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The *ACE* Gene and Human Performance
12 Years On

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Abstract

Some 12 years ago, a polymorphism of the angiotensin I-converting enzyme (*ACE*) gene became the first genetic element shown to impact substantially on human physical performance. The renin-angiotensin system (RAS) exists not just as an endocrine regulator, but also within local tissue and cells, where it serves a variety of functions. Functional genetic polymorphic variants have been identified for most components of RAS, of which the best known and studied is a polymorphism of the *ACE* gene. The *ACE* insertion/deletion (I/D) polymorphism has been associated with improvements in performance and exercise duration in a variety of populations. The I allele has been consistently demonstrated to be associated with endurance-orientated events, notably, in triathlons. Meanwhile, the D allele is associated with strength- and power-orientated performance, and has been found in significant excess among elite swimmers. Exceptions to these associations do exist, and are discussed.

In theory, associations with ACE genotype may be due to functional variants in nearby loci, and/or related genetic polymorphism such as the angiotensin receptor, growth hormone and bradykinin genes. Studies of growth hormone gene variants have not shown significant associations with performance in studies involving both triathletes and military recruits. The angiotensin type-1 receptor has two functional polymorphisms that have not been shown to be associated with performance, although studies of hypoxic

ascent have yielded conflicting results. *ACE* genotype influences bradykinin levels, and a common gene variant in the bradykinin 2 receptor exists. The high kinin activity haplotye has been associated with increased endurance performance at an Olympic level, and similar results of metabolic efficiency have been demonstrated in triathletes.

Whilst the *ACE* genotype is associated with overall performance ability, at a single organ level, the *ACE* genotype and related polymorphism have significant associations. In cardiac muscle, *ACE* genotype has associations with left ventricular mass changes in response to stimulus, in both the health and diseased states. The D allele is associated with an exaggerated response to training, and the I allele with the lowest cardiac growth response. In light of the I-allele association with endurance performance, it seems likely that other regulatory mechanisms exist. Similarly in skeletal muscle, the D allele is associated with greater strength gains in response to training, in both healthy individuals and chronic disease states. As in overall performance, those genetic polymorphisms related to the *ACE* genotype, such as the bradykinin 2 gene, also influence skeletal muscle strength.

Finally, the *ACE* genotype may influence metabolic efficiency, and elite mountaineers have demonstrated an excess of I alleles and I/I genotype frequency in comparison to controls. Interestingly, this was not seen in amateur climbers. Corroboratory evidence exists among high-altitude settlements in both South America and India, where the I allele exists in greater frequency in those who migrated from the lowlands. Unfortunately, if the *ACE* genotype does influence metabolic efficiency, associations with peak maximal oxygen consumption have yet to be rigorously demonstrated.

The *ACE* genotype is an important but single factor in the determinant of sporting phenotype. Much of the mechanisms underlying this remain unexplored despite 12 years of research.

1. Renin-Angiotensin Systems (RAS) and Human Performance

Some 12 years ago, a polymorphism of the angiotensin I-converting enzyme (*ACE*) gene became the first genetic element shown to impact substantially on human physical performance.[1] This article reviews what we have learned in the intervening period.

1.1 Methods

This article does not represent a formal, structured systematic review, but is rather a contextual discussion of the field and of the key relevant papers. As such, we used PubMed, MEDLINE and Google Scholar to identify articles of relevance published between 1 May 1998 (the first published report of the *ACE* genotype being associated with physical performance) and 1 March 2010. The primary search terms were 'ACE/angiotensin converting enzyme/angiotensin 1-converting enzyme', with 'genotype/polymorphism'. Search results were then narrowed using terms relevant to performance phenotypes, including 'performance', 'power', strength', 'athlete', '$\dot{V}O_{2max}$', 'altitude', 'hypoxia' and 'elite'. Studies were excluded if no English language translation were available. Only human studies were sought.

1.2 Human RAS

The endocrine renin-angiotensin system (RAS) was long considered a key regulator of circulatory homeostasis. Here, renin (a 37 kDa aspartyl protease) cleaves hepatically derived angiotensinogen to yield decapeptide angiotensin I. This, in

turn, is acted upon by the peptidyl dipeptidase ACE to generate octapeptide angiotensin II (Ang II). Agonist action of Ang II at the angiotensin type-1 receptor (AT_1R) causes an elevation in arterial blood pressure through direct arterial vasoconstriction, and through salt and water retention provoked by adrenal aldosterone release. The vascular role of other receptors for Ang II (such as AT_2R) and its degradation products (e.g. the AT_4R) are less well characterized.[2] Meanwhile, ACE also cleaves bradykinin, a 9-amino acid peptide member of the kinin-kallikrein system, which is a potent vasodilator.[3] Bradykinin acts on the two receptors, bradykinin type-1 receptor (BK_1R) and BK_2R.[4] Bradykinin levels are therefore inversely related to ACE activity.[5,6] Increasing, ACE activity therefore drives hypertensive responses (increased AT_1R activation) and diminishes hypotensive responses (reduced BK_2R activation), thereby playing a crucial role in the regulation of human blood pressure and salt and water balance.[7]

In addition to this endocrine RAS, however, local tissue and cellular RAS (paracrine, autocrine and intracrine) also exist in diverse tissues where they serve a variety of functions, many of which are related to the regulation of tissue growth and injury responses.[8-10]

Functional genetic polymorphic variants have been identified for most components of the RAS, including renin, angiotensinogen, and the Ang II and bradykinin receptors (table I). By far the best known (and best studied) is a polymorphism in the human *ACE* gene. Plasma ACE levels are very stable within individuals, but marked inter-individual variations exist.[27] The absence (deletion [D]) rather than the presence (insertion [I]) of a 287-base pair (bp) *Alu* repeat sequence within intron 16 of the *ACE* gene is associated with elevated plasma[11] and tissue[12,13] ACE activity; those homozygous for the deletion allele demonstrate cardiac and monocyte ACE activity almost >75% than that found in those of I/I or I/D genotypes.[12,13]

