Original Research

The Effect of Angiotensin Converting Enzyme Genotype on Aerobic Capacity Following High Intensity Interval Training

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ABSTRACT

International Journal of Exercise Science 7(3) : 250-259, 2014. Obesity increases the risk of developing type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Physical activity can reduce T2DM and CVD risk, and increase aerobic capacity, a significant predictor of all-cause mortality and morbidity. High intensity interval training (HIIT) produces similar improvements in aerobic capacity to continuous moderate exercise (CME). Different genotypes of angiotensin converting enzyme (ACE) have been implicated in improving aerobic capacity and therefore predicted health. This study investigated the effects of different ACE genotypes on the impact of 6 weeks of HIIT on aerobic capacity, and thus health status. 20 young adults were recruited for this study; test subjects completed 6 weeks of HIIT 3 times a week. VO_{2max} was tested to determine aerobic capacity pre- and post-HIIT and DNA collected from saliva for determination ACE genotype. After 6 weeks of HIIT there was no significant change in VO_{2max}; when subjects were separated into responder categories, high responders significantly increased their aerobic capacity whilst there was a large but non-significant decrease in non-responders. Subjects carrying a D-allele showed a significant increase in VO_{2max} following HIIT indicating specific ACE genotypes may be associated with differing VO_{2max} responses. These preliminary results suggest that HIIT can significantly reduce the time required for exercise whilst still achieving notable improvements in aerobic capacity in high responders; they also indicate that ACE D-allele carriers who would not usually be expected to show large VO_{2max} responses following CME may yield equivalent aerobic capacity improvements following HIIT, and thereby reduce their overall morbidity and mortality disease risk.

KEY WORDS: HIIT, ACE, high responders

INTRODUCTION

Obesity (BMI ≥30) is rapidly becoming one of the world's biggest health care problems (40), increasing the risk of type 2 diabetes mellitus (T2DM), (18), and is one of the five leading global risks for mortality. Treatment of T2DM is achieved through a combination of medication, diet modification and exercise in order to promote weight loss and increase cardiorespiratory fitness, and engaging in such physical activity even after leading a sedentary lifestyle or increasing activity to recommended levels can delay all-cause mortality (10).

Myers et al. (23) showed that for every 1-MET (metabolic equivalent) increase in exercise capacity survival improved by 12%. The importance of cardiorespiratory fitness is highlighted by Garber et al. (10) who observed that in both men and women mortality risk reduction was greater in subjects with a higher cardiorespiratory fitness than in those that met the recommended guidelines of physical activity. The gold standard measure of cardiorespiratory fitness is VO_{2max} (aerobic capacity), which represents the maximal amount of oxygen per unit time that can be delivered to the peripheral organs, in particular the skeletal muscle during exercise (2). The most accurate measure of VO_{2max} is analysis of expired air during graduated intense exercise (32).

To increase aerobic capacity the recommend weekly exercise for adults is a minimum of 30 minutes of moderate intensity aerobic exercise 5 days per week. Alternatively, vigorous aerobic exercise may be performed 3 days a week or the 2 types of exercise may be combined (15). Despite the benefits of exercise being numerous, well documented and public health messages persuading people to be more active, approximately 65% of UK adults do not meet recommended health guidelines. The "perceived lack of time" is frequently cited as the main cause of insufficient physical activity and compliance to exercise (28). Therefore there has been increased interest in the use of low volume, high intensity interval training and its possible health benefits (25).

High-intensity interval training (HIIT) is the term used to describe short bursts of maximal intensity exercise separated by periods of rest or low intensity exercise (11). Most HIIT sessions take approximately 10 to 15 minutes in comparison to a minimum of 30 minutes of CME (11, 15), and studies varying from two to eight weeks have shown improvements in VO_{2max} and decreases in disease risk factors (1, 21, 34). Studies have also shown that in both health and disease HIIT can induce comparable or better changes in exercise performance when compared to CME (27), and 16 weeks of HIIT was more effective at increasing aerobic capacity in individuals with metabolic syndrome, increasing VO_{2max} by 35% compared to a 16% increase with continuous exercise (37). Aerobic capacity is suggested to be a better risk marker for all-cause mortality and morbidity than traditional risk factors such as T2DM and hypertension and regular exercise induces many changes including increased aerobic capacity and increased muscle strength (10). The extent of these changes differs between individuals often depending on the type of exercise, lifestyle and genetic factors, leading to the observation that different individuals can be termed as positive or non-responders to performance or specific risk factors, including aerobic capacity (4, 35).

