mice* package was used to impute these missing values (38), and results were pooled from all imputed iterations for both mixed models as well as parallel growth models with *miceadds* package (39). All analyses were performed using the R software version 4.0.2.

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Conflict of Interest

The authors have no conflict of interests to declare.

403 Authors Contributions

404 The contribution from each author were as follows: MJ, NE, XY - conception or design of the
405 work;

406 MJ, SL, JA, MS, EM, SV, AG - acquisition, analysis or interpretation of data for the work; MJ,
407 SL, JA, XY, AG, DH, MS, EM, AH, SV, NE - drafting the work or revising it critically for
408 important intellectual content. We confirm that all authors: approved the final version of the
409 manuscript, agreed to be accountable for all aspects of the work in ensuring that questions
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411 resolved, and all persons designated as authors qualify for authorship, and all those who qualify
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	Longitudinal Intervention (12 weeks)						
	Pre	4WP	8WP	12WP	Δ(4WP-Pre	Δ(8WP-Pre	Δ(12WP-Pre
N	20	19	18	16	19	18	16
Age (years)	33.1±9.0	-	-	-	-	-	-
BMI (kg.m-2)	26.4±4.2	-	-	26.4±3.8	-	-	-
Wpeak (W/kg)	3.48±0.97	3.76±0.96	3.88±0.95	4.06±0.94	0.27±0.16	0.40±0.24	0.56±0.33
LT (W/kg)	2.38±0.74	2.63±0.79	2.71±0.76	2.76±0.70	0.27±0.22	0.33±0.32	0.37±0.35
VO2max (mL.min-1.kg-1)	51.0±10.6	53.1±10.3	54.5±11.1	55.3±10.7	1.76±3.04	3.36±5.83	3.81±6.13

584 **Table 1:** Group characteristics with Delta Changes. Values are presented as mean ± SD.

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586

	Covariates								
	Intercept & Intercept			Slope & Slope			Intercept & Slope		
Variable	Estimate	Std. Error	P-Value	Estimate	Std. Error	P-Value	Estimate	Std. Error	P-Value
W_{peak} & LT	**0.57**	**0.21**	**0.006**	**0.01**	**0.006**	**0.05**	-0.009	0.04	0.83
W_{peak} & VO_{2peak}	**7.45**	**2.72**	**0.006**	0.06	0.06	0.34	-0.11	0.54	0.84
W_{peak} & CS	0.92	0.73	0.21	0.002	0.02	0.08	-0.16	0.22	0.48
W_{peak} & COX	0.45	0.36	0.22	-0.02	0.05	0.76	0.14	0.41	0.78
W_{peak} & SDH	1.96	2.22	0.38	0.13	0.14	0.34	76.87	43.99	0.08

W_{peak} & mtCN	0.18	0.41	0.67	-0.002	0.02	0.89	0.02	0.17	0.93
W_{peak} & MHI	0.42	0.29	0.16	0.03	0.04	0.48	-0.006	0.03	0.85
LT & VO_{2peak}	**6.03**	**2.26**	**0.008**	0.12	0.14	0.39	-0.25	0.49	0.62
LT & CS	0.75	0.61	0.22	0.04	0.05	0.41	-0.15	0.19	0.42
LT & COX	0.54	0.32	0.09	0.02	0.09	0.83	-0.004	0.34	0.99
LT & SDH	1.99	1.86	0.28	0.06	0.26	0.83	-0.59	0.94	-0.08
LT & mtCN	0.32	0.99	0.32	0.05	-0.79	0.44	0.19	-0.82	0.42
LT & MHI	0.23	1.16	0.25	0.008	-0.16	0.87	0.04	0.13	0.89
VO_{2peak} & CS	3.78	8.11	0.64	0.88	0.76	0.25	-1.48	2.58	0.57
VO_{2peak} & COX	3.35	3.83	0.38	-1.59	1.40	0.26	3.69	4.57	0.42
VO_{2peak} & SDH	13.69	22.65	0.55	1.02	1.51	0.49	-7.74	12.25	0.53
VO_{2peak} & mtCN	1.17	4.78	0.81	0.25	0.68	0.72	-2.10	2.95	0.49
VO_{2peak} & MHI	2.65	2.93	0.37	-0.07	0.22	0.77	0.29	0.79	0.72
CS & COX	**2.87**	**1.46**	**0.05**	0.91	0.79	0.25	**-3.58**	**1.76**	**0.04**
CS & SDH	6.97	8.45	0.41	0.88	1.45	0.54	76.87	48.17	0.11

CS & mtCN	0.49	1.44	0.73	0.08	0.14	0.58	-0.49	0.46	0.29
COX & SDH	6.79	4.47	0.13	-1.03	2.24	0.65	-0.53	4.72	0.91
COX & mtCN	0.09	0.06	0.13	-0.13	0.09	0.19	-0.09	0.07	0.19
SDH & mtCN	-0.09	0.37	0.80	-0.006	0.07	0.93	0.29	0.22	0.19

Table 2: Bivariate growth models: The Intercept & Intercept informs if variables have similar baseline values, Slope & Slope informs if variables have similar rate of changes, and Intercept & Slope informs if baseline values of one variable affects the rate of change of the other variable.

Figure 1: Individual changes (i.e. subject-by-training interaction) in physiological measures (W_{peak}, LT, and VO_{2peak}) after 4, 8 and 12 weeks of HIIT. Each participant is represented by a different colour. Red squares represent mean values for each variable in each timepoint.

Figure 2: Example of subject by training interaction and comparison between whole body and molecular markers. The box plots represent individual confidence intervals for changes in VO_{2peak}. Confidence interval: response estimate ± 1.96 x SD of segmental changes.

Figure 3: Individual changes in mitochondrial health index (MHI) for each 4-week segment up to 12 weeks. Each participant is represented by an individual colour.