1.3 The Angiotensin I-Converting Enzyme (ACE) Insertion/Deletion Polymorphism and Human Physical Performance

The *ACE* I/D polymorphism was the first specific gene variant to be associated with human physical performance.[1] Maximum duration of a standardized repetitive elbow flexion exercise (using a 15 kg barbell) was recorded in 78 Caucasian military recruits before and after 10 weeks of identical military training. Baseline performance was independent of *ACE* genotype, unlike improvements in exercise duration with training, which were strongly genotype-dependent; gains of 79.4 ± 25.2 and 24.7 ± 8.8 seconds were seen in those of I/I and I/D genotype, respectively ($p = 0.005$ and 0.007), but not in D/D homozygotes (7.1 ± 14.9 seconds; $p = 0.642$). The I/I homozygotes thus showed an 11-fold greater improvement than those of D/D genotype.

In addition, the association of the *ACE* genotype with performance did not seem limited to the

Table I. Selected polymorphisms of the renin-angiotensin system and associated receptors

Gene	Polymorphism/alleles	Gene location	Functional effect	References
ACE (17q22-24)	287 bp I/D	Intron 16	Protein levels	11-14
	C > T (position 4656)	3′ UTR	Protein levels	
Angiotensinogen (1q42-q43)	M > T (position 235)	Exon2 (+704)	Protein levels	15-21
	A > C (position 20)	5′ UTR promotor	Protein levels	
	A > G (position 6)	5′ UTR promotor	Protein levels	
	C > T (position 532)	5′ UTR	Protein levels	
Renin (1q32-q32)	rs5707 (T > G)	Intron 4	Protein levels	22
Angiotensin II type-1 receptor (3q21–q25)	A > C (position 1166)	3′ UTR	Receptor sensitivity	23-25
	T > A (position 810)	Promoter	Unknown	
Angiotensin II type-2 receptor (Xq22–q23)	G > A (position 1675)	Intron 1	Protein levels	26

bp = base pair; **D** = deletion; **I** = insertion; **UTR** = untranslated region.

young Caucasian male. In 83 postmenopausal women randomized to receive hormone replacement therapy rather than placebo,[28] those of I/I genotype showed greater increases in adductor pollicis muscle strength than those of I/D or D/D genotype (mean ± standard error 16.0 ± 1.53%, 14.3 ± 2.67% and 7.76 ± 4.13%, respectively; $p = 0.017$ for gene effect, $p = 0.004$ for I allele effect).

Since then, a wealth of other studies has supported an association of the *ACE* genotype with sporting performance. In general, the I allele seems associated with endurance-orientated events.[9] Thus, in a study of 91 British Olympic-standard runners (79 Caucasian), I allele frequency increased with competitive distance, from 0.35 to 0.53 and 0.62 for the three distance groups ≤200 m, 400–3000 m and ≥5000 m, respectively ($p = 0.009$ for linear trend).[29] This was noted to hold true in the subanalysis of the 79 Caucasians. Meanwhile, in a study of 35 truly elite ultra-distance swimmers, genotype frequencies differed ($p < 0.01$) for those classified as better at 1- to 10-km distances (6% I/I vs 47% I/D vs 47% D/D) when compared with those who were best at 25-km races (18.8% I/I vs 75% I/D vs 6.2% D/D). I-allele frequency was 0.29 for the shorter distance swimmers and 0.59 for the 25-km group.[30] Similarly, an excess of the I allele was identified amongst the 64 members of the Australian Olympic rowing squad in the 1996 Atlanta games ($p < 0.02$),[31] amongst long-distance cyclists and Russian endurance athletes,[9,32] and amongst the fastest 100 South African-born finishers (103 I [51.5%] and 97 D [48.5%]), and of the 2000 and 2001 South African Ironman triathlons (140 I [42.2%] and 192 D [57.8%]; $p = 0.036$).[33]

While the I allele seems associated with endurance-orientated events, the D allele seems associated with strength- and power-orientated performance.[9] The majority of swimming events are undertaken in <2 minutes[29] and thus power rather than endurance is likely to be key to success. In Olympic level swimmers, an excess of the D allele was noted compared with controls (0.60 vs 0.51; $p = 0.034$),[29] a finding replicated amongst elite Caucasian swimmers from European and Commonwealth championships ($p = 0.004$), where a significant excess of the D allele was noted, especially in those competing over shorter distances ($p = 0.005$ for ≤400 m).[34] Further studies have demonstrated this association, but only in the elite short-distance swimmers (<200 m).[30,35]

Conflicting data do exist, but generally seem explained by the study of heterogeneous (often mixed race and sex, and sporting discipline) subject groups.[36-42] While one must be cautious, in that association does not imply causality, the reports have generally maintained consistency. It should be acknowledged that the majority of earlier studies were population studies, with little physiological data, this being more common in more recent studies. Homogeneity is desirable in these genetic performance studies as the *ACE* gene is only one of many genetic factors affecting performance, and the addition of other (non-genetic factors) such as age, sex and sporting discipline, which may themselves interact with genotype, often results in too great a variance in phenotype to be able to discern the role of genotype. Notable exceptions do exist, and a well designed study in 121 Israeli runners reported a positive association between the D/D genotype and elite endurance performance.[42] One explanation for this atypical finding is that it represents artefact related to the heterogeneous nature of the Israeli Caucasian Jewish population.[43] Alternatively, this may be a true reflection of the Israeli population, though greater numbers would be needed to clarify this. A large Korean-based study showed a decreasing frequency of the D allele with advancing levels of performance in power-orientated athletes (in track and field, and weight lifting).[44] Whilst the subjects were from mixed disciplines and the numbers in each discipline were small, this study (just as those in Israeli runners) is noteworthy in its conflict with the prevailing view.