The angiotensin-converting enzyme (ACE) gene has been studied extensively and is believed to play a role in aerobic capacity and exercise performance (7, 16), with VO_{2max} showing an approximate 12% inter-individual variation due to ACE genotype (14) The human ACE genotypes consist of an insertion (I) or absence (D) of a 287 base pair alanine sequence in intron 16. It has been reported that approximately 23% of the population have the ACE-II genotype and have lower circulating ACE levels (31); the I-allele is associated with increased maximal heart rate and VO_{2max} and

therefore elite endurance performance following medium duration aerobic training. In contrast the D-allele is associated with high circulating ACE levels and a 10% increased risk of cardiovascular disease; approximately 28% of the population have the ACE-DD genotype (32). Individuals carrying the D-allele tend to show greater improvements following shorter duration high intensity exercise (7).

As VO_{2max} is an independent risk marker for CVD and overall morbidity and mortality (3, 21, 30), the ACE genotype (DD in particular) may, partially at least, exert its effect on CV disease due to untrained individuals having lower VO_{2max} values. As described above, short periods of HIIT can significantly increase VO_{2max}, and ACE D-allele individuals respond particularly well to high intensity-type exercise. One could therefore predict that those who carry one or both copies of the D allele may gain greater improvements in aerobic capacity following a relatively short period of high intensity interval training than those carrying the I allele. Therefore, this study investigated whether ACE genotype affected the response of 6 weeks of HIIT on aerobic capacity, and therefore overall health, in young healthy individuals.

METHODS

Participants

Many studies involving HIIT have used less than 20 subjects. In this study 20 recreationally active healthy adults were recruited to participate; subjects were divided into control (n=7) and test (n=13) groups. Test subjects completed all experimental procedures at pre and post-HIIT, and following a 6 week detraining period where no HIIT was performed. Controls did not perform HIIT and completed experimental procedures at pre and post-HIIT. Subjects were requested to maintain their normal diet and physical activity levels throughout the duration of the study. Subjects were informed of the experimental protocol before signing a written consent form. Genetic and physiological information was stored by anonymous code and stored with a person not involved with the study. The experimental protocol was approved by Cardiff University institutional ethics committee.

Protocol

A VO_{2max} test was performed on a cycle ergometers (Seca cardiotest 100, model 545). After a warm up at low resistance (30 watts) for 5 minutes at 60-65 rpm, the resistance was increased in increments of 30 watts every 2 minutes with the subject maintaining a pace above 60 rpm. Incremental increases continued until exhaustion or when subjects could no longer maintain pace above 60 rpm. Maximal wattage achieved before exhaustion (breaking wattage) was then used as the starting wattage for HIIT. VO_{2max} was determine by a gas analysis system and was determined as the highest value achieved over a 20 second period using LabChart 7 (ADI instruments).

HIIT consisted of exercising 3 times per week with no more than 2 consecutive days rest, for 6 weeks on cycle ergometers (Seca cardiotest 100, model 545). Each session consisted of a 5 minute warm up, 3 x 1 minute maximum intensity intervals (>120rpm) at breaking wattage, interspersed with a 2 minute working recovery and a 3 minute cool down. Those who completed the 3 intervals on the 1st

HIIT session had their wattage adjusted upward by 10% increments based on performance and perceived effort, whilst for those unable to maintain the required >120 rpm for any interval, wattage was adjusted down in 10% increments based on the same criteria. During the 6 weeks of HIIT if a subject completed 3 intervals maintaining >120 rpm on 2 consecutive sessions, wattage was adjusted upward in 10% increments to ensure maximum intensity was being exerted during each session.

