Title

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Abstract

Angiotensin converting enzyme (ACE) and bradykinin receptor B2 (B2R) genetic variation may affect thirst due to effects on angiotensin II production and bradykinin activity respectively. To examine this, 45 healthy Caucasian men completed 60 minutes of cycle exercise at 62 ± 5% 2peak in a room heated to 30.5 ± 0.3°C with ad libitum fluid intake. Blood samples were collected pre-, mid-, and immediately post-cycle. Fluid intake, body mass loss (BML), sweat loss (determined via changes in body mass and fluid intake) and thirst sensation were recorded. All participants were genotyped for the ACE insert fragment (I) and the B2R insert sequence (P). Participants were homozygous for the wild type allele (WW or MM), heterozygous (WI or MP) or homozygous for the insert (II or PP). No differences between genotype groups were found in mean (± SD) voluntary fluid intake (WW: 613 ± 388, WI: 753 ± 385, II: 862 ± 421 mL, P = 0.31; MM: 599 ± 322, MP: 745 ± 374, PP: 870 ± 459 mL, P = 0.20), percentage BML or any other fluid balance variables for both the ACE and B2R genes, respectively. Mean thirst perception in the B2R PP group, however, was higher (P < 0.05) than both MM and MP at 30, 45 and 60 minutes. In conclusion, the results of this study suggest that voluntary fluid intake and fluid balance in healthy men performing 60 minutes of moderate intensity exercise in the heat are not predominantly influenced by ACE or B2R genetic variation.

Key Words
Thirst; Angiotensin converting enzyme; Bradykinin receptor B2; Dipsogenic; Exercise; Fluid Balance
**Introduction**

It has long been observed that the majority of humans performing physical work or exercising do not voluntarily drink enough water to replace sweat losses despite adequate fluid supply (Pitts *et al.* 1944). This phenomenon has been termed ‘voluntary dehydration’ (Rothstein *et al.* 1947) and has been found to frequently occur in athletes (Maughan *et al.* 2004; Passe *et al.* 2007) and the general population even in favourable conditions for fluid intake. Based on these observations, it is apparent that relying on thirst mechanisms does not always guarantee sufficient total water intake (Armstrong and Grandjean 2007).

Intracranial and systemic administration of physiological doses of angiotensin II, a component of the renin-angiotensin aldosterone system (RAAS), has been shown to promote drinking in animals (Fitzsimons 1972) and humans (Fitzsimons 1998). Angiotensin II is produced from the cleavage of angiotensin I by angiotensin converting enzyme (ACE). A 287 base pair (bp) Alu repeat insert within intron 16 of the *ACE* gene has been identified as a common allelic variant (Rigat *et al.* 1990) unique to humans (Montgomery *et al.* 2002). The wild type allele (*W*) is significantly associated with both higher tissue (Danser *et al.* 1995), and serum (Rigat *et al.* 1990) ACE activity.

Another known function of ACE is the degradation of vasodilator kinins within the kallikrein kinin system, particularly bradykinin (Dzau *et al.* 1988). Bradykinin exerts its effects on bradykinin B2 receptors (B2R) and has been shown to be a powerful dipsogen. Endogenous bradykinin (Cadnapaphornchai *et al.* 2004) and exogenous infusion of bradykinin (Fregly and Rowland 1991), during acute ACE inhibition has been observed to cause polydipsia and polyuria. Furthermore, the antagonism of bradykinin receptors reverses such effects (Cadnapaphornchai *et al.* 2004). An allelic variant containing a nine bp repeat
insertion (P) has been identified in exon 1 of the gene encoding for B2R (Braun et al. 1996). The wild type allele (M) is significantly associated with higher gene transcriptional and receptor activity (Braun et al. 1996; Lung et al. 1997). It is possible, therefore, that variants in ACE and B2R genes may affect thirst and fluid intake in humans. Understanding the potential role of these, and other, dipsogenic gene variants on thirst and fluid intake may explain some of the large differences in these variables that are often observed in athletes and recreational exercisers.

