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The effects of ACE gene polymorphisms on ACE content before and after High-Intensity Interval Exercise

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Objective Angiotensin Converting Enzyme (ACE) is expressed in human skeletal muscle. The *ACE* I/D polymorphism (rs4341) has been associated with athletic performance in some studies. Studies suggested that the *ACE* I/D gene polymorphism is associated with ACE enzyme content in serum, however, the effect of *ACE* I/D on ACE protein content in human skeletal muscle in unclear. Angiotensin-converting enzyme 2 (ACE2) is a new component of the renin-angiotensin system (RAS), which is counter-regulatory to the ACE enzyme. The polymorphisms in the *ACE2* gene (rs1978124 and rs2285666) have been reported to be associated with hypertension, however, their effects on ACE content in the blood and in skeletal muscle have yet to be explored. Utilising the Gene SMART cohort (n=81), we investigated whether the ACE I/D gene polymorphism (rs4341) and two ACE2 gene polymorphisms (rs1978124 and rs2285666) were associated with ACE enzyme content in the blood and skeletal muscle at baseline, and following a single session of High-Intensity Interval Exercise (HIIE).

Methods *ACE* and *ACE2* gene polymorphisms were determined using the TaqMan SNP assay (Applied Biosystems, Foster City, California, United States) by Mastercycler® ep realplex2 (Eppendorf, Hamburg, Germany), and QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, Foster City, California, United States). For quantitation of ACE content in the plasma, Abcam Human ELISA Kit (ab119577 –ACE (CD143)) was used (Abcam, Cambridge, United Kingdom). Western blots were used to measure ACE content in skeletal muscle. We used robust linear models adjusted for age to test the effect of the ACE I/D polymorphism on outcomes at baseline, using the MASS package in the R statistical software. p-values were adjusted for multiple comparisons using the Benjamini and Hochberg method, and all reported p-values are adjusted p-values. An adjusted p value < 0.005 was considered significant.

Results We found that the *ACE* I/D gene polymorphism was associated with ACE content in the blood ($p<0.005$) at baseline, but not the ACE protein content in skeletal muscle at baseline. The *ACE2* polymorphisms (rs1978124 and rs2285666) were not associated with ACE enzyme content in the blood or in skeletal muscle at baseline. A single session of HIIE tended ($0.005 < p < 0.05$) to increase blood ACE content immediately post exercise, while skeletal muscle ACE protein content was lower 3 hours post a single session of HIIE ($p<0.005$). However, those changes were not related to *ACE* I/D or *ACE2* polymorphisms.

Conclusions The *ACE* I/D gene polymorphism influences ACE enzyme content in the blood but not the ACE protein content of human skeletal muscle. *ACE* I/D gene polymorphism does not influence the changes of ACE content after a single session of HIIE. *ACE2* gene polymorphisms seem to have no effect on ACE content in the blood and skeletal muscle, before or after a session of HIIE.