Associations between CD36 gene polymorphisms and metabolic response to a short-term endurance training program in a young-adult population

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Complete List of Authors:	Jayewardene, Avindra; University of Sydney, Exercise Health and Performance Faculty Research Group, Faculty of Health Sciences Mavros, Yorgi; University of Sydney, Exercise Health and Performance Faculty Research Group, Faculty of Health Sciences Gwinn, Tom; University of Sydney, Exercise Health and Performance Faculty Research Group, Faculty of Health Sciences Hancock, Dale; University of Sydney, School of Molecular Biosciences, Faculty of Science Rooney, Kieron; University of Sydney, Exercise Health and Performance Faculty Research Group, Faculty of Health Sciences
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Associations between CD36 gene polymorphisms and metabolic response to a short-term endurance training program in a young-adult population

Authors:

Avindra F. Jayewardene ^{a*} *BAppSc*
Yorgi Mavros ^a *PhD* (yorgi.mavros@sydney.edu.au)
Tom Gwinn ^a *BAppSc, BSc* (tom.gwinn@sydney.edu.au)
Dale P. Hancock ^b *PhD* (dale.hancock@sydney.edu.au)
Kieron B. Rooney ^a *PhD* (kieron.rooney@sydney.edu.au)

Institutions:

^a *Exercise Health and Performance Faculty Research Group, Faculty of Health Sciences, University of Sydney, Lidcombe, NSW, Australia.*
^b *School of Molecular Biosciences, Faculty of Science, University of Sydney, Camperdown, NSW, Australia.*

Corresponding Author:

Avindra F. Jayewardene
Address: C42 - Cumberland Campus,
Faculty of Health Sciences,
The University of Sydney,
Lidcombe, NSW, 2141, Australia.
Ph: +61 2 9351 9403; Fax: +61 2 9351 9204; E-mail: ajay5611@uni.sydney.edu.au

Abstract

Purpose: Recent studies have shown that CD36 gene variants are associated with an increased prevalence of chronic disease. Although a genetic component to trainability has been proven, no data is available on the influence of CD36 on training response.

Methods: Two single nucleotide polymorphisms (SNPs) (rs1527479 and rs1984112) were assessed for associations with whole-body substrate oxidation, response to a 75g dextrose oral glucose tolerance test (OGTT), fasting plasma lipids and CVD risk factors in a young healthy cohort, both using cross-sectional analysis and following a 4-week endurance exercise training program. Genotyping was performed using real-time polymerase chain reaction (PCR).

Results: Cross-sectional data was collected in 34 individuals (22.7 ± 3.5 y), with 17 completing the training program. At baseline, TT SNP carriers at rs1527479 and wild-type (WT) GG carriers at rs1984112 were associated with significantly greater whole-body rate of fat oxidation (Fat_{ox}) during submaximal exercise ($P < 0.05$), whilst AA carriers at the same position were associated with elevated triglyceride (TG) levels. A significant genotype x time interaction in Fat_{ox} at SNP rs1984112 was identified at rest. Significant genotype x time interactions were present at rs1527479, with TT carriers exhibiting favourable response to training when compared to C-Allele carriers for fasting TG, diastolic blood pressure (DBP) and mean arterial pressure (MAP).

Conclusion: Cross-sectional assessment identified associations with Fat_{ox} and TG. Training response at both SNPs identified “at-risk” genotypes responding favourably to the training stimulus in Fat_{ox} , TG, DBP and MAP. Although these data show potential pleiotropic influence of CD36 SNPs, assessment in a larger cohort is warranted.

Keywords

Lipid metabolism, SNPs, Fatty Acid Translocase, Intervention, CVD Risk

Introduction:

Long-chain fatty acids (LCFAs) are the predominant substrate source for ATP re-synthesis both at rest, and during light to moderate exercise in healthy individuals. Fatty acid translocase (FAT)/cluster of differentiation 36 (CD36) is a multifunctional 88kDa glycoprotein that has been shown to be the predominant transporter in the highly regulated process by which LCFAs are transported from adipose tissue into the heart and skeletal muscle cells, as well as the mitochondria (Ibrahimi et al. 1999; Bonen et al. 2007; Yanai et al. 2007; Nickerson et al. 2009). CD36 is unique, as it is the only identified fatty acid (FA) transport protein that modulates lipid uptake across the sarcolemma and into the mitochondria (Bezair et al. 2006; Holloway et al. 2006; Smith et al. 2012), particularly during muscle contraction. Gene variants within the CD36 gene have previously been associated with multiple metabolic health indicators related to cardiovascular disease (CVD) (Lepretre et al. 2004; Ma et al. 2004; Febbraio and Silverstein 2007; Noel et al. 2010), highlighting a pleiotropic influence. Moreover, particular single nucleotide polymorphisms (SNPs) within the CD36 gene have also been directly associated with metabolic conditions, such as type II diabetes mellitus (T2DM), in older cohorts (Corpeleijn et al. 2006).

Exercise training induces an increased capacity for the uptake and oxidation of FAs (Kiens et al. 1993), but the exact mechanisms by which this occurs are still to be elucidated in their entirety. The key role of CD36 in the training-induced up-regulation of whole body fat oxidation (Fat_{ox}) has been established, via both increased CD36 protein content on the plasma membrane, and elevated protein content coimmunoprecipitation with carnitine palmitoyltransferase I on the mitochondrial membrane (Schenk and Horowitz 2006; Talanian et al. 2010; McFarlan et al. 2012). There is evidence of considerable inter-individual variation in the response to exercise training, with a genetic component to trainability strongly supported by numerous data (Bouchard and Rankinen 2001; Bouchard 2012). We have recently published the only CD36 genetic variability data in a young Australian population and identified associations between SNP rs1527479 with Fat_{ox} during submaximal exercise, as well as between SNPs rs1527479 and

rs1984112 with diastolic blood pressure (DBP) and resting heart rate (RHR) (Jayewardene et al. 2014). Whilst this pilot data was limited by a small sample size and the cross-sectional nature of the study, it remains undetermined whether CD36 SNPs influence individual response to an aerobic training intervention, as there is no data available at present.

Individual variability in FA metabolism can be proposed to be attributable in part to polymorphisms in genes that encode membrane proteins responsible for FA transport. Prior to our previously reported pilot data (Jayewardene et al. 2014), there was only one study relating CD36 polymorphisms to FA metabolism during exercise, with their focus on CD36-deficient participants supporting the role of CD36 in LCFA mobilisation and metabolism in human participants (Yanai et al. 2007). Identification of genetic markers that have a significant influence on training response could lead to the development of individualized interventions that would allow for optimal lipid oxidation and favourable cardiometabolic response (Jeukendrup and Wallis 2005).

We conducted a prospective study to assess CD36 pleiotropic response to a supervised training intervention. We recruited a young, otherwise healthy population, similar to that in which we previously reported CD36 associated dysregulation of Fat_{ox} during exercise. Dependent variables tested for associations with CD36 genotype included Fat_{ox} , fasting plasma lipids, resting CVD risk factors and response to an oral glucose tolerance test (OGTT). Focus was placed on the two SNPs (rs1527479 and rs1984112) previously investigated in our cross-sectional study, which had been associated with disease states in older Caucasian populations (Ma et al. 2004; Corpeleijn et al. 2006; Rodrigues et al. 2013). We hypothesised that carriers of the at-risk SNP genotypes (TT carriers at rs1527479; AA carriers at rs1984112) will have an attenuated response to the training program, as well as unfavourable CVD risk profile when compared to the wild-type (WT) and heterozygous carriers, which could explain their associations with chronic disease evident in older morbid cohorts.

Methods:

Study participants and design

This study comprised of two stages. All participants completed stage 1, a cross-sectional screening analysis of genotype and metabolic health indicators. Participants were then given the option to participate for stage 2, which included (as outlined below) a 4-week supervised endurance training intervention and repeat testing.

34 physically active participants (22 males, 12 females; 22.7 ± 0.6 y) volunteered for the cross-sectional screening component of the study from May 2012 to December 2013. All were between 18 and 30 years old, non-smokers who were capable of performing an incremental cycling test to exhaustion. This study complied with the ethical guidelines laid down for human research by the Australian NHMRC and was approved by the University of Sydney Human Research Ethics Committee. Before taking part in the study, all participants were made aware of experimental procedures, and gave written consent to participate.