To date, only one study has previously investigated the influence of these allelic variants on thirst and voluntary dehydration. Saunders et al. (2006) investigated the association of these allelic variants with weight changes in 428 Caucasian male ironman triathletes during competition. The authors concluded that the B2R PP genotype was associated with greater weight loss during the ironman triathlon with a significant linear trend for the distribution of this genotype across weight loss groups. On the other hand, no association was found with ACE genotype.

The aim of the present study was to conduct a controlled laboratory study investigating the influence of ACE and B2R allelic variations on thirst, voluntary fluid intake and fluid balance during moderate intensity exercise in the heat. It was hypothesized that individuals homozygous for the wild type ACE or B2R allele would drink more fluid during exercise in the heat.

Materials and methods

Participants
Forty-five Caucasian men aged 18-45 years (mean ± SD, age 28 ± 8 y, height 178.3 ± 6.7 cm, body mass 78.93 ± 14.29 kg, body mass index 24.74 ± 3.59 kg.m⁻², and peak oxygen uptake 50.95 ± 10.10 ml.kg⁻¹.min⁻¹) volunteered to participate in the present investigation. All participants were healthy non-smokers, not concurrently taking medication, and not supplementing with creatine. Verbal and written explanations of the experimental procedures were given before participants provided written consent and completed a medical screening questionnaire. The study had prior approval from the Manchester Metropolitan University’s Faculty of Science and Engineering ethical advisory committee, in accordance with the Declaration of Helsinki.

**Preliminary trials**

Each participant completed two preliminary trials, separated by a minimum of 48 hours, prior to their main experimental trial. In the first of these, peak oxygen uptake ($\dot{V}_{\text{peak}}$) was assessed through the performance of a continuous, incremental cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) test to volitional fatigue in a thermoneutral environment (21.4 ± 1.0°C; 33 ± 7% relative humidity). Pulmonary gas-exchange (Cosmed K4b², Italy) and heart rate (HR) (Polar FS2c, Kempele, Finland) were continuously analysed and recorded using a breath-by-breath system. Rating of perceived exertion (RPE) (Borg, 1998) was obtained every 2 minutes. Utilising the rolling mean of 10 breaths and work rate, the work-rate to oxygen uptake ($\dot{V}$) relationship was determined. The highest mean value of the rolling 10 breath oxygen consumption values was accepted as $\dot{V}_{\text{peak}}$. The desired work rate eliciting 55% of $\dot{V}_{\text{peak}}$ was then calculated, with account taken for the mean response time for $\dot{V}$ during ramp exercise (i.e. two thirds of the ramp rate was deducted from the calculated work rate) (Whipp et al. 1981).
The second preliminary trial involved familiarising the participants with the experimental trial protocol. All procedures undertaken in the main trial, described in detail below, were performed in a room heated to 30°C, with the exception of body mass (BM) weighing and urine and blood sampling.

**Experimental protocol**

In the 24 h period preceding the experimental trial, participants refrained from strenuous exercise and abstained from alcohol and caffeine ingestion. In addition, participants were also asked to fast from 2100 hours the evening before the experimental trial, and to consume 500 mL of water approximately 90 minutes prior to arrival at the laboratory in an effort to ensure an adequate level of hydration.

All participants reported to the laboratory for their experimental trial between 0800 and 1000 hours. Upon arrival at the laboratory, participants were asked to completely empty their bladder into a container from which a 5 mL urine sample was retained for future analysis. Participants were then seated in an upright position for 15 minutes in an air-conditioned environment maintained at approximately 20°C before a 9 mL blood sample was obtained by venous puncture of an antecubital vein. Mid- and post-exercise blood samples were taken with the participants seated on the cycle ergometer in an upright position in an attempt to reduce the influence posture has on circulating blood and plasma volumes (Hagan *et al*. 1978). During the 15 minute seating period, resting HR and thirst sensation (on adapted Borg scales) were recorded.