Genomic DNA isolation from saliva was carried out using a DNA preparation kit QIAamp® DNA Blood Mini Kit (QIAGEN, no: 51104) according to the Manufacturers' instructions. Following isolation DNA concentrations were normalised to 50µg/ml; samples were subsequently prepared for PCR at the same time using the primers 5'CTGGAGACCACTCCCATCCTTTCT 3' (forward) and 5'GATGTGGCCATCACATTCGTCACGAT 3' (reverse) (Invitrogen Customer Primers Life technologies™). PCR conditions were as described previously (Movva et al., 2007). Briefly, 1µl of each primer (100pmol/µl), 1µl isolated DNA, 12.5µl BioMix Red including Taq DNA polymerase (Bioline, UK) and 8.5µl distilled water were incubated under the following conditions using a Techne flexigene for thermal cycling (Staffordshire, UK): 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds. A final extension at 72°C for 5 minutes was performed. Gel electrophoresis was carried out using a 2% agarose gel (EDTA) stained with RedSafe Nucleic Acid staining solution (Intron Biotechnology). Quickload 2-log DNA ladder (New England Biolabs, Herts, UK) was used to identify DNA fragments. PCR amplification showed a 490-bp product (I allele) and/or 190-bp product (D allele) depending on the presence or absence of the insertion of a 287-bp fragment.

Statistical Analysis

Statistical analysis was performed using Minitab Statistical software (version 15) and Microsoft Excel. Data was tested for normality using Anderson-Darling tests. Appropriate transformations were performed if needed before One-Way ANOVAs or Student T-Test were performed. For correlation analysis, regression analysis and Pearson's correlations were conducted. If data could not be normalised, a Mann-Whitney test was performed. Statistical significance was accepted when $p<0.05$, all data are presented as means ± SEM unless otherwise stated.

RESULTS

VO_{2max} is widely used in exercise physiology and is associated with exercise performance and risk factors of both CVD and metabolic disorders (5). At the start of the study VO_{2max} results of the test (n=13) and control groups (n=7) were not significantly different to one another (48.6 vs 43.9 ml/kg/min; p=0.41), and the control subjects mean VO_{2max} did not significantly change after 6 weeks (43.9 to 43.9 ml/kg/min; p=0.85) (figure 1). 6 weeks of HIIT in test subjects increased aerobic capacity by 12.6% however this was not significant (48.6 to 54.7 ml/kg/min; p= 0.08) (figure 1); there was no significant

change in VO_{2max} following a 6 week detraining period.

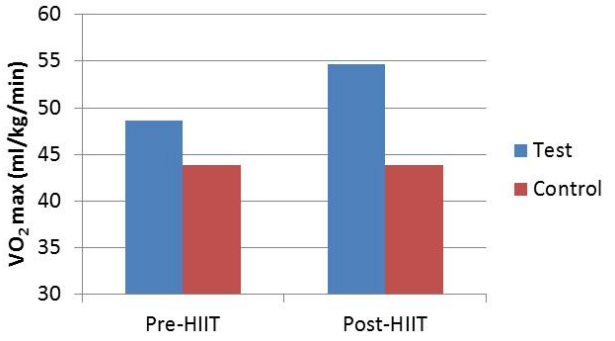

Figure 1. Average VO_{2max} values of test and control groups at pre- and post-HIIT. Pre-HIIT values of VO_{2max} for test subjects (n=13) and controls (n=7) were not significantly different (48.6 ± 3.0 vs 43.9 ± 5.2 ml/kg/min; p= 0.41). Post-HIIT values of VO_{2max} in controls were not significantly different form pre-HIT values (43.9 ± 5.2 to 43.9 ± 4.8 ml/kg/min; p=0.85). Post-HIT values of VO_{2max} were not significantly different from pre-HIIT for the test group (48.6 ± 3.0 to 54.7 ±3.3 ml/kg/min; p=0.08).

Individual test subjects responded differently to HIIT; training adaptions to VO_{2max} ranged from a 29% decrease to a 60% increase from pre-HIIT levels. This is in line with other studies (40). VO_{2max} responses were categorised into high and non-responders. Based on Bouchard et al. (4), high responders were defined as subjects with a VO_{2max} increase greater than 2 standard errors of the mean (2xSEM) (positive effect of exercise) from pre-HIIT values, whilst non-responders were those who showed no positive effect greater than 2xSEM (figure 2).

High responders (n=9) to HIIT showed a significant average 27% increase in their VO_{2max} (47.0 to 59.7 ml/kg/min; p<0.001); interestingly, 6 weeks of detraining (ie. No HIIT) significantly decreased VO_{2max} by 11% in these high responders (59.7 to 52.1 ml/kg/min; p= 0.008), however this was still higher than pre-HIIT values (p=0.06). This decrease in VO_{2max} after detraining suggests that maintaining HIIT or some other form of exercise is important. Non-responders (n=4) showed a non-significant 16.8% decrease in aerobic capacity post-HIT (52.2 to 43.4 ml/kg/min; p=0.06). Following the 6 week detraining period, non-responder VO_{2max} levels increased back to near pre-HIIT values (50.4 ml/kg/min; p=0.23), although this was increase not significant. This suggests that HIIT may have a negative effect on aerobic capacity in some individuals but that decreasing this form of training may allow it to improve again over a relatively short period.