Stage 1 - Cross-sectional screening

Stage 1 consisted of two sessions. Participants reported to the laboratory on each occasion between the hours of 0700 and 0900 having fasted for ≥ 10 -hrs, and having been instructed to abstain from consumption of alcohol, caffeine and to refrain from strenuous exercise for the 24hr prior to each session.

Session one involved the collection of a blood sample for DNA extraction, anthropometric and body composition measurements, fasting blood metabolites, an oral glucose tolerance test (OGTT) and an assessment of maximal oxidative capacity (VO_{2peak}).

Session two was performed 1 - 4 weeks after session one, and involved measurement of resting CVD risk factors (resting heart rate (RHR), blood pressure (BP) and rate-pressure product (RPP)) and assessment of Fat_{ox} at rest and during exercise. Brachial BP and radial RHR were measured in duplicate and in accordance with consensus recommendations (Palatini et al. 2006; National Heart Foundation 2010). RPP was calculated as the product of RHR and systolic BP (SBP).

Anthropometry and body composition

Morning fasting height (wall-mounted Holtain stadiometer; Holtain Ltd, Crymych Pembs, UK) and naked weight (weight in clothes [kg] - weight of clothes [kg]) were measured in triplicate to the nearest 0.1 cm and 0.1 kg, respectively. Waist and hip circumference were measured in duplicate and in accordance with consensus recommendation (Dalton et al. 2003).

Body fat percentage (%BF) was determined using bioelectrical impedance assessment (BIA) (RJL Systems Inc., Clinton, MI, USA) using sex-dependent equations (Sun et al. 2003).

Fasting blood metabolites

Fasting blood metabolites were assessed at a similar time of the morning for all participants. Plasma blood glucose was measured using fingerprick capillary samples on a glucose oxidase auto-analyser (Coefficient of Variation (CV) 2.5 - 2.9%; Accu-Chek Go, Roche Diagnostics, Basel, Switzerland). Triglyceride (TG) (CV 4.3%), total cholesterol (TC) (CV 2.3%) and high-density lipoprotein cholesterol (HDL-C) (CV 1.9%) were measured using a CardioChek® Analyser (CardioChek®, Indianna, USA).

Low-density lipoprotein cholesterol (LDL-C) was estimated using two separate formulae dependent on fasting TG levels (Friedewald et al. 1972; Ahmadi et al. 2008).

Oral glucose tolerance test

A 75-g dextrose OGTT was performed, with blood glucose (BG) measured using fingerprick capillary samples on a glucose oxidase auto-analyser (Accu-Chek Go, Roche Diagnostics, Basel, Switzerland). Samples were obtained at 0, 30, 60, 90 and 120-min. The trapezoidal rule was used to calculate the area under the curve of glucose response (AUC).

Exercise testing

Exercise assessments were conducted on a Lode Corival cycle ergometer (Lode BV, Netherlands). Fractional percentages of O₂ and CO₂ were measured using a Parvo Medics TrueOne 2400 metabolic system (Parvo Medics, UT, USA). Female participants performed the assessments during the early to mid-follicular phase (Days 1 - 14) of their menstrual cycle to account for changes in circulating estradiol levels between the follicular and luteal phases which influences substrate utilisation (Wenz et al. 1997; Zderic et al. 2001).

Maximal oxidative capacity (VO_{2peak})

A ramp protocol was utilised to assess VO_{2peak} , with all participants cycling at 75 W for the first three minutes of the assessment. Ramp test increments varied between 1 W per 2 - 4 seconds, determined by the assessor based on sex and following a review of participant's habitual PA levels (Baecke et al. 1982). Ventilatory gases were analysed throughout the course of the assessment, until the participant reached exhaustion. VO_{2peak} was assessed using a 30 sec average.

Whole body substrate utilisation

Participants were asked to follow a 1-day controlled diet developed based on weight [175kJ/kg; 4.6g CHO/kg; 1.6g TF/kg; 2.5g protein/kg] on the day prior to this assessment in order to reduce the component of variability in substrate metabolism attributable to differences in dietary intake (Jeacocke

and Burke 2010; Krishnan and Cooper 2014). A 3-day food diary was also provided to monitor dietary intake and exercise patterns prior to testing. Six submaximal stages of 6 min representing 20 - 70% $\text{VO}_{2\text{peak}}$ were used to assess Fat_{ox} . Continuous ventilatory gas collection occurred using a Parvo Medics TrueOne 2400 metabolic system (Parvo Medics, UT, USA), with data analysed for 2 min between minutes 4 and 6 at each stage. Fat_{ox} and whole-body rate of carbohydrate oxidation (CHO_{ox}) were calculated using non-protein respiratory quotient (RQ) (Peronnet and Massicotte 1991):

$$\text{Fat oxidation (g.min}^{-1}\text{)} = 1.695 \cdot \text{VO}_2 - 1.701 \cdot \text{VCO}_2$$

$$\text{Carbohydrate oxidation (g.min}^{-1}\text{)} = 4.585 \cdot \text{VCO}_2 - 3.226 \cdot \text{VO}_2$$

Stage 2 - Training protocol

Following the initial screening, 17 participants (13 males, 4 females; 22.7 ± 0.7 y) volunteered to complete the endurance training intervention component of the study. The training protocol consisted of three supervised submaximal cycling sessions per week for 4 weeks, each lasting 45 min. The intensity at which participants trained was determined, relative to their initial $\text{VO}_{2\text{peak}}$ and increased incrementally over the 4 week training period such that participants cycled at 60% $\text{VO}_{2\text{peak}}$ for weeks 1 and 2, 70% $\text{VO}_{2\text{peak}}$ for week 3 and 75% $\text{VO}_{2\text{peak}}$ for week 4. Participants were instructed to maintain pre-study dietary and habitual PA patterns over the course of the training intervention.

SNP Genotyping

Genomic DNA was extracted from peripheral whole blood using a Promega wizard® genomic DNA purification kit (Promega, Madison, WI, USA). Genotyping of SNP rs1527479 in the upstream promoter region (intron 1B, -3489 bp relative to the translation start site), as well as SNP rs1984112 (5' flanking exon 1A, -33137 bp relative to the translation start site) were performed using custom Taqman® real-time polymerase chain reaction (PCR) technology (VIC® and FAM™ labelled-dyes). The probes were

designed using the Applied Biosystems® Taqman® design tool (Applied Biosystems®, Foster City, CA, USA). The primer and probe sequences used for rs1527479 were as follows:

Forward 5'-GGGAAAAGGCCAGATAGATTCA-3'
Reverse 5'-ATCTGGAGAAGGGCTAATATATGCA-3'
Probe [VIC/FAM] 5'-AACTAGGTTGTGGCA[C/T]AG-3'

The primer and probe sequences for rs1984112 were as follows:

Forward 5'-TTTACTGAACAGGAAACTG-3';
Reverse 5'-GTAAAAATCACAGTGAAAAATT-3';
Probe [VIC/FAM] 5'-AGGAAACTGTAGTTA[A/G]GA-3'

The PCR reaction was performed in a total volume of 25 µL, containing 2.5 µL of DNA template, 12.5 µL of 2X Applied Biosystems® Taqman® Genotyping Master mix and 1.25 µL 20X Taqman® probe mix specific for each SNP. The reactions were carried out using an Applied Biosystems® 7500FAST Real-Time PCR System. The amplification consisted of initial denaturation (95°C, 10 min); 40 cycles consisting of denaturation (95°C, 15 s), annealing and extension (60°C, 1 min).