Following the pre-exercise blood sample, participants were weighed nude to the nearest 0.01 kg (Adam Equipment Co Ltd., GFK 150, Milton Keynes, UK) before initiation of the exercise protocol. Participants cycled at a constant work-rate equivalent to an initial load of 55% of $P_{\text{peak}}$ for 60 minutes on an electronically braked cycle ergometer (Lode...
Excalibur Sport, Groningen, Netherlands), with a three to five minute rest interval at 30 minutes for blood sample collection. Breath-by-breath expired air samples, analysed for oxygen uptake and HR, were collected for 3 minutes pre-exercise and at every 15 minutes of cycling. Ratings of perceived exertion and thirst sensations were recorded pre-exercise then every 15 minutes of cycling. Post-exercise nude BM was recorded after participants towel dried.

During the 60 minute cycle, participants were provided with constant access to a commercially available hypotonic sports drink containing 2% carbohydrate. The participants were given a choice of two flavours and asked to consume *ad libitum*. Drinks were provided in specialised drinks bottles (CamelBak Better Bottle™) kept in opaque insulating sleeves to prevent participants from viewing the volume of fluid contained and aimed to maintain drinks at regular room temperature. Drink bottles were also changed every 15 minutes. The volume of fluid consumed was determined by weighing of drinks bottles. A 5 mL sample of the drink was obtained for analysis. Total sweat loss was calculated through change in BM corrected for fluid intake. For the purposes of this investigation, it was assumed that all mass loss during exercise was due to sweating since respiratory water loss and mass loss due to substrate exchange would have accounted for only a small component of total mass loss (Maughan *et al*. 2007).

**Biochemical analysis**

Drink and urine samples were stored at 4°C until analysis for osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Berlin, Germany). EDTA blood samples were analysed for haemoglobin concentration by the cyanmethaemoglobin method, packed-cell volume by microcentrifugation. The haemoglobin and packed-cell volume values were used to estimate percentage changes in blood, erythrocyte and plasma volumes,
as described by Dill and Costill (1974). Remaining whole blood samples were stored at -80°C until DNA extraction. Serum tube blood samples were centrifuged at 1500 g for 15 min at 4°C before serum was removed and kept at 4°C until the analysis of osmolality using the method described previously. All analyses were performed in duplicate, with the exception of the packed-cell volume measurements, which were made in triplicate.

**Genotyping**

Genomic DNA was extracted from 5 mL of EDTA whole blood using Qiagen FlexiGene DNA Kit (West Sussex, UK) according to the manufacturer’s instructions. The participants were genotyped for the $W/I$ allelic variants within intron 16 of the $ACE$ gene, and the $M/P$ allelic variants within exon 1 of the $B_2R$ gene. For each gene to be assayed, primers unless stated, were designed using publicly available genomic sequences obtained through GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the public domain primer design software Primer3 (http://frodo.wi.mit.edu/primer3; Rozen and Skaletsky 2000). Target gene sequences to be amplified within whole gene sequences were identified from published literature. Primers were designed with Primer3 using the human mispriming library.