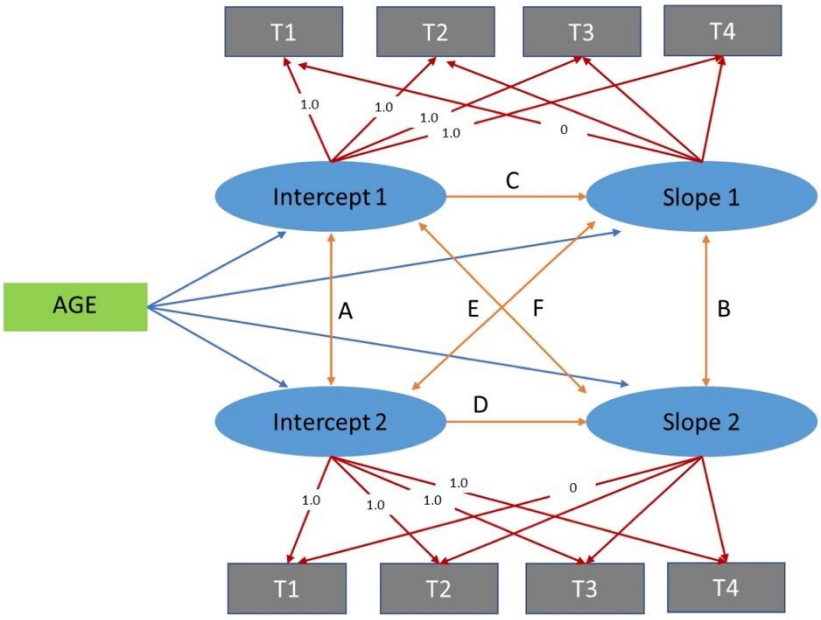
Figure 4: Distribution of fibre type separated by timepoint. Although minor changes are observed in the distribution overtime, those were not significant for either fibre type proportion or expression following 4, 8 and 12 weeks of HIIT. Cycle threshold (Ct).

Figure 5: Study design. Tests and muscle biopsies were interspaced by 48 hours.

Supplementary Files

Individual and Parallel Growth models. Raw means were graphed in order to verify trajectory of each variable. We estimated single and parallel Latent Growth Models (LGM) in R using the lavaan package (27). Before conduction the parallel LGMs we performed analyses in the individual variables to establish best model fit. Linear, quadratic, and piecewise models were estimated as appropriate for each variable to ensure best fit (CFI >0.95, SRMR <0.08 and chi-

square p-value >0.05). A careful evaluation of the resulting parameter estimates indicates that all variances were positive and resulting parameter estimates were plausible (e.g., no Heywood cases). Once best fitting single models were estimated we progressed to conduct the parallel analyses. A figure bellow provides a conceptual diagram of the estimated models with the tracks branded as reference. The loadings for the timepoints measurements on the slope factor were fixed to 0.0 for the pre timepoint, thereby setting the start of the study as the intercept. Of most interest in this study is the covariance between the slope factors (i.e., growth rates), indicating the degree to which change in physiological and molecular markers are associated with each other (Track B in Figure 2). The estimated relationship between intercepts (Track A) captures the cross-sectional association between markers at the start of the study. The estimated growth models included a series of important covariates. First, slope factors within each system were regressed on the corresponding intercepts in order to control for any spurious association in the relationship between the growth factors (e.g., regression to the mean; Track C and D). Once this was verified slopes and intercepts were allowed to covary in order to investigate if baseline measures (intercept) affected the rate of change of participants (slope). When appropriate (i.e. better fit for the model) age was included as a covariate of the trajectories of change by regressing the latent growth factors on each. Age of entry was included to account for potentially important individual heterogeneity in development at the outset of the study because standard LGMs treat all individuals as starting at the same time. Finally, we tested whether initial status on markers had an effect on the rate of change in the other after controlling for the above-mentioned covariates (Track E and F). Individual models were required because attempts to estimate a model with growth in all variables simultaneously led to problems with specification (i.e., negative residual variances), which is not uncommon in very large structural models (28).



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638 Conceptual diagram of parallel latent growth model.

639 Circles represent the latent variables; squares represent the manifest variables measured at each
640 of the five timepoints. T1-T4: Timepoints Pre, 4WP, 8WP and 12WP; Single headed arrows
641 denote regression paths, double headed arrows denote covariances; Track A: Covariance
642 between Intercept 1 and 2; Track B: Covariance between Slope 1 and 2; Track C: Slope 1
643 regressed on Intercept 1; Track D: Slope 2 regressed on Intercept 2; Track E: Covariance
644 between Slope 2 and Intercept 1; Track F: Covariance between Slope 1 and Intercept 2.

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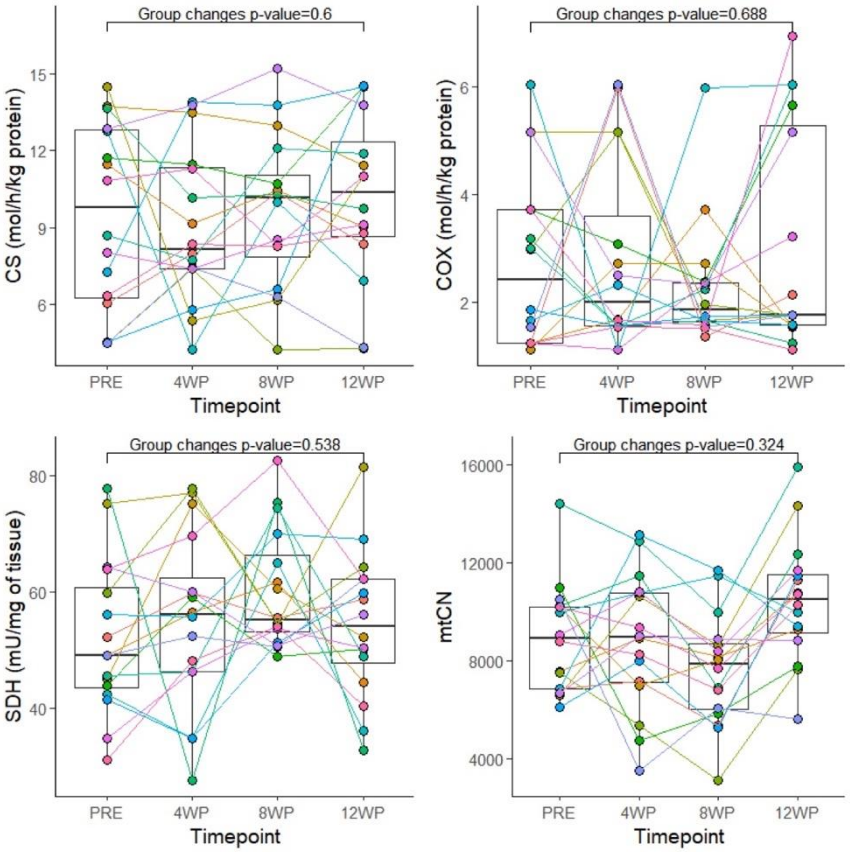

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648 **Supplementary Figure 1:** Individual changes in Citrate Synthase (CS), the Cytochrome-C Oxidase(COX),
649 Succinate Dehydrogenase (SDH), Mitochondrial Copy Number (mtCN), and Mitochondrial Health Index (MHI)
650 for each 4 weeks up to 12 weeks.