Statistical Analyses

All data were assessed for normality using histograms and descriptive statistics. Normally distributed data are presented as Mean ± SD. Non-normally distributed data were log transformed prior to use in parametric statistics if possible and presented as median (range). Both SNP loci were tested for departure from Hardy – Weinberg equilibrium using a Chi-square (χ^2) analysis. Baseline data was analysed using sequential one-way Analysis of Covariance (ANCOVA). Dominant-allele analyses were also performed (SNP carriers vs. non-carriers) on baseline data and used to assess all training outcomes, due to the limited sample size and adequate distribution amongst genotypes. All dependent variables were adjusted

222 for sex, age and VO_{2peak} . A split-plot repeated measures ANCOVA was used to determine time effect (p_t)
223 and genotype x time (p_{gxt}) interaction for normally distributed data. Wilcoxon signed rank test were used
224 if normalisation of data were not possible via log transformation. Training data was adjusted for
225 covariates, which included sex, age, VO_{2peak} and baseline value. A value of $P < 0.05$ was considered
226 statistically significant as all hypotheses were specified a priori. Post hoc analyses were considered for all
227 ANCOVA models where $P < 0.1$, due to the limited sample size. Under peer-review, the suggestion was
228 made to include the alternate SNP to that being assessed as a covariate to both the one-way and repeated
229 measures ANCOVA models. The changes to the original statistical outputs are provided in the
230 supplementary material. Statistical analyses were performed using SPSS version 21.0 software (SPSS
231 Inc., Chicago, IL, USA), and bias-corrected effect sizes (Hedges' g) often used for small sample size
232 (Hedges and Olkin 2014).

Results:

Participant characteristics at the first stage are presented in Table S1. Genotype distributions were in Hardy-Weinberg equilibrium at both SNP loci. Three participants did not complete all baseline requirements, and were excluded from analyses altogether.

Cross-sectional data

TT carriers of rs1527479 had significantly greater Fat_{ox} compared to C-Allele carriers at 40% VO_{2peak} ($P = 0.033$; $g = -0.8$) and tended towards higher Fat_{ox} at 60% ($P = 0.061$; $g = -0.5$) (Figure 1(a)). No difference was observed in Fat_{ox} during dominant model analysis of the screening data at rs1984112 ($P > 0.05$) (Figure 1(c)).

At rs1527479, the TT genotype tended towards being greater at fasting blood glucose (FBG) ($P = 0.066$; $g = -0.5$) (Table 1). Genotypic variation in the cross-sectional TG screening data at rs1984112 was present, with AA carriers significantly greater than G-allele carriers ($P = 0.01$; $g = -0.9$) (Table 1). No other significant differences were evident in OGTT and fasting plasma lipid markers at baseline ($P > 0.05$; Figure 2 (a) & (c)).

No significant difference in VO_{2peak} or W_{peak} was observed at either SNP at screening (Tables 1).

Training Effect

Time effect analyses are included in the supplementary material (Supplementary Table S4& S5). Two participants dropped out due to time constraints, and were assessed in the cross-sectional analyses only. Excluding the two dropouts, adherence to the training intervention was 100% for all participants. No adverse events were reported over the course of this study.

Whole body fuel utilisation analysis at SNP rs1984112 identified genotype x time interactions at rest ($P_{\text{gxt}} = 0.011$; $g = -0.6$) (Figure 1 (d)), with AA carriers experiencing significantly greater increases in Fat_{ox} when compared to G-Allele carriers. No genotype x time interaction was observed at rs1527479 (Figure 1(b)).

TT carriers of SNP rs1527479 reduced DBP ($P_{\text{gxt}} = 0.016$; $g = 0.9$) and MAP ($P_{\text{gxt}} = 0.028$; $g = 0.8$) when compared to C-allele carriers (Table 2). Post-training assessment showed that TT carriers also experienced a decrease in TG levels compared to C-Allele carriers ($P_{\text{gxt}} = 0.009$; $g = 1.1$) (Table 2). At SNP rs1984112, a significant genotype x time interaction was present in HDL-C ($P_{\text{gxt}} = 0.024$) (Table 3). No clinically relevant changes in OGTT data were present at either SNP (Figures 2 (b) & (d)).

No training induced group-time interactions in $\text{VO}_{2\text{peak}}$ and W_{peak} were evident at either SNP ($P_{\text{gxt}} > 0.05$) (Tables 2 & 3).

Discussion:

In the present study, we were able to find a number of interactions of note. Cross-sectional analysis at stage 1 identified associations with our primary dependent variable, Fat_{ox} , at both SNPs. Investigation of the impact of a short-term endurance-training program identified significant time effects for Fat_{ox} , along with MAP and FBG. Possible influence of CD36 gene variants on training response were also identified, with genotype x time interactions observed in Fat_{ox} , and documented metabolic health indicators TG, HDL-C, DBP and MAP. With particular emphasis on a young, healthy cohort in this functional genetic study, we endeavoured to minimise secondary phenotypic influence from overt pathology commonly present in older cohorts, allowing for identification of early indicators in a hypothesised “at-risk” population, allowing for a better understanding of the potential confounding influence CD36 gene variants.

Genetic variants within key metabolic genes could be proposed to alter either encoded protein function and/or abundance, depending on the location and influence of the SNP. This could be a possible mechanism behind the previously mentioned associations between the CD36 SNPs investigated in our research and chronic disease states and risk factors identified in older cohorts (Ma et al. 2004; Corpeleijn et al. 2006; Rodrigues et al. 2013). Whilst significant differences in Fat_{ox} were evident during cross-sectional analysis at stage 1 using both models of analysis at both SNPs, only analysis at rs1984112 was consistent with our hypothesis, with the GG WT genotype exhibiting higher Fat_{ox} at 20 - 40% VO_{2peak} (Supplementary Table S2).

When training data were analysed using the dominant model at both SNPs, a gene-exercise interaction effect was only evident at rs1984112 at rest. Additional analysis performed in an attempt to possibly reduce the confounding influence of the alternate SNP strengthened this association ($P_{gxt} = 0.001$, Supplementary Table S11). A similar analysis on SNP rs1527479 data also observed a significant

genotype x time interaction at resting Fat_{ox} ($P_{\text{gxt}} = 0.414$ to $P_{\text{gxt}} = 0.012$). Large effect sizes were also present at both SNPs ($|g| = 0.6 - 1.1$). Significant increases in Fat_{ox} were evident as a result of the training program prior to covariate adjustment, predominantly present at training specific exercise intensities (50 - 70% $\text{VO}_{2\text{peak}}$). With CHO_{ox} decreasing across all intensities at both SNPs, our data is consistent with the notion that there is a preferential switch to fat oxidation following endurance training. Research investigating the ameliorating effects of exercise training on metabolic impairments, such as FA accumulation, as a result of genetic factors in rat models showed that the influence of selective breeding resulting in reduced expression of a number of key metabolic genes could be overturned as a result of exercise-training (Lessard et al. 2011; McFarlan et al. 2012). Exercise-induced up-regulation of key metabolic genes is believed to be the mechanism responsible for this result. Similar increases in proteins involved in LCFA transport as a result of exercise training (FABP_{pm} , FATP1 , FATP4), particularly CD36, have been confirmed in numerous human models (Arkininstall et al. 2004; Roepstorff et al. 2004; Talanian et al. 2010), with a number of varying exercise modalities.

Cross-sectional assessment between participants in the present study observed that AA SNP carriers at rs1984112 had significantly elevated TG, an association reinforced following the addition of rs1527479 as a covariate in ANCOVA model ($P = 0.008$, Supplementary Table S10). Ma and colleagues (2004) identified a haplotype, including SNP rs1984112, which was associated with not only elevated TG, but also free-fatty acids (FFA) (Ma et al. 2004). With the authors suggesting their results were consistent with those seen in CD36 knockout mouse models (Febbraio et al. 1999), they postulate that the increased plasma FFA associated with decreased clearance, as a result of decreased CD36 expression, would lead to increased hepatic FFA uptake, leading to increased production of TGs. Unfortunately, FFA was not collected in the present study to test whether this hypothesis was present, but its inclusion for analysis in future work would be of great interest. However, the association evident in our pre-clinical cohort is of

great interest, particularly with the documented importance of TGs as a biomarker for CVD risk and all-cause mortality (Miller et al. 2011; Nordestgaard and Varbo 2014).

