ACE Activity and Endurance Performance during the South African Ironman Triathlons

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Key words
- angiotensin-converting enzyme
- athlete
- phenotype
- RAAS
- RAS

Abstract

The insertion allele of the angiotensin-converting enzyme (*ACE*) gene has been associated with endurance performance. Since a large portion of the variance seen in circulatory ACE levels is unaccounted for by the insertion/deletion polymorphism it is likely that the ACE phenotype would serve as a more informative marker in assessing elite endurance performance. The aim of this study was to correlate plasma ACE activity with performance of a homogenous population of South African-born Caucasian male triathletes. Plasma ACE activity was determined in 145 triathletes, representing the fastest and slowest subgroups who completed either the 2000 or 2001 South African Ironman Triathlon. There was a trend for lower mean plasma ACE activity in the fastest (28.85 ± 8.60 mU/ml) when compared to the slowest finisher subgroup (31.65 ± 8.75 mU/ml, $P=0.055$). There was a significant positive correlation between plasma ACE activity and overall finishing time within the participants who completed the event in under 15 h ($r=0.192$, $P=0.029$). There was also a positive correlation with cycle ($r=0.195$, $P=0.034$) and run ($r=0.184$, $P=0.040$) stages but not the swim stage ($r=0.084$, $P=0.353$). In conclusion, this is the first study to report a relationship between plasma ACE activity and endurance performance in humans.

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Introduction

Athletic performance is a multifactorial phenotype, which is determined by poorly understood complex interactions of multiple environmental and genetic factors [4]. However, the insertion/deletion (I/D) polymorphism within intron 16 of the gene encoding the angiotensin-converting enzyme (ACE), an enzyme essential in both the renin-angiotensin-aldosterone (RAAS) and kallikrein-kinin systems (KKS) [15], is associated with athletic performance in some Caucasian populations [22].

Several studies have demonstrated an association of the D allele of the *ACE* gene with elite performance in sprinting and short distance swimming [23,24,37]. The I allele and/or the II genotype is, on the other hand, generally overrepresented amongst endurance disciplines [2,13,23]. Although genetic association studies should be evaluated with caution as initially reported associations are often not confirmed in follow-up studies, the majority of studies within the literature report that the *ACE* I/D polymorphism is most commonly associated with elite performance in athletes of European descent [25]. In support of this, Collins and colleagues [5] reported a significantly higher occurrence of the I allele in the fastest 100 South African-born Caucasian finishers of the South African Ironman Triathlon when compared to a South African-born Caucasian control population. In addition there was also a significant linear trend for the I allele distribution among the fastest 100 finishers (51.5%), slowest 100 finishers (47.5%), and control (42.2%) population.

The I/D polymorphism has been the most extensively studied and characterised of all the polymorphisms present in both the RAAS and KKS pathways and has been associated with numerous physiological conditions [15]. Although the function of this intronic polymorphism has yet to be elucidated, it accounts for half of the total phenotypic variance seen in circulating ACE concentration in Caucasians and is one of the major loci in regulating *ACE* gene expression in both the systemic and local systems [7,9,10,37]. Serum ACE levels have been shown to be lowest in subjects possessing the II genotype and highest in subjects with the DD genotype [1,30,34]

Table 1 Physiological and performance characteristics of the fastest and slowest South African-born male triathlete groups and subgroups who completed either the 2000 and/or the 2001 South African Ironman Triathlons.

	Fastest Group (n = 100)	Fastest Subgroup (n = 72)	P-value[1]	Slowest Group (n = 100)	Slowest Subgroup (n = 73)	P-value[2]
age (years)	31.8 ± 6.3 (100)	33.1 ± 6.0 (72)	0.170	33.7 ± 8.0 (100)	32.9 ± 7.9 (73)	0.520
height (cm)	180.6 ± 6.5 (91)	180.5 ± 6.8 (66)	0.943	181.3 ± 7.0 (93)	181.3 ± 7.7 (69)	0.934
weight (kg)	76.3 ± 7.3 (100)	76.5 ± 7.8 (72)	0.829	81.0 ± 10.3 (100)	81.1 ± 10.7 (73)	0.865
BMI (kg/m^2)	23.4 ± 1.6 (91)	23.4 ± 1.6 (66)	0.776	24.7 ± 2.5 (93)	24.9 ± 2.4 (69)	0.643
overall time (min)	688 ± 39 (100)	689 ± 37 (72)	0.828	857 ± 47 (100)	860 ± 47 (73)	0.703
swim time (min)	64 ± 9 (94)	64 ± 10 (68)	0.886	78 ± 12 (98)	78 ± 13 (71)	0.889
cycle time (min)	363 ± 22 (92)	365 ± 21 (66)	0.629	429 ± 29 (93)	430 ± 28 (66)	0.861
run time (min)	252 ± 25 (98)	253 ± 26 (70)	0.967	331 ± 33 (96)	332 ± 33 (70)	0.768

