The plateau at $\dot{V}O_{2max}$ is associated with anaerobic alleles

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Abstract

Objectives: This study tests the hypothesis that individuals who achieve a plateau at $\dot{V}O_{2max}$ ($\dot{V}O_{2plat}$) are more likely to possess alleles, associated with anaerobic capacity, than those who do not.

Design: A literature survey, physiological testing and genetic analysis was used to determine any association between the aerobic and anaerobic polymorphisms of 40 genes and $\dot{V}O_{2plat}$.

Methods: 34, healthy, Caucasian volunteers, completed an exercise test to determine $\dot{V}O_{2max,}$ and $\dot{V}O_{2plat}$. 28 of the volunteers agreed to DNA testing and 26 were successfully genotyped. A literature search was used to determine whether the 40 polymorphisms analysed were associated with aerobic, or anaerobic exercise performance.

Results: The literature survey enabled classification of the 40 target alleles as aerobic [11], anaerobic [24], or having no apparent association (NAA) [5] with exercise performance. It also found no previous studies linking a genetic component with the ability to achieve $\dot{V}O_{2plat}$. Independent *t*-tests showed a significant difference ($p < 0.001$) in the ability to achieve $\dot{V}O_{2plat}$, but no other measured physiological variable was significantly different. Pearson's χ^2 testing demonstrated a highly significant association ($p = 0.008$) between anaerobic allele frequency and $\dot{V}O_{2plat}$, but not with $\dot{V}O_{2max}$. There was no association between aerobic alleles and $\dot{V}O_{2plat}$, or $\dot{V}O_{2max}$. Finally there were no significant differences in the allelic frequencies, observed in this study and those expected of Northern and Western European Caucasians.

Conclusion: These results support the hypothesis that the ability to achieve $\dot{V}O_{2plat}$ is associated with alleles linked to anaerobic exercise capacity.

Key Words;

Polymorphism

Oxygen consumption,

Anaerobic capacity

Exercise performance

1. Introduction

Factors contributing to athletic performance are complex, involving the interaction of numerous factors including training methods, psychology, technology, diet, and genetics.[1] Of these, genetic factors are a major component, with overall heritability of athletic status estimated at *ca*. 66%. Despite this no single gene, or polymorphism, has been definitely associated with elite athletes, in any given sport.[2] Accordingly identifying genetic variants that contribute to athletic success has been challenging with at least 200 genetic polymorphisms, both nuclear and mitochondrial, been associated with athletic achievement.[3] Recent studies have primarily focused on either endurance,[3] or power (sprint) performance [4] and their associated polymorphisms. Typically such studies have concentrated on relatively few genes (e.g. ACE, ACTN3, AMPD1, NOS3, PGC1A, PPARG), however conflicting findings, even within the same populations, means their exact influence has not been fully resolved.[1]

An important contributor to athletic performance is maximal oxygen uptake ($\dot{V}O_{2max}$), which is a measure of aerobic power and cardio-respiratory fitness.[3,5] Classically $\dot{V}O_{2max}$ is based on the levelling off, or plateau, in oxygen uptake, despite a continued increase in exercise intensity.[5] However many participants fail to reach $\dot{V}O_{2plat}$, for a variety of reasons, including experimental methodology, modelling approaches and populations tested.[5] Previous research has also attributed the ability to attain a $\dot{V}O_{2plat}$ to a greater reliance, in some individuals, on oxygen-independent (anaerobic) metabolism,[6] also referred to as "anaerobic capacity". Green[7] defined anaerobic capacity as "the maximal amount of ATP resynthesised *via* anaerobic metabolism during a specific type of short duration maximal exercise". Examination

of power–duration relationships shows that exercise, above $\dot{V}O_{2max,}$ involves an increased recruitment of type II muscle fibres, to maintain power output.[8] Accordingly it is reasonable to expect that individuals with greater anaerobic capacity and/or an increased proportion of type II muscle fibres will perform better in short duration sprint-type activities. Moreover they should also be able to increase their power output, when already working at $\dot{V}O_{2max}$ and more readily achieve a $\dot{V}O_{2plat.}$[7,8] Clearly the ability to increase work rate when already at $\dot{V}O_{2max}$, using anaerobic metabolism, can be an important determinant of athletic performance. Thus in 5km and 8km races, finish place and run-time is correlated with anaerobic, rather than aerobic capacity.[9] Hence it is surprising that there are no studies, to date, which have investigated a genetic component to the ability to achieve a $\dot{V}O_{2plat.}$[1,3]

Accordingly the aim of this study is to test the hypothesis that individuals who achieve $\dot{V}O_{2plat}$ are more likely to possess polymorphisms, associated with increased anaerobic capacity, than those who do not.