Specificity in prescribing interventions aimed at reducing such CVD risk factors requires further investigation. A significant gene-exercise interaction was evident in our data at rs1527479, with the high-risk TT genotype group exhibiting a protective reduction in TG following exercise-training when compared to C-allele carriers, an interaction present independent of SNP rs1984112. Previous research investigating the effect of exercise-training on TG levels suggest that in the absence of concurrent weight-loss and/or reduced energy intake (Duncan et al. 2005; Fontana et al. 2007), there would be a minimal reduction in plasma TGs, whilst elevated baseline TG level (>1.69 mmol/L) was also a determining factor (Durstine et al. 2002). In the present data, baseline grouped means were below this range, participants were instructed not to alter dietary patterns over the course of the intervention and no significant difference in weight loss between these groups were identified. With these factors considered, the genotype x time interactions evident could well be related to the impact of the training stimulus on CD36 protein function and/or abundance. Further investigation of this potential protective gene-exercise interaction on plasma TG is warranted and required to make any significant and appropriate interpretation of this data however, with the greater control of diet via meal provisions throughout the training intervention merited, in order to ensure there would be no increase in caloric intake often associated with exercise (Laan et al. 2010).

Whilst no significant difference in BP measures were evident in the present cross-sectional and baseline data, significant genotype x time interactions were present at both SNP loci in our training data. Rice and colleagues (2002) investigated heritability of HR and BP response to exercise training in the HERITAGE family cohort (Rice et al. 2002). Following a 20-week exercise-training program, they reported that the trainability of SBP in hypertensive families appears to be determined by genetic factors, this observation

was not present in the normotensive sub-group, whilst DBP response was believed to be a result of environmental factors as opposed to genetic. In our cohort, DBP and MAP values at rs1527479 exhibited favourable adaptation for TT carriers when compared to C-allele carriers ($g = 0.9$ and $g = 0.8$, respectively), opposing our hypothesis that this “at-risk” group would not respond as well. DBP and MAP response when participants were stratified at rs1984112 were not expected, with an increase in DBP in both G-allele and AA groups, whilst AA carriers increased MAP and no change was seen in the G-allele group. If a genetic component of DBP and MAP trainability were to be considered, our data is consistent with the belief that the AA group would not respond as well to the training stimulus. Previous assessments of genetic variance and BP response to an exercise-training program have investigated a handful of genes, including ACE, ApoE, LDL and AGT genes (Hagberg et al. 1999; Rauramaa et al. 2002; Zhang et al. 2002). Current data suggests further examination of the influence of CD36 gene variants on BP response in a larger sample size is warranted. The role of CD36 in BP determination has been shown in spontaneous hypertensive rat (SHR) models, with a strong inverse correlation inverse correlation of renal expression of CD36 with both SBP and DBP (Pravenec et al. 2008). It is postulated that CD36 mutations may influence the regulation of BP through nitric oxide-related pathways in the kidney (Zou and Cowley 1999; Pravenec et al. 2008), as CD36 is known to co-localise with endothelial nitric oxide synthase (eNOS) in the caveolae of endothelial cells in the renal medulla (Bordessoule et al. 1993), and is a determinant of eNOS activation by FAs.

The variance in Fat_{ox} identified at stage 1 analysis at both SNP loci in the present study were contrary to previous cross-sectional pilot data collected by this lab in a separate study cohort (Jayewardene et al. 2014), where C-allele carriers at rs1527479 shown to have elevated Fat_{ox} when compared to TT carriers, and no difference evident at SNP rs1984112 in that particular data set. As we investigated similar associations in a slightly larger cohort, with the only change being a shift towards lower exercise intensities, due to potential inaccuracies associated with higher exercise intensities (Jeukendrup and

Wallis 2005), it may suggest that the current results are a more accurate representation of the genotypic associations present within our cohort (comparisons presented in Table 4).

Of the significant genotype x time interactions identified in our stage 2 data, a number of the more favourable responses to the training program were present in the “at-risk” genotypes for Fat_{ox}, TG, and BP variables between both SNPs. Whilst these findings are not consistent with our hypothesis, it may suggest that these particular individuals may have a greater capacity for positive metabolic flexibility when an external stimuli is exerted. The implications of this theory are that the adverse associations previously described at both SNPs in older, comorbid populations (Ma et al. 2004; Corpeleijn et al. 2006; Rodrigues et al. 2013) are primarily a result of chronic inactivity over the course of a lifetime.

Although our study focuses on the role of CD36 as a transport protein in skeletal muscle during exercise, Febbraio and Silverstein (2007) identified the intrinsic difficulty in investigating CD36 is that the protein is expressed in multiple tissues, as well as multiple cell types within a single tissue (Febbraio and Silverstein 2007). Furthermore, the lack of a non-exercise control group for the training intervention means that we are unable to definitively rule out the confounding influence of external factors on dependent variables included in our study.

Whilst a larger sample size would increase the power of this study, our preliminary data supports further investigation in this study population. Additionally, broadening the scope of the study to include specific dependent variables to investigate associations identified in this study would be beneficial to understand possible mechanistic influences, which were beyond the scope of this particular study. Investigation of genotypic influence on CD36 protein expression within the active muscle during exercise would be of particular interest.

Conclusion

In conclusion, cross-sectional analysis identified associations between CD36 genotype and dependent variables Fat_{ox} and TG. The training data suggests that a short-term endurance training program would be sufficient to elicit significant increases in Fat_{ox} at intensities 40 - 70% $\text{VO}_{2\text{peak}}$. A significant genotype x time interaction at rs1984112 in Fat_{ox} was observed, however the response was not consistent with our hypothesis following training, with the “at-risk” AA SNP carriers experiencing a greater increase when compared to G-Allele carriers. Training response at SNP rs1527479 was contrary to our hypothesis as well, with TT SNP carriers exhibiting favourable response in fasting TG, DBP and MAP. These data may suggest that these “at-risk” groups exhibit greater metabolic flexibility, allowing for preferential adaptation to a training stimulus. Whilst these data show the potential pleiotropic influence of CD36 SNPs rs1527479 and rs1984112 on training response, further assessment in a larger cohort is warranted.

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Statements

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest: The authors declare that they have no conflict of interest.

References:

- Ahmadi, S.-A., Boroumand, M.-A., Gohari-Moghaddam, K., Tajik, P., and Dibaj, S.-M. 2008. The impact of low serum triglyceride on LDL-cholesterol estimation. *Archives of Iranian Medicine* **11**: 318-21. PMID:18426324.
- Arkinstall, M.J., Bruce, C.R., Clark, S.A., Rickards, C.A., Burke, L.M., and Hawley, J.A. 2004. Regulation of fuel metabolism by preexercise muscle glycogen content and exercise intensity. *Journal of Applied Physiology* **97**: 2275-2283. doi:10.1152/ajpendo.00557.2003.
- Baecke, J.A., Burema, J., and Frijters, J.E. 1982. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *American Journal of Clinical Nutrition* **36**: 936-42. PMID:7137077.
- Bezaire, V., Bruce, C.R., Heigenhauser, G.J.F., Tandon, N.N., Glatz, J.F.C., Luiken, J. et al. 2006. Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. *American Journal of Physiology-Endocrinology and Metabolism* **290**: E509-E515. doi:10.1152/ajpendo.00312.2005.
- Bonen, A., Han, X.-X., Habets, D.D.J., Febbraio, M., Glatz, J.F.C., and Luiken, J.J.F.P. 2007. A null mutation in skeletal muscle FAT/CD36 reveals its essential role in insulin- and AICAR-stimulated fatty acid metabolism. *American Journal of Physiology - Endocrinology & Metabolism* **292**: E1740-9. doi:10.1152/ajpendo.00579.2006. PMID:17264223.
- Bordessoule, D., Jones, M., Gatter, K.C., and Mason, D.Y. 1993. Immunohistological patterns of myeloid antigens: tissue distribution of CD13, CD14, CD16, CD31, CD36, CD65, CD66 and CD67. *British Journal of Haematology* **83**: 370-83. doi:10.1111/j.1365-2141.1993.tb04659.x. PMID:7683483.
- Bouchard, C. 2012. Genomic predictors of trainability. *Experimental Physiology* **97**: 347-52. doi:10.1113/expphysiol.2011.058735. 21967902.