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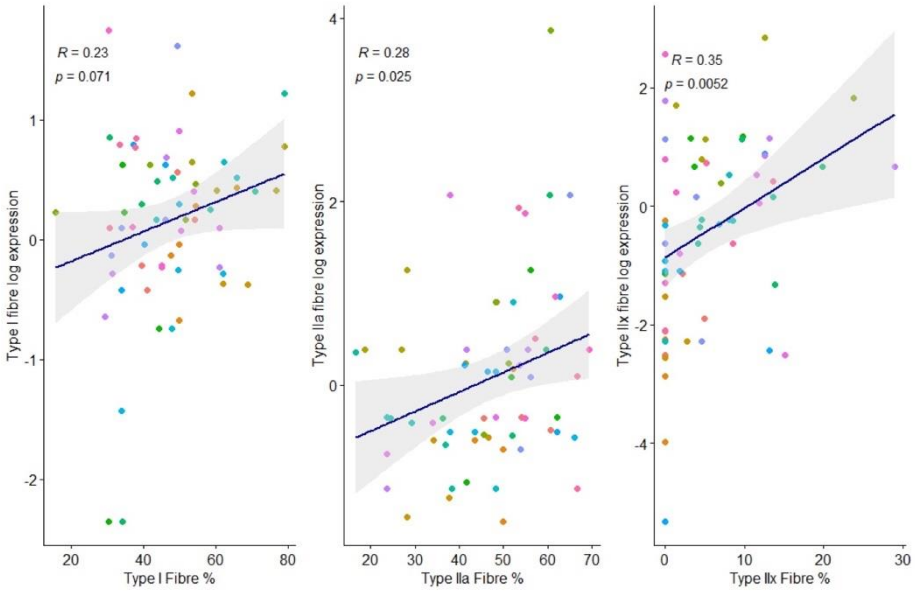

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653 **Supplementary Figure 2**: Fibre type % and expression correlations

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	Fixed Effects - Group Level				Random Effects - Individual Level				Confidence Intervals
	Variable	Estimate	Standard error	p-value	Variable	Variance	Standard error	p-value	(2.5% - 97.5%)

W_{peak} (W/kg)	Time point	0.16	0.04	**0.0003**	ID x Timepoint	0.02	0.14	**0.0005**	0.09 – 0.24
					Residual	0.14	0.12		
LT (W/kg)	Time point	0.12	0.03	**0.0007**	ID x Timepoint	0.009	0.09	**0.05**	0.06 - 0.18
					Residual	0.027	0.16		
VO_{2max} (mL/min/kg)	Time point	1.39	0.51	**0.0148**	ID x Timepoint	3.81	1.95	**0.0002**	0.27 - 2.32
					Residual	4.54	2.13		

Supplementary Table 1: Results from linear mixed model for physiological variables: Variable ~ Timepoint + random intercept (ID) + random slope (ID x Timepoint). Where Variable = Measurement value, Timepoint = PRE – 4WP – 8WP – 12WP. Fixed effects represent changes at the group level, while random effects represent changes at the individual level (trainability)

	Fixed Effects - Group Level				Random Effects - Individual Level			
	Variable	Estimate	Standard error	p-value	Variable	Variance	Standard error	p-value
CS	Timepoint	0.32	0.27	0.24	ID x Timepoint	0.52	0.45	0.25
	Age	0.03	0.07	0.66	Residual	1.81	0.28	
COX	Timepoint	0.09	0.19	0.65	ID	0.42	8.32	0.96

	Age	-0.002	0.027	0.93	Residual	1.40	0.19	
SDH	Timepoint	1.62	1.42	0.25	ID	4.7	2.34	0.06
	Age	-0.25	0.22	0.25	Residual	11.54	1.23	
mtCN	Timepoint	311.24	263.18	0.24	ID	775.16	535.21	0.15
	Age	130.09	41.25	**0.002**	Residual	2232.52	248.25	
MHI	Timepoint	21.19	35.67	0.56	ID x Timepoint	6671	81.68	0.096
	Age	4.11	4.65	0.88	Residual	68412	261.56	

Supplementary Table 2: Results from linear mixed model for mitochondrial measures. Linear mixed model: Variable ~ Timepoint + random intercept (ID) + random slope (ID x Timepoint). Where Variable = Measurement value, Timepoint = PRE – 4WP – 8WP – 12WP. Fixed effects represent changes at the group level, while random effects represent changes at the individual level (trainability)

	Fixed Effects - Group Level				Random Effects - Individual Level			
	Variable	Regression coefficient	Standard error	p-value	Variable	Variance	Standard error	p-value
Fibre Type I %	Time point	-0.97	1.27	0.45	ID x Timepoint	2.67	1.64	0.79
	W_{peak}	4.67	2.41	0.07	Residual	94.83	9.74	

	Time point	-0.88	1.19	0.47	ID x Timepoint	0.91	0.95	0.83
	LT	6.39	2.77	**0.03**	Residual	96.38	9.82	
	Time point	-0.85	0.21	0.46	ID x Timepoint	0.26	0.51	0.94
	VO_{2peak}	0.58	0.21	**0.01**	Residual	93.32	9.66	
Fibre Type IIa %	Time point	1.86	1.36	0.18	ID x Timepoint	0.84	0.92	0.79
	W_{peak}	-6.03	1.91	**0.005**	Residual	127.62	11.29	
	Time point	1.86	1.17	0.12	ID x Timepoint	0.39	0.62	0.91
	LT	-5.66	2.71	**0.05**	Residual	94.51	9.72	
	Time point	1.81	1.14	0.12	ID x Timepoint	0.29	0.54	0.93
	VO_{2peak}	-0.5	0.19	**0.02**	Residual	94.02	9.69	

Fibre Type IIx %	Time point	-0.98	4.46	0.196	ID x Timepoint	1.01	0.91	0.27
	W_{peak}	0.43	1.22	0.722	Residual	5.86	0.70	
	Time point	-0.94	0.78	0.23	ID x Timepoint	0.98	0.92	0.29
	LT	-0.31	1.45	0.83	Residual	5.87	0.71	
	Time point	-0.89	0.78	0.25	ID x Timepoint	0.97	0.89	0.27
	VO_{2peak}	-0.07	0.11	0.55	Residual	5.83	0.70	
Fibre Type I (log expression)	Time point	-0.005	0.09	0.95	ID x Timepoint	0.004	0.06	0.95
	W_{peak}	-0.005	0.11	0.96	Residual	0.53	0.73	
	Time point	-0.007	0.08	0.93	ID x Timepoint	0.003	0.06	0.96
	LT	0.003	0.14	0.98	Residual	0.53	0.73	