2. Methods

Following institutional ethical approval (Faculty of Science and Engineering Research Ethics Panel, Anglia Ruskin University, UK. FST/FREP/12/339), 34 recreationally competitive, Caucasians (29 males, 5 females; age = 27.5 ± 3.29 years, mass = 73.1 ± 10.8 kg, stature = 179 ± 8 cm), recruited from a student population, volunteered for this study. All participants were provided with full, written, information about the experimental procedures and any associated risks before signing an informed consent form, as per the Helsinki declaration 1975 (revised 2013), to permit their participation. Participants also completed a pre-exercise medical questionnaire to eliminate any with history of cardiopulmonary diseases, diabetes, or recent (within 3

126 months) musculoskeletal injuries. Finally all participants were asked to read and sign
127 a second informed consent form giving permission to sample and test their DNA for
128 specific polymorphisms.
129 Before testing participants were instructed that they should not eat for 3 hours before
130 testing and ensure that they arrived in a well-hydrated state, without having consumed
131 alcohol, or caffeine, for 24 hours. They were also requested not to complete any
132 heavy training sessions, within 48 hours, either side of testing. All participants
133 attended a laboratory habituation visit to familiarise themselves with test equipment
134 and procedures. During this visit each participant's preferred seat and handlebar
135 heights was recorded for their subsequent test visit.
136 Prior to all trials a metabolic cart (Metalyzer 3B, Cortex, Leipzig, Germany)
137 was calibrated for both volume and flow using a 3 L syringe (Hans Rudolph, Kansas,
138 USA), to establish linearity and reproducibility. Additionally a two-point gas
139 calibration was undertaken using 15% CO_2 and 0% O_2 in balanced nitrogen (BOC,
140 Nottingham, UK) and ambient O_2.[5,6] All exercise testing was performed using a pre-
141 calibrated cycle ergometer (Lode, Excalibur Sport, Groningen, Netherlands. A low
142 resistance turbine and facemask was used to determine respiratory volumes and flow
143 rates. Using a sampling rate of 60 $ml \cdot min^{-1}$, expired O_2, CO_2 and N_2 concentrations
144 were measured, while being drawn, directly, from the turbine assembly, into the
145 metabolic cart. Gas concentrations and respiratory kinetics were aligned using custom
146 metabolic cart software, allowing calculation of gas exchange variables ($\dot{V}O_2$, $\dot{V}CO_2$,
147 $\dot{V}_E$ and RER). Heart rate was continually monitored, throughout each exercise trial,
148 using a short-range telemetric monitoring system (Polar 810s, Kemple, Finland).[5]
149 Immediately after each $\dot{V}O_{2max}$ trial capillary blood samples (5 µl) were collected, for
150 lactate analysis (GM7 Micro-Stat analyser, Analox Instruments, UK). As before, the

Micro-Stat analyser was calibrated, as per manufacturers' instructions, before analysis, with all samples being measured immediately upon collection.[10]

To determine $\dot{V}O_{2max}$ and associated cardio-respiratory responses, participants completed an incremental exercise stress test, to volitional exhaustion, on a pre-calibrated cycle ergometer, using a ramp rate of 0.42 $W \cdot s^{-1}$, with a starting resistance of 50 W (females), 100 W (males), at a minimum cadence of 60 rpm. Tests were terminated, either through volitional withdrawal, or if cadence decreased by > 5 rpm of that prescribed, despite strong verbal encouragement. Throughout the course of the test, expired air and gas exchange variables were recorded on a breath-by-breath basis. Prior to the incremental test the participants undertook a self-selected warm-up with a duration of 5.2 ± 0.8 min. All testing protocols were in accordance with previous work.[5,6,10] A confirmation of $\dot{V}O_{2max}$ was determined by the participant recording a $\Delta\dot{V}O_2$ of ≤ 1.5 $ml \cdot kg^{-1} \cdot min^{-1}$ across the final 2, consecutive, 30-breath sampling periods.[5,10] Additional (secondary) methods were employed to confirm a maximal effort, namely a respiratory exchange ratio (RER) ≥ 1.15; maximal heart rate (HR_{max}) of > 205.9 – 0.685·age and peak blood lactate (pBLa) ≥ 8.0 mmol.[5,10]

For DNA testing participants were instructed not to eat/drink/smoke/clean teeth for 3 h prior to sampling. On arrival they were given a coded, sterile, plastic tube and a cotton wool swab-stick (FitnessGenes Ltd, DiagnOx Laboratory, 77 Heyford Park, Bicester, OX255HD, UK). This was used to collect a sample of buccal cells, by rubbing it against the inside of the cheek for 1 min, before sealing in the coded tubes, which were immediately sent to FitnessGenes Ltd., for genetic analysis.