Values are expressed as mean ± standard deviation, with the number of subjects (*n*) with non-missing data in parentheses ([1] Fastest Group vs. Fastest Subgroup; [2] Slowset Group vs. Slowest Subgroup)

suggesting that Ang II production and BK degradation would be the lowest in individuals possessing the II genotype. Consequently lower serum Ang II levels and reduced BK degradation have demonstrated a variety of effects on skeletal muscle believed to be associated with endurance performance [18].

Previous studies have suggested that ACE activity is the more informative marker in assessing the role of this enzyme in human cardiovascular disease rather than the genotype [21]. Considering the variance seen in circulatory ACE levels is largely unaccounted for by the I/D polymorphism, we hypothesize that plasma ACE activity would be of greater predictive value than the *ACE* genotype. Thus, this study is the first to compare plasma ACE activity with endurance performance in humans.

Materials and Methods

Participants

The 145 male participants included in this study were a subgroup of the previously recruited athletes who completed the 2000 and 2001 South African Ironman Triathlon held in Gordons Bay, Cape Town [5]. The event is an ultra-endurance event and consists of a 3.8 km swim, 180 km cycle and a 42.2 km run. 72 and 73 of the participants were originally included in the fastest and slowest 100 South African-born finishers previously genotyped for the *ACE* I/D polymorphism. These subgroups were included in this study due to the unavailability of plasma samples for all of the previously genotyped subjects for the *ACE* I/D polymorphism [5]. The age, height, weight and BMI of the 2 subgroups were similarly matched to their respective fastest or slowest groups (**Table 1**). Similarly, event and stage completion times of the subgroups were not significantly different to those of the parent groups (**Table 1**).

The triathletes completed an informed consent and personal particulars questionnaire during the registration period prior to the race from which age, height, country of birth and ethnicity were determined. Either the measured body weight at race registration or the self-reported body weight from completed questionnaires was used during this study [5, 33]. BMI was calculated as weight (kg) divided by height in meters squared (m^2). Race results were obtained from the race organisers. Approval for this study was obtained from the Human Research and Ethics Committee of the Faculty of Health Sciences, University of Cape Town and meets the ethical standards of the International Journal of Sports Medicine [14]. The South African Weather Service provided details of the environmental conditions on the 2 race days.

Blood sample collection

Approximately 5 ml of venous blood was obtained from each participant by venipuncture of a forearm vein and collected into vacutainers (BD Vacutainer® CPT™ Cell Preparation Tubes) with Sodium Heparin^N during the race registration period. Samples were immediately centrifuged at 1 200 × *g* at 4 °C for 15 min and the plasma was dispensed into 1.5 ml microfuge tubes and stored at −20 °C during the registration period. The plasma samples were subsequently transferred to −80 °C storage until analysis. It has however been shown that plasma ACE activity remains stable after prolonged storage at −20 or −80 °C [16]. Fresh plasma samples, which were stored immediately at −80 °C after centrifugation of the blood samples, were also obtained from local participants for comparison with plasma samples collected at the events. There were no significant differences in ACE activities when the fresh samples were compared to the respective stored samples obtained during the event registration period (data not shown).

Determination of ACE activity, concentration and genotype

Residual plasma ACE enzyme activity was determined as previously described with additional precipitation and centrifugation steps to minimise the effect of insoluble plasma proteins on the fluorescence readings [32]. Sample fluorescence was assessed using a Cary ECLIPSE Fluorescence Spectrophotometer (Varian, Walnut Creek, CA) at an excitation and emission wavelength (λ) at 360 and 486 nm, respectively, using 10 nm wide entrance and exit slits (mean ± standard deviation, $n = 3$).