	Time point	-0.01	0.08	0.87	ID x Timepoint	0.001	0.04	0.98
	VO_{2peak}	0.005	0.01	0.61	Residual	0.53	0.73	
Fibre Type IIa (log expression)	Time point	0.12	0.11	0.32	ID x Timepoint	0.02	0.15	0.89
	W_{peak}	-0.18	0.14	0.21	Residual	0.88	0.94	
	Time point	0.12	0.11	0.31	ID x Timepoint	0.02	0.14	0.88
	LT	-0.29	0.17	0.11	Residual	0.87	0.93	
	Time point	0.10	0.11	0.36	ID x Timepoint	0.01	0.11	0.97
	VO_{2peak}	-0.02	0.01	0.25	Residual	0.91	0.95	
Fibre Type IIx (log expression)	Time point	-0.05	0.16	0.76	ID x Timepoint	0.03	0.18	0.57
	W_{peak}	0.23	0.03	0.46	Residual	1.55	1.24	

	Time point	-0.02	0.15	0.90	ID x Timepoint	0.03	0.16	0.65
	LT	0.09	0.36	0.79	Residual	1.57	1.25	
	Time point	-0.02	0.15	0.87	ID x Timepoint	0.03	0.17	0.62
	VO_{2peak}	0.01	0.03	0.63	Residual	1.56	1.25	

Supplementary Table 3: Results from linear mixed model for fibre type measures. Linear mixed model: Variable ~ Timepoint + random intercept (ID) + random slope (ID x Timepoint). Where Variable = Measurement value, Timepoint = PRE – 4WP – 8WP – 12WP. Fixed effects represent changes at the group level, while random effects represent changes at the individual level (trainability)

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RESEARCH ARTICLE

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Mitochondrial respiration variability and simulations in human skeletal muscle: The Gene SMART study

Macsue Jacques[1] | Jujiao Kuang[1] | David J. Bishop[1] | Xu Yan[1] | Javier Alvarez-Romero[1] | Fiona Munson[1] | Andrew Garnham[1] | Ioannis Papadimitriou[1] | Sarah Voisin[1] | Nir Eynon[1,2]

[1]Institute for Health and Sport (iHeS), Victoria University, Melbourne, Victoria, Australia

[2]Murdoch Children's Research Institute, Melbourne, Victoria, Australia

Correspondence
Nir Eynon, Institute for Health and Sport (iHeS), Victoria University, PO Box 14428, Melbourne, VIC 8001, Australia.
Email: Nir.Eynon@vu.edu.au

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Nir Eynon, Grant/Award Number: NHMRC CDF#APP1140644; Sarah Voisin, Grant/Award Number: NHMRC CDF#APP115777321

Abstract

Mitochondrial respiration using the oxygraph-2k respirometer (Oroboros) is widely used to estimate mitochondrial capacity in human skeletal muscle. Here, we measured mitochondrial respiration variability, in a relatively large sample, and for the first time, using statistical simulations, we provide the sample size required to detect meaningful respiration changes following lifestyle intervention. Muscle biopsies were taken from healthy, young men from the Gene SMART cohort, at multiple time points. We utilized samples for each measurement with two technical repeats using two respirometer chambers (n = 160 pairs of same muscle after removal of low-quality samples). We measured the Technical Error of measurement (TE_M) and the coefficient of variation (CV) for each mitochondrial complex. There was a high correlation between measurements from the two chambers ($R > 0.7$ $P < .001$) for all complexes, but the TE_M was large (7.9-27 pmol s^{-1} mg^{-1}; complex dependent), and the CV was >15% for all complexes. We performed statistical simulations of a range of effect sizes at 80% power and found that 75 participants (with duplicate measurements) are required to detect a 6% change in mitochondrial respiration after an intervention, while for interventions with 11% effect size, ~24 participants are sufficient. The high variability in respiration suggests that the typical sample sizes in exercise studies may not be sufficient to capture exercise-induced changes.

KEYWORDS

exercise, intervention, mitochondria, OXPHOS, oxygen consumption

Abbreviations: ATP, adenosine triphosphate; CI, complex I, electron input through CI; CV, coefficient of variation; CI_P, oxidative phosphorylation (OXPHOS) capacity (P) through CI; ETS, electron transport system; CI+CII, convergent electron input through CI and CII CI+CII_E, capacity (E) through CI+CII; CI+CII_P, complex II (CII) linked respiration; E, ETS capacity; ETS, measurement of electron transport system; FCCP, p-trifluoromethoxyphenylhydrazone; Gene SMART, genes and skeletal muscle adaptive response to training; Inv-RCR, inverse of respiratory control ratio (CIL/CI+II_P); L, leak respiration; LCR, leak control ratio (CIL/CI+II_E); OXPHOS, oxidative phosphorylation; P, oxphos capacity; PCR, phosphorylation control ratio (CI+II_P/CI+II_E); ROX, residual oxygen consumption; SCR, substrate control ratio at constant P (CIP/CI+II_P); TCA, tricarboxylic acid; TE_M, technical error of measurement.

Sarah Voisin and Nir Eynon are Co-senior authorship.

1 | INTRODUCTION

The mitochondrion is a membrane-enclosed organelle found in eukaryotic cells. With its five specialized complexes, it produces adenosine triphosphate (ATP) and thus constitutes the powerhouse of the cell.[1] ATP is produced during oxidative phosphorylation (OXPHOS) via the tricarboxylic acid (TCA) cycle inside the mitochondrial matrix, and via the electron transport system (ETS) along the inner mitochondrial membrane.[2] Mitochondrial respiration measured by maximal oxygen consumption in skeletal muscle fibers, is currently the primary way of assessing mitochondrial capacity.[3,4] The most common method in human skeletal muscle uses fibers permeabilized by saponin.[5,6] Using a combination of different substrates, this technique is able to mimic the processes (ie, TCA cycle and ETS) occurring within the mitochondrion.