- 447 Bouchard, C. and Rankinen, T. 2001. Individual differences in response to regular physical activity.
448 *Medicine & Science in Sports & Exercise* **33**: S446-51; discussion S452-3. PMID:11427769.
- 449 Corpeleijn, E., van der Kallen, C.J.H., Kruijshoop, M., Magagnin, M.G.P., de Bruin, T.W.A., Feskens,
450 E.J.M. et al. 2006. Direct association of a promoter polymorphism in the CD36/FAT fatty acid
451 transporter gene with Type 2 diabetes mellitus and insulin resistance. *Diabetic Medicine* **23**: 907-
452 11. doi:10.1111/j.1464-5491.2006.01888.x. PMID:16911630.
- 453 Dalton, M., Cameron, A.J., Zimmet, P.Z., Shaw, J.E., Jolley, D., Dunstan, D.W. et al. 2003. Waist
454 circumference, waist-hip ratio and body mass index and their correlation with cardiovascular
455 disease risk factors in Australian adults. *Journal of Internal Medicine* **254**: 555-563.
456 doi:10.1111/j.1365-2796.2003.01229.x.
- 457 Duncan, G.E., Anton, S.D., Sydeaman, S.J., Newton, R.L., Jr., Corsica, J.A., Durning, P.E. et al. 2005.
458 Prescribing exercise at varied levels of intensity and frequency: a randomized trial. *Archives of*
459 *Internal Medicine* **165**: 2362-9. doi:10.1001/archinte.165.20.2362. PMID:16287765.
- 460 Durstine, J.L., Grandjean, P.W., Cox, C.A., and Thompson, P.D. 2002. Lipids, lipoproteins, and exercise.
461 *Journal of Cardiopulmonary Rehabilitation* **22**: 385-398. doi:10.1097/00008483-200211000-
462 00002. PMID:12464825.
- 463 Febbraio, M., Abumrad, N.A., Hajjar, D.P., Sharma, K., Cheng, W., Pearce, S.F. et al. 1999. A null
464 mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism.
465 *Journal of Biological Chemistry* **274**: 19055-62. doi:10.1074/jbc.274.27.19055. PMID:10383407.
- 466 Febbraio, M. and Silverstein, R.L. 2007. CD36: implications in cardiovascular disease. *International*
467 *Journal of Biochemistry & Cell Biology* **39**: 2012-30. doi:10.1016/j.biocel.2007.03.012.
468 PMID:17466567.
- 469 Fontana, L., Villareal, D.T., Weiss, E.P., Racette, S.B., Steger-May, K., Klein, S. et al. 2007. Calorie
470 restriction or exercise: effects on coronary heart disease risk factors. A randomized, controlled

- 471 trial. American Journal of Physiology - Endocrinology & Metabolism **293**: E197-202.
472 doi:10.1152/ajpendo.00102.2007. PMID:17389710.
- 473 Friedewald, W.T., Levy, R.I., and Fredrickson, D.S. 1972. Estimation of the concentration of low-density
474 lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical
475 Chemistry **18**: 499-502. PMID:4337382.
- 476 Hagberg, J.M., Ferrell, R.E., Dengel, D.R., and Wilund, K.R. 1999. Exercise training-induced blood
477 pressure and plasma lipid improvements in hypertensives may be genotype dependent.
478 Hypertension **34**: 18-23. doi:10.1161/01.HYP.34.1.18. PMID:10406818.
- 479 Hedges, L.V. and Olkin, I. (2014). Statistical method for meta-analysis. Academic press:
- 480 Holloway, G.P., Bezaire, V., Heigenhauser, G.J.F., Tandon, N.N., Glatz, J.F.C., Luiken, J.J.F.P. et al.
481 2006. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/ CD36 content and
482 carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. Journal
483 of Physiology **571**: 201-210. PMID:16357012.
- 484 Ibrahim, A., Bonen, A., Blinn, W.D., Hajri, T., Li, X., Zhong, K. et al. 1999. Muscle-specific
485 overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces
486 plasma triglycerides and fatty acids, and increases plasma glucose and insulin. Journal of
487 Biological Chemistry **274**: 26761-6. doi:10.1074/jbc.274.38.26761. PMID:10480880.
- 488 Jayewardene, A.F., Gwinn, T., Hancock, D.P., Mavros, Y., and Rooney, K.B. 2014. The associations
489 between polymorphisms in the CD36 gene, fat oxidation and cardiovascular disease risk factors
490 in a young adult Australian population: a pilot study. Obes Res Clin Pract **8**: e618-21.
491 doi:10.1016/j.orcp.2014.09.001. PMID:25277110.
- 492 Jeacocke, N.A. and Burke, L.M. 2010. Methods to standardize dietary intake before performance testing.
493 International Journal of Sport Nutrition & Exercise Metabolism **20**: 87-103. PMID:20479482.

- 494 Jeukendrup, A.E. and Wallis, G.A. 2005. Measurement of substrate oxidation during exercise by means
495 of gas exchange measurements. *International Journal of Sports Medicine* **26 Suppl 1**: S28-37.
496 doi:10.1055/s-2004-830512. PMID:15702454.
- 497 Kiens, B., Essengustavsson, B., Christensen, N.J., and Saltin, B. 1993. Skeletal muscle substrate
498 utilization during submaximal exercise in man: effect of endurance training. *Journal of*
499 *Physiology-London* **469**: 459-478. doi:10.1113/jphysiol.1993.sp019823.
- 500 Krishnan, S. and Cooper, J.A. 2014. Effect of dietary fatty acid composition on substrate utilization and
501 body weight maintenance in humans. *European Journal of Nutrition* **53**: 691-710.
502 doi:10.1007/s00394-013-0638-z. PMID:24363161.
- 503 Laan, D.J., Leidy, H.J., Lim, E., and Campbell, W.W. 2010. Effects and reproducibility of aerobic and
504 resistance exercise on appetite and energy intake in young, physically active adults. *Appl Physiol*
505 *Nutr Metab* **35**: 842-7. doi:10.1139/h10-072. PMID:21164556.
- 506 Lepretre, F., Vasseur, F., Vaxillaire, M., Scherer, P.E., Ali, S., Linton, K. et al. 2004. A CD36 nonsense
507 mutation associated with insulin resistance and familial type 2 diabetes. *Human Mutation* **24**:
508 104. doi:10.1002/humu.9256. PMID:15221799.
- 509 Lessard, S.J., Rivas, D.A., Stephenson, E.J., Yaspelkis, B.B., III, Koch, L.G., Britton, S.L. et al. 2011.
510 Exercise training reverses impaired skeletal muscle metabolism induced by artificial selection for
511 low aerobic capacity. *American Journal of Physiology-Regulatory Integrative and Comparative*
512 *Physiology* **300**: R175-R182. doi:10.1152/ajpregu.00338.2010.
- 513 Ma, X., Bacci, S., Mlynarski, W., Gottardo, L., Soccio, T., Menzaghi, C. et al. 2004. A common
514 haplotype at the CD36 locus is associated with high free fatty acid levels and increased
515 cardiovascular risk in Caucasians.[Erratum appears in *Hum Mol Genet.* 2005 Dec
516 15;14(24):3973]. *Human Molecular Genetics* **13**: 2197-205. doi:10.1093/hmg/ddh233.
517 PMID:15282206.

