Dipsogenic genes associated with weight changes during Ironman Triathlons

Colleen J. Saunders2, Liesl de Milander2, Tamara Hew-Butler2, Stavroulla L. Xenophontos3, Marios A. Cariolou3, Lakis C. Anastassiades4, Timothy D. Noakes2 and Malcolm Collins1,2,*

1Medical Research Council of South Africa and 2Department of Human Biology, MRC/UCT Research Unit for Exercise Science and Sports Medicine, University of Cape Town, South Africa, 3Molecular Genetics Department B and Laboratory of Forensic Genetics, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus and 4Cardiovascular Diagnostic Centre, Nicosia, Cyprus

Received July 12, 2006; Revised August 17, 2006; Accepted August 24, 2006

Thirst is regulated by a complex interaction of signalling pathways within the central nervous system, including components of the renin–angiotensin and kalikrein kinin systems, as well as the serotonergic pathways. The aim of this study was to determine whether there were any associations between polymorphisms within the ACE, BDKRB2, NOS3 and/or 5-HTT genes with weight changes during the 2000 and 2001 226 km South African Ironman Triathlons. Pre- and post-race serum [Na+] and body weights, as well as genotype data, were collected from 428 (61.1%) Caucasian male triathletes who were divided into three groups according to their relative weight loss during the triathlon (0–3, 3–5 and >5%). There was a significant linear trend for the distribution of both the BDKRB2 +9/+9 genotype and the 5-HTT SS genotype between the three weight loss groups, with the >5% group having the highest percentage of athletes with the +9/+9 genotype ($\chi^2 = 5.3, P = 0.021$) and the highest percentage of athletes with the SS genotype ($\chi^2 = 5.8, P = 0.016$). Likewise, the >5% group had the highest percentage of athletes with the combined SS 5-HTT and/or +9/+9 BDKRB2 genotypes ($\chi^2 = 7.4, P = 0.007$). In conclusion, the functional SS genotype of the serotonin transporter-linked polymorphic region (5-HTTLPR) within the 5-HTT gene and the functional +9/+9 genotype of the BDKBR2 gene were associated with larger weight losses during the Ironman Triathlons. These findings suggest the involvement of the serotonergic pathways in the control of thirst and drinking behaviour and provide further evidence for the dipsogenic effect of circulating bradykinin.

INTRODUCTION

In 1938, Adolph and Dill (1) reported that humans walking in the desert heat for many hours did not drink sufficiently to prevent some degree of weight loss during exercise, subsequently termed ‘voluntary dehydration’ (2). The authors concluded that this voluntary dehydration occurred because of a time delay before sweat losses were replaced, causing a temporary reduction in total body water (TBW) content. The authors further concluded that this loss in body weight resulted from voluntary under-drinking during exercise, although they emphasized ‘the accuracy with which thirst fulfils its function of securing water to make up all deficits’ as it was sufficient to maintain daily water balance (1). Others have used the term ‘involuntary dehydration’ (reviewed in 2) to describe ‘the lag in voluntary fluid intake with dehydration’. More recently, the concept that voluntary dehydration is detrimental during exercise has been promoted (3), and as a result, it is argued that thirst is an inadequate index of fluid requirements during exercise (4). Instead, athletes are now advised to ignore thirst and to drink ‘the maximal amount that can be tolerated’ in order to prevent any weight loss during exercise (4).

An unexpected consequence of this advice was the increase in the incidence of exercise-associated hyponatraemia (EAH), and its potentially fatal complication, exercise-associated hyponatraemic encephalopathy (EAHE), in subjects who over-drink during exercise (5). These subjects retain fluid because of a failure to suppress appropriately the secretion of the antidiuretic hormone, arginine vasopressin (AVP), in response to sustained high rates of fluid intake (5).
rates of fluid ingestion, AVP increases TBW by prohibiting urinary free water clearance, thereby causing a dilutional hyponatraemia (5). In our own studies of EAH and EAHE, we observed a wide variation in post-race serum sodium concentrations in subjects completing marathon and ultramarathon endurance events such as the 226 km Ironman Triathlon that combines swimming (3.8 km), cycling (180 km) and running (42.2 km) (5). There was also a wide variation in the weight changes experienced by athletes during these events, varying from weight gain in excess of 8.5% to weight losses greater than 12.5% (5). Similarly, there is a wide variation in post-race sodium concentrations and weight changes during the individual events (6). These data indicate that both voluntary dehydration and voluntary overhydration occur during exercise, even though within each individual event athletes received the same advice on how much they should drink during exercise. Since both these conditions occur in the same events, fluid availability cannot explain the presence of voluntary dehydration in the majority of exercise. Rather, this finding suggests that drinking behaviour may be regulated not purely by fluid availability but, more likely, by individual choice which might have a genetic component.