Table II summarizes studies relating the *ACE* genotype to sporting performance in the last 12 years.

In theory, such associations with the *ACE* genotype might be due to linkage of the *ACE* I/D polymorphic site with functional variation in an adjacent locus, such as the nearby growth hormone (GH).[76] The T > A variant in intron 4 of the *GH1* gene has been associated with lower levels of GH and insulin-like growth factor-I.[77]

Table II. Studies in athletes associating the *ACE* insertion/deletion (I/D) genotype with sporting performance

Study	Cohort	No. of subjects and ethnicity	Performance[a]	Outcome measure[b]	Association with performance	I/D associations
Amir et al.[42]	Runners	121 Israeli	Elite	Performance	Yes	D and endurance
Cam et al.[45]	Sprinters	88 Caucasian	Non-elite	Performance	Yes	D and short distance
Cerit et al.[46]	Army	186 Caucasian	Army	Performance	Yes	D and short duration
Colakoglu et al.[47]	Athletes	99 Caucasian	Non-elite	Performance	Yes	D and strength
Juffer et al.[48]	Footballers	52 mixed	Elite	Prevalence	Yes	D more prevalent
Lucia et al.[49]	Cyclists	50 Caucasian	Elite	Performance	Yes	D/D and endurance
Munisea et al.[50]	Mixed	141 mixed	Elite	Prevalence	Yes	D/D and endurance rowers
Winnicki et al.[51]	Mixed	233 mixed	Sedentary	Performance	Yes	D/D and sedentary lifestyle
Nazarov et al.[9]	Mixed	217 Caucasian	Elite	Sport performance	Yes	D/D and short distance
Costa et al.[52]	Swimmers	72 Caucasian	Elite	Prevalence and performance	Yes	D/D and short distance
Woods et al.[34]	Swimmers	102 Caucasian	Elite	Prevalence and performance	Yes	D/D and short distance
Giaccaglia et al.[53]	Elderly	213 mixed	Sedentary	Training	Yes	D/D and strength
Zhao et al.[54]	Army	67 Chinese	Army	Performance	Yes	D/D and $\dot{V}O_{2max}$
Tsianos et al.[55]	Climbers	284 mixed	Elite	Performance	Yes	I and high ascent
Gayagay et al.[31]	Rowers	64 Caucasian	Elite	Prevalence	Yes	I and endurance
Myerson et al.[29]	Runners	91 Caucasian	Elite	Sport performance	Yes	I and endurance
Montgomery et al.[1]	Army	78 Caucasian	Army	Performance	Yes	I and endurance
Collins et al.[33]	Triathletes	166 Caucasian	Elite	Performance	Yes	I and endurance
Hruskovicova et al.[56]	Runners	445 Caucasian	Elite	Performance	Yes	I and endurance
Cieszczyk et al.[57]	Rowers	55 Caucasian	Elite	Prevalence	Yes	I and endurance
Min et al.[58]	Track and field	277 Japanese	Non-elite	Prevalence	Yes	I and endurance
Rankinen et al.[36]	Mixed	192 Caucasian	Elite	Prevalence and performance	Mixed	I and endurance

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Table II. Contd

Study	Cohort	No. of subjects and ethnicity	Performance[a]	Outcome measure[b]	Association with performance	I/D associations
Tsianos et al.[30]	Swimmers	35 Caucasian	Elite	Performance	Yes	I and endurance, D and short distance
Cam et al.[59]	Runners	55 Caucasian	Non-elite	Performance	Yes	I and endurance, D and short distance
Moran et al.[60]	Mixed	1027 Caucasian	Adolescents	Performance	Yes	I and endurance, D and strength
Thompson et al.[61]	Climbers	139 Caucasian	Elite	Prevalence and performance	Yes	I and high ascent
Hurlbut et al.[62]	Sedentary	40 Caucasian	Sedentary	Training	Yes	I and insulin requirements
Dekany et al.[63]	Mixed	50 Caucasian	Elite	Performance	Yes	I and metabolic efficiency
Williams et al.[64]	Army	58 Caucasian	Army	Training	Yes	I and metabolic efficiency
Kim et al.[44]	Power athletes	155 Korean	Elite	Sport performance	Yes	I and power
Kritchevsky et al.[65]	Elderly	3075 Caucasian	Sedentary	Activity	Yes	I/I and mobility limitation
Goh et al.[66]	Rugby players	17 Singaporean	Non-elite	Performance	Yes	I/I and $\dot{V}O_{2max}$
Alvarez et al.[32]	Mixed	60 Caucasian	Elite	Prevalence	Yes	I and elite status
Turgut G et al.[67]	Mixed	160 Turkish	Athletes and sedentary	Prevalence	Yes	I and athletes
Scott et al.[68]	Sprinters	230 African-Americans	Elite	Sport performance	No	
Sonna et al.[39]	Army	148 mixed	Army	Training	No	
Frederiksen et al.[69]	Elderly	684 Caucasian	Sedentary	Activity	No	
Scott et al.[70]	Runners	271 Africans	Elite	Performance	No	
Day et al.[71]	Sedentary	62 Caucasian	Sedentary	Performance	No	
Oh[72]	Mixed	139 Korean	Elite	Performance	No	
Papadimitriou et al.[73]	Track and field	101 Greek	Elite	Prevalence	No	
Thomis et al.[74]	Twins	54 Caucasian	Sedentary	Training	No	
McCauley et al.[75]	Active	79 Caucasian	Active	Performance	No	
Frederiksen et al.[69]	Elderly	203 Caucasian	Sedentary	Performance	No	

a Elite performance status refers to athletes involved in competition at an international level.

b Training as an outcome measure refers to an alteration in a pre-determined set of training exercises.

D = deletion; **I** = insertion; $\dot{\mathbf{V}}\mathbf{O_{2max}}$ = maximal oxygen consumption.