The ACE ELISA Kit™ (CHEMICON® International, Inc., Temecula, CA) was used to determine plasma ACE protein concentration. Experimental and standard protein concentrations were determined as per manufacturer's instructions. The optical density of the samples was determined on an Anthos Labtec AR 2001 Microplate Reader (Anthos Labtec Instruments GmbH, Salzburg, Austria) with a wavelength (λ) filter of 450 nm with a reference filter at 620 nm (mean ± standard deviation, $n = 2$). Protein concentrations were calculated using non-linear regression of a second order polynomial curve obtained from the standard ACE samples.

The blood samples drawn from participants were previously used to genotype the participants for the I/D polymorphism within intron 16 of the *ACE* gene [5].

Statistical analysis

Data were analysed using the STATISTICA version 10.0 (StatSoft Inc., Tulsa, OK) and GraphPad Prism version 5.0d for Mac OS X (GraphPad Software, San Diego, CA, USA) programs. Where

applicable, data are presented as means ± standard deviations with the number of participants with non-missing data in parentheses. A 1-way ANOVA was used to determine any significant differences between the characteristics of the triathlete subgroups. Any significant differences in endurance performance between the fast and slow triathlete subgroups were adjusted for BMI. In addition, a Tukey's honest significant difference post hoc test was used to identify specific differences between groups when applicable. Pearson's correlation analysis was used to determine relationships between plasma ACE activity and other continuous variables, as well as, between performance variables. Statistical significance was accepted when $P=0.05$.

Results

Participant characteristics and environmental conditions

The *ACE* ID genotype distributions of the fastest ($n=72$) and slowest ($n=73$) subgroups included in this study were similar to those of the larger faster ($n=100$) and slower ($n=100$) groups reported in a previous study [5]. There were no significant differences in the mean ages and heights between the 2 triathlete subgroups (**Table 2**). However, the participants within the fastest subgroup were significantly lighter than the slowest subgroup ($P=0.004$) and this difference was reflected in the BMI of the 2 subgroups ($P<0.001$).

The triathletes in the fastest subgroup finished the event within 689 ± 37 min whereas the slowest subgroup finished within 860 ± 47 min (unadjusted $P<0.001$, P-value adjusted for BMI <0.001) (**Table 2**). As expected, the fastest subgroup also completed the swim, cycle and run disciplines of the triathlon in significantly faster times than the slow subgroup (unadjusted $P<0.001$, P value adjusted for BMI <0.001).

The average dry bulb temperature during the 2000 and 2001 events was 20.5 °C (range 17.0–23.9 °C, midday temperature 21.7 °C) and 17.2 °C (range 15.6–20.9 °C, midday temperature 20.0 °C) respectively. The average humidity during the 2000 and 2001 events was 68 % (range 46–87 %) and 63 % (range 48–79 %) respectively. The sea temperature during 2000 was 16 °C and 15 °C during 2001. The average wind speed during the 2000 event was 4.6 m/s (0 m/s at 0700 h to 7.1 m/s at 2300 h), while the average wind speed during 2001 was 6.4 m/s with a maximum gust of 22.3 m/s (81 km/h) at 2051 h.

Plasma ACE activity in triathlete subgroups

There was a tendency ($P=0.055$) for the mean plasma ACE activities to be lower in the fastest triathlete finisher subgroup (28.85 ± 8.60 mU/ml, $n=72$) when compared to the slowest triathlete finisher subgroup (31.65 ± 8.75 mU/ml, $n=73$) (**Fig. 1a**). In the combined cohort, the II homozygous triathletes displayed a significantly lower ACE activity (23.47 ± 7.58 mU/ml, $n=38$) than the heterozygous ID (31.36 ± 7.49 mU/ml, $n=70$) and homozygous DD (35.15 ± 8.06 mU/ml, $n=37$) triathletes (II vs. ID, $P<0.001$; II vs. DD, $P<0.001$; ID vs. DD, $P=0.040$) (**Fig. 1b**). The pooled ID genotype participants displayed a plasma ACE activity in between the homozygote levels. These findings were mirrored once the activity data were split into the fastest and slowest triathlete finisher subgroups (**Fig. 1c**). No significant differences were observed when the plasma enzyme activities of the respective genotypes were compared between the 2 subgroups (Fast II vs. Slow II, $P=0.111$; Fast ID vs. Slow ID, $P=0.183$; Fast DD vs. Slow DD, $P=0.869$) (data not shown).