Thirst is regulated by a complex interaction of several signalling pathways within the central nervous system (reviewed in 7,8). Components of the renin–angiotensin (RAS) and kalikrein kinin (KKS) (9) systems, as well as the serotonergic pathways, have among other pathways been shown to be dipsogenic i.e. agents that stimulate thirst. Cerebral serotonin (5-HT) has been found to exert an inhibitory action on water intake (10) and injections of methysergide, a 5-HT receptor antagonist, into the lateral parabrachial nucleus (PPBN) of the hindbrain of rats increase both water and salt intake in response to pharmacological and physiological stimuli (11–13). The serotonin transporter protein (5-HTT) is responsible for the reuptake of serotonin in the synaptic cleft, an action which effectively decreases the amount of active serotonin available to its receptors. Since serotonin inhibits thirst, individuals with a higher activity of the 5-HTT protein would be expected to have a higher water intake. The serotonin transporter-linked polymorphic region (5-HTTLPR) located at −1.4 kb within the transcriptional region of the 5-HTT (SLC6A4) gene is associated with expression of this gene. The long, or L allele, of this gene is associated with higher levels of 5-HTT expression than the short, or S allele (14).

Within the RAS, angiotensin II is produced from angiotensin I converting enzyme (ACE) and is powerfully dipsogenic (15). ACE is also a component of the KKS in which it degrades kinins into inactive peptide fragments. There is suggestive evidence that this system acts via kinin B2 receptors to oppose the anti-diuretic effects of AVP (16). In particular, in the presence of ACE inhibition, bradykinin is powerfully dipsogenic in rats (17). We therefore hypothesised that individuals with a higher kinin B2 receptor activity would maintain higher levels of hydration during prolonged exercise. If the KKS is involved in the regulation of thirst, it is logical to expect associations of several components of this system with the maintenance of fluid balance during exercise. The insertion (I allele) and deletion (D allele) of a 287 bp Alu repeat sequence within intron 16 of the ACE gene are associated with lower and higher levels of circulating ACE levels, respectively (18), whereas the absence (−9), rather than the presence (+9), of a 9 bp repeat sequence in exon 1 of the BDKRB2 gene, which encodes for the kinin B2 receptor, is associated with increased bradykinin B2 receptor activity (19,20). In addition, the activation of the bradykinin B2 receptor results in the production of nitric oxide by the NOS enzymes (21) and the NOS3 gene, which encodes endothelial constitutive nitric oxide synthase, contains a missense Glu298Asp (G894T) polymorphism within exon 7 (22).

The aim of this study was, therefore, to determine whether there were any associations between polymorphisms within the ACE, BDKRB2, NOS3 and/or 5-HTT genes with the athlete’s weight changes, a proxy of hydration status, during the 2000 and 2001 South African Ironman Triathlons.

RESULTS

Subject characteristics

Seven hundred and one male athletes completed the 2000 and/or the 2001 South African Ironman Triathlons (23). There was a strong correlation \((r = 0.859)\) between the 2000 and 2001 finishing times of the 115 athletes who completed both events. Pre- and post-race weights, as well as complete or partial genotype data, were obtained from 428 (61.1%) of the Caucasian male athletes, 126 from the 2000 and 302 from the 2001 event. These athletes were representative of the entire field of athletes (data not shown). The 2001 data were used for those athletes with complete sets of data for both years. There were significant linear relationships between the athletes’ age \((r = 0.149, n = 428, P = 0.002)\), pre-race weight \((r = 0.207, n = 428, P < 0.001)\), relative weight change during the event \((r = 0.213, n = 428, P < 0.001)\) and pre-race \([\text{Na}^+]\) \((r = 0.123, n = 370, P = 0.018)\) with their overall race time. The athletes’ weight immediately after the race and their BMI were also positively correlated with their overall finishing time (data not shown).

