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CFTR genotype-related body water and electrolyte balance during a marathon

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The aim of this investigation was to determine the influence of CFTR genotype on body water and electrolyte balance during a marathon. Fifty-one experienced runners completed a marathon race. Before and after the race, body mass and a sample of venous blood were obtained. During the race, sweat samples were collected using sweat patches, and fluid and electrolyte intake were obtained using self-reported questionnaires. Thirty-eight participants (74.5% of the total) were 7T/7T homozygotes, 11 (21.6%) were 7T/9T heterozygotes, and one participant presented the rare genotype 5T/7T. Another participant with 9T/9T presented the mutation p.L206W. Participants with 7T/7T showed higher sweat sodium concentrations (42.2 ± 21.6 mmol/L) than 7T/9T (29.0 ± 24.7 mmol/L; $P = 0.04$). The runner with the 5T/7T genotype (10.2 mmol/L) and the participant with the p.L206W mutation (20.5 mmol/L) exhibited low-range sweat sodium concentrations. However, post-race serum sodium concentration was similar in 7T/7T and 7T/9T (142.1 ± 1.3 and 142.4 ± 1.6 mmol/L, respectively; $P = 0.27$) and did not show abnormalities in participants with the 5T/7T genotype (140.0 mmol/L) and the p.L206W mutation (143.0 mmol/L). Runners with the CFTR-7T/7T genotype exhibited increased sweat sodium concentrations during a marathon. However, this phenotype was not related with increased likelihood of suffering body water and electrolyte imbalances during real competitions.

During exercise-induced thermoregulatory sweating, the secretory portion of the sweat glands produces a precursor fluid with a tonicity similar to the plasma/serum upon cholinergic stimulation (Sato et al., 1989). From this precursor fluid, Na^+ and Cl^- are partially reabsorbed during its passage through the sweat duct, finally resulting in excreted sweat that is hypotonic with respect to the plasma/serum (Brown et al., 2011a). Despite isolating factors that contribute to sweat electrolyte concentration, such as diet, acclimatization, training status, and collection techniques, the excreted sweat greatly varies between individuals (from 20 to 100 mmol/L for sweat sodium concentration; Sawka et al., 2007; Godek et al., 2010). Serum electrolyte concentration is very similar in healthy individuals (as well as the precursor fluid; Hamouti et al., 2011) and thus, the inter-individual variability in sweat electrolyte concentration must be related to electrolyte reabsorption within the sweat duct (Sato et al., 1989). Although it is still not well understood, polymorphic variants in specific genes might contribute to the variability in the amount of salt lost by sweating during exercise.

The cystic fibrosis transmembrane protein (e.g., CFTR), encoded by the CFTR gene, plays a fundamental role in ion reabsorption in sweat glands (Eichner, 2008). Sweat ducts are impermeable to chloride and this protein acts as a cAMP-activated Cl^- channel to reabsorb Cl^- from the precursor fluid during its passage through the sweat gland duct. The CFTR protein also influences other membrane transport proteins such as epithelial Na^+ channels (ENaC; Muhammad et al., 2011) to reabsorb Na^+ from sweat. A defect in the CFTR protein limits both Na^+ and Cl^- reabsorption in the sweat duct and produces excretion of sweat with Na^+ and Cl^- concentrations several times above the normal levels (Brown et al., 2011a). This is the case of cystic fibrosis, an autosomal recessive disease associated with mutations in the CFTR gene. Cystic fibrosis results in the loss and/or malfunction of the CFTR protein ultimately affecting the amount of electrolytes reabsorbed within the sweat duct. Consequently, patients with cystic fibrosis typically have abnormal sweat electrolyte concentrations with sweat sodium values > 60 mmol/L (Wheatley et al., 2011) but that can be as high as 130 mmol/L (Brown et al., 2011a). In fact, it has been found that individuals with cystic fibrosis might develop hyponatremia during exercise due to the high amount of sodium lost through sweating (Wheatley et al., 2011).