- 518 McFarlan, J.T., Yoshida, Y., Jain, S.S., Han, X.X., Snook, L.A., Lally, J. et al. 2012. In vivo, fatty acid
519 translocase (CD36) critically regulates skeletal muscle fuel selection, exercise performance, and
520 training-induced adaptation of fatty acid oxidation. *Journal of Biological Chemistry* **287**: 23502-
521 23516. doi:10.1074/jbc.M111.315358. PMID:22584574.
- 522 Miller, M., Stone, N.J., Ballantyne, C., Bittner, V., Criqui, M.H., Ginsberg, H.N. et al. 2011.
523 Triglycerides and cardiovascular disease: A scientific statement from the American Heart
524 Association. *Circulation* **123**: 2292-2333. doi:10.1161/CIR.0b013e3182160726.
525 PMID:21502576.
- 526 National Heart Foundation (2010). Guide to management of hypertension 2008.
527 [http://www.heartfoundation.org.au/SiteCollectionDocuments/HypertensionGuidelines2008to2010](http://www.heartfoundation.org.au/SiteCollectionDocuments/HypertensionGuidelines2008to2010Update.pdf)
528 [Update.pdf](http://www.heartfoundation.org.au/SiteCollectionDocuments/HypertensionGuidelines2008to2010Update.pdf): National Heart Foundation.
- 529 Nickerson, J.G., Alkhateeb, H., Benton, C.R., Lally, J., Nickerson, J., Han, X.-X. et al. 2009. Greater
530 transport efficiencies of the membrane fatty acid transporters FAT/CD36 and FATP4 compared
531 with FABPpm and FATP1 and differential effects on fatty acid esterification and oxidation in rat
532 skeletal muscle. *Journal of Biological Chemistry* **284**: 16522-30. doi:10.1074/jbc.M109.004788.
533 PMID:19380575.
- 534 Noel, S.E., Lai, C.-Q., Mattei, J., Parnell, L.D., Ordovas, J.M., and Tucker, K.L. 2010. Variants of the
535 CD36 gene and metabolic syndrome in Boston Puerto Rican adults. *Atherosclerosis* **211**: 210-5.
536 doi:10.1016/j.atherosclerosis.2010.02.009. PMID:20223461.
- 537 Nordestgaard, B.G. and Varbo, A. 2014. Triglycerides and cardiovascular disease. *Lancet* **384**: 626-35.
538 doi:10.1016/S0140-6736(14)61177-6. PMID:25131982.
- 539 Palatini, P., Benetos, A., Grassi, G., Julius, S., Kjeldsen, S.E., Mancia, G. et al. 2006. Identification and
540 management of the hypertensive patient with elevated heart rate: statement of a European Society
541 of Hypertension Consensus Meeting. *Journal of Hypertension* **24**: 603-10.
542 doi:10.1097/01.hjh.0000217838.49842.1e. PMID:16531784.

- 543 Peronnet, F. and Massicotte, D. 1991. Table of nonprotein respiratory quotient: an update. Canadian
544 Journal of Sport Sciences **16**: 23-9. PMID:1645211.
- 545 Pravenec, M., Churchill, P.C., Churchill, M.C., Viklicky, O., Kazdova, L., Aitman, T.J. et al. 2008.
546 Identification of renal Cd36 as a determinant of blood pressure and risk for hypertension. Nature
547 Genetics **40**: 952-4. doi:10.1038/ng.164. PMID:18587397.
- 548 Rauramaa, R., Kuhanen, R., Lakka, T.A., Vaisanen, S.B., Halonen, P., Alen, M. et al. 2002. Physical
549 exercise and blood pressure with reference to the angiotensinogen M235T polymorphism.
550 Physiological Genomics **10**: 71-7. doi:10.1152/physiolgenomics.00050.2002. PMID:12181364.
- 551 Rice, T., An, P., Gagnon, J., Leon, A.S., Skinner, J.S., Wilmore, J.H. et al. 2002. Heritability of HR and
552 BP response to exercise training in the HERITAGE Family Study. Medicine & Science in Sports
553 & Exercise **34**: 972-9. doi:10.1097/00005768-200206000-00011. PMID:12048324.
- 554 Rodrigues, A.C., Sobrino, B., Genvigir, F.D.V., Willrich, M.A.V., Arazi, S.S., Dorea, E.L. et al. 2013.
555 Genetic variants in genes related to lipid metabolism and atherosclerosis, dyslipidemia and
556 atorvastatin response. Clinica Chimica Acta **417**: 8-11. doi:10.1016/j.cca.2012.11.028.
557 PMID:23247049.
- 558 Roepstorff, C., Vistisen, B., Roepstorff, K., and Kiens, B. 2004. Regulation of plasma long-chain fatty
559 acid oxidation in relation to uptake in human skeletal muscle during exercise. American Journal
560 of Physiology-Endocrinology and Metabolism **287**: E696-E705.
561 doi:10.1152/ajpendo.00001.2004.
- 562 Schenk, S. and Horowitz, J.F. 2006. Coimmunoprecipitation of FAT/CD36 and CPT I in skeletal muscle
563 increases proportionally with fat oxidation after endurance exercise training. American Journal of
564 Physiology-Endocrinology and Metabolism **291**: E254-E260. doi:10.1152/ajpendo.00051.2006.
- 565 Smith, B.K., Bonen, A., and Holloway, G.P. 2012. A Dual Mechanism of Action for Skeletal Muscle
566 FAT/CD36 During Exercise. Exercise & Sport Sciences Reviews **40**: 211-217.
567 doi:10.1097/JES.0b013e31825eb263. PMID:22653277.

- Sun, S.S., Chumlea, W.C., Heymsfield, S.B., Lukaski, H.C., Schoeller, D., Friedl, K. et al. 2003. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. *American Journal of Clinical Nutrition* **77**: 331-40. PMID:12540391.
- Talanian, J.L., Holloway, G.P., Snook, L.A., Heigenhauser, G.J.F., Bonen, A., and Spriet, L.L. 2010. Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. *American Journal of Physiology - Endocrinology & Metabolism* **299**: E180-8. doi:10.1152/ajpendo.00073.2010. PMID:20484014.
- Wenz, M., Berend, J.Z., Lynch, N.A., Chappell, S., and Hackney, A.C. 1997. Substrate oxidation at rest and during exercise: effects of menstrual cycle phase and diet composition. *Journal of Physiology & Pharmacology* **48**: 851-60. PMID:9444630.
- Yanai, H., Watanabe, I., Ishii, K., Morimoto, M., Fujiwara, H., Yoshida, S. et al. 2007. Attenuated aerobic exercise capacity in CD36 deficiency. *Journal of Medical Genetics* **44**: 445-447. doi:10.1136/jmg.2007.050070.
- Zderic, T.W., Coggan, A.R., and Ruby, B.C. 2001. Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *Journal of Applied Physiology* **90**: 447-53. PMID:11160041.
- Zhang, B., Sakai, T., Miura, S., Kiyonaga, A., Tanaka, H., Shindo, M. et al. 2002. Association of angiotensin-converting-enzyme gene polymorphism with the depressor response to mild exercise therapy in patients with mild to moderate essential hypertension. *Clinical Genetics* **62**: 328-33. doi:10.1034/j.1399-0004.2002.620414.x. PMID:12372063.
- Zou, A.P. and Cowley, A.W., Jr. 1999. Role of nitric oxide in the control of renal function and salt sensitivity. *Current Hypertension Reports* **1**: 178-86. doi:10.1007/s11906-999-0016-7. PMID:10981063.

593 **Tables**

Draft

594 **Table 1** - Stage 1 clinical characteristics of all participants according to dominant model analysis at both rs1984112 and rs1527479