This variant was examined in the aforementioned South African Ironman, but no association was seen with performance.[78] There was, however a differential in the post-race temperature, with the AA genotypes having a significantly higher temperature (37.7 ± 0.8°C vs 37.2 ± 0.8°C; p = 0.019), consistent with a genetic predisposition to metabolic efficiency (see sections 1.5 and 1.6). A study in 847 British military recruits failed to demonstrate a GH-dependant effect on left ventricular (LV) mass by training, suggesting the effects of ACE I/D on exercise-induced LV growth (see section 1.4) are not mediated through linkage to the GH site.[79]

Alternatively, these effects of the *ACE* genotype may be mediated through changes in Ang II activity. Ang II acts on the AT_1R, the gene for which itself has two polymorphisms[23,80] that appear functional.[40,81,82] However, these do not seem to be associated with differences in performance,[32,83] although one small Korean study (17 females) has suggested a possible association with training-related gains in maximal oxygen consumption ($\dot{V}O_{2max}$).[84] Studies of hypoxic ascent (see section 1.5) have yielded conflicting results.[85,86]

Finally, *ACE* genotype does influence bradykinin levels (see section 1.2). The ACE I allele is associated with higher kinin activity, while a common gene variant in the BK_2R exists: the absence (–9) rather than the presence (+9) of a 9-bp fragment is associated with greater receptor response and gene transcription.[87,88] In a study of running distance in 81 Olympic-standard track athletes, the 'high-kinin receptor activity' haplotype (*ACE* I/*BK2BR* –9) was associated with endurance performance (p = 0.003). This suggests that at least part of the association of ACE with performance phenotypes is mediated through changes in kinin activity at its BK_2R.[89] Similarly, in the 2000 and 2001 South African Ironman triathlons, the *B2BKR* –9/–9 genotype occurred at a significantly higher frequency amongst the triathletes (27.0%) when compared with controls (19.3%; p = 0.035), the –9 allele being associated with shorter finishing times.[90] Interestingly the –9 allele was also associated with reduced weight loss among the triathletes, consistent with an effect on metabolic efficiency (see section 1.5).[91]

Thus, *ACE* genotype is associated with differences in athletic performance; in part through modulation of kinin levels (although a role for altered Ang II activity at its receptors cannot be excluded). But through what physiological processes might such biochemical effects be exerting their influence?

1.4 ACE Polymorphism and Cardiac Muscle

Ang II acts as a trophic agent on cardiac muscle[92,93] in both normal and diseased states.[94] The expression of myocardial RAS components rises during LV hypertrophy (LVH),[95] which is likely to be mediated through the increased synthesis of Ang II and subsequent AT_1R activation.[92,93,96] ACE inhibition in animal models attenuates LVH and also leads to a greater regression in LV mass than other similarly hypotensive agents.[97-99] The rise in racehorse LV mass with training correlates with athletic performance.[100] Positive correlations have also been seen between LV mass, aerobic performance and race jumping ability.[101,102] In humans, an exaggerated LV growth response to training is associated with the ACE D allele.[103] Thus, in 140 Caucasian male military recruits, the rise in echocardiographically assessed LV mass associated with 10 weeks of physical training was +2.0, +38.5 and +42.3 g in the I/I, I/D and D/D groups, respectively (p < 0.0001). Furthermore, the rise in prevalence of ECG-defined LVH was also *ACE* genotype dependent (p < 0.01).[103] These findings were replicated in a cohort of 74 male endurance athletes,[104] in whom athletes of D/D genotype had significantly higher LV mass indices (LVMI) as well as a higher prevalence of LVH (LVMI >131 g/m^2). However, the highest LVMI (150 ± 23 g/m^2) was seen in the 15 athletes with both ACE D/D and AT_1R AC/CC genotypes. The association of the ACE D allele with LV mass has since been confirmed in elite wrestlers,[105] elite football players[83] and endurance athletes.[106] Studies that have examined subjects exposed to multiple hypertrophic stimuli at different timepoints, or diverse population groups have, however, (perhaps unsurprisingly) failed to identify this association.[40,107-111] Nonetheless, the ACE D allele does seem associated

with LVH in disease states such as non-insulin-dependent diabetes mellitus,[112] hypertension,[113] hypertrophic cardiomyopathy[114] and calcific aortic stenosis.[115] Rather counter intuitively, however, I/I (compared with D/D) genotype has been associated with a significantly greater impairment in fractional shortening in response to ultra-endurance exercise.[116] The extent of extra-cardiac adaptation (in response to training) has also been associated with the *ACE* genotype.[117] In a study of 56 athletes, the I/I genotype was associated with increased aortic compliance in response to training. Alterations on aortic compliance affect LV work, thus influencing LV mass indirectly.

Such effects, in humans, may be less dependent upon *ACE* genotype-dependent differences in Ang II activity at the AT_1R. The administration of sub-hypotensive doses of the AT_1R antagonist losartan to 141 British Army recruits did not alter the LV growth response to exercise.[118] In a study of 90 patients undergoing anti-hypertensive therapy, *B2BKR* +9/+9 genotype was associated with poor LV mass regression.[119] This was uncorrected for the patients *ACE* genotype. In a group of 109 military recruits undergoing 10 weeks of basic physical training, both the *ACE* and *B2BKR* genotypes interacted biologically in an additive way, with those of a genotype likely to be associated with lowest kinin activity (ACE D/D, *B2BKR* +9/+9) exhibiting the greatest LV growth. In these, mean LV growth was 15.7 g, compared with −1.37 g in those homozygous for ACE I and *B2BKR* −9 alleles ($p = 0.003$ for trend across genotypes).[120]

Sheer increases in LV size, however, do not automatically lead to increased performance. Further, the ACE I allele is associated with the lowest cardiac growth response but also with the endurance-exercise phenotype. It thus seems likely that other mechanisms exist.