Regular exercise has a beneficial effect on mitochondrial function.[7] Endurance exercise training improves mitochondrial respiration,[8-13] with high-intensity exercise training leading to larger improvements (up to 40%)[8,10-12] than moderate continuous exercise training (up to 20%).[14,15] However, changes in mitochondrial respiration using permeabilized muscle fibers following exercise training have been assessed in relatively small sample sizes, typically within a range of n = 8-15,[16] and without assessing respiration changes in a control group.[17] Mitochondrial respiration values, as well as improvements in mitochondrial respiration following exercise training are also quite variable between studies.[3,10,14,16,18,19] For example, Irving et al[18] observed a ~1.5-fold change in mitochondrial respiration after moderate intensity endurance training (n = 34), while Robach et al[20] did not observe any change after a similar intervention (n = 17). Mitochondrial respiration capacity in human exercise intervention studies is not exclusive to skeletal muscle samples. Although such investigations are less common, different studies have measured respiration capacity in adipose tissue,[21,22] and blood cells (including lymphocytes and platelet).[23-26]

Tests run in duplicates (or more) enable researchers to capture technical variability, and/or biological day-to-day variability within participants.[17] The typical error of measurement (TE_M), also called "within-subject standard deviation," provides an estimate of such variability.[17] In the only study to date investigating the reliability of mitochondrial respiration measurement,[3] the TE_M between two fiber bundles from the same biopsy was 10.5 pmol s^{-1} mg^{-1} in the maximal oxidative phosphorylation (OXPHOS) and the coefficient of variations (CVs) were worryingly high (15.2% between two fiber bundles, 23.9% between legs, and 33.1% at different time points).[3] More studies are required to confirm whether such variability is consistently high, but, more importantly, this technical variability needs to be put into perspective with typical mitochondrial respiration changes following interventions (eg, exercise training interventions). The qualifiers "high" and "low" variability only make sense when compared with the magnitude of the intervention-induced changes, which will determine how likely we are to detect those changes.

Therefore, the aim of the present study was to investigate the reliability of mitochondrial respiration measurements in human *vastus lateralis* muscle using large number of duplicate fiber bundles (160 pairs) collected from a range of participants at different time points. We correlated mitochondrial respiration values between two chambers containing bundles of same muscle sample for complexes CI_P, $CI + CII_P$ and $CI + CII_E$, and calculated the TE_M and the CV between experiments. Using the estimated TE_M and CV, we performed simulations to determine the minimum number of participants required to detect meaningful mitochondrial respiration changes of various effect sizes following a hypothetical intervention, at 80% power.

2 | MATERIALS AND METHODS

2.1 | Participants

Participants were from the Genes and Skeletal Muscle Adaptive Response to Training (Gene SMART) cohort. The full study methodology has been previously described elsewhere.[27] 68 apparently healthy, Caucasian men (age = 31.4 ± 8.2 years old; BMI = 25.2 ± 3.2 kg/m^2) participated in the study and signed a written informed consent. Participants were excluded if they had any preexisting heart condition, health issues, and/or preexisting injury that could potentially impair exercise capacity. The study was approved by the Human Ethics Research Committee at Victoria University (HRE13-223).

2.2 | Muscle biopsies

A controlled diet for 48 hours prior to the muscle biopsies was provided to the participants, according to the guidelines of the Australian National Health & Medical Research Council (NHMRC). Muscle biopsies were taken by an experienced medical doctor from the *vastus lateralis* muscle of the participants' dominant leg, following local anesthesia (2 mL, 1% Lidocaine (Lignocaine)). The needle was inserted in the participant leg and manual suction was applied for muscle collection. Care was taken not to contaminate the muscle samples with local anesthetic during the biopsy. Approximately 2-6 mg of muscle was then immediately placed in ice-cold BIOPS for determination of mitochondrial respiration in two individual chambers (duplicates).

2.3 | Mitochondrial respiration

Immediately after each biopsy (within max 30 minutes of collection), 2-6 mg of muscle fibers were mechanically separated using pointed forceps under a binocular microscope in 2-mL ice-cold biopsy preservation solution on ice (BIOPS, 2.77 mM CaK_2EGTA, 7.23 mM K_2EGTA, 5.77 mM Na_2ATP, 6.56 mM $MgCl_2 \bullet 6H_2O$, 20 mM Taurine, 15 mM Na_2Phosphocreatine, 20 mM Imidazole, 0.5 mM Dithiothreitol, and 50 mM K^+-MES at pH 7.1).[6] Permeabilization of the plasma membrane occurred in the same solution with 50 µg/ml of saponin (Sigma-Aldrich, St Louis, USA) for 30 minutes rotating on ice. This was followed by rinsing the muscle fibers for 3 × 7 minutes in mitochondrial respiration medium (MiR05, 0.5 mM EGTA, 3 mM $MgCl_2 \bullet 6H_2O$, 60 mM K-lactobionate, 20 mM Taurine, 10 mM KH_2PO_4, 20 mM Hepes, 110 mM sucrose, and 1 g/L bovine serum albumin at pH 7.1)[6] on ice. Mitochondrial respiration was measured in duplicates in washed muscle fibers (between 1 and 3 mg wet weight of muscle fibers/chamber) in MiR05 at 37°C using the high-resolution Oxygraph-2k (Oroboros, Innsbruck,Austria), with additional substrates. Oxygen concentration (mM) and flux (pmol × s^{-1} × mg^{-1}) were recorded using DatLab software. Reoxygenation by direct syringe injection of O_2 was necessary to maintain O_2 levels between 275 and 450 mM and to avoid potential oxygen diffusion limitation. A substrate-uncoupler-inhibition tritation sequence was used. The following substrates were added (final concentration): malate (2 mM) and pyruvate (5 mM) were added to measure the LEAK respiration (L) through Complex I (CI) (CI_L), followed by $MgCl_2$ (3mM) and ADP (5 mM) to measure oxidative phosphorylation (OXPHOS) capacity (P) through CI (CI_P), followed by succinate (10 mM) to measure P through CI + Complex II (CII) linked respiration ($CI + CII_P$).[28] This respiration state represents the maximal respiratory capacity in the respirometer chamber.[28] Cytochrome *c* (10 µM) was used to test the integrity of the outer mitochondrial membrane, in this step if the respiration increased >10% when cytochrome *c* was added, values from that chamber were removed due to a damaged membrane. A series of steps (steps of 0.5 µM) p-trifluoromethoxyphenylhydrazone (FCCP) titrations followed for measurement of electron transport system (ETS) capacity (E) through CI + CII ($CI + CII_E$). Antimycin (3.75 µM) was added to block the activity of complex III and to measure the residual oxygen consumption (ROX) indicative of non-mitochondrial oxygen consumption. Substrate and coupling control ratios were calculated from the different titration steps obtained from the protocol used.[7] A background calibration for the Oroboros machine was performed every 3 months, and air calibrations were performed before each experiment. The results were pasted into the excel spreadsheet supplied by the manufacturer (Oroboros). If air calibrations presented more than 5% deviation in the results, membranes were changed, and new background calibration was done. Instrument backgrounds were performed in MiR05, and oxygen levels were at 450 nmol/mL. Highly variable graphs are indicative of poor quality, as shown on the O2K software, were removed.