$ACE$ genotype was ascertained using two separate PCR reactions. Firstly, the following forward (5’-GGGGACTCTGTAAGCCACTG-3’) and reverse (5’-TCGGGTAAAACTGGAGGATG-3’) primers were used to detect the presence of a $W$ allele. The presence of a $W$ allele resulted in an approximate 300 bp PCR product whilst the absence of a $W$ allele resulted in no PCR products being formed. Due to the preferential amplification of the $W$ allele observed with the above primers and furthermore owing to the preferential amplification of the $W$ allele in heterozygous samples (Shanmugam et al. 1993), a second insertion specific PCR was performed on all samples using the following forward (5’-TGGGACCACAGCGCCCGCCACTAC-3’) and reverse (5’-
TCGCCAGCCCTCCCATGCCCATAAC-3’) primers as previously utilised by Settin et al. (2009). The presence of an I allele resulted in an approximate 350 bp PCR product whilst for samples homozygous for the W allele, no products were visualised. The PCR reactions were carried out in a total volume of 20 µl containing 20 ng of DNA, 1 x NH₄, 1.5 mM MgCl₂, 25 µM each of dATP, dTTP, dCTP and dGTP, 250 nM of each primer, and 0.02 units of Taq DNA polymerase. For the first ACE PCR reaction, the PCR conditions consisted of an initial denaturing step at 95°C for 5 min; followed by 10 cycles of denaturing at 95°C for 30 seconds, annealing for 30 seconds at 58°C and extension for 45 seconds at 72°C; then 30 cycles of denaturing at 89°C for 20 seconds, annealing for 30 seconds at 58°C and extension for 45 seconds at 72°C; and a final extension step at 72°C for 5 min. The ACE insertion specific PCR conditions consisted of these identical steps except for an annealing temperature of 59°C. The amplified fragments were resolved by electrophoresis on 2% agarose gel stained with ethidium bromide and visualised under ultra violet (UV) light.

B₂R genotype was ascertained using the following forward (5’-CACTCCAGCTCTGGCTTCTG-3’) and reverse (5’-TTCAGTCGCTCCCTGGTACT-3’) primers to produce approximately 100 and/or 90 bp fragments. The PCR reactions were carried out in volumes and concentrations as above. The PCR conditions were also as above except for an annealing temperature of 55°C. The amplified fragments were subsequently resolved on 4.5% microfagarose gel stained with ethidium bromide and visualised under UV light. All fragments were sized using a molecular weight marker (Hyperladder™ V, Bioline, UK).

**Statistical analysis**

Differences in participant characteristics, environmental conditions during exercise, and pre-exercise hydration status between genotype groups were examined using one-way ANOVA. One-way ANOVA were also used to examine differences in percentage BML, fluid
intake, sweat loss, sweat rate, and drink osmolality between genotype groups. Significant F-tests were followed by multiple independent Student’s t-tests. The false discovery rate procedure was applied to control for type 1 error. Pre and post BM data within groups was analysed using paired Student’s t-test. Differences in subjective measures during the 60 minute submaximal exercise between genotype groups were determined using two-way mixed model ANOVA (time x group). Two-way mixed model ANOVA (time x group) were also used to examine differences in serum osmolality, blood volume, and plasma volume. Sphericity for repeated measures was assessed and where appropriate, Greenhouse-Geisser corrections were applied for epsilon <0.75, and the Huynh-Feldt correction adopted for less severe asphericity. Significant F-tests were followed with one-way ANOVA at time points and multiple independent Student’s t-tests. The false discover rate procedure was applied to control for type 1 error. All data were analysed using SPSS for Windows version 14.0 (Chicago, IL). Statistical significance was accepted at the 5% level and results presented as mean ± standard deviation (SD).

Results

Subject characteristics and environmental conditions

The genotype distributions for ACE and B2R of the participants in this study were in Hardy-Weinberg equilibrium. Participant characteristics and exercise conditions grouped according to genotype are presented in Table 1 (ACE) and Table 2 (B2R). Genotype groups for both variants were not different in age, height, body mass, BMI, peak, average exercise intensity or pre-exercise urine osmolality (P > 0.05). Six participants were classed as dehydrated at the start of exercise with pre-exercise urine osmolalities of >900 mOsm.kg⁻¹. There were no
differences in the environmental exercise conditions between the ACE genotypes. However, for B_2R, ambient temperature for the MP genotype group was significantly higher than the PP group (P < 0.05). Relative humidity was also significantly higher for PP compared to MM and MP (P < 0.05). Data is presented in Tables 1 and 2.

Mean exercise intensity and HR significantly increased over time (P < 0.05) but no interaction effect or main effect of genotype was observed in any of these variables (P > 0.05) for both variants.