DNA was extracted using Qiagen DNA Blood Mini Kits. Samples were analysed, using allele-specific PCR[11], for total of 40 putative exercise-associated genes. Primers (Appendix A.1) were designed using Oligo Explorer 1.5 software and

checked for uniqueness using the NCBI BLASTW search engine (https://blast.ncbi.nlm.nih.gov/Blast.cgi). PCR was performed using a thermal cycler (Eppendorf Mastercycler Gradient; Eppendorf, Hamburg, Germany). The final volume of all PCR protocols was 25 µl. PCR conditions were as follows: initial denaturing at 95°C, 10 min.; 35 cycles at 95°C, 1 min.; 52 °C, 45 s; 72°C, 1 min.; with final extension at 72°C, 5 min. PCR products were subject to restriction enzyme digest and visualised by gel electrophoresis, using 1.2% agarose gels, for verification. Replicate samples were checked and if there was a mismatch, the genotyping was repeated. If, on repetition, no match was found, the sample was excluded from the final dataset. For the insertion/deletion (I/D) ACE polymorphism an indirect detection method was used, based on genotyping rs4341 (C/G; Appendix A.1), which is in total linkage disequilibrium with the I/D polymorphism.[12]

A literature search was made of journals in PubMed, Google Scholar and Web of Science databases to determine which alleles of the 40 target alleles were associated with aerobic, or anaerobic performance, or whether they had NAA with exercise performance. Key words included the names of each gene and their SNPs (Appendix A.1), together with the terms: athlete, sport, exercise, physical performance, endurance, muscle, power, strength, sprint, aerobic, anaerobic, $\dot{V}O_{2max}$, plateau and maximal oxygen consumption. Exclusion criteria were animal-based studies, articles not published in English and articles published before the year 2000.

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS; Version 21.0, Chicago, Illinois, USA). Shapiro-Wilks and Levine tests showed all physiological and gas exchange data was normally distributed and with the exception of $\Delta\dot{V}O_2$, to display homogeneity of variance. Two-tailed independent *t*-tests were used to test the null hypothesis that there were no differences

between physiological data from $\dot{V}O_{2plat}$ achievers and non-achievers. A further independent *t*-test was used to test the same null hypothesis for $\Delta\dot{V}O_{2,}$ but assuming unequal variance. Power calculations ($\alpha = 0.05$, n = 34) suggested a statistical power > 95% for these analyses. Frequencies of alternative alleles, for each gene, were calculated using SPSS "Crosstabs" function. Crosstabs also allowed Pearson's χ^2 tests to determine whether the observed allelic frequencies of the participants differed from those of a wider, comparable, European population. Here expected allelic frequencies, calculated from data for Caucasians of Northern and Western European ancestry (HapMap-CEU genetic database: https://www.ncbi.nlm.nih.gov/SNP/index.html), were compared with the allelic frequencies found in this study. Allelic frequencies, for each gene, were also compared with $\dot{V}O_{2plat}$ and $\dot{V}O_{2max}$, using Pearson's χ^2 tests on, either 3 × 2 contingency tables, where all 3 possible allelic combinations were present, or 2 x 2 contingency tables for genes where only 2 of the possible 3 allelic combinations were observed. These analysis tested the null hypotheses that each gene's allelic frequency had no association with either $\dot{V}O_{2plat}$ achievement, or with achievement of a "low", or "high" $\dot{V}O_{2max}$. The latter been defined as being lower, or higher, than the final group's mean $\dot{V}O_{2max}$ (53.6 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$). Results from these analyses enabled the 40 polymorphisms to be tabulated in order of the magnitude of their p - values, from lowest to highest, with respect to the allele's association with $\dot{V}O_{2plat}$ achievement and $\dot{V}O_{2max}$ magnitude. Further 3 × 2 (2 x 2, excluding those genes with NAA) χ^2 tests were performed to assess the distribution of aerobic and anaerobic alleles within the first 20 and second 20 positions, within these tables, with respect to p - values, for both $\dot{V}O_{2plat}$ and magnitude of $\dot{V}O_{2max}$. Finally a regression analysis was performed to determine any relationship between the values of $\dot{V}O_{2max}$ and $\Delta\dot{V}O_{2.}$