Plasma ACE concentration in triathlete subgroups

ACE concentrations were determined using a sensitive ACE ELISA kit with a human ACE-specific polyclonal antibody. A cohort of triathlete plasma samples ($n=58$), with an equal distribution of the 3 genotypes present within the 2 subgroups (Pearson Chi-square = 0.357, $P=0.982$), was used to compare plasma ACE activity and total ACE protein concentration. The mean plasma ACE concentration of the fastest finisher subgroup (375 ± 112 ng/ml, $n=30$) did not differ from that obtained for the slowest finisher subgroup (384 ± 107 ng/ml, $n=28$) ($P=0.755$). Plasma ACE protein concentrations of these samples were dependent on the *ACE* genotype with DD individuals displaying the highest overall protein concentration (453 ± 127 ng/ml, $n=19$) compared to ID (374 ± 67 ng/ml, $n=20$) and II (310 ± 77 ng/ml, $n=19$) finishers (II vs. ID, $P=0.009$; II vs. DD, $P<0.001$; ID vs. DD, $P=0.019$). There was a strong positive correlation ($r=0.776$, $P<0.001$, $n=58$) between plasma ACE enzyme activity and ACE protein concentration (**Fig. 1d**).

Plasma ACE activity and endurance performance

Although there were no significant differences ($P=0.143$) in plasma ACE activity when the triathletes were divided into 4 seedings (9–11 h, 11–13 h, 13–15 h and 15–17 h) based on their individual finishing times (**Fig. 2**), there was a linear increase in the average plasma ACE activity within the 3 fastest seeding groups. Because of the possible linear relationship between completion time and the ACE phenotype within the triathletes who completed the race within 15 h, plasma ACE activity was correlated to individual triathlete performance during the South African Ironman triathlons. There was a significant positive correlation between plasma ACE activity and overall finishing time ($r=0.192$, $P=0.029$, $n=130$) (**Fig. 3a**), cycle time ($r=0.195$, $P=0.034$, $n=119$) (**Fig. 3c**) and run time ($r=0.184$, $P=0.040$, $n=125$) (**Fig. 3d**) when only the 130 triathletes who completed the race sub-15 h were analysed. Those triathletes with

	Fastest Triathletes (n = 72)	Slowest Triathletes (n = 73)	P-value
age (years)	33.1 ± 6.0 (72)	32.9 ± 7.9 (73)	0.869
height (cm)	180.5 ± 6.8 (66)	181.3 ± 7.7 (69)	0.533
weight (kg)	76.5 ± 7.8 (72)	81.1 ± 10.7 (73)	0.004
BMI (kg/m^2)	23.4 ± 1.6 (66)	24.9 ± 2.4 (69)	< 0.001
overall time (min)	689 ± 37 (72)	860 ± 47 (73)	< 0.001
swim time (min)	64 ± 10 (68)	78 ± 13 (71)	< 0.001
cycle time (min)	365 ± 21 (66)	430 ± 28 (66)	< 0.001
run time (min)	253 ± 26 (70)	332 ± 33 (70)	< 0.001

Values are expressed as mean ± standard deviation, with the number of subjects (*n*) with non-missing data in parentheses

Table 2 Physiological and performance characteristics of the fastest and slowest South African-born male triathlete subgroups who completed either the 2000 and/or the 2001 South African Ironman Triathlons.