### Fluid intake and fluid balance

#### ACE

Both the WW and WI genotype groups exhibited a significant decrease in body mass at the end of the 60 minute cycle. The II group also tended to a significant decrease in body mass (P = 0.07). There were no differences in mean percentage BML, fluid intake, and total sweat loss (Table 1).

#### B_2R

A significant decrease in body mass occurred in all three genotype groups at the end of 60 minute cycle (P < 0.05). There were no differences in mean percentage BML, fluid intake, and total sweat loss between genotypes.

#### Blood variables

#### ACE

Plasma volume significantly decreased by 9.1 ± 5.5, 6.0 ± 7.8 and 7.8 ± 6.6% from pre to post for WW, WI, and II, respectively. No interaction effect (P = 0.61) or main effect of
genotype ($P = 0.91$) was found. Serum osmolality (Figure 1a) significantly increased over time ($P < 0.05$) but no interaction effect ($P > 0.05$) or main effect of genotype ($P > 0.05$) was present.

$B_2R$

Plasma volume significantly decreased by 6.0 ± 8.4, 6.8 ± 6.0 and 9.0 ± 6.7% from pre to post for MM, MP, and PP, respectively. No interaction effect ($P = 0.69$) or main effect of genotype ($P = 0.51$) was found. Serum osmolality (Figure 1b) significantly increased over time ($P < 0.05$) but no interaction effect ($P > 0.05$) or main effect of genotype ($P > 0.05$) was present.

Subjective measures

$ACE$

Ratings of perceived exertion and thirst (Figure 2a) significantly increased over time ($P < 0.05$). However, no interaction effects ($P = 0.92$, $P = 0.81$) or main effects of genotype ($P = 0.57$, $P = 0.83$), were found for these subjective measures, respectively.

$B_2R$

Ratings of perceived exertion and thirst (Figure 2b) significantly increased over time ($P < 0.05$). No interaction effect ($P = 0.22$) or main effect of genotype ($P = 0.20$) were found for RPE. Both an interaction effect and main effect of genotype ($P < 0.05$) was found for thirst, however. Thirst was significantly higher ($P < 0.05$) in the PP genotype group compared to both MM and MP in the latter half of the 60 minute cycle at 27-30 min, 42-45 min, and 57-60 min.
Discussion

The primary finding of the present investigation was that both ACE and B2R allelic variation did not significantly influence voluntary fluid intake or fluid balance during moderate intensity exercise in the heat. All six genotype groups exhibited indications of the voluntary dehydration phenomena however, with five of the groups manifesting decreases in body mass from pre-exercise that reached significance. Furthermore, the pattern of results surprisingly appeared the reverse of that expected. Participants with the ACE II genotype and participants with the B2R PP genotype did not exhibit the greatest level of voluntary dehydration but rather exhibited the intermediate and lowest level of dehydration, respectively.

With regards to ACE, the findings of the present study lend support to those of Saunders et al. (2006) who reported no significant association of ACE genotype and weight change in competing Caucasian male ironman triathletes. The present study was the first controlled laboratory study to be conducted that confirms this previous finding to the wider population of healthy Caucasian males partaking in a period of moderate intensity exercise in the heat.

It is acknowledged that a limitation of this study is sample size and that more participants should be investigated or added to the dataset for firm conclusions to be drawn. However, it is worth noting that previous studies such as de Souza et al. (2013), Folland et al. (2000), Santana et al. (2011) and Williams et al. (2011) have found positive ACE results/associations with sample sizes smaller than in the present study (range n = 27 to n = 41).

Given the influences of ACE genotype on inter-individual variation in circulating ACE concentrations (Day et al. 2007; Rigat et al. 1990), it was theorised that ACE allelic variation may have a resulting effect on angiotensin II concentrations and thus thirst and fluid intake.
during exercise. The results of the present study suggest that this may not be the case. Instead, other rate limiting steps in the production of angiotensin II or its activity such as angiotensin I concentration or angiotensin II receptor activity may be responsible for inter-individual differences in thirst, voluntary fluid intake and voluntary dehydration.