1.5 *ACE* Genotype and Skeletal Muscle

The *ACE* genotype may also influence human skeletal muscle growth. Certainly, Ang II transduces mechanical load to yield growth responses,[121] which might translate to greater strength gains. In keeping with 33 healthy subjects undergoing a 9-week training regimen, greater gains in quadriceps strength (both isometric and dynamic) were seen in D-allele carriers.[121] Other work supports the role of the *ACE* genotype in the regulation of muscle strength.[122] In 103 patients with chronic obstructive pulmonary disease (COPD), the D allele was associated with greater quadriceps strength, using non-volitional testing.[123] These effects may, in part, be mediated by genotype-dependent differences in skeletal muscle growth, but may also be mediated by differences in muscle fibre type. In 41 untrained healthy volunteers, the I allele was associated with a predominance of type-I muscle fibre (fatigue resistant, 'slow twitch') when compared with the D allele.[124]

Just as in the heart, this association of genotype with muscle performance may be partly mediated through changes in bradykinin activity at the BK_2R. Bradykinin modulates the action of insulin on skeletal muscle and fat.[125] Animal models have demonstrated improved insulin-dependent glucose transport with ACE inhibitors.[126,127] Other metabolic influences of bradykinin on muscle substrates and transport include those on glycogen levels, lactate concentration,[128] the availability of glucose/free fatty acid substrates,[129] and the expression of the GLUT4 glucose transporter.[130] In 110 patients with COPD, reduced BK_2R activity was associated with reduced quadriceps strength.[131]

The *ACE* genotype may also be associated with differences in the mechanical/metabolic efficiency of skeletal muscle.[132] In a study of Caucasian male military recruits undergoing an 11-week physical training programme, baseline delta efficiency (DE; defined as the change in work performed per minute to the change in energy expended per minute) was independent of genotype.[64] However, recruits of I/I genotype showed a significant increase in DE (an absolute change of +1.87%, representing a proportional increase of 8.62% relative to baseline) not seen in recruits of D/D genotype (absolute change of −0.39%). In keeping with such an effect on 'metabolic efficiency', the I allele seemed to be associated with a relative anabolic response (in terms of both muscle and fat mass) amongst military recruits under conditions of high calorie expenditure

during intensive physical training.[133] As before, subjects of different genotype were phenotypically indistinguishable prior to engaging in training. Such effects may, in part, be mediated through *ACE*-genotype-dependent modulations in kinin activity. In a study of 115 healthy men and women, DE was strongly associated with BK_2R genotype (23.84 ± 2.41% vs 24.25 ± 2.81% vs 26.05 ± 2.26% for those of +9/+9 vs +9/–9 vs –9/–9 genotypes; p = 0.0008).[89] This study also found evidence of interaction with the *ACE* I/D genotype. Subjects who were of *ACE* I/I and *B2BKR* –9/–9 genotype had the highest baseline DE.[89] Further, the D allele was associated with greater rises in core temperature during a standardized heat-exertion test.[134] The *ACE* genotype may also affect metabolic efficiency via systemic effects, although these are less well documented.[135,136]

If *ACE* genotype influences metabolic efficiency, then one might anticipate a marked association of genotype with performance in hypoxic environments. Elite British male mountaineers who have ascended beyond 7000 m without the use of supplemental oxygen, demonstrate a significant excess in I allele (and I/I genotype) frequency when compared with controls.[137] The same finding was made in 139 mountaineers attempting an ascent to 8000 m, in whom the I allele was associated with maximal altitudes achieved (8079 ± 947 m for D/Ds, 8107 ± 653 m for I/Ds, and 8559 ± 565 m for I/Is; p = 0.007).[61]

Whilst a role for genotype-dependent differences in muscle metabolic efficiency may underpin these findings, other mechanisms may also play a role. Whilst not identified in 126 tourist climbers ascending Mount Kilimanjaro (5895 m) or in a high-altitude pulmonary oedema study of 164 climbers at 4559 m,[138,139] others have suggested that part of this association may be mediated through an increased risk of acute mountain sickness being associated with the D allele.[140,141] Meanwhile, the ACE I allele seems to be associated with an enhanced exertional ventilatory response to acute hypoxia,[142] and thus with the preservation of arterial oxygenation at high altitude in rapid ascent.[143] Corroboratory epidemiological evidence exists among the Quechua-speaking native people living above 3000 m in South America, and those in the Ladakh region of India living above 3600 m.[144,145] The I allele was found in greater frequency in those who had migrated from the lowlands. The relationship is unclear, and negative studies exist. González et al.[146] studied 63 athletes exposed to an altitude of 2200 m, and failed to demonstrate a relationship between *ACE* genotype and the erythropoietic response to altitude.

Finally, *ACE* genotype may be associated with differences in the muscle injury response. In a study of 70 physically active subjects undergoing eccentric exercise, the strongest independent determinant of peak creatine kinase (CK) levels was *ACE* genotype.[91] Here, the I/I genotype was associated with a greater CK rise (adjusted odds ratio 1.3; 95% CI 1.03, 1.64; p = 0.02). Others have failed to replicate these findings (peak changes in serum CK levels being non-significantly lower amongst those of I/I genotype).[147] However, the use of a racially heterogeneous cohort might explain this finding, as might the study of 'loaded squats' rather than upper-limb loading, as in the previous study.[147]

Currently, observational data suggest that the use of ACE inhibitors may modulate muscle metabolism to a indicate where mass (and metabolic efficiency) are altered to a measurable level.[148] High-quality randomized trials in both physiological and pathophysiological states are now warranted to determine the effects of RAS modulation on muscle mass, function and ultimately on global physical performance.