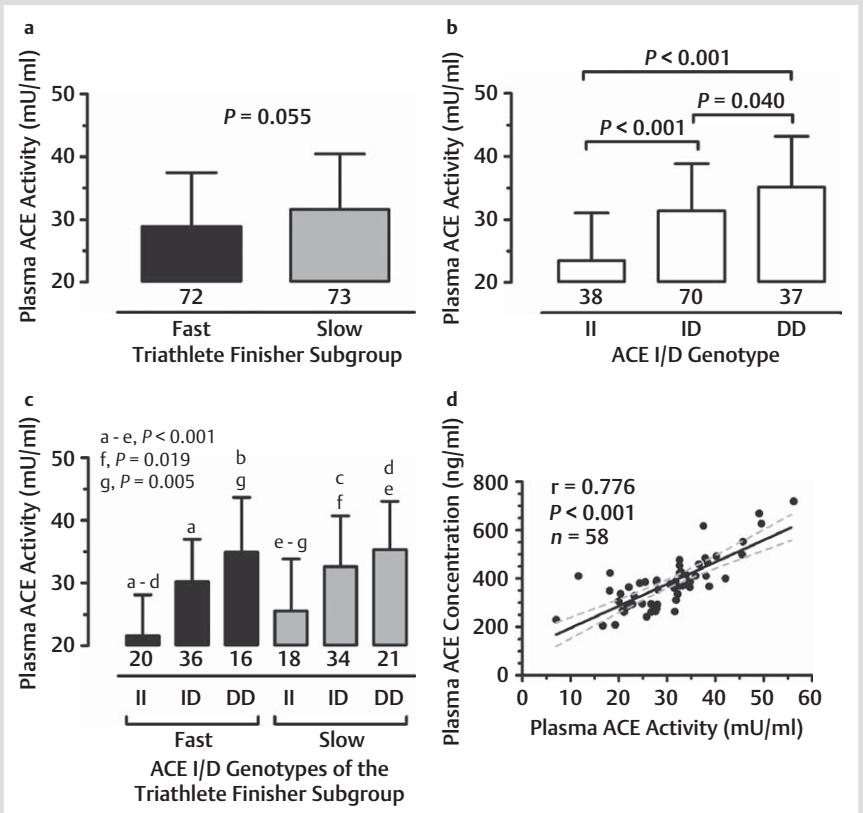

Fig. 1 Plasma ACE activities in a cohort of South African-born triathletes who completed the 2000 and/or 2001 South African Ironman Triathlon. **a** Average plasma ACE activity ± standard deviation of the fastest and slowest finisher subgroups. **b** Average plasma ACE activity ± standard deviation of the 3 *ACE* I/D genotype groups. **c** Average plasma ACE activity ± standard deviation of the 3 *ACE* I/D genotype groups within the fastest finishers (black bars) and slowest finishers (grey bars). **d** Correlation of 58 triathletes plasma ACE enzyme activity (mU/ml) and plasma ACE concentration (ng/ml). The solid line is the line of best fit and the grey dashed lines are the 95 % confidence intervals. The numbers under each bar in **a–c** indicate the number of triathletes (*n*) in each group.

the lowest plasma ACE activity completed the cycle, run and overall race in the fastest times. There was however no significant correlation (r = 0.084, $P = 0.353$, $n = 125$) between plasma ACE activity and the swim time (**Fig. 3b**). Interestingly there were strong positive correlations between the triathletes' overall finishing time and the cycle (r = 0.897, $P < 0.001$, $n = 131$) and run (r = 0.901, $P < 0.001$, $n = 140$) times. Although weaker, there was still a significant positive correlation between the triathletes' overall finishing and swim times (r = 0.574, $P < 0.001$, $n = 139$). There was however no significant correlation of ACE activity and overall finishing time (r = 0.117, $P = 0.161$, $n = 145$), swim time (r = 0.066, $P = 0.438$, $n = 139$), cycle time (r = 0.132, $P = 0.133$, $n = 132$) and run time (r = 0.103, $P = 0.225$, $n = 140$) when the 15 triathletes who completed the events during the last 2 h (16 and 17 h) before the cut off times were included in the analysis.