3. Results

A total of 2627 articles were identified. After deduplication, 2073 articles were scanned, based on title and abstract, following which a further 1986 articles were excluded. The remaining 87 articles enabled the 40 different polymorphisms to be classified either as primarily aerobic (11), anaerobic (24), or having NAA on exercise performance (5) (Table A.1). Alleles associated with muscle size/power and/or sprint performance were classified as "anaerobic", whilst those associated with endurance performance, or increased $\dot{V}O_{2max}$, were classified as "aerobic". For some genes (e.g. ACE and ACTN3) their alternative alleles could be classified as either aerobic, or anaerobic.[20] In this instance classification was made on the basis of the allele that appeared to have the greatest influence. Critically no references were found linking any genes, or their alleles, with $\dot{V}O_{2plat}$ achievement.

TABLE A.1

Of the 34 participants 19 (56%) achieved $\dot{V}O_{2plat}$, with both achievers and non-achievers also meeting the various secondary criteria (RER_{max}, HR_{max} and pBLa), to confirm $\dot{V}O_{2max}$. Only $\Delta\dot{V}O_2$ was significantly different ($p < 0.001$) for achievers (0.8 ± 0.39 $ml.kg^{-1}.min^{-1}$) and non-achievers (2.2 ± 0.68 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$), respectively (Table A.2). Furthermore there were no significant differences for any other measured response variable: Time at $\dot{V}O_{2max}$ (s); $\dot{V}CO_{2max}$ ($l{\cdot}min^{-1}$); $\dot{V}CO_{2max}$ ($ml{\cdot}kg^{-1}{\cdot}min^{-1}$); Time at $\dot{V}CO_{2max}$ (s); RER at $\dot{V}O_{2max}$; Time at RER_{max} (s); V_{Emax} ($l{\cdot}min^{-1}$); End time (s) (data not shown). Regression analysis (data not shown) found no relationship ($r^2 = 0.044$) between the magnitude of $\dot{V}O_{2max}$ and $\Delta\dot{V}O_2$.

TABLE A.2

28 participants agreed to DNA testing and of these 26 participants were successfully genotyped (14 plateau achievers (54%) and 12 non-plateau achievers). This final group was identical to the original group in terms of their physiological responses (Table A.2). Crucially comparisons of their allelic frequencies, with frequencies obtained from the HapMap-CEU genetic database showed no significant differences (Table A.1).

Table A.3 shows that individuals who achieve $\dot{V}O_{2plat}$ are much more likely to possess polymorphisms associated with anaerobic performance than those who do not. Thus of the first 20 of the 40 genes tested (Table A.3) 16 had alleles classified as "anaerobic", 2 "aerobic" and 2 "NAA", whilst in the second 20, 8 alleles are classified as "anaerobic", 9 "aerobic" and 3 "NAA" ($\chi^2 = 7.32$; $p = 0.026$). Excluding those genes classified as having NAA ($\chi^2 = 7.10$; $p = 0.008$) demonstrated that such a distribution of anaerobic alleles was highly unlikely to occur by chance.

TABLE A.3

Manual inspection of the association of the alleles of genes (ACTN3, IL6, ADRB213, PPARG: Table A.3) with a significant, or very close to a significant association, with $\dot{V}O_{2plat}$, (Table A.3) showed that for ACTN3 (C), IL6 (G) and PPARG (G), it was the anaerobic allele that predominated. Thus for ACTN3 the homozygous, CC, genotype was associated with 11 plateau achievers and only 3 non-achievers. For IL6 the homozygous, GG, genotype was associated with 9 plateau achievers and only 3 non-achievers. In the case of PPARG, where only 2 of the 3

possible genotypes (CC and CG) were recorded, it was the CG genotype that was associated with plateau achievement. Finally the “aerobic” "ADRB213, (Table A.1), also showed a significant relationship with $\dot{V}O_{2plat}$ (Table A.3), where the AA (3) and GA (10) genotypes were associated with $\dot{V}O_{2plat}$, compared with 5 non-achievers.

In contrast to the results for $\dot{V}O_{2plat}$, there was no significant association of aerobic alleles with $\dot{V}O_{2max}$ ($\chi^2 = 0.29$; $p = 0.86$) (Table A.3). Excluding those genes with NAA had no effect ($\chi^2 = 0.06$; $p = 0.81$). Only CYP1A2 showed a significant relationship with a higher than average $\dot{V}O_{2max}$, with the aerobic PCG1A and ACE genes, being close to significance. For CYP1A2 the AA genotype was present in 13 above average $\dot{V}O_{2max}$ performers and one below average performer.