The CFTR gene contains a tract of polyT (IVS8-Tn) with allelic 3 variants: 5T, 7T, and 9T. This polyT tract is

located in intron 8 of the CFTR gene and it affects the accuracy of exon 9 splicing and, hence, the expression of a functional CFTR protein (Andrieux et al., 2002). These three polymorphic variants in the polyT tract are considered *per se* normal and they are not directly related to any physiological dysfunction or medical condition. However, appropriate exon 9 expression is directly related to the disease penetrance of CFTR mutations with the 5T variant commonly associated with more severe disease and 7T and 9T often being associated with normal CFTR function even in the presence of mutations. Besides, it is possible to find a CFTR malfunctioning protein when an individual is homozygous for 5T or heterozygous for 5T plus a mutation (Dumur et al., 1996).

Interestingly, some healthy individuals (typically known as "salty sweaters") show sweat Na^+ and Cl^- concentrations that approach those patients with cystic fibrosis, without any other signs/symptoms of this disease (Brown et al., 2011b). It has been speculated that some abnormalities in the CFTR protein can produce salty sweat without developing cystic fibrosis or other CFTR-related diseases in healthy individuals (Montain et al., 2001; Lewis et al., 2014). Recently, two investigations have studied the potential association between athletes with salty sweat and mutations in the CFTR gene. In a laboratory setting, Brown et al. (2011a) observed that both healthy salty sweaters ($n = 6$) and patients with cystic fibrosis ($n = 6$) had lower expressions of the CFTR protein in the ductal luminal membrane of the sweat glands when compared with healthy athletes with typical sweat. Interestingly, none of the healthy salty sweaters was a carrier of mutations in the CFTR gene. On the other hand, using an ecological experiment, Lewis et al. (2014) investigated the genetic profile of 31 ultra endurance runners with electrolyte imbalances after a competition. None of the 31 participants in this previous study had CFTR mutations; however, sweat sodium concentration was not measured and thus ultra endurance athletes might have developed electrolyte deficits because of other factors different from excessive sweat sodium loss (such as excessive rehydration during exercise). Besides, none of these investigations measured intron 8 polymorphism of CFTR gene and, thus, the influence of the 5T, 7T, and 9T variants on the sweat electrolyte concentration during exercise has not been tested yet.

Exercise-associated hyponatremia (EAH) is defined as a serum or plasma sodium concentration below the normal range of 135 mmol/L that occurs during or up to 24 h after prolonged exercise. This electrolyte imbalance is one of the most common life-threatening disorders that can happen during an endurance event (Bennett et al., 2014) and its prevalence is between 2% and 27% in athletes competing in endurance events (Montain et al., 2001; Rosner, 2009). EAH is mainly caused by water or hypotonic fluid intake well above sweat losses combined with inadequate suppression of antidiuretic hormone secretion (Noakes et al., 2005). However, other factors in the pathogenesis of EAH include high sweat sodium losses, inability to mobilize exchangeable sodium stores and production of metabolic water (Lewis et al., 2014). Mathematical models have suggested that sodium loss in healthy "salty sweaters" might play a key role in the development of EAH (Montain et al., 2006). Furthermore, the amount of sweat sodium lost during exercise is not only related to the prevalence of EAH but it can also negatively impact sports performance (Coso et al., 2008; Montain et al., 2006) and produce a higher prevalence of heat cramping (Horswill et al., 2009).

Although previous investigations suggest that there is no association between high values of Na^+ and Cl^- in thermoregulatory sweat and mutations in the CFTR gene, the low number of participants investigated (Brown et al., 2011a) and the absence of a relationship between genotype and phenotype (Lewis et al., 2014) require further investigation to confirm this hypothesis. The purpose of our study was to determine the influence of the CFTR genotype on body water and electrolyte balance during a marathon with special relevance to the changes in serum sodium concentration. We fulfilled this aim by simultaneously measuring 50 of the most common cystic fibrosis mutations in the European population, the three variants in a tract of polyT (IVS8-Tn) in intron 8 of the CFTR gene and sweat electrolyte concentrations in a sample of endurance-trained runners. We hypothesized that CFTR genotype would affect the amount of salt (Na^+ and Cl^-) lost by sweating during a marathon.

Methods

Subjects

Fifty-one healthy and experienced marathon runners volunteered to participate in this study. Most participants were contacted by phone and email from a group of runners that had participated in previous investigations while other runners were recruited at the race registration. Before enrolling in the investigation, participants underwent a medical examination and completed a questionnaire about previous training, running experience, and best race time in the marathon. Age and main morphological and physical variables of the participants in this investigation are shown in Table 1. Each