2.4 | Citrate synthase activity

Intrinsic changes in the mitochondria can be determined by quantitative measurements of specific markers. Such measurements can estimate the content of the mitochondria and are commonly used to normalize global measurements of mitochondrial function. The most commonly used measurement is the citrate synthase (CS) enzyme activity.[29] Thus, we have normalized our results by CS activity.

Complete enzyme extractions, from small pieces of frozen tissues, were performed in an ice-cold buffer (KH_2PO_4 & K_2HPO_4) using a TissueLyser II (Qiagen, Hilden, Germany). Protein concentration was assessed using the bicinchoninic acid assay. Total citrate synthase (CS) activity was measured (30°C, pH 7.5) using standard spectrophotometric assays. CS activity is presented in international units (IU).

2.5 | Statistical analyses

We calculated three different metrics to show the reliability of mitochondrial respiration measurements. First, we calculated the correlation between duplicates from the two chambers, for each complex, using non-parametric Spearman's test to downweight the influence of outliers, and a stringent P value < .005 for significance. Then, we calculated the within-subject standard deviation, also called typical (or technical) error of measurement (TE_M)[17]:

$$TE_M = \frac{\sqrt{\sum_1^n (x_{i1} - x_{i2})^2}}{2n},$$

where n is the number of pairs of duplicates and x is the respiration measurement.

We calculated the coefficient of variability (CV) estimated by:

$CV = 100 \times \frac{TE_M}{\mu}$, where µ is the mean respiration across all duplicates and all samples. While TE_M is expressed in the units of mitochondrial respiration (pmol s^{-1} mg^{-1}), CV is a percentage.

Lastly, we performed simulations based on the TE_M for the $CI + CII_P$ and $CI + CII_E$ respiration values. We simulated increases of 1%-50% in mitochondrial respiration in each participant after a hypothetical intervention and estimated the

sample size (number of participants) required to detect this change at 80% power.

All analyses were performed using the R software.

3 | RESULTS

3.1 | Large technical error in mitochondrial respiration measurement

Two fiber bundles from the same muscle biopsy were run simultaneously in two chambers totaling 160 duplicate pairs after removal of results that indicated damaged membrane (ie, cyt-c increased >10%). Respiration measurements were correlated between chambers ($R \geq 0.71$-0.75, $P < .005$ for all) (Figure 1). Yet when compared with correlations obtained for gene expression data (generally $R > 0.9$),[30,31] the correlation values obtained here are rather low.

The poor correlation between chambers was consistent with high TE_M and CV estimates for all complexes (Table 1), as all complexes showed a CV ≥ 15%. When reporting the Flux Control Ratios (FCRs), to account for lab-to-lab variability,[7] the TE_M and CV estimates were also significantly elevated, with some reaching more than 100%.

The poor correlation along with high TEM and CV (> 15%) estimates for all complexes was still observed when we normalized mitochondrial respiration with CS activity, (Table 2).

3.2 | Simulations to estimate the sample size required to detect changes in mitochondrial respiration at 80% power

We performed simulations for both the CI + CII_P and CI + II_E respiration values since they are the most commonly reported respiration measurements in the literature, as well as the PCR and SCR ratios. We estimated the sample size required to detect true changes in mitochondrial respiration at 80% power. Since TE_M and CV values for mitochondrial respiration and mitochondrial respiration/CS were similar we have not conducted simulations for the latter. However, we have attached the code for this calculation in the supplementary data.

For the coupled and uncoupled respiration, the minimum sample size required to observe a percentage increase at 80% power is shown on Figure 2. An intervention that increases mitochondrial respiration by 10% at the group level requires

TABLE 1 Chamber-specific respiration values and FCRs, typical error of measurement and coefficient of variation for each substrate

	Mean ± SD			
	Chamber 1	Chamber 2	TE_M	CV (%)
CI_P (pmol s^{-1} mg^{-1})	82.9 ± 30.6	85.1 ± 28.7	14.9	17.5
CI + CII_P (pmol s^{-1} mg^{-1})	123.1 ± 39.0	123.0 ± 36.0	19.0	15.3
CI + II_E (pmol s^{-1} mg^{-1})	154.9 ± 48.8	150.6 ± 44.1	24.4	15.9
LCR (CI_L/CI + II_E)	0.08 ± 0.08	0.09 ± 0.07	0.04	50.8
PCR (CI + II_P/CI + II_E)	0.80 ± 0.12	0.82 ± 0.11	0.07	9.0
RCR (CI + II_P/CI_L)	11.1 ± 31.3	11.6 ± 16.7	22.8	193.7
Inv_RCR (CI_L/CI + II_P)	0.11 ± 0.09	0.11 ± 0.08	0.05	48.6
SCR (CI_P/CI + II_P)	0.67 ± 0.11	0.69 ± 0.08	0.07	10.8

Abbreviations: CI, complex I; CI, electron input through CI; CI+II, convergent electron input through CI and CII; CI+CII, complex I & II; E, ETS capacity; L, leak respiration; Inv-RCR, inverse of respiratory control ratio (CI_L/CI+II_P); LCR, leak control ratio (CI_L/CI+II_E); P, oxphos capacity; PCR, phosphorylation control ratio (CI+II_P/CI+II_E); RCR, respiratory control ratio (CI+II_P/CI_L); SCR, substrate control ratio at constant P (CI_P/CI+II_P).

FCR were calculated from mass-specific mitochondrial respiration measurements in permeabilized muscle fibers (vastus lateralis).

TABLE 2 Chamber-specific respiration values normalized by CS activity, typical error of measurement, and coefficient of variation for each substrate

	Mean ± SD			
	Chamber 1	Chamber 2	TE_M	CV (%)
CI_P*/CS**	5.38 ± 1.92	5.60 ± 1.79	1.02	18.5
CI+CII_P*/CS**	8.13 ± 2.73	8.24 ± 2.52	1.25	15.2
CI+II_E*/CS**	10.35 ± 3.65	10.22 ± 3.32	1.57	15.3

Note: Results based on 128 muscle samples.

*(pmol s^{-1} mg^{-1}); **(mIU × mg $protein^{-1}$).

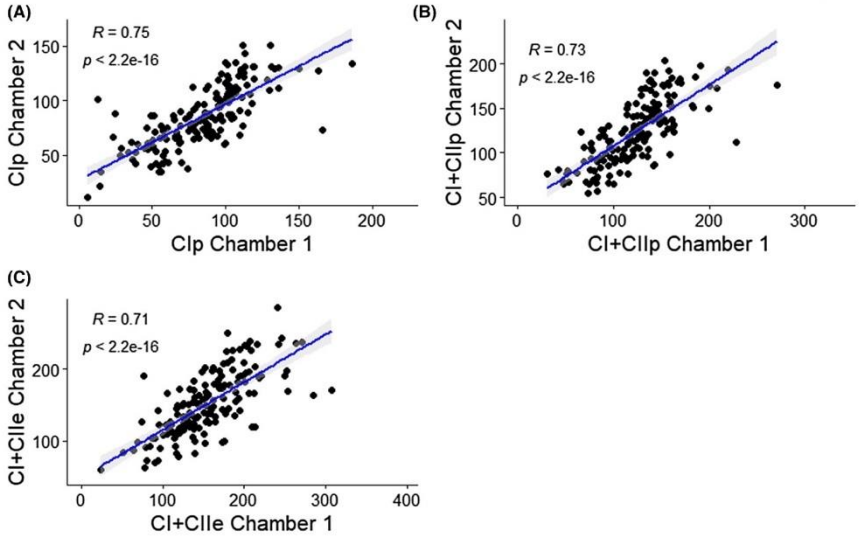

FIGURE 1 Spearman's correlation between chambers after the addition of (A) oxidative phosphorylation (OXPHOS) capacity (P) through Complex I (CI_P), (B), measure P through CI+Complex II (CII) linked respiration ($CI+CII_P$), (C) electron transport system (ETS) capacity (E) through CI+CII ($CI+CII_E$). All values are in pmol s^{-1} mg^{-1}

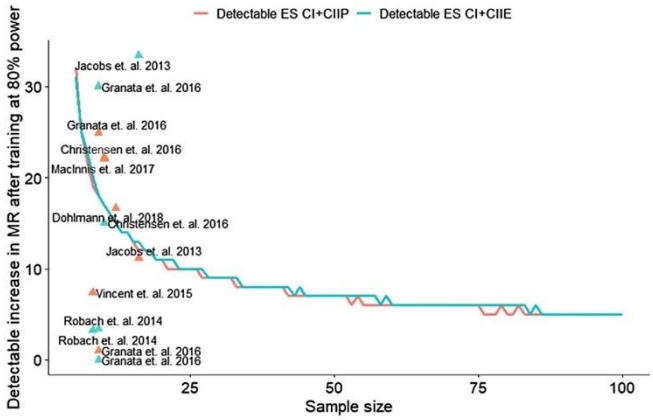

FIGURE 2 Minimum sample size required to detect increases in mitochondrial respiration (MR) after training at 80% power. A minimum of ~55 ($CI+CII_P$) and ~60 ($CI+CII_E$) pairs of duplicate samples are necessary to detect an increase of 6% in mitochondrial respiration at the group level, at 80% power. An intervention with ~20 samples/individuals would require a change of at least 11% in mitochondrial respiration to achieve 80% power for both $CI+CII_P$ and $CI+CII_E$ respiration. Experiments with less than 15 individuals would require a change of at least 13% to achieve 80% power. The triangles represent real effect sizes and sample sizes reported in different studies [8,10-12,14,20,35]

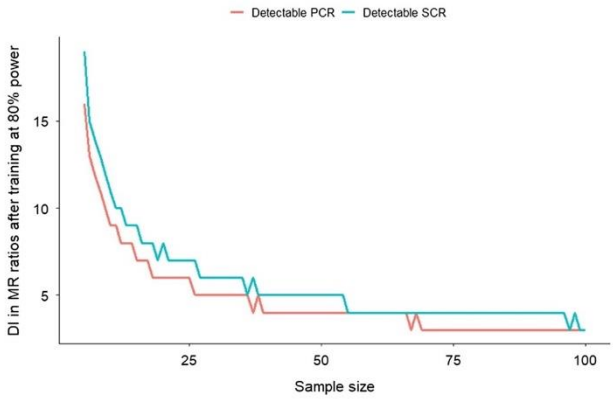

FIGURE 3 Minimum sample size required to detect increases (DI) in mitochondrial respiration (MR) ratios after training at 80% power. A minimum of ~26 (PCR) and ~38 (SCR) pairs of duplicate samples are necessary to detect an increase of 5% in mitochondrial respiration at the group level, at 80% power. An intervention with ~20 samples/individuals would require a change of at least 6% and 8% in mitochondrial respiration to achieve 80% power for PCR and SCR ratios, respectively. Experiments with less than 20 individuals would require a change of at least 7%-10% to achieve 80% power. Due to inconsistencies in the literature in which ratios each study calculates we have not included data from published studies in our ratios graph

a minimum of 23 participants to detect changes for CI + CII_P and CI + CII_E at 80% power. Our results suggest that with the typical sample size in exercise training studies (n = 12), only changes of >15% in mitochondrial respiration following training would be detectable at 80% power.

For the PCR and SCR respiration ratios, the minimum sample size required to observe a percentage increase at 80% power is shown on Figure 3. An intervention that increases mitochondrial respiration by 10%, at the group level, requires a minimum of 11 participants to detect changes for PCR ratio and 22 participants for the SCR ratio at 80% power.

We have also simulated percentage increases after hypothetical exercise training intervention in a cohort of 20 individuals (Figure 4A). We observed that an increase of ~11% or more in mitochondrial respiration is necessary for changes to be detected at 80% statistical power if each participant had duplicate respiration measurements. While for ratios (Figure 4B), a minimum of ~6% increase for PCR phosphorylation control ratio (CI + II_P/CI + II_E) and ~7% increase for SCR substrate control ratio at constant P (CI_P/CI + II_P) is required to be detected at 80% power, if each participant had duplicate respiration measurements.

4 | DISCUSSION

In the present study, we reported the TE_M and CV for measurements of mitochondrial respiration for CIp, CI + CII_P, and CI + CII_E, using the OROBOROS equipment, and in a large sample (n = 160 pairs of duplicate respiration measurements). We also performed statistical simulations to uncover the minimum number of participants required to detect an intervention-induced change in mitochondrial respiration at 80% power. We found a very large variability in all measurements (CV > 15%), suggesting that this measurement may only be appropriate in studies using large sample sizes (n ≥ 55) or that detect large effect sizes (>15%). To account for between-lab (lab-by-lab) variability, we have also computed the TE_M and CV for mitochondrial respiration ratios including: LCR (L/E), PCR (P/E), RCR (P/L), Inv RCR (L/P), and SCR (constant P). Not surprisingly, the TE_M and CV remained >9%, and the statistical simulations suggest a sample size of ≥ 26 is required to achieve 80% power. Mitochondrial respirations values were also normalized by CS activity, but no significant changes in TE_M or CVs were observed. In other words, the type of training should be carefully selected to achieve effect sizes in mitochondrial respiration experiments if sample size is a limitation. For example, participants who did sprint interval training (SIT) (n = 9) presented >19% change in mitochondrial respiration after 4 weeks of training, while participants who did only moderate intensity (n = 9) or high intensity interval training (n = 11) did not show any changes in mitochondrial respiration after 4 weeks of training.[10] Higher numbers of technical replicates (ie, number of chambers used for the same muscle—here we used two) could potentially lower the TE_M, in which case the required sample size would be lower to detect a given effect size.

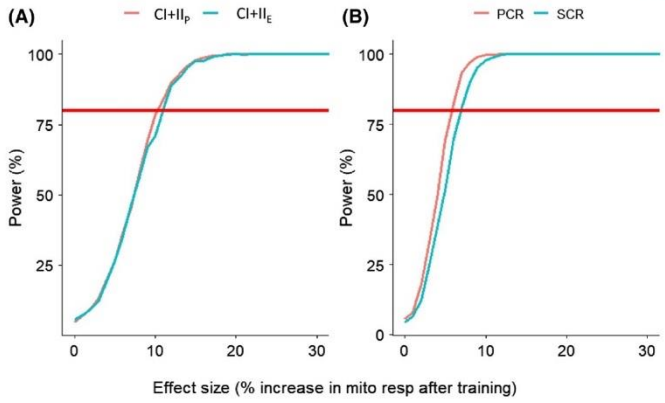

FIGURE 4 A, Power to detect percentage change in mitochondrial respiration (effect size) after a training intervention with n = 20 participants. A minimum of ~11% increase in mitochondrial respiration is needed to be detected at 80% power for both the CI+CII$_P$ and CI+CII$_E$, if each participant had duplicate respiration measurements. B, Power to detect percentage change in mitochondrial respiration ratios (effect size) after a training intervention with n = 20 participants. A minimum of ~6% increase for PCR phosphorylation control ratio (CI+II$_P$/CI+II$_E$) and ~7% increase for SCR substrate control ratio at constant P (CI$_P$/CI+II$_P$) is required to be detected at 80% power, if each participant had duplicate respiration measurements

TE$_M$ includes variability due to machine calibration and human error, and is potentially specific to each research facility.[17] However, Cardinale et al[3] recently reported a similarly high CV (15.2%), suggesting that the high variability we observed occurs across research facilities and is intrinsic to the OROBOROS equipment. Permeabilization of muscle pieces involves taking a small piece of muscle (typically 2-6 mg) and placing it in a dish with BIOPS solution; then, a technician uses two pairs of sharp forceps to separate individual fiber bundles.[32] Since this process is complicated, it is recommended that the same person performs the procedure in a given study to avoid variability between technicians.[33] The degree of fiber separation determines the amount of mitochondria present after the permeabilization, thus affecting the respiration measurements.[33] It is plausible that technicians vary in their ability to efficiently separate fibers, and this could lead to higher respiration values and potentially higher variability as well. Thus, different technical staff/researchers conducting the experiment can explain why experiments are variable. The largest variability in measurements, recently reported by Cardinale et al[3] was in experiments conducted by two different technicians working on the same piece of muscle (mean ± SD =31.3 ± 7.1 vs 26.3 ± 8.1 pmol s^{-1} mg^{-1}, P = .12). In the present study, some of the experiments were conducted by one technician, and some by another technician (ie, the two technicians never handled the same piece of muscle), which might explain some of the variability we reported. Unfortunately, we are unable to calculate the variability due to technicians in this study as they worked on different muscle samples. It should also be noted that it is common to use creatine in the respiration chambers when working with permeabilized muscle. However, several papers have not used creatine in their experiments and have reported valid and replicable results.[7,8,10,12]

To the best of our knowledge, the smallest meaningful change in mitochondrial respiration after training or other lifestyle interventions has never been reported, since this is dependent on the overall aim of each study.[10] In the present study, we calculated the minimum number of participants required to detect a change in mitochondrial respiration at 80% power. Our results suggest that with the typical sample size in exercise training studies (n = 12), only changes of >18% in mitochondrial respiration following training would be detectable at 80% power. This means that it would be a challenge to observe true changes in mitochondrial capacity using the OROBOROS technology, since most studies do not report such large increases following exercise training (−9% to 20%).[18,19,32,34-38] We acknowledge that some of the studies have observed significant changes in mitochondrial respiration without reaching the effect sizes we presented here. This implies that although such studies were significant, the sample sizes were too low to detect the magnitude of changes they reported at 80% power. The simulations presented in this paper provide important information for planning experiments investigating mitochondrial capacity. Future studies can use this data and the code we provided (see Supplementary File 1) as a guide to determine the number of participants required to

detect changes in mitochondrial respiration in their study. Alternatively, if the number of participants is a limitation, then a careful consideration of the exercise intervention duration is recommended, to trigger changes that are large enough to achieve 80% power.

In conclusion, we found very large variability in mitochondrial respiration measurements, reflected by TE_M and CV, and calculated the required sample size necessary for studies aimed at detecting changes in mitochondrial respiration. We recommend that future studies utilizing this method in skeletal muscle would follow the guidelines we provided here to detect significant changes in mitochondrial capacity, following lifestyle interventions. Finally, it should be noted that mitochondrial respiration is only one measure of mitochondrial function. The use of integrative approaches,[1,16,32] such as mitochondrial protein expression, mitochondrial content quantification, mitochondrial DNA sequencing, and appropriate statistical methods[17] may allow discoveries in the complex and integrative nature of exercise adaptations[16,39] as well as strengthen results from mitochondrial respiration measurements.

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CONFLICT OF INTEREST

The authors have no conflict of interest on the production of this manuscript.

AUTHOR CONTRIBUTIONS

M Jacques performed the experiments, analyzed the data, and drafted the manuscript; J Kuang performed the experiments and revised the manuscript; DJ Bishop revised the manuscript; X Yan participated in the research design and revised the manuscript; J Alvarez-Romero performed the experiments; F Munson, I Papadimitriou; and A Garnham performed and collected the muscle biopsies and revised the manuscript; S Voisin performed the data analyses and assisted in drafting the manuscript; and N Eynon designed the research and assisted in drafting the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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