It may be that the RAAS plays little role in the regulation of fluid intake and balance during exercise in the heat. Greenleaf et al. (1983) have suggested the RAAS is influenced by reductions in total body water and plasma volume is the predominant stimulus to thirst during exercise in the heat. Maresh et al. (2004), on the other hand, suggest that the RAAS has a minor role whilst the AVP system has a dominant role in regulating thirst during exercise heat challenge. The lack of influence of ACE genotype on thirst and voluntary fluid intake found in the present study appears to support the conclusion of Maresh et al. (2004).

The greater role and importance of the AVP system in regulating thirst and fluid balance during exercise in the heat is supported by serum osmolality data presented in this investigation. In healthy humans, AVP is released in response to small increases in blood plasma osmolality. Mid-exercise serum osmolality in the present study increased a significant amount over time to elicit enhanced AVP secretion and stimulated thirst response. It is also worth noting that only three participants exhibited a serum osmolality above 295 mOsm.kg$^{-1}$ at any point of measurement, suggesting that serum osmolality was generally well regulated within the normal range of 280-295 mOsm.kg$^{-1}$ when free access to fluid was available.

The $B_2R$ results of the present study are in disagreement to the findings of Saunders et al. (2006) who found a significant linear trend for the distribution of the $PP$ genotype across three percentage weight loss groups. The lack of genotype influence obtained in the present
study compared to Saunders et al. (2006) is likely due to the difference in study population and exercise stimulus. With a few exceptions, the group of healthy participants who volunteered for the present study were active recreational exercisers who were relatively fit. The 60 minutes of moderate intensity exercise at 30°C and approximately 30% relative humidity would have therefore been no doubt challenging and hard, but comparably less physiologically challenging than an ultra-endurance event lasting 10-15 h in a hot and humid climate. It may therefore only be in such extreme situations that an influence of $B_2R$ variation is observed.

Despite the absence of a significant influence of genotype for voluntary fluid intake and fluid balance in the present study, subjective perception of thirst was significantly higher in the $PP$ genotype group compared to the others in the latter half of the 60 minute cycle. Since serum osmolality was regulated within normal range, there was no significant difference in serum osmolality and no significant differences in blood or plasma volume decrease between genotypes, it is unlikely that the excess thirst experienced by this group was either osmotic or hypovolemic thirst due to a greater physiological need for water. This finding supports the perception that thirst in humans is not solely influenced by physiological need but other factors such as habit and psychology may also play a role. The results of the other subjective measures are suggestive towards this.

Sweat loss during this study (approximately 1.1 L/h) was relatively low compared to other studies. Byrne et al. (2006) observed a sweat rate of 1.47 ± 0.34 L/h in runners participating in a half marathon in environmental conditions of 25.6 - 27.3 °C and a relative humidity of 75 – 90%. Similarly, Lee et al. (2010) observed a sweat rate of 1.45 ± 0.32 °C during a run at 26.4 °C and 81% humidity and O’Neal et al. (2014) observed a sweat rate of 1.35 ± 0.4 L/h
during a run at 20 ± 3 °C and 54 ± 14% humidity. This difference could be attributed to the relatively low humidity in the present study. Rate of fluid intake amounted to 0.37 ± 0.26 L/h and 0.075 ± 0.062 L/h in the studies by Byrne et al. (2006) and Lee et al. (2010) which are much lower than in the present study (approximately 0.74 L/h). A likely reason for this difference in ingestion rate is the mode of exercise. Cycling allows for greater ingestion due to easier access to fluid and also less likely to suffer from gastrointestinal comfort compared to running. Furthermore, fluid intake is highly variable between individuals and subject to the effect of a number of variables including palatability of fluid provided (Minehan et al. 2002). A greater volume of fluid is ingested during exercise if the drink is considered to be acceptable in comparison to less acceptable or to water (Passe et al. 2000). Similarly, greater fluid intake during exercise is observed with ingestion of a carbohydrate electrolyte solution compared to water and other solutions that are rated less palatable (Passe et al. 2004; Rivera-Brown et al. 1999). Some of the difference in fluid ingestion volume may therefore be attributed to greater subjective acceptability and palatability of the flavoured 2% carbohydrate drink provided in this present study compared to the water and 6% carbohydrate electrolyte solutions provided in the studies by Byrne et al. (2004) and Lee et al. (2010).
In conclusion, the results of the present study suggests that \textit{ACE} and \textit{B}_2\textit{R} allelic variation do not play a major role in governing voluntary fluid intake and fluid balance in normal healthy males partaking in a 60 minute period of moderate intensity exercise in the heat. It appears that other factors such as subjective feeling and subjective perception of the exercise environment may be more important in determining thirst and fluid intake in exercise of this nature and in this population. Additional or a larger number of participants should be investigated whilst future work should also examine the responses of hormones and enzymes involved in body fluid regulation in order to elucidate the underlying mechanism involved and attempt to explain the observed individual differences in thirst, fluid intake and fluid balance. \textbf{References}