There was a significant \((P < 0.001)\) negative linear relationship \((r = −0.352, n = 317)\) between the post-race serum \([\text{Na}^+]\) versus the relative changes in body weight during the South African Ironman Triathlons (Fig. 1). As summarized in Table 1, the athletes completed the triathlon with various combinations of percentage weight change, used as an indirect measure of hydration status (dehydration, euhydration and overhydration) and sodium concentrations. In order to test for linear trends for the genotype effects on weight loss during the triathlon \((0–3, 3–5 and >5\%\)) and/or the 2001 South African Ironman Triathlons (23). There was a strong correlation \((r = 0.859)\) between the 2000 and 2001 finishing times of the 115 athletes who completed both events. Pre- and post-race weights, as well as complete or partial genotype data, were obtained from 428 (61.1%) of the Caucasian male athletes, 126 from the 2000 and 302 from the 2001 event. These athletes were representative of the entire field of athletes (data not shown). The 2001 data were used for those athletes with complete sets of data for both years. There were significant linear relationships between the athletes’ age \((r = 0.149, n = 428, P = 0.002)\), pre-race weight \((r = 0.207, n = 428, P < 0.001)\), relative weight change during the event \((r = 0.213, n = 428, P < 0.001)\) and pre-race \([\text{Na}^+]\) \((r = 0.123, n = 370, P = 0.018)\) with their overall race time. The athletes’ weight immediately after the race and their BMI were also positively correlated with their overall finishing time (data not shown).
group were similarly matched for age, height, actual weight prior to and after the event, BMI and pre-race serum [Na\(^+\)] and country of birth (Table 2). As expected, there were significant differences in both the absolute and relative weight changes during the triathlon between the groups, with the 0–3, 3–5 and >5% weight loss groups having average relative weight changes of \(-2.2 \pm 0.7\%\) (range: \(-0.13\) to \(-2.98\%\)), \(-4.0 \pm 0.6\%\) (range: \(-3.03\) to \(-4.99\%\)) and \(-6.2 \pm 1.5\%\) (range: \(-5.01\) to \(-16.89\%\)) respectively. In addition, there were significant differences in the post-race serum [Na\(^+\)], the overall race and split (i.e. swim, cycle and run) times between the three groups. On average, the group which lost the most weight during the triathlon, and were considered to be the most dehydrated, finished the event with the fastest time and highest post-race serum [Na\(^+\)] (Table 2).

### Genotype effects on weight loss independent of post-race serum [Na\(^+\)]

The ACE, BDKRB2, NOS3 and 5-HTT genotype distributions of the triathletes included in this study were in Hardy–Weinberg equilibrium. There was a significant linear trend for the distribution of the +9/9 genotype of the BDKRB2 gene between the three weight loss groups, with the >5% group having the highest percentage of athletes with the +9/9 genotype (33.3%, 37 +9/9+9 genotype; 66.7%, 74 −9 allele combination), the 3–5% group with the intermediate percentage of athletes (24.5%, 47 +9/9+9 genotype; 75.5%, 145 −9 allele combination) and the 0–3% group with the least percentage of athletes with the +9/9 +9 genotype (19.1%, 16 +9/9+9 genotype; 81.0%, 68 −9 allele combination) \((\chi^2 = 5.3, P = 0.021)\) (Table 3). There were no significant genotype effects of either the ACE I/D polymorphism, or the G894T polymorphism within the NOS3 gene, on weight loss during the triathlons (Table 3).

There was however a significant linear trend for the distribution of the SS genotype of the 5-HTTLPR polymorphism upstream of the 5-HTT gene among the three weight loss groups, with the >5% group (21.1% SS) having the highest percentage of athletes with a SS genotype and the 0–3% group (8.9% SS) with the least \((\chi^2 = 5.8, P = 0.016)\) (Table 3). Similar results were obtained when only the South African-born individuals were included in the analysis \((\chi^2 = 5.5, P = 0.018)\) (data not shown).

There was also a significant difference in the genotype distribution among the three groups when the subjects with either SS 5-HTT and/or +9/9 BDKRB2 genotype (SS and +9/9, SS and −9/+9, SS and −9/−9, LL and +9/9 and LS and +9/+9 genotypes) were combined together and compared to the rest of the athletes \((\chi^2 = 7.4, P = 0.025)\) (Fig. 2). In addition, there was a linear trend for the distribution of the combined SS 5-HTT and/or +9/9 BDKRB2 genotypes between the three weight loss groups, with the >5% group (43.2% SS and/or +9/9 genotype, \(n = 48\)) having the highest percentage of athletes, the 3–5% group (34.4% SS and/or +9/9 genotype, \(n = 64\)) with the intermediate percentage of athletes and the 0–3% group (24.4% SS and/or +9/9 genotype, \(n = 20\)) with the least percentage of athletes with this combination \((\chi^2 = 7.4, P = 0.007)\) (Fig. 2). Similar results were obtained when only the South African-born athletes were analysed \((\chi^2 = 4.5, P = 0.034)\) (data not shown).