4. Discussion

It is well-established that the ability to attain $\dot{V}O_{2plat}$ is inconsistent, with different studies reporting considerable variation in attainment. Possible explanations include methodology, such as $\dot{V}O_2$ sampling intervals, protocol duration, modelling approaches and populations tested.[5,6] There is also evidence that the ability to achieve $\dot{V}O_{2plat}$ has a physiological component, namely anaerobic capacity.[6] Such a physiological component would, by necessity, be underpinned by a genetic component.[4,17] The results of this study strongly support this hypothesis by demonstrating that possession of anaerobic alleles showed a highly significant association with $\dot{V}O_{2plat}$ attainment (Table A.3). Of those genes showing a significant relationship, ACTN3 [4,13] showed the highest level of significance ($p = 0.012$). ACTN3 codes for α-Actinin-3, a protein expressed in fast glycolytic type II fibres, which are responsible for rapid and powerful contractions during anaerobic activities, such as

sprinting and weightlifting.[13] A common variant R577X (rs1815739 C/T) of this gene results in the replacement of an arginine codon (Arg or R) with a stop codon, (X), producing a non-functional α-actinin-3 protein. This slows the anaerobic metabolism of type II fibres, causing a shift toward increased oxidative metabolism.[27] Since achieving $\dot{V}O_{2plat}$ means increasing work rate, without further increase in oxygen consumption,[5] it is not surprising that type II fibres, which can function under hypoxic conditions, such as are encountered at $\dot{V}O_{2plat}$, could contribute to $\dot{V}O_{2plat}$ achievement.

IL6, which also showed a significant association with $\dot{V}O_{2plat,}$ is expressed in muscle cells, where it appears to have a role in hypertrophic muscle growth.[17] As with this study, previous work reports that frequencies of the G allele were significantly higher in power athletes, compared with endurance athletes.[17]

The ability of muscles to operate under hypoxic conditions also provides an explanation for the significant association seen between ADRB213 and $\dot{V}O_{2plat}$. Although ADRB213 was classified as "aerobic" (TableA.1),[1,2] there is good evidence to suggest that it also confers an advantage when exercising under hypoxic conditions,[28] such as those that will occur at $\dot{V}O_{2plat}$. Here Tsianos et al.[28], studying the Mount Olympus Marathon, which reaches an altitude of 2,690 m and represents a significant hypoxic challenge, found that A-allele of ADRB213 was associated with faster completion times.

In contrast to the strong association between anaerobic alleles and $\dot{V}O_{2plat}$ (Table A.3), there was no significant association with aerobic allelic frequency and $\dot{V}O_{2max}$ (Table A.3). Only one gene, CYP1A2, showed a significant association with $\dot{V}O_{2max}$ (Table A.3). CYP1A2 polymorphisms have been the subject of numerous studies because of their role in the metabolism of caffeine.[29]. Here the SNP in the CYP1A2 gene (163C>A; rs762551) is responsible for the haplotype which confers a faster capacity

to metabolize caffeine on AA homozygotes.[30] Rapid production of these metabolites is believed to be responsible for performance-enhancing effects of caffeine among AA homozygotes.[29] With respect to the significant association with CYP1A2 and $\dot{V}O_{2max}$ seen in this study, specifically the AA genotype, there is also increasing evidence that the AA genotype is overrepresented in endurance athletes,[20] supporting the findings of this study.

Finally the allelic distribution of participants, in this study, did not differ significantly from those of Caucasians of Northern and Western European ancestry. This suggests the findings, described above, are likely applicable to this wider population group. Accordingly further research is required to confirm these findings and to determine whether they apply to different population groups.

5. Conclusion

This study has demonstrated that:

- Allelic frequencies, of the participants, are representative of the wider Northern and Western European Caucasian population.
- There is a statistically significant association of anaerobic alleles with $\dot{V}O_{2plat}$ attainment.
- Hence the ability to $\dot{V}O_{2plat}$. has a genetic component.

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Author Declaration of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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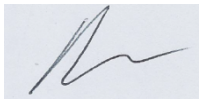
Don Keiller 

Dan Gordon 

Table A.1 Genes, single nucleotide polymorphisms (SNP) and classification according to whether a specific nucleotide is associated with anaerobic, aerobic performance, or having no apparent association (NAA). A, T, G and C are the SNP-specific nucleotide bases. Performance associated bases in bold. χ^2 values (χ^2 Freq) and p - values are for the allelic frequencies obtained in this study, compared with expected Northern and Western European Caucasian frequencies.