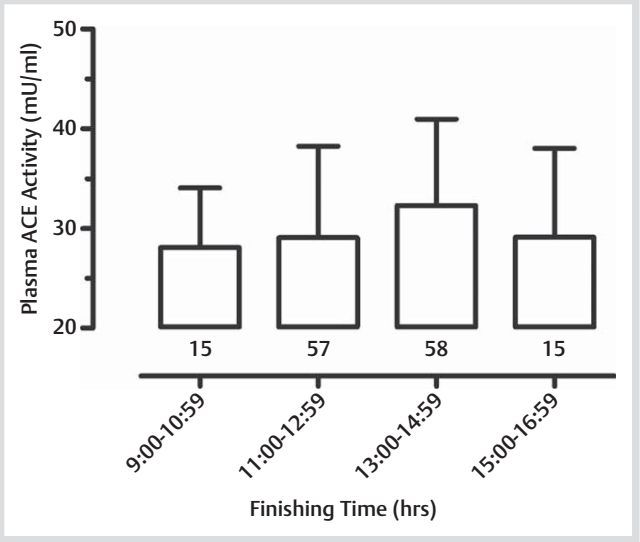

Fig. 2 2-h period finishing times and plasma ACE activities in a cohort of South African-born triathletes who completed the 2000 and/or 2001 South African Ironman Triathlon. Plasma ACE activities within each 2-h period is represented as a mean ± standard deviation with the number of triathletes (*n*) within each time period indicated under the bars.

Discussion

We have previously reported that the I allele of the *ACE* gene was over-represented in the fastest South African-born finishers of the Ironman triathlon [5]. In the present study we show that a positive correlation exists between plasma ACE activity and the overall finishing times of male Caucasian South African-born participants in the 2000 and 2001 South African Ironman Triathlons when the 15 triathletes who finished the race during the last 2 h were excluded from the analysis. Plasma ACE activity was generally lower in the triathletes who completed the cycle, run and overall race in the fastest times. Furthermore, a similar relationship between ACE activity and performance was demonstrated in the cycle and run stages of the race, but not for the swim. This is, to our knowledge, the first study to report a relationship between plasma ACE activity and performance in an ultra-endurance event.

The mean triathlete plasma enzyme activities for the I/D genotypes corresponded well to those determined previously [7, 12, 34]. There was also a strong correlation between plasma ACE protein concentration and ACE activity within the cohort in which ACE protein concentrations were measured. Plasma ACE protein concentration in Ironman athletes was significantly ele-

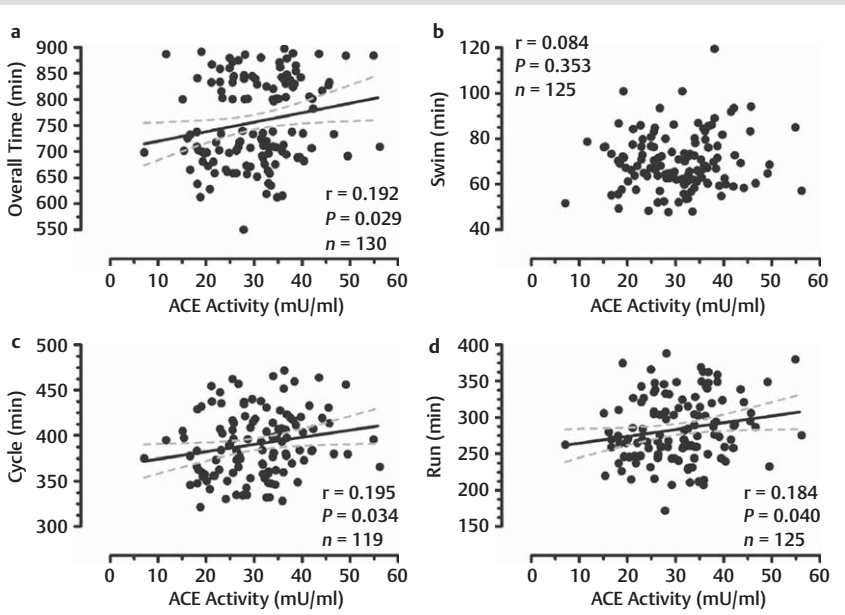

Fig. 3 Relationship between plasma ACE activity and **a** overall time, **b** swimming time, **c** cycling time and **d** running time for a cohort of South African-born triathletes who completed the 2000 and/or 2001 South African Ironman Triathlon. The solid line is the line of best fit and the grey dashed lines are the 95% confidence intervals.

vated in the pooled DD genotype when compared to the ID and II genotypes as observed with healthy subjects [8,30]. Together, these results highlight the fact that plasma ACE activity is a useful indicator of the circulating ACE phenotype.