The ACE, BDKRB2, NOS3 and 5-HTT genotypes were not significantly associated with the performance of the athletes included in this study.

### 5-HTT genotype effects on physiological characteristics during the Ironman Triathlon

The athletes were divided into two groups based on their 5-HTT genotype: those with the SS genotype and those with either the LL or LS genotypes (L allele). As shown in Table 4, there were no significant differences in age, height, absolute weight, BMI, serum [Na\(^+\)], country of birth or the overall and split times when the athletes were divided into the L allele and SS genotype groups. There was, however, a significant difference in absolute \((P = 0.049)\) and relative \((P = 0.039)\) weight changes between the two groups (Table 4). The athletes with an L allele lost on average \(4.1 \pm 1.8\% \quad (n = 349)\) of their pre-race weight, whereas

![Figure 1. Relationship between post-race serum [Na\(^+\)] and relative changes in body weight during the triathlons.](image-url)
BMI (kg/m²) 23.7
Height (cm) 181
Age (years) 34.7

Ironman Triathlons

Pre-race \[\text{Na}^+)\] (mmol/l) 140.8
Post-race \[\text{Na}^+)\] (mmol/l) 140.1

Overall time (min) 791 ± 96 (92)
Swim time (min) 73 ± 14 (91)
Bike time (min) 402 ± 41 (86)
Run time (min) 302 ± 52 (91)

Pre-race weight (kg) 78.4 ± 9.8 (92)
Post-race weight (kg) 76.7 ± 9.6 (92)

South African born (%) 66.3 (92)

Age (years) 34.7
Height (cm) 181
BMI (kg/m²) 23.7

Table 2. General characteristics of the three groups of athletes differing in their extent of weight loss (0–3, 3–5 and >5%) during the 2000 or 2001 South African Ironman Triathlons

<table>
<thead>
<tr>
<th>Variable</th>
<th>Weight loss (0–3%)</th>
<th>Weight loss (3–5%)</th>
<th>Weight loss (&gt;5%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.7 ± 9.4 (92)</td>
<td>34.1 ± 7.8 (206)</td>
<td>35.1 ± 7.6 (126)</td>
<td>0.583</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181 ± 7 (83)</td>
<td>180 ± 7 (189)</td>
<td>181 ± 6 (115)</td>
<td>0.601</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 2.2 (82)</td>
<td>24.1 ± 2.3 (188)</td>
<td>23.8 ± 2.0 (115)</td>
<td>0.300</td>
</tr>
<tr>
<td>South African born (%)</td>
<td>66.3 (92)</td>
<td>65.7 (204)</td>
<td>54.2 (120)</td>
<td>0.082</td>
</tr>
<tr>
<td>Pre-race weight (kg)</td>
<td>78.4 ± 9.8 (92)</td>
<td>79.6 ± 9.2 (206)</td>
<td>79.0 ± 8.0 (126)</td>
<td>0.595</td>
</tr>
<tr>
<td>Post-race weight (kg)</td>
<td>76.7 ± 9.6 (92)</td>
<td>76.4 ± 8.9 (206)</td>
<td>74.1 ± 7.8 (126)</td>
<td>0.081a</td>
</tr>
<tr>
<td>Absolute weight change (kg)</td>
<td>-1.7 ± 0.6 (92)</td>
<td>-3.2 ± 0.6 (206)</td>
<td>-4.9 ± 1.2 (126)</td>
<td>&lt;0.001b</td>
</tr>
<tr>
<td>Relative weight change (%)</td>
<td>-2.2 ± 0.7 (92)</td>
<td>-4.0 ± 0.6 (206)</td>
<td>-6.2 ± 1.5 (126)</td>
<td>&lt;0.001c</td>
</tr>
<tr>
<td>Pre-race [\text{Na}^+)] (mmol/l)</td>
<td>140.8 ± 1.6 (82)</td>
<td>140.9 ± 1.6 (182)</td>
<td>140.7 ± 1.4 (102)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Post-race [\text{Na}^+)] (mmol/l)</td>
<td>140.1 ± 2.3 (68)</td>
<td>141.4 ± 2.4 (150)</td>
<td>142.3 ± 2.8 (95)</td>
<td>&lt;0.014b</td>
</tr>
<tr>
<td>Overall time (min)</td>
<td>791 ± 96 (92)</td>
<td>758 ± 85 (206)</td>
<td>733 ± 92 (126)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Swim time (min)</td>
<td>73 ± 14 (91)</td>
<td>71 ± 13 (195)</td>
<td>67 ± 11 (120)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Bike time (min)</td>
<td>402 ± 41 (86)</td>
<td>393 ± 40 (188)</td>
<td>380 ± 42 (118)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Run time (min)</td>
<td>302 ± 52 (91)</td>
<td>282 ± 43 (191)</td>
<td>276 ± 47 (122)</td>
<td>0.003c</td>
</tr>
</tbody>
</table>

Variables are expressed as average ± standard deviation, with the number of subjects in parentheses. Athletes with a weight loss between 0 and 3% were considered to be euhydrated, and athletes with a weight loss >3% were considered to be dehydrated.