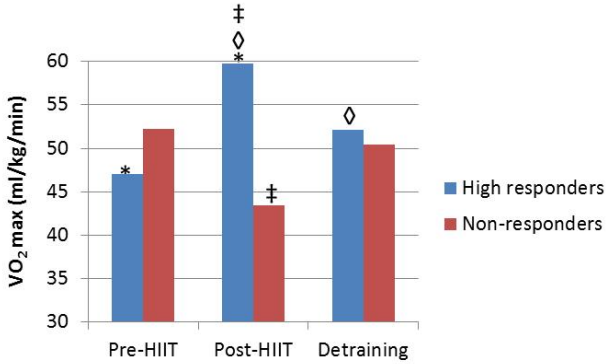

Figure 2. VO_{2max} results of high responders (n=9) and non-responders (n=4). Aerobic capacity was significantly increased in high responders (47.0 ± 4.3 to 59.7 ± 3.6 ml/kg/min; *p<0.001) at post-HIIT. This was followed by a significant decrease in aerobic capacity after 6 weeks of detraining (59.7 ± 3.6 to 53.2 ± 3.3 ml/kg/min; ◊p=0.008). In non-responders aerobic capacity decreased (52.2 ± 2.3 to 43.4 ± 2.8 ml/kg/min; p=0.06) and increased following detraining (to 50.4 ± 2.4 ml/kg/min; p=0.23). There was a significant difference between positive and non-responder VO_{2max} values post-HIIT (59.7 vs 43.4 ml/kg/min; ‡p=0.005).

Interestingly, there was a significant difference between positive and non-responder VO_{2max} values post-HIIT (59.7 vs 43.4 ml/kg/min; p=0.005), although this significance vanished following the detraining period (52.1 vs 50.4 ml/kg/min). In addition, positive responder pre-HIIT

VO_{2max} values were higher than the corresponding non-responder levels, however this was insignificant (p=0.30).

Distribution of ACE genotypes in test subjects was 20% II, n=2; 60% ID, n=6: 20% DD, n=2, and previous work has showed a 23% II, 49% ID and 28% DD distribution (31).

Table 1. Differences in VO_{2max} in the dominant and recessive model ACE genotype groups. There was a significant increase in VO_{2max} in the dominant model group (ID and DD) following HIIT (*p=0.033). There was no significant difference between the two groups pre-HIIT.

	Dominant model ACE ID / DD (n=8)	Recessive model ACE ID / II (n=8)
Pre-HIIT VO_{2max} (ml/kg/min)	47.8 ± 4.25*	44.1 ± 2.48
Post-HIIT VO_{2max} (ml/kg/min)	55.8 ± 4.12*	49.6 ± 2.49
Response (change in VO_{2max}) (ml/kg/min)	+ 8.0 ± 3.1	+ 5.6 ± 4.1
P value (pre-HIIT to post-HIIT)	0.033	0.21

Based on a dominant model (DD+ID) (38, 42), and as we were most interested to see how subjects containing the 'high intensity' D-allele responded to HIIT, subjects were separated into groups containing DD/ID and II/ID alleles; table 1 shows that in response to HIIT the subjects carrying the D-allele had a relatively large 8.05 ml/min/kg (16.9%) increase in VO_{2max} (p=0.033). When subjects were separated based on a recessive model (II+ID) there was a small but insignificant increase in VO_{2max} (table 1). Therefore, VO_{2max} response to HIIT may be related to the ACE genotype as D-allele carriers showed a significant increase in VO_{2max} following HIIT.

DISCUSSION

This study demonstrated that individual VO_{2max} responses to HIIT was heterogeneous; VO_{2max} was significantly increased in high responders (27%) whilst it decreased non-significantly in non-responders. Other studies of VO_{2max} have reported high and low/non-responders with results ranging from a 2% decrease to >30% increase (35, 40).