1.6 ACE and Maximal Oxygen Consumption

$\dot{V}O_{2max}$ is a physiological characteristic bounded by the parametric limits of the Fick equation:

(LV end-diastolic volume − LV end-systolic volume) × heart rate × arterio-venous oxygen difference

Elite endurance athletes have a high $\dot{V}O_{2max}$ due primarily to a high cardiac output from a large compliant cardiac chamber (including the myocardium and pericardium), which relaxes quickly and fills to a large end-diastolic volume.[149] Peak $\dot{V}O_{2max}$ has been associated with performance

in competitive endurance-based sport.[150] To date, any putative association between *ACE* genotype and peak $\dot{V}O_{2max}$ remains unproven. Abraham et al.[151] studied 57 patients, stratified by *ACE* genotype, with impaired LV function of ischaemic origin. No differences in baseline LV function were noted across *ACE* genotypes, although *ACE* D/D genotype was associated with a decreased mean $\dot{V}O_{2max}$. Similarly, amongst 47 postmenopausal women, those subjects of I/I genotype demonstrated a 6.3 mL/kg/min higher $\dot{V}O_{2max}$ than those of D/D genotype and a 3.3 mL/kg/min higher $\dot{V}O_{2max}$ ($p < 0.05$) than the *ACE* I/D genotype group.[152] However, other studies cast doubt upon these data. Two studies, one in sedentary females and the other in both active and sedentary females, using cycle ergometry and maximal treadmill exercise tests, found no relationship between *ACE* genotype and peak $\dot{V}O_{2max}$.[71,153] However, both groups have relatively small cohorts (62 and 77, respectively). The contribution of cardiac contractile performance to $\dot{V}O_{2max}$ is likely to be limited in those with impaired cardiac contratile function (such as those with cardiac disease *per se*, or in the elderly in whom systolic and diastolic function may be limited when compared with younger populations). Elderly females, just as those with heart disease, may be receiving a range of medications that might confound reliable observation. A much larger cohort of US army recruits undergoing 8 weeks of basic training, found no significant association between *ACE* genotype, peak $\dot{V}O_{2max}$ or other measures of performance.[39] However, this study was also flawed; the 147 recruits included had varying baseline fitness levels, and were of both sexes, diverse ages and were drawn from a spread of ethnic groups, making reliable interpretation difficult. Further studies in army recruits do, however, suggest that the cardiopulmonary response to training does not seem *ACE*-genotype dependent.[154] Bouchard et al.[155,156] studied 99 families with 415 pairs of siblings, and found no association of the *ACE* locus (17q23) with baseline $\dot{V}O_{2max}$ or its response to a 20-week standardized endurance training programme. Studies in COPD have also yielded conflicting results.[157,158] A single study in post-myocardial infarction patients has demonstrated a differential increase in $\dot{V}O_{2max}$ as a result of training between the I/I and D/D genotypes.[159]

Overall, further studies are required of the response to different training regimens (intensity and duration), in populations of homogeneous race, sex and age, and disease state if this issue is to be satisfactorily addressed. Other questions also need to be addressed. Is the discrepancy in the propensity to gain muscle mass in the I/I homozygotes in some studies and D/Ds in others the result of competing effects of RAS on muscle growth and metabolic efficiency? Or is it of changes in substrate use, in which a dietary influence might play a role? Is the association of the *ACE* I allele with performance of mountaineers mediated through the same mechanisms as those for elite endurance performance at sea level? Can one mimic the training effects of I/I homozygotes in D/D homozygotes with the use of ACE inhibitors? Finally, the relative role of Ang II and bradykinin (and of their specific receptors) needs clarification.

2. Conclusions

Human sporting phenotypes result from the interaction of genetic variation with environmental stimuli. The *ACE* I/D polymorphism is but one such genetic factor – the D allele tending to be associated with power/sprint performance, and the I allele with endurance sports. The mechanisms underlying such observations remain inadequately explored, as does the role for specific RAS antagonists in modulating such performance.

The prevalence of both the D and I alleles in populations worldwide suggest that they may both have offered different survival advantages. That of the I allele may relate to improved endurance performance, and enhanced oxygen utilization in times of both exercise and illness. The D allele, being associated with gains in strength with training, may offer separate advantages related directly to strength itself, but also to the acquisition of increased muscle bulk in response to muscle strength training/high loading. In addition, however, *ACE* genotype influences a variety of other phenotypes, such as haemorrhage response[160] to

the outcome from infection[161] – all of which may offer separate evolutionary selection pressures beyond those exerted through 'fitness phenotypes' alone.

Indeed, such issues remind us of the reason for study. Whilst research in the field of sports genetics might raise the spectre of drug doping,[162] it has intrinsic scientific value, and may also suggest possible therapeutic targets.

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REVIEW ARTICLE

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Effect of Mouth-Rinsing Carbohydrate Solutions on Endurance Performance

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Contents

Abstract

Ingesting carbohydrate-electrolyte solutions during exercise has been reported to benefit self-paced time-trial performance. The mechanism responsible for this ergogenic effect is unclear. For example, during short duration (≤1 hour), intense (>70% maximal oxygen consumption) exercise, euglycaemia is rarely challenged and adequate muscle glycogen remains at the cessation of exercise. The absence of a clear metabolic explanation has led authors to speculate that ingesting carbohydrate solutions during exercise may have a 'non-metabolic' or 'central effect' on endurance performance. This hypothesis has been explored by studies investigating the performance responses of subjects when carbohydrate solutions are mouth rinsed during exercise. The solution is expectorated before ingestion, thus removing the provision of carbohydrate to the peripheral circulation. Studies using this method have reported that simply having carbohydrate in the mouth is associated with improvements in endurance performance. However, the performance response appears to be dependent upon the pre-exercise nutritional status of the subject. Furthermore, the ability to identify a central effect of a carbohydrate mouth rinse maybe affected by the protocol used to assess its impact on performance. Studies using functional MRI and transcranial stimulation have provided evidence that carbohydrate in the mouth stimulates reward centres in the brain and increases corticomotor excitability, respectively. However, further research is needed to determine whether the central effects of mouth-rinsing carbohydrates, which have been seen at rest and during fatiguing exercise, are responsible for improved endurance performance.