Weight loss (0–3%) vs. weight loss (>5%) P-value 0.001b
Weight loss (3–5%) vs. weight loss (>5%) P-value 0.001a
Weight loss (0–3%) vs. weight loss (3–5%) P-value 0.001a

Those with an SS genotype lost on average 4.6 ± 1.7% (n = 66) of their pre-race weight. Similarly, there was also only a significant difference in the absolute (L allele: -3.2 ± 1.2 kg, n = 218 versus SS genotype: -3.8 ± 1.5 kg, n = 37; P = 0.004) and relative (L allele: -4.0 ± 1.4%, n = 218 versus SS genotype: -4.8 ± 1.7%, n = 37; P = 0.003) weight changes between the two genotype groups when only the South African-born athletes were analysed (data not shown). There were no BDKBR2, NOS3 or ACE genotype effects on the physiological characteristics of the athletes finishing the 2000 or 2001 South African Ironman Triathlons (data not shown).

DISCUSSION

Approximately 60% of the human body’s weight is made up by water in a 2:1 ratio of intracellular versus extracellular fluid (ECF) (7). These two fluid compartments are in a state of osmotic equilibrium determined by the relative concentrations of ions impermeable to the cell membrane. This osmotic equilibrium may be disturbed by the loss of water or ions from either compartment, which results in the activation of reflex mechanisms aimed at minimizing the changes in fluid volume. These reflex mechanisms serve to preserve cardiovascular function. However, fluid losses must be replaced in order to restore fluid balance (8). The regulation of fluid balance and the neural mechanisms responsible for thirst and salt appetite have been extensively reviewed by McKinley and Johnson (8) and Johnson and Thunhorst (7). Exercise causes the loss of sodium and body water through sweating, and exercise-induced hypovolaemia will therefore activate both reflex and hormonal responses, resulting in behaviours aimed at restoring fluid balance.

The most important finding in this study was that the functional serotonin transporter-linked polymorphic region (5-HTTLP), located within the distal transcriptional region of the 5-HTT gene, was associated with the relative weight changes of the triathletes during the 2000 and/or 2001 South African Ironman Triathlons. Athletes with an SS genotype lost significantly more weight during the event than did athletes with either the LS or LL genotypes. Similar results were obtained when only the South African-born athletes were analysed, excluding any possible effects of population stratification. The SS genotype of the gene is associated with lower levels of 5-HTT expression and, consequently, the maintenance of higher 5-HT levels within the synaptic cleft (14). We hypothesized that individuals with an SS genotype would experience less thirst as a result of cerebral serotonin’s inhibition of the perception of thirst. Consequently, they would allow a larger decrease in ECF volume during the triathlon, resulting in increased weight loss during the event.

As reviewed by McKinley and Johnson (8) and Johnson and Thunhorst (7), visceral information regarding ECF volume...
Table 3. The ACE I/D, BDKRB2 −9/+9, NOS3 missense Glu298Asp (G894T) polymorphisms and the serotonin transporter (5-HTT) linked polymorphic region genotype distributions within the three groups of athletes differing in their extent of weight loss (0–3, 3–5 and >5%) during the 2000 or 2001 South African Ironman Triathlons independent of post-race serum [Na+]