Variable	rs1527479		<i>P</i>	<i>g</i>	rs1984112		<i>P</i>	<i>g</i>
	C-Allele	TT			G-Allele	AA		
<i>n</i>	24	10			21	13		
Anthropometric measures								
Age (yrs)	22.6±3.6	22.7±3.4	0.955	-	22.0±3.2	23.8 ±3.7	0.14	-
Height (cm)	175.3±10.1	168.7 ± 7.0	0.011	-	173.0 ±10.8	174.0±8.0	0.55	-
Weight (kg)	69.5(46.4)	72.8(24.9)	0.11	0.3	70.5(39.7)	71.9(32.6)	0.244	-0.4
BMI (kg.m ⁻²)	23.4±2.7	23.9±2.1	0.511	-0.2	23.5(9.3)	23.2(7.9)	0.33	-0.5
% Body fat	20.1±8.0	21.4±5.7	0.228	-0.2	18.1(21.8)	23.0(26.9)	0.124	-0.2
WC (cm)	78.0±6.7	78.2±6.2	0.98	0.0	76.3(18.0)	75.4(25.7)	0.72	-0.4
HC (cm)	98.0±5.3	96.2±6.1	0.402	0.3	96.6±5.6	98.9±5.3	0.433	-0.4
WHR	0.80±0.04	0.81±0.05	0.284	-0.2	0.80±0.04	0.80±0.05	0.743	0.0
SBP (mmHg)	114±10	120±7	0.1	-0.6	116±8	114±11	0.806	0.2
DBP (mmHg)	72±6	72±9	0.673	0.0	72±7	72±6	0.741	0.0
MAP (mmHg)	86±6	88±7	0.664	-0.3	87±6	86±7	0.885	0.2
RHR (bpm)	53±9	55±8	0.447	-0.2	56±9	50±8	0.139	0.7
RPP (mmHg.bpm)	6072±1269	6585±860	0.237	-0.4	6504±1135	5770±1135	0.203	0.6
Fasting Plasma^a								
TC (mmol.L ⁻¹)	3.98±0.63	3.81±0.66	0.571	0.3	3.87±0.64	4.02±0.63	0.993	-0.2
HDL-C (mmol.L ⁻¹)	1.36±0.30	1.29±0.44	0.727	0.2	1.35±0.32	1.32±0.40	0.351	0.1
LDL-C (mmol.L ⁻¹)	2.08±0.55	2.00±0.40	0.69	0.2	1.96±0.45	2.22±0.56	0.347	-0.5
TG (mmol.L ⁻¹)	0.80±0.18	0.80±0.19	0.849	0.0	0.73(0.59)	0.82(0.67)	0.01	-0.9
Oral glucose tolerance test (OGTT)								
BG ₀ (mmol.L ⁻¹)	4.9±0.4	5.1±0.3	0.066	-0.5	5.0±0.4	4.9±0.4	0.182	0.2

BG ₁₂₀ (mmol.L ⁻¹)	6.0±1.0	5.8±1.0	0.634	0.2	6.1±1.0	5.6±0.9	0.353	0.5
AUC (mmol.L ⁻¹ .hr ⁻¹)	14.2±1.5	13.5±2.2	0.282	0.4	14.0±1.9	14.0±1.5	0.854	0.0
Exercise variables								
VO _{2peak} (L.min ⁻¹)	3.1±0.6	3.1±0.8	0.95	0.0	3.0±0.7	3.2±0.7	0.362	-0.3
W _{peak} (Watts)	258(221)	271(229)	0.978	-0.1	258±65	269±64	0.996	-0.2

595 **Note:** Data presented as means ± SDs. Non-normally distributed data presented as median (range). BMI, body mass index; WC, waist
596 circumference; HC, hip circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial
597 pressure; RHR, resting heart rate; RPP, rate-pressure product; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-
598 density lipoprotein cholesterol; TG, triglyceride; FBG, fasting blood glucose; BG₁₂₀, blood glucose at 120-min of the OGTT; VO_{2peak}, peak
599 oxidative capacity; W_{peak}, peak power output; Fat_{ox0}, whole body fat oxidation rate at rest. ^aMetabolite readings outside the range of the
600 CardioChek® analyser were entered as the closest limit value for the respective assay.

601 **Table 2** - Training-induced adaptations of dependent variables for dominant model analysis at rs1527479.

Variable <i>n</i>	C-Allele 11	TT 6	Mean Difference (95% CI)	<i>P</i> _{gxt}	<i>g</i>
Anthropometric measures					
Weight (kg)					
<i>Baseline</i>	76.2±12.7	69.1±10.0			
<i>Response</i>	0.4±2.3	-1.4±2.3	-1.8 (-11.1, 14.6)	0.233	-0.1
BMI (kg.m⁻²)					
<i>Baseline</i>	24.0±3.2	23.6±2.1			
<i>Response</i>	0.1±0.7	-0.5±0.8	0.6 (-2.6, 3.7)	0.164	-0.1
% Body fat					
<i>Baseline</i>	18.3±8.4	21.0±4.6			
<i>Response</i>	0.6±1.8	-0.1±3.9	0.7 (-7.2, 8.6)	0.69	0.1
WC (cm)					
<i>Baseline</i>	80.8±8.2	77.8±7.7			
<i>Response</i>	0.0±2.7	-1.8±2.5	1.8 (-6.8, 10.6)	0.279	0.2
HC (cm)					
<i>Baseline</i>	99.0±6.5	96.3±4.0			
<i>Response</i>	-1.0±3.2	-1.4±2.9	0.4 (-5.8, 6.6)	0.616	0.1
WHR					
<i>Baseline</i>	0.82±0.03	0.82±0.06			
<i>Response</i>	0.01±0.03	-0.01±0.01	0.02 (-0.03, 0.06)	0.346	0.4
SBP (mmHg)					
<i>Baseline</i>	120±7	120±6			
<i>Response</i>	-1±5	-2±7	1 (-6, 8)	0.625	0.1
DBP (mmHg)					
<i>Baseline</i>	74±7	74±8			
<i>Response</i>	4±9	-3±6	7 (-1, 15)	0.016	0.9
MAP (mmHg)					
<i>Baseline</i>	89±5	90±7			
<i>Response</i>	3±6	-3±6	6 (-1, 11)	0.028	0.8
RHR (bpm)					
<i>Baseline</i>	54±7	56±9			
<i>Response</i>	-2±5	-2±8	0 (-8, 9)	0.954	0.1
RPP (mmHg.bpm)					

	Baseline	6549±964	6710±927			
	Response	-265±718	-362±1022	106 (-932, 1127)	0.959	0.1
Fasting plasma ^a						
TC (mmol.L ⁻¹)						
	Baseline	3.98±0.70	3.83±0.76			
	Response	-0.39±0.50	-0.04±0.59	-0.35 (-1.14, 0.43)	0.394	-0.5
HDL-C (mmol.L ⁻¹)						
	Baseline	1.24±0.23	1.35±0.57			
	Response	-0.06±0.09	0.02±0.28	-0.08 (-0.48, 0.34)	0.22	-0.2
LDL-C (mmol.L ⁻¹)						
	Baseline	2.22±0.72	2.00±0.49			
	Response	-0.28±0.58	-0.05±0.76	-0.23 (-0.95, 0.47)	0.725	-0.3
TG (mmol.L ⁻¹)						
	Baseline	0.86±0.23	0.84±0.21			
	Response	0.17±0.24	-0.08±0.17	0.25 (0.01, 0.49)	0.009	1.1
Oral glucose tolerance test (OGTT)						
BG ₀ (mmol.L ⁻¹)						
	Baseline	4.8±0.4	5.1±0.3			
	Response	0.2±0.6	-0.3±0.5	0.5 (0.0, 0.9)	0.807	1.1
BG ₁₂₀ (mmol.L ⁻¹)						
	Baseline	6.0±0.9	5.9±0.5			
	Response	-0.4±1.0	-0.1±1.1	-0.3 (-1.2, 0.5)	0.682	-0.4
AUC (mmol.L ⁻¹ .hr ⁻¹)						
	Baseline	14.2±1.3	13.6±1.2			
	Response	0.2±1.1	0.2±1.0	0.0 (-1.3, 1.4)	0.567	0.0
Exercise Variables						
VO _{2peak} (L.min ⁻¹)						
	Baseline	3.2±0.6	3.1±0.8			
	Response	0.2±0.3	0.1±0.2	0.1 (-0.6, 0.9)	0.149	0.2
W _{peak} (Watts)						
	Baseline	272±60	259±69			
	Response	28±19	21±13	7 (-61, 75)	0.459	0.1

Note: Data presented as means ± SDs. Non-normally distributed data presented as median (range). BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; RHR, resting heart

605 rate; RPP, rate-pressure product; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol;
606 LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; BG, blood glucose; BG_{XX}, blood glucose at
607 XX min of the OGTT; VO_{2peak}, peak oxidative capacity; W_{peak}, peak power output. ^aMetabolite readings
608 outside the range of the CardioChek® analyser were entered as the closest limit value for the respective
609 assay. Adjustment was for age, sex, VO_{2peak} and baseline reading of the corresponding dependent
610 variable.

611

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612 **Table 3** - Training-induced adaptations of dependent variables for dominant model analysis at rs1984112.