Negative responders do occur (4), however additional VO_{2max} testing would confirm if these were anomalous results due to external factors, for example fatigue during testing. An increase in VO_{2max} resulting from HIIT has been observed in numerous studies (12, 19) and as little as 2 weeks of HIIT has been reported to increase aerobic capacity by 9.5% (41), and (6) 6 weeks of HIIT has been shown to increase VO_{2max} as effectively as CME despite a significant reduction in the volume and time commitment of the training (6). In addition it has been demonstrated that during a 24 week HIIT study intensity of training was particularly important in increasing aerobic capacity (24).

The mechanisms behind this increase in aerobic capacity remain to be comprehensively examined (11). It is likely that the increase in aerobic capacity involves improvements to the cardiorespiratory system. Studies of HIIT in rats have shown comparable changes in VO_{2max} to human studies (18). These studies have allowed the examination of the adaptations to cardiac function seen with HIIT, and have shown that in rats HIIT

improves cardiac myocyte contractility, calcium handling and increased hypertrophy to a greater extent than lower intensity exercise. In addition, 10 weeks HIIT in rats produced adaptations that improved cardiac efficacy which were not observed with CME (13).

Increasing VO_{2max} is particularly important as low aerobic capacity is a strong predictor of mortality and is associated with increased risk of CVD and T2DM (10, 29). Improvements in VO_{2max} following HIIT suggests that if the goal is to maximise cardiorespiratory fitness, HIIT may be more effective in high responders than CME. It is tempting to assume that adverse responders should not perform HIIT as it results in a decrease in their aerobic capacity, therefore increasing the risk of CVD (23). However, a recent large review showed that an adverse response in one variable does not necessarily mean negative responses in other variables (4). It is also interesting to note that in our study once non-responders to VO_{2max} ceased HIIT their VO_{2max} values returned to near pre-HIIT values very quickly. It will be interesting to see in the future whether VO_{2max} adverse responders to HIIT also have deleterious responses in exercise performance tests.

ACE is a gene that is often associated with the trainability of aerobic capacity, however several studies have not observed any difference in VO_{2max} trainability among the ACE genotypes (30, 33). ACE genotype is not normally associated with endurance performance in an untrained state, and its effects require a period of gene-environment interaction (16). Following medium duration aerobic training presence of the I-allele is associated with endurance-orientated events, and affects bradykinin concentration; the I-allele is associated with increased endurance performance at an Olympic level (26), increased maximal heart rate and VO_{2max} (14); evidence suggests the ACE-I allele effectiveness has a threshold value of between 10-30 minutes with lasting maximal exercises (9). In contrast, the D-allele is associated with increased left ventricular muscle mass, and improvements in strength and power orientated performance following high intensity exercise over shorter periods (7, 26).

Importantly, ACE genotype is also associated with disease risk, for example the presence of the dominant ID/DD model increases risk of the metabolic syndrome (42), and there is an increased frequency of the D risk allele in coronary patients (8).

Following on from this evidence, we hypothesised that individuals carrying the ACE D-allele may gain significant improvements in VO_{2max} following HIIT, and therefore potentially gain health benefits. Interestingly, our preliminary data seem to support this hypothesis; based on a dominant model (38, 42), D-allele carriers showed a significant increase in VO_{2max} that was not observed in the recessive model. As mentioned above, the D-allele is associated with greater strength gains in skeletal muscle following training in both healthy individuals and those with chronic disease (26). It is therefore not surprising that ACE D-allele carriers showed positive VO_{2max} improvements following HIIT, and it may be expected that their aerobic capacity would not adapt as positively to CME. In contrast, ACE I-allele carriers would be expected to gain greater VO_{2max} improvements following CME than HIIT. However there are also many other

candidate genes for the individual differences in trainability of VO_{2max}. Recently, a system was established to predict VO_{2max} responses following exercise based on 29 predictor genes of which ACE was not included (36).

In conclusion, this preliminary study demonstrates that HIIT can significantly reduce the time required for exercise whilst still achieving notable improvements in aerobic capacity in high responders and those carrying the ACE D-allele. These findings have important implications for both every day and prescribed exercise, and indicate that future large scale studies comparing the effects of CME and HIIT on VO_{2max} in individuals with the ACE I and D alleles would be appropriate. Long term follow up studies may also indicate whether such training regimes lead to health benefits in different sub-populations, Overall HIIT may prove to yield more favourable results in some individuals than CME.

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