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Weight loss (0–3%)</th>
<th>Weight loss (3–5%)</th>
<th>Weight loss (&gt;5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>n = 88</td>
<td>n = 200</td>
<td>n = 121</td>
</tr>
<tr>
<td>II</td>
<td>21.6 (19)</td>
<td>21.5 (43)</td>
<td>23.1 (28)</td>
</tr>
<tr>
<td>ID</td>
<td>52.3 (46)</td>
<td>51.0 (102)</td>
<td>50.4 (61)</td>
</tr>
<tr>
<td>DD</td>
<td>26.1 (23)</td>
<td>27.5 (55)</td>
<td>26.5 (32)</td>
</tr>
<tr>
<td>BDKRB2</td>
<td>n = 84</td>
<td>n = 192</td>
<td>n = 111</td>
</tr>
<tr>
<td>−9/−9</td>
<td>32.1 (27)</td>
<td>26.6 (51)</td>
<td>27.0 (30)</td>
</tr>
<tr>
<td>−9/+9</td>
<td>48.8 (41)</td>
<td>49.0 (94)</td>
<td>39.6 (44)</td>
</tr>
<tr>
<td>+9/+9</td>
<td>19.1 (16)</td>
<td>24.5 (47)</td>
<td>33.3 (37)</td>
</tr>
<tr>
<td>NOS3</td>
<td>n = 86</td>
<td>n = 195</td>
<td>n = 123</td>
</tr>
<tr>
<td>GG</td>
<td>36.1 (31)</td>
<td>39.0 (76)</td>
<td>42.3 (52)</td>
</tr>
<tr>
<td>GT</td>
<td>50.0 (43)</td>
<td>46.7 (91)</td>
<td>43.9 (54)</td>
</tr>
<tr>
<td>TT</td>
<td>14.0 (12)</td>
<td>14.4 (28)</td>
<td>13.8 (17)</td>
</tr>
<tr>
<td>5-HTT</td>
<td>n = 90</td>
<td>n = 198</td>
<td>n = 123</td>
</tr>
<tr>
<td>SS</td>
<td>8.9 (8)</td>
<td>15.7 (31)</td>
<td>21.1 (26)</td>
</tr>
<tr>
<td>LS</td>
<td>55.6 (50)</td>
<td>55.1 (109)</td>
<td>43.1 (53)</td>
</tr>
<tr>
<td>LL</td>
<td>35.6 (32)</td>
<td>29.3 (58)</td>
<td>35.8 (44)</td>
</tr>
</tbody>
</table>

Values are expressed as percentage, with the number of subjects in parentheses. Athletes with a weight of loss between 0 and 3% were considered to be euhydrated, and athletes with a weight loss of >3% were considered to be dehydrated. Genotype distributions for (i) ACE, $\chi^2 = 0.2$, $P = 0.996$; (ii) BDKRB2, $\chi^2 = 6.2$, $P = 0.189$; (iii) NOS3, $\chi^2 = 0.9$, $P = 0.919$ and (iv) 5-HTT, $\chi^2 = 8.7$, $P = 0.070$. BDKRB2 +9/+9 genotype versus −9 allele (−9/−9 or −9/+9 genotype) distribution linear trend $\chi^2 = 5.3$, $P = 0.021$. 5-HTT SS genotype and L allele (LS and LL genotypes) distribution linear trend $\chi^2 = 5.8$, $P = 0.016$.

arises from vascular baroreceptors and volume receptors and travels via the IXth and Xth cranial nerves to the nucleus of the tractus solitarius (NTS) in the hindbrain. Inhibitory serotonergic pathways originating in the medullary NTS and the area postrema project to the LPBN. It has been proposed that the LPBN receives and integrates information reflecting the ECF volume (24). It has also been suggested that serotonin released into the LPBN inhibits behaviours, such as drinking, which cause an expansion of the ECF (7). The results of this study suggest that individuals with the SS genotype of the 5-HTT gene, which is associated with higher expression of the gene and, by implication, increased serotonin levels in the synaptic cleft, are more likely to produce exercise-induced hypovolaemia because sweat losses are less likely to be replaced during exercise. This corroborates the prevailing evidence that serotonergic pathways are involved in the control of thirst and drinking behaviour.

The second important finding in this study was that the functional +9/−9 polymorphism within exon 1 of BDKRB2 gene, but neither the functional I/D ACE polymorphism nor the G894T NOS3 polymorphism, was also associated with the percentage weight loss during the Ironman Triathlons. The group of triathletes that lost the most weight (≥5%) group had the highest frequency of the +9/+9 genotype. This finding should, however, be interpreted with caution, given that the possible effects of population stratification could not be excluded.

The presence (+9), rather than the absence (−9), of a 9 base pair repeat sequence in exon 1 of the BDKRB2 gene is associated with decreased gene transcription (20) and lower BDKRB2 mRNA expression (19). The +9/+9 genotype, therefore, results in lower bradykinin β2 receptor activity and, subsequently, less marked effects of endogenous bradykinin.