1. Introduction

The endogenous stores of carbohydrate are finite. Prolonged fixed-intensity exercise to fatigue is associated with the depletion of muscle glycogen and/or hypoglycaemia. Thus, it is widely accepted that providing carbohydrate during exercise can improve endurance capacity by preventing hypoglycaemia and can provide a ready fuel for the working muscles (for reviews see Coyle[1] and Tsintzas and Williams[2]). A common method of providing carbohydrate during exercise is in the form of carbohydrate-electrolyte solutions. Providing carbohydrate in the form of a carbohydrate-electrolyte solution supplies fuel as well as providing fluid and electrolytes that are lost as a consequence of sweating. Ingesting fluid during exercise has been reported to reduce cardiovascular stress and hyperthermia associated with exercise-induced dehydration.[3] In addition, fluid ingestion has been reported to have a profound metabolic effect during exercise. For example, ingesting fluid alone has been reported to improve endurance capacity[4] by reducing the utilization of muscle glycogen.[5] Therefore, it is not surprising that most laboratory studies have examined the influences of ingesting carbohydrate-electrolyte solutions on endurance capacity (>1 hour) rather than endurance performance (i.e. time trials). This is because the associated increases in core temperature and heart rate during short-duration exercise are not as pronounced as exercise of greater durations (>1 hour).[3,6] In addition, hypoglycaemia and severe depletion of muscle glycogen have not been reported following short periods (≤1 hour) of intense (>70% maximal oxygen consumption [$\dot{V}O_{2max}$]) exercise.[7]

The metabolic effect of carbohydrate ingestion appears to differ depending upon the mode of exercise. For example, blood glucose concentrations during prolonged treadmill running do not decrease to the same extent as with prolonged cycling.[2] It is important to note that the majority of studies investigating the impact of carbohydrate ingestion on endurance performance have used cycling rather than running as the mode of exercise.

McConell et al.[8] reported that during high-intensity cycling, only a small percentage (26%) of the total carbohydrate ingested actually enters the peripheral circulation during exercise. In addition, ingesting glucose has been reported to have no effect on carbohydrate oxidation, muscle metabolism or performance when cycling to fatigue at approximately 80% $\dot{V}O_{2max}$.[8] Furthermore, when glucose was infused directly into the circulation (60 g/h), the rate of muscle glycogen oxidation was unaffected. Exogenous carbohydrate was reported to contribute to only 9 g of the 54 g of carbohydrate oxidized in the final quarter of a 1-hour cycling time trial.[9] However, in running, the ingestion of 50 g of carbohydrate in a 5.5% solution has been reported to result in a 28% sparing of glycogen in the vastus lateralis muscle during a 60-minute treadmill run. The ingestion of the carbohydrate solution resulted in a 42% sparing of glycogen in the type I muscle fibres, with type II muscle fibres unaffected. The amount of glycogen spared was directly related to the magnitude of serum insulin increase within the first 20 minutes of exercise.[10] Nevertheless, adequate concentrations of glycogen remained in the muscle following the 60-minute treadmill run at 70% $\dot{V}O_{2max}$ in both the carbohydrate and placebo trials. For a comprehensive review on muscle glycogen metabolism during both running and cycling exercise, consult Tsintzas and Williams.[2] To our knowledge, no studies have measured muscle glycogen concentrations in response to mouth rinsing with a carbohydrate solution.

Despite the absence of a clear metabolic rationale, both fluid and carbohydrate ingestion have been reported to independently improve time-trial performance.[6] Below et al.[6] asked subjects to cycle at a constant intensity (80% $\dot{V}O_{2max}$) for 50 minutes followed by a 10-minute time trial, in which the task was to complete a fixed amount of work as quickly as possible. Providing both fluid and carbohydrate improved time-trial performance by approximately 6%. Furthermore, the beneficial independent effects of fluid and carbohydrate ingestion on performance were reported to be additive. The improvements in performance with fluid ingestion was attributed to maintaining a higher cardiac output and attenuating the increases in core temperature and heart rate, which were observed when no fluid was ingested. However,

there was no evidence that carbohydrate ingestion influenced either core temperature or heart rate. Furthermore, ingesting carbohydrate did not appear to have a significant effect on blood glucose concentrations or carbohydrate oxidation. Thus, an explanation by which carbohydrate improved performance in this study was reported to be 'unclear'.[6]

It is important to note that not all studies have reported a benefit following the ingestion of fluid[11] or carbohydrate[12,13] on time-trial performance. Nevertheless, there is substantial evidence showing that ingesting appropriate carbohydrate-electrolyte solutions during exercise can improve endurance performance of approximately 1 hour in duration. Benefits to performance have been reported in both cycling[14-17] and running.[18,19] However, a mechanism to explain this improvement in performance remains to be established.

Intriguingly, the absence of a clear metabolic benefit when subjects ingest carbohydrate has led authors to speculate that carbohydrate may influence 'central' or 'non-metabolic' pathways during exercise. To this end, this review will include a consideration of those studies that have investigated the potential 'central' effect of carbohydrate on performance. Studies testing this hypothesis have removed the provision of glucose or fluid to the peripheral circulation by requiring their subjects to simply mouth rinse the carbohydrate solution without ingestion. Thus, this review will focus on the performance response to simply having carbohydrate in the mouth and potential mechanisms by which this may exert an ergogenic effect. The impact of pre-exercise nutritional status, mode of exercising testing, concentration and type of carbohydrate in the rinsed solution will be discussed separately. The importance of the method and protocols used to detect a possible ergogenic effect of mouth-rinsing carbohydrate-electrolyte solutions will also be considered. The literature cited in this review was retrieved using online search databases (i.e. PubMed and SportDiscus®). Key search terms used included 'carbohydrate', 'mouth rinse', 'performance', 'oral', 'central' and 'exercise (running and cycling)'.