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### Tables

#### Table 1: Participant characteristics, environmental conditions during exercise, and fluid balance grouped according to angiotensin converting enzyme (ACE) genotype. *WW*; homozygous wild type, *WI*; heterozygous, *II*; homozygous insertion. Values are mean ± SD.

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>WW</em></th>
<th><em>WI</em></th>
<th><em>II</em></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>21</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>30 ± 9</td>
<td>27 ± 8</td>
<td>28 ± 7</td>
<td>0.50</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.2 ± 5.9</td>
<td>178.6 ± 6.1</td>
<td>178.8 ± 8.7</td>
<td>0.82</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>76.73 ± 14.84</td>
<td>82.79 ± 15.15</td>
<td>74.39 ± 11.10</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>24.31 ± 3.54</td>
<td>25.87 ± 3.94</td>
<td>23.19 ± 2.38</td>
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<tr>
<td>2peak (mL.min⁻¹.kg⁻¹)</td>
<td>49.98 ± 12.83</td>
<td>48.44 ± 8.50</td>
<td>56.30 ± 8.24</td>
<td>0.09</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.4 ± 0.3</td>
<td>30.5 ± 0.2</td>
<td>30.5 ± 0.3</td>
<td>0.50</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>30 ± 7</td>
<td>30 ± 6</td>
<td>27 ± 8</td>
<td>0.48</td>
</tr>
<tr>
<td>% 2peak</td>
<td>63.0 ± 3.2</td>
<td>61.7 ± 5.1</td>
<td>59.7 ± 4.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Urine osmolality (mOsmol.kg⁻¹)</td>
<td>459 ± 298</td>
<td>502 ± 291</td>
<td>472 ± 311</td>
<td>0.92</td>
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<tr>
<td>Drink osmolality (mOsmol.kg⁻¹)</td>
<td>116 ± 4</td>
<td>116 ± 4</td>
<td>115 ± 3</td>
<td>0.70</td>
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<tr>
<td>Body mass loss (Kg)</td>
<td>0.40 ± 0.48</td>
<td>0.30 ± 0.37</td>
<td>0.40 ± 0.68</td>
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<td>Body mass loss (%)</td>
<td>0.51 ± 0.63</td>
<td>0.35 ± 0.48</td>
<td>0.46 ± 0.90</td>
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<td>Sweat loss (L)</td>
<td>1.013 ± 0.257</td>
<td>1.048 ± 0.254</td>
<td>1.257 ± 0.674</td>
<td>0.28</td>
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<tr>
<td>Fluid intake (L)</td>
<td>0.613 ± 0.388</td>
<td>0.753 ± 0.385</td>
<td>0.862 ± 0.421</td>
<td>0.31</td>
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</table>
Table 2: Participant characteristics, environmental conditions during exercise, and fluid balance grouped according to bradykinin receptor B2 (B2R) genotype. MM; homozygous wild type, MP; heterozygous, PP; homozygous insertion. Values are mean ± SD.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MM</th>
<th>MP</th>
<th>PP</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>17</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>31 ± 9</td>
<td>26 ± 8</td>