Components of both the circulating and brain RAS are also associated with polydipsia and polypria (15,25). The substrate, angiotensin I, and the product, angiotensin II, of the ACE are diphasic (reviewed in 7,8). Although the functional ACE I/D polymorphism was not associated with relative weight changes in this study, several ACE inhibitors are associated with polydipsia and polypria (26). The observed effects of ACE inhibitors on urine production and the stimulation of water intake suggest the possibility that bradykinin, which is degraded by ACE to produce inactive peptide fragments, mediates these physiological effects on water balance (9). In support of this, the infusion of bradykinin in the presence of ACE inhibition results in both polydipsia and polypria in rats (17). Recently, Cadnapaphornchai et al. (9), have shown that, in the presence of ACE inhibition by captopril (which effectively increases circulating bradykinin levels), bradykinin acts via its β2 receptor to suppress the effects of AVP, causing polypria. They further showed that, although bradykinin results in polypria, it should be considered primarily a diphasic agent.

Bradykinin produces polypria through its action on β2 receptors to block AVP-induced re-absorption of water in the collecting ducts of the kidneys (16,27). Given this diuretic effect of bradykinin, athletes with the −9/−9 genotype of the BDKRB2 gene should, in theory, excrete more fluid and show the greatest weight loss. However, our results show the opposite effect and suggest a different explanation.

During cardiovascular stress, such as produced by prolonged exercise, vascular flow to the kidneys is reduced and results in a decreased glomerular filtration rate. There is, therefore, a decrease in urine production which would effectively attenuate any diuretic effect of bradykinin. It could, therefore, be postulated that during exercise the diuretic effect of bradykinin is masked by both the decrease in renal perfusion and the
In conclusion, the SS genotype of the serotonin transporter-linked polymorphic region (5-HTTLPR) within the 5-HTT gene and the +9/+9 genotype of the BDKBR2 gene were associated with larger weight losses during the 2000 and 2001 South African Ironman Triathlons. These findings suggest that the serotonergic pathways are involved in the control of thirst and drinking behaviour and provide further evidence for the dipsogenic effect of circulating bradykinin.

METHODS

Subjects

Participants were recruited from the 701 male triathletes who completed either the 2000 (272 finishers) and/or the 2001 (544 finishers) South African Ironman Triathlons, of which 115 completed both events as previously described (23). Both triathlons, held outside Cape Town, were multiple phase endurance events consisting consecutively of 3.8 km swimming, 180 km cycling and 42.2 km running. Prior to the event, each competitor was sent a detailed explanation of the study and invited to participate. At race registration, those triathletes who agreed to participate in the study completed an informed consent form and personal particulars questionnaire. Four hundred and twenty-eight of the consenting male Caucasian triathletes were included in this study. Approval for this study was obtained from the Research and Ethics Committee of the Faculty of Health Sciences, University of Cape Town.

DNA extraction and genotyping

Approximately 4.5 ml of venous blood was collected from each subject into an EDTA vacutainer tube by venupuncture of a forearm vein. These samples were stored at 4°C until DNA extraction was performed as described by Lahiri and Nurnberger (30). The extracted DNA was stored at 4°C until subsequent genotyping.

The subjects were previously genotyped for the insertion (I)/deletion (D) polymorphism within intron 16 of the ACE

In this study, weight changes during the triathlon events were used as an indirect measure of changes in body fluid volume. All athletes used in this study were male and exposed to the same environmental conditions, although differences in metabolic rates and heat dissipation could not be excluded even though the BMI’s across the three weight loss groups were similar. It should also be noted that not all weight loss during exercise is due to fluid loss through sweating. Muscle fuel depletion, urination, defecation and respiratory water loss also account for some of the weight lost during an exercise bout. Again it was assumed that all athletes competing in the triathlons experienced similar levels of fuel depletion and similar weight loss due to these factors. The difference in weight before and after the events was therefore used as an indirect measure of changes in body fluid volumes. The restrictions of this study are acknowledged, as there was limited control of confounding factors and the observed results need to be confirmed in strictly controlled laboratory studies.

Although the triathletes who lost the most weight during the triathlons also completed the event in the fastest time, it is highly unlikely that the observed association of the SS 5-HTT and +9/+9 BDKBR2 genotypes with the athletes’ relative weight loss was confounded by their performance. First, we have not shown any association of the SS genotype of the 5-HTT gene with the performance of the triathletes during the 2000 and 2001 South African Ironman Triathlons (L. de Milander, unpublished observations). Secondly, we and others have shown that the −9, rather than the +9, allele of the BDKBR2 gene is associated with endurance performance (28,29).