Gene	SNP	Anaerobic	Aerobic	Reference	χ^2 Freq	p-value
ACE	rs4341	G	**C**	13	2.57	0.28
ACTN3	rs1815739	**C**	T	13	3.62	0.16
ACVR1B*	rs2854464	**A**	G	14	0.02	0.99
ADRB213	rs1042713	G	**A**	2	0.08	0.96
ADRB214	rs1042714	**G**	C	2	0.09	0.96
AGT	rs699	**C**	T	14	5.69	0.06
AKT1	rs1130214	G	**T**	4	0.09	0.96
AMPD1	rs17602729	**C**	T	15	0.49	0.78
APOA2	rs5082	NAA	NAA	16	1.68	0.43
APOA5*	rs662799	NAA	NAA	16	2.06	0.36
BDKRB2	rs1799722	C	**T**	17	0.09	0.96
CKM	rs8111989	**G**	A	18	1.18	0.55
Clock	rs1801260	NAA	NAA	19	0.51	0.79
CNTF	rs1800169	**G**	A	14	0.49	0.78
CYP1A2	rs762551	C	**A**	20	0.50	0.78
ESR1	rs722208	**A**	G	21	0.25	0.88
FTO	rs9939609	**T**	A	22	2.32	0.31
HIF1A	rs11549465	**T**	C	17	1.2	0.55
IGF1-35*	rs35767	**T**	C	2	2.4	0.30
IGF1-71	rs7136446	**C**	T	23	0.81	0.67
IGFBP-3	rs2854744	**C**	A	23	2.62	0.27
IL15RA	rs2296135	**A**	C	17	2.41	0.31
IL6	rs1800795	**G**	C	17	3.32	0.19
IL6R	rs2228145	**C**	A	17	0.92	0.63
MCM6*	rs4988235	NAA	NAA	24	3.18	0.34
MSTN*	rs1805086	**G**	A	25	1.34	0.51
MTHFR	rs1801131	**C**	A	17	0.24	0.88
MTR	rs1805087	**G**	A	2	1.94	0.38
MTRR	rs1801394	**G**	A	2	2.75	0.25
NOS3	rs2070744	**T**	C	4	3.21	0.20
PGC1A	rs8192678	G	**A**	2	0.68	0.71
PPARA	rs4253778	C	**G**	2	0.08	0.95

PPARG*	rs1801282	**G**	C	2	1.23	0.54
SHBG	rs1799941	**A**	G	21	1.02	0.61
SLC16A1	rs1049434	T	**A**	1	2.93	0.63
UCP1*	rs6536991	NAA	NAA	26	1.47	0.48
UCP2	rs660339	C	**T**	1	0.16	0.92
UCP3	rs1800849	C	**T**	1	0.08	0.96
VDR	rs2228570	**T**	C	14	3.54	0.17
VEGFA*	rs2010963	G	**C**	2	1.99	0.37

* Only 2 of the possible 3 allelic combinations present.

Table A.2. Physiological data for participants classified as plateau achievers, or non-achievers, as described in methodology for all 34 participants (Original) and those 26 participants (Final) who provided a DNA sample that was successfully analysed.

Parameter	Group	Plateau	Non Plateau	p - value
Mass (kg)	Original Final	72.4 ± 9.86 72.9 ± 10.22	73.7 ± 12.03 74.3 ± 13.24	0.73 0.62
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	Original Final	54.5 ± 9.75 52.3 ± 10.98	53.0 ± 9.11 55.1±12.53	0.79 0.48
$\Delta\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	Original Final	0.76 ± 0.387 0.69 ± 0.418	2.21 ± 0.682 2.32 ± 0.740	< 0.001[a] < 0.001[a]
RER_{max}	Original Final	1.18 ± 0.072 1.19 ± 0.079	1.19 ± 0.076 1.18 ± 0.081	0.75 0.58
HR_{max} (bpm)	Original Final	192.8 ± 8.41 192.2 ± 8.60	193.3 ± 9.71 194.1 ± 10.72	0.66 0.81
pBLa (mmol)	Original Final	9.6 ± 2.39 10.7 ± 1.64	9.9 ± 2.30 10.5 ± 1.71	0.59 0.72

Data are presented as means ± SD.

[a] Significant at the 99.9% level.