2. Evidence: Performance Studies

To our knowledge, only six studies have investigated the influence of mouth rinsing with a carbohydrate solution on endurance performance. The purpose of this section is to review the studies in the order in which they were published. Furthermore, the impact that mouth rinsing with a carbohydrate solution has on performance, has only been investigated using cycling and running. Therefore, the original studies completed in cycling will be reviewed first followed by the studies that used running as a mode of exercise.

2.1 Cycle Time Trials

Carter et al.[20] were the first to provide evidence that mouth rinsing with a carbohydrate solution during exercise could improve cycle time-trial performances of approximately 1 hour in duration. In this study, seven male and two female cyclists completed two experimental trials, where the task was to complete a fixed amount of work (914 ± 40 kJ) as quickly as possible. In the two trials, which were separated by 1 week, the subjects mouth rinsed with either a 6.4% maltodextrin solution or water (25 mL) at every 12.5% of the time trial completed. The solutions were rinsed in the mouth for approximately 5 seconds before being expectorated. The trials were completed following a 4-hour postprandial period. However, the exact composition of the pre-exercise meal was not stated. The mean power output was significantly greater when mouth rinsing with carbohydrate than with water (259 ± 16 W vs 252 ± 16 W, respectively). Of note, an increase in power output was observed during the first three-quarters of the time trial. Eight of the nine cyclists improved their performance during the carbohydrate trial. Thus, time to complete the fixed amount of work was reduced on average by 2.9% when cyclists mouth rinsed with carbohydrate rather than water (59.57 ± 1.50 minutes vs 61.37 ± 1.56 minutes, respectively). There was no difference in heart rate (172 ± 1 beats/min and 171 ± 1 beats/min) or ratings of perceived exertion (RPE) [16 ± 1] when mouth rinsing with carbohydrate or water, respectively. Unfortunately, the volume of expectorate

was not measured and there was no blood sampling during exercise because the investigators did not want to disrupt the time-trial performance. Thus, whether or not any carbohydrate was inadvertently ingested could not be established.

Pottier et al.[21] investigated performance responses of 12 male cyclists to the same trial conditions reported by Carter et al.[20] In this study, each cyclist was required to complete the time trial (975 ± 85 kJ) on four occasions separated by 48 hours. The four experimental conditions involved either mouth rinsing with a placebo or carbohydrate-electrolyte solution, or ingesting a placebo or carbohydrate-electrolyte solution. The total quantity of solution rinsed/ingested was 14 mL/kg body mass. The subjects received 2 mL/kg body mass of the solution before the 5-minute warm up (100 W). The subjects then received 1.5 mL/kg body mass immediately before and at every 12.5% of the time trial completed. During the mouth-rinse trials, subjects mouth rinsed the solution for 5 seconds before the solution was expectorated. The carbohydrate-electrolyte solution was a commercially available sports drink. The placebo solution was identical in formulation except that it contained no carbohydrate. The cyclists completed the time trial significantly faster (3.7%) when mouth rinsing with the carbohydrate-electrolyte solution (61.7 ± 5.1 minutes) than with mouth rinsing the placebo (64.1 ± 6.5 minutes). However, ingesting the carbohydrate-electrolyte solution was reported not to improve performance (63.2 ± 6.9 minutes) over the ingestion of the placebo solution (62.5 ± 6.9 minutes). Surprisingly, mouth rinsing with the carbohydrate-electrolyte solution resulted in a greater improvement in performance than when ingesting the same solution. The authors suggest that performance was improved due to the presence of carbohydrate in the oral cavity. However, they speculate that this performance benefit may be lost due to the short oral transit time when the carbohydrate-electrolyte solution is ingested.[21] There were no clear differences in the physiological variables (blood lactate, blood glucose, heart rate) or RPE recorded during exercise. However, an interesting observation is that when ingesting or mouth rinsing with the carbohydrate-electrolyte solution, subjects began their exercise with higher blood glucose concentrations than subjects in either placebo trial. In this study, subjects were requested to consume a carbohydrate-rich meal 3 hours before the test and consume a carbohydrate-rich diet (400 g carbohydrate) the day before the trial. Unfortunately, the actual values and subject compliance to these dietary requests are not reported. It is important to note that different day-to-day dietary preparation for the time trials would have large effects on performance.[17] For example, the effect of mouth rinsing with a carbohydrate solution was investigated in cyclists who had consumed a standardized breakfast 2 hours before completing a time trial.[22] The breakfast provided before exercise contained 2.4 g of carbohydrate per kg of the subjects body mass. Ingesting similar quantities of carbohydrate 3 hours before exercise has been reported to increase muscle glycogen by 11–15%.[23,24] Fourteen male endurance-trained cyclists completed the same time trial as that used in previous cycling performance studies.[20,21] Identical to Carter et al.,[20] cyclists mouth rinsed with a 6.4% maltodextrin solution or water immediately before and every 12.5% of the time trial completed. Eight of 14 cyclists completed the time trial faster when mouth rinsing with the maltodextrin solution than with water. However, in this study, the performance time (68.14 ± 1.14 minutes vs 67.52 ± 1.00 minutes) and average power output (265 ± 5 W vs 266 ± 5 W) did not differ between the carbohydrate or water trials, respectively. There were no differences in heart rate reported between trials. Unfortunately, no blood samples or expired air was collected during exercise.

Chambers et al.[25] reported the results of two separate cycling time-trial performance studies that used the same protocols as described by Carter et al.[20] In both studies, the time trials were completed following an overnight fast, with each trial separated by at least 3 days. Subjects mouth rinsed with either a carbohydrate or placebo solution immediately before and every 12.5% of the time trial completed. In these studies, the test solutions were mouth rinsed for approximately 10 seconds (double the duration of previous studies) before being expectorated into a bowl.