Researchers have previously reported an excess of the D allele in elite swimmers competing over short distances (less than 400 m) but not over extended distances [6,23,35,38]. However, no correlation was observed between plasma ACE activity and the 3.8 km swim time of the Ironman triathlon. As the swim stage is the opening and shortest event of the race, it is plausible that pacing strategies of participants may have obscured any possible relationship between ACE activity and swim completion time. Future work is therefore required to investigate the possible relationship between ACE activity and completion time in pure endurance swimming events.

Participant training and injury data was not readily available for this study. The negative effects of under-training, over-training or physical injury on the actual performance times of the triathletes could therefore not be determined. It is tempting to speculate that these effects could have been larger in the triathletes who completed the race during the last 2 h. This is therefore a possible explanation why the ACE activity within the slowest seeding group was lower than expected. Accurate training and injury data should therefore be collected and analysed in future work.

While the I/D polymorphism has been associated with numerous diseases and endurance phenotypes in matched Caucasian populations, these genetic associations of the *ACE* I/D polymorphism have not been demonstrated in other ethnicities. The I/D polymorphism has been shown to account for 47% of the total phenotypic variance seen in circulating ACE concentration and is one of the major loci in regulating *ACE* gene expression in both the systemic and local systems in Caucasian populations. This association has not been demonstrated for any other ethnicity and indicates that other means of *ACE* genetic regulation may exist. As the ethnic variation in the genetic regulation of plasma ACE is poorly understood at present, evaluation of circulating ACE activity, i.e., the ACE phenotype and not genotype, for all athletes, regardless of ethnicity or nationality, may be informative. We propose that if the ACE phenotype is an important determinant of athletic ability; nationality and population groups which are influenced by *ACE* genotypes should not affect overall activity. Further studies should also assess the ACE activities of all event finishers and not only those in the fastest and slowest tiers or a single population group.

Numerous potential mechanisms that may explain the role of the *ACE* genotype, and hence plasma ACE concentration, in athletic performance have been reviewed previously [25]. Interestingly epigenetic regulation of the *ACE* gene, such as DNA methylation and histone acetylation, has been shown to modulate the expression of ACE mRNA and serum protein concentration [31,39]. Although the role of *ACE* epigenetic regulation in endurance performance remains to be elucidated, it has nevertheless been suggested that the epigenetic regulation of this gene might be more relevant to endurance physiology than the I/D polymorphism [26]. Plasma ACE has been thought to play a role in the systemic RAAS pathway by improving cardiovascular fitness by reducing blood pressure and maintaining fluid balance through the action of Ang II and BK on their cognate receptors. However, studies demonstrating no association between enhanced respiratory capability and ACE cast some doubt on this mechanism of ACE activity in respect of athletic performance [11,28,38]. Alternatively, the I/D polymorphism has also been thought to be in linkage disequilibrium with another gene present on chromosome 17q23 and that it is the phenotype of the linked gene that is responsible for the perceived effect of superior endurance ability. It is unlikely that the linked gene would be involved in the cardiovascular capacities of the participants [17] as to date none of the genes linked to VO_2 max, the standard measure of aerobic fitness and maximal oxygen consumption, has been mapped to the chromosome that harbours the *ACE* gene [27]. Lastly, the involvement of local RAAS pathways within numerous tissues including cardiac and skeletal muscle has seemed the most plausible causative cellular effect of the *ACE* I/D polymorphism. The I allele, associated with lower plasma expression, has been implicated in increased muscle efficiency [19], stimulating the growth of type-1 muscle fibres (partially involved in endurance performance) [29] and increased metabolic efficiency [3]. Conversely the D allele, associated with

increased plasma expression, has been linked to hypertrophy, hyperplasia [20] and also thought to reroute blood flow from type-1 muscle fibres to type-2 muscle fibres resulting in strength gains in athletes participating in power-orientated sports [29,36].

It should be noted that endurance performance is a multifactorial phenotype. At the biological level, a single enzyme within a single pathway is not solely responsible for determining the endurance phenotype. This phenotype is determined by the interaction of multiple enzymes across numerous biological pathways in several physiological systems, which may include ACE, the RAAS, the KKS and skeletal muscle.

In conclusion, these findings corroborate the hypothesis that ACE activity might be a better marker of human endurance performance than the *ACE* I/D polymorphism.

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