Table A.3. Results of χ^2 analyses to determine; (**A**) the gene's association with allelic frequency and presence/absence of $\dot{V}O_{2plat}$ (χ^2 $\dot{V}O_{2plat}$) and (**B**) to determine the gene's allelic frequency association (χ^2 $\dot{V}O_{2max}$) with low (< 53.6 ml·kg^{-1}·min^{-1}) and high $\dot{V}O_{2max}$ (> 53.6 ml·kg^{-1}·min^{-1}). Genes are ordered with respect to increasing p -values for χ^2 $\dot{V}O_{2plat}$ and χ^2 $\dot{V}O_{2max,}$ respectively. Class = Classification: anaerobic (An), aerobic (A), or no apparent association (NAA), as defined in Table1.

A (gene)	χ^2 $\dot{V}O_{2plat}$	p-value	Class	**B** (gene)	χ^2 $\dot{V}O_{2max}$	p-value	Class
ACTN3	8.82	0.012[a]	An	CYP1A2	8.94	0.011[a]	A
IL6*	6.37	0.041[a]	An	PGC1A	4.53	0.10	A
ADRB213	6.04	0.049[a]	A	IL15RA	4.57	0.10	An
PPARG*	3.69	0.055	An	ACE	3.72	0.15	A
IGFBP-3	4.1	0.13	An	IGF1-71	3.49	0.17	An
VDR	3.74	0.15	An	FTO	3.51	0.17	An
ESR1	3.75	0.15	An	AKT1	3.48	0.17	A
CKM	3.75	0.15	An	UCP1*	1.64	0.20	NAA
MTHFR	3.11	0.21	An	BDKRB2	3.16	0.21	A
IGF1-71	2.85	0.24	An	MTR	2.99	0.22	An
AGT	2.83	0.24	An	ADRB214	2.94	0.23	An
HIF1A	2.82	0.24	An	ADRB213	2.88	0.24	A
AMPD1	2.76	0.25	An	MTHFR	2.83	0.24	An
FTO	2.69	0.26	An	MSTN*	1.27	0.26	An
ACVR1B*	1.21	0.27	An	AGT	2.67	0.26	An
MTR	2.51	0.28	An	IGFBP-3	2.40	0.30	An
MCM6*	0.91	0.34	NAA	NOS3	1.92	0.38	An
CNTF	2.16	0.32	An	MTRR	1.86	0.39	An
UCP3	1.92	0.38	A	APOA5*	0.65	0.42	NAA
APOA2	1.78	0.41	NAA	**VDR**	1.66	0.44	An
CLOCK	1.67	0.43	NAA	CNTF	1.63	0.44	An
NOS3	1.66	0.43	An	PPARA	1.45	0.48	A
MTRR	1.65	0.44	An	UCP3	1.44	0.49	A
UCP1*	0.52	0.47	NAA	ESR1	1.37	0.50	An
APOA5*	0.48	0.49	NAA	HIF1A	1.35	0.51	An
IL6R	1.41	0.49	An	MCM6*	0.39	0.53	NAA
SLC16	1.41	0.49	A	IGF1-35*	0.39	0.55	An
CYP1A2	1.36	0.51	A	IL6R	1.17	0.56	An
VEGFA*	0.34	0.56	A	CKM	1.13	0.57	An
ADBR214	1.36	0.51	An	UCP2	1.13	0.57	A
PPARA	1.14	0.56	A	CLOCK	1.05	0.59	NAA

BDKRB2	1.09	0.58	A	IL6*	0.97	0.60	An
AKT1	0.75	0.68	A	APOA2	0.96	0.62	NAA
PGC1A	0.56	0.75	A	SLC16	0.92	0.62	A
IGF1-35*	0.07	0.79	An	PPARG*	0.23	0.63	An
UCP2	0.36	0.83	A	SHBG	0.65	0.72	An
ACE	0.36	0.83	A	ACVR1B*	0.46	0.79	An
SHBG	0.36	0.84	An	AMPD1	0.41	0.81	An
IL15RA	0.20	0.90	An	ACTN3	0.08	0.96	An
MSTN*	0.01	0.92	An	VEGFA*	0.06	0.97	A

* Only 2 of the possible 3 allelic combinations present.

Appendix A.1. Genes, SNPs and primers used, in this study, together with primer orientation.