In conclusion, the SS genotype of the serotonin transporter-linked polymorphic region (5-HTTLPR) within the 5-HTT gene and the +9/+9 genotype of the BDKBR2 gene were associated with larger weight losses during the 2000 and 2001 South African Ironman Triathlons. These findings suggest that the serotonergic pathways are involved in the control of thirst and drinking behaviour and provide further evidence for the dipsogenic effect of circulating bradykinin.
gene, the −9/+9 polymorphism within exon 1 of the \( BDKRB2 \) gene and the missense Glu298Asp (G894T) polymorphism in exon 7 of the \( NOS3 \) gene (29,31).

In addition, the subjects were genotyped for the serotonin transporter-linked polymorphic region (5-HTTLPR) of the 5-HTT gene, with slight modifications to methods previously described by Heils et al. (14). Briefly, either a short (S) 484 bp or long (L) 528 bp fragment was PCR amplified using the following forward, 5′-GGC GTC GCT AAT TGC -3′, and reverse, 5′-GAG GGA CTG AGC TGC ACA ACC CAC -3′, primers. The PCR reactions were carried out in a final volume of 50μl containing at least 100 ng of DNA, 1× Tris–KCl, 0.5 mM MgCl₂, 25 μM each of dATP, dTTP, dCTP, dGTP, 20 pmol of each primer, 5% dimethyl sulfoxide and 0.5 units of Taq DNA polymerase. The PCR conditions consisted of an initial denaturing step for 3 min at 95°C, followed by 35 cycles of denaturing for 30 s at 95°C, annealing for 30 s at 61°C and extension for 30 s at 72°C and a final extension step at 72°C for 7 min. The amplified fragments were resolved on 4% polyacrylamide gels and visualized under UV light after ethidium bromide staining.

Biochemical analysis and weight determinations

For pre- and post-race serum \([\text{Na}^{+}]\) determinations, ~4.5 ml of venous blood was collected from each subject into a lithium heparin vacutainer tube by venupuncture of a forearm vein at race registration (1 to 3 days prior to the race) and within 10 min of completing the triathlon. The samples were analysed using an Easylyte PLUS Na/K/Cl analyzer (Medica Corporation, Bedford, MA, USA) as previously described (6).

The subjects were also weighed prior to the triathlon in their swimming costume and immediately after the triathlon in their running gear without shoes, as previously described (6,23). The weights were corrected for the standard clothing worn at the time of measurement. Percentage body weight lost or gained was calculated as the difference between the pre- and post-race weights divided by the pre-race weight and expressed as a percentage. Although weight loss during exercise is significantly influenced by fuel utilization (32–35), the weight changes during the Ironman were nevertheless used as an indirect measure of the athletes hydration status after the event, since the group of athletes with the greatest percentage of weight loss would also, presumably, have been the most dehydrated (23). As previously described, athletes who gained weight during the event were considered to be overhydrated, those who lost between 0 and 3% body weight were considered to be ehydrated and those who lost more than 3% of their pre-race body weight were considered to be dehydrated (5). Only the athletes who were considered to have completed the race ehydrated (0 and 3% weight loss) or dehydrated (>3% weight loss), irrespective of their post-race serum \([\text{Na}^{+}]\), were analysed in this study. The athletes with a >3% weight loss were further divided into two groups: those with a weight loss of between 3 and 5% and those with a weight loss of >5%.

Statistical analysis

Data were analysed with the STATISTICA version 7 (StatSoft Inc., Tulsa, OK, USA) and GraphPad InStat version 2.05a (GraphPad Software, San Diego, CA, USA) statistical programmes. Differences in genotype frequencies between the triathlete weight loss groups were determined by Pearson chi-square (\( \chi^2 \)) analysis. The \( \chi^2 \) test of linear trend was determined using the GraphPad InStat software. Any significant differences between the characteristics of the triathlete groups were determined by a one-way analysis of variance (ANOVA). When the overall F-value was significant, a Tukey’s honest significance post hoc test was used to determine specific differences. Statistical significance was accepted when \( P < 0.05 \). Hardy–Weinberg equilibrium was established using the Genepop web version 3.4 program (http://www.medic. curtin.edu.au/genepop/).

ACKNOWLEDGEMENTS

Research at the 2000 and 2001 South African Ironman Triathlons was funded by dedicated grants from the race organizers, with support from the University of Cape Town, the South African Medical Research Council and Discovery Health. Special thanks to the staff and students from the UCT/MRC Research Unit for Exercise Science and Sports Medicine as well as individuals from Body iQ Corporate Wellness, Pathnet Laboratories and the Shosholoza Outreach and Development Programme of the Sports Science Institute of South Africa who assisted in collection of the data and samples for this project.

Conflict of Interest statement. The authors declare no conflict of interest.

REFERENCES