<td>27 ± 8</td>
<td>0.36</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.9 ± 5.7</td>
<td>180.0 ± 7.5</td>
<td>177.4 ± 6.6</td>
<td>0.42</td>
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<tr>
<td>Body Mass (kg)</td>
<td>77.87 ± 15.58</td>
<td>78.89 ± 15.78</td>
<td>79.89 ± 12.13</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI (kg.m^2)</td>
<td>24.78 ± 4.43</td>
<td>24.21 ± 3.66</td>
<td>25.30 ± 2.79</td>
<td>0.70</td>
</tr>
<tr>
<td>2peak (mL.min^−1.kg^−1)</td>
<td>49.45 ± 10.12</td>
<td>50.64 ± 11.68</td>
<td>52.59 ± 8.49</td>
<td>0.71</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.5 ± 0.3</td>
<td>30.6 ± 0.2*</td>
<td>30.3 ± 0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>27 ± 6</td>
<td>27 ± 6</td>
<td>33 ± 6*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>% 2peak</td>
<td>61.3 ± 4.6</td>
<td>62.1 ± 5.2</td>
<td>61.0 ± 3.8</td>
<td>0.78</td>
</tr>
<tr>
<td>Urine osmolality (mOsmol.kg^−1)</td>
<td>477 ± 288</td>
<td>436 ± 270</td>
<td>537 ± 329</td>
<td>0.63</td>
</tr>
<tr>
<td>Drink osmolality (mOsmol.kg^−1)</td>
<td>117 ± 5</td>
<td>116 ± 4</td>
<td>116 ± 3</td>
<td>0.57</td>
</tr>
<tr>
<td>Body mass loss (Kg)</td>
<td>0.39 ± 0.36</td>
<td>0.34 ± 0.50</td>
<td>0.33 ± 0.58</td>
<td>0.95</td>
</tr>
<tr>
<td>Body mass loss (%)</td>
<td>0.50 ± 0.49</td>
<td>0.41 ± 0.64</td>
<td>0.37 ± 0.77</td>
<td>0.87</td>
</tr>
<tr>
<td>Sweat loss (L)</td>
<td>0.986 ± 0.254</td>
<td>1.089 ± 0.401</td>
<td>1.195 ± 0.521</td>
<td>0.41</td>
</tr>
<tr>
<td>Fluid intake (L)</td>
<td>0.599 ± 0.322</td>
<td>0.745 ± 0.374</td>
<td>0.870 ± 0.459</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*Significantly higher than PP; †Significantly higher than MM and MP
Figure captions

Fig. 1: (a) Serum osmolality pre, mid and immediately post 60 min of cycling for each angiotensin converting enzyme (ACE) genotype group. WW, homozygous wild type; WI, heterozygous; II, homozygous insertion. (b) Serum osmolality pre, mid and immediately post 60 min of cycling for each bradykinin receptor B2 (B2R) genotype group. MM, homozygous wild type; MP, heterozygous; PP, homozygous insertion. Values are mean ± SD. *Significant increase from baseline pre-exercise for ACE WW and WI genotype groups and all B2R genotypes (P < 0.05). #Significant increase from baseline pre-exercise for MM genotype group (P < 0.05).

Fig. 2: (a) Subjective feeling of thirst at baseline, pre-exercise and 15 min intervals throughout 60 min of cycling for each angiotensin converting enzyme (ACE) genotype group. WW, homozygous wild type; WI, heterozygous; II, homozygous insertion. (b) Subjective feeling of thirst at baseline, pre-exercise and 15 min intervals throughout 60 min of cycling for each bradykinin receptor B2 (B2R) genotype group. MM, homozygous wild type; MP, heterozygous; PP, homozygous insertion. Values are mean ± SD. *Significant difference between PP and MM and MP (P < 0.05).