Gene	SNP	Primer	Orientation
ACE	rs4341	GGGCTGGAGCTCAAG[C/G]CATTCAAACCCCTA	Forward
ACTN3	rs1815739	CTGCCCGAGGCTGAC[C/T]GAGAGCGAGGTGCC	Forward
ACVR1B	rs2854464	GTGTTAGTGTCAGCC[A/G]TGGGAAATGAGCCA	Forward
ADRB213	rs1042713	TTGCTGGCACCCAAT[A/G]GAAGCCATGCGCCG	Forward
ADRB214	rs1042714	CACGACGTCACGCAG[C/G]AAAGGGACGAGGTG	Forward
AGT	rs699	CTGGCTGCTCCCTGA[T/C]GGGAGCCAGTGTGG	Forward
AKT1	rs1130214	CCCAGGAGGTTTTTG[G/T]GCTTGCGCTGGAGG	Forward
AMPD1	rs17602729	TAATGCAATACTCAC[A/G]TTTCTCTTCAGCTG	Reverse
APOA2	rs5082	GGTCCTTGGACTTGA[A/G]TGCAACAGGAAGCA	Reverse
APOA5	rs662799	AACTGGAGCGAAAGT[A/G]AGATTTGCCCCATG	Forward
BDKRB2	rs1799722	AGGCTGATGACATCA[C/T]TACCCAGCCCTTGA	Forward
CKM	rs8111989	AGAAATGGGGAGCCA[G/A]GGCAGGTTCTTGAG	Forward
Clock	rs1801260	GTGATCATAGGGGCA[C/T]AGCCAGTTCTGACA	Forward
CNTF	rs1800169	TTTTCCTGTATCCTC[A/G]GCCAGGTGAAGCAT	Forward
CYP1A2	rs762551	GTGAGCTCTGTGGGC[A/C]CAGGACGCATGGTA	Forward
ESR1	rs722208	GGTGGGGTGGAAGAC[A/G]CTGAAATGAATTTT	Forward
FTO	rs9939609	GACTGCTGTGAATTT[A/T]GTGATGCACTTGGA	Forward
HIF1A	rs11549465	TTCGATCAGTTGTCA[C/T]CATTAGAAAGCAGT	Forward
IGF1-35	rs35767	TTTTTTTTTTTTCC[A/G]CATGACTCTCAGGG	Reverse
IGF1-71	rs7136446	CACTGCCCTAAGTGC[C/T]GCGTAGTATGTGAA	Forward
IGFBP-3	rs2854744	CGGGCTCCGGGCGTG[A/C]GCACGAGGAGCAGG	Forward
IL15RA	rs2296135	TTTCTCTGTGAACTG[A/C]AAGTTAGGATGAGG	Forward
IL6	rs1800795	CTAGTTGTGTCTTNC[C/G]ATGCTAAAGGACGT	Forward
IL6R	rs2228145	TTAACCTAGTGCAAG[A/C]TTCTTCTTCAGTAC	Forward
MCM6	rs4988235	GATAAGATAANGTAG[C/T]CCCTGGCCTCAAAG	Forward
MSTN	rs1805086	ACAATAAAGTAGTAA[A/G]GGCCCAACTATGGA	Forward
MTHFR	rs1801131	AGCTGACCAGTGAAG[A/C]AAGTGTCTTTGAAG	Forward
MTR	rs1805087	AAGATATTAGACAGG[A/G]CCATTATGAGTCTC	Forward
MTRR	rs1801394	CATNGCAGAAGAAAT[A/G]TGTGAGCAAGCTGT	Forward
NOS3	rs2070744	AAGCTCTTCCCTGGC[T/C]GGCTGACCCTGCCT	Forward
PGC1A	rs8192678	GAAGCAGACAAGACC[A/G]GTGAACTGAGGGAC	Forward
PPARA	rs4253778	CTTGATATCTAGTTT[C/G]GATTCAAAAGCTTC	Forward
PPARG	rs1801282	GATTCTCCTATTGAC[C/G]CAGAAAGCGATTCC	Forward
SHBG	rs1799941	CTCCACCGCCCACAC[A/G]CAAGGCTGCCTGCC	Forward
SLC16A1	rs1049434	CCAGAAAGACACAGA[A/T]GGAGGGCCCAAGGA	Forward
UCP1	rs6536991	CCCAAAACATGTCTT[C/T]TCTTCACTGACATG	Forward
UCP2	rs660339	CGGTACTGGGCGCTG[A/G]CTGTAGCGCGCACT	Reverse
UCP3	rs1800849	TGGTCTTATACACAC[A/G]GGCTGACCTGAAAC	Reverse
VDR	rs2228570	CTGTTCTTACAGGGA[C/T]GGAGGCAATGGCGG	Forward
VEGFA	rs2010963	TGCGAGCAGCGAAAG[C/G]GACAGGGGCAAAGT	Forward