Variable		G-Allele	AA	Mean Difference	P_{gxt}	g
n		11	6	(95% CI)		
Anthropometric measures						
Weight (kg)						
	Baseline	69.7±12	80.9±8			
	Response	-0.1±2.9	-0.3±1.2	0.2 (-11.8, 12.1)	0.823	0.4
BMI (kg.m ⁻²)						
	Baseline	22.7±2.0	26.0±3.0			
	Response	-0.1±0.9	-0.1±0.4	0.0 (-2.6, 2.6)	0.693	0.0
% Body fat						
	Baseline	18.3±4.8	21.1±10.7			
	Response	-0.1±2.7	1.2±2.5	-1.3 (-9.2, 6.6)	0.461	-0.2
WC (cm)						
	Baseline	77.1±6.2	84.7±8.9			
	Response	-1.0±2.8	0.1±2.5	-1.1 (-8.9, 6.7)	0.56	-0.2
HC (cm)						
	Baseline	96.2±5.0	95.0±5.9			
	Response	-1.2±3.7	-1.1±1.4	-0.1 (-5.8, 5.6)	0.993	0.0
WHR						
	Baseline	0.80±0.04	0.83±0.05			
	Response	-0.00±0.03	0.01±0.02	-0.01 (-0.05, 0.04)	0.366	-0.2
SBP (mmHg)						
	Baseline	119±6	122±6			
	Response	-1±6	-2±5	1 (-6, 7)	0.72	0.1
DBP (mmHg)						
	Baseline	74±7	75±8			
	Response	1±8	3±11	-2 (-10, 6)	0.444	-0.3
MAP (mmHg)						
	Baseline	89±6	90±6			
	Response	0±6	2±7	-2 (-8, 5)	0.559	-0.2
RHR (bpm)						
	Baseline	58±6	49±6*			
	Response	-1±6	-3±6	2 (-5, 8)	0.365	0.2
RPP (mmHg.bpm)						

	<i>Baseline</i>	6922±802	6026±910			
	<i>Response</i>	-224±873	-436±724	212 (-696, 1120)	0.278	0.2
Fasting plasma^a						
TC (mmol.L⁻¹)						
	<i>Baseline</i>	3.86±0.72	4.05±0.72			
	<i>Response</i>	-0.17±0.49	-0.46±0.63	0.29 (-0.49, 1.07)	0.208	0.4
HDL-C (mmol.L⁻¹)						
	<i>Baseline</i>	1.38±0.38	1.09±0.31			
	<i>Response</i>	-0.03±0.21	-0.03±0.11	0.00 (-0.39, 0.38)	0.024	0.0
LDL-C (mmol.L⁻¹)						
	<i>Baseline</i>	1.96±0.58	2.48±0.68			
	<i>Response</i>	-0.08±0.55	-0.42±0.78	0.34 (-0.32, 1.01)	0.636	0.5
TG (mmol.L⁻¹)						
	<i>Baseline</i>	0.77±0.15	1.00±0.25†			
	<i>Response</i>	0.03±0.21	0.18±0.28	-0.15 (-0.36, 0.05)	0.336	-0.8
Oral glucose tolerance test (OGTT)						
BG₀ (mmol.L⁻¹)						
	<i>Baseline</i>	4.8±0.5	5.0±0.3			
	<i>Response</i>	-0.0±0.6	0.1±0.6	-0.1 (-0.6, 0.3)	0.646	-0.3
BG₁₂₀ (mmol.L⁻¹)						
	<i>Baseline</i>	6.2±0.8	5.4±0.5			
	<i>Response</i>	-0.2±1.1	-0.4±0.9	0.2 (-0.6, 1.0)	0.423	0.3
AUC (mmol.L⁻¹.hr⁻¹)						
	<i>Baseline</i>	13.7±1.1	14.6±1.5			
	<i>Response</i>	0.0±1.0	0.5±1.1	-0.5 (-1.8, 0.8)	0.261	-0.4
Exercise Variables						
VO_{2peak} (L.min⁻¹)						
	<i>Baseline</i>	3.0±0.8	3.4±0.4			
	<i>Response</i>	0.2±0.2	0.2±0.3	0.0 (-0.7, 0.7)	0.862	0.0
W_{peak} (Watts)						
	<i>Baseline</i>	253±68	294±40			
	<i>Response</i>	26±19	23±15	3 (-62, 68)	0.951	0.1

Note: Data presented as means ± SDs. Non-normally distributed data presented as median (range). **P* =

0.027; †*P* = 0.029, BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR,

waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial

pressure; RHR, resting heart rate; RPP, rate-pressure product; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; BG, blood glucose; BG_{XX}, blood glucose at XX min of the OGTT; VO_{2peak}, peak oxidative capacity; W_{peak}, peak power output. ^aMetabolite readings outside the range of the CardioChek® analyser were entered as the closest limit value for the respective assay. Adjustment was for age, sex, VO_{2peak} and baseline reading of the corresponding dependent variable.

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Table 4 - Summary of significant cross-sectional associations between SNPs rs1527479 and rs1984112 in a young-adult Australian cohort to date - similarities and differences from a previous data in an identically recruited cohort.

Current Study (N = 34)	Jayewardene et al. (2014) (N=22)
rs1984112	
• Fat_{ox20} : GG ↑ GA*	• Fat_{ox20} : NS
• Fat_{ox30} : GG ↑ GA*	• Fat_{ox30} : NS
• Fat_{ox40} : GG ↑ GA & AA*	• Fat_{ox40} : NS
• DBP : NS	• DBP : AA ↓ GA & G-Allele
• RHR : NS	• RHR : GG ↑ GA & AA
rs1527479	
• Fat_{ox40} : TT ↑ C-allele & CT*	• Fat_{ox40} : TT ↓ CC
• RHR : NS	• RHR : TT ↑ C-allele
• RPP : TT tended ↑ CC & CT*	• RPP : TT ↑ C-allele

Note: DBP, diastolic blood pressure; Fat_{oxXX}, whole body fat oxidation rate at XX% VO_{2peak}; NS, not significant; RHR, resting heart rate; RPP, rate-pressure product. *Genotypic analysis included in the supplementary material.

Figure Captions

Fig. 1 Whole body rate of fat oxidation (Fat_{ox}) data stratified by dominant-allele model analysis; **(a)** Stage 1 cross-sectional absolute data for C-Allele ($n = 24$) and TT carriers ($n = 10$) at rs1527479, $*P < 0.05$; **(b)** Change (Δ) data following training intervention stratified at C-Allele ($n = 11$) and TT carriers ($n = 6$) at rs1527479; **(c)** Stage 1 cross-sectional absolute data for G-Allele ($n = 21$) and AA carriers ($n = 13$) at rs1984112; **(d)** Change (Δ) data following training intervention stratified at G-Allele ($n = 11$) and AA carriers ($n = 6$) at rs1984112, $**P_{\text{ext}} < 0.05$. Data presented as means $\pm$ SD. Non-normally distributed data presented as median (range). $\text{Fat}_{\text{ox}0}$, whole body fat oxidation rate at rest; $\text{Fat}_{\text{ox}XX}$, whole body fat oxidation rate at XX% $\text{VO}_{2\text{peak}}$. Adjustment was for age, sex, $\text{VO}_{2\text{peak}}$ and baseline reading of the corresponding dependent variable

Fig. 2 Oral glucose tolerance (OGTT) data stratified by dominant-allele model analysis; **(a)** Stage 1 cross-sectional absolute data for C-Allele ($n = 24$) and TT carriers ($n = 10$) at rs1527479, $*P < 0.05$; **(b)** Change (Δ) data following training intervention stratified at in C-Allele ($n = 11$) and TT carriers ($n = 6$) at rs1527479; **(c)** Stage 1 cross-sectional absolute data for G-Allele ($n = 21$) and AA carriers ($n = 13$) at rs1984112; **(d)** Change (Δ) data following training intervention stratified at G-Allele ($n = 11$) and AA carriers ($n = 6$) at rs1984112, $**P_{\text{ext}} < 0.05$. Data presented as means $\pm$ SD. Non-normally distributed data presented as median (range). Adjustment was for age, sex, $\text{VO}_{2\text{peak}}$ and baseline reading of the corresponding dependent variable

144x114mm (300 x 300 DPI)

143x112mm (300 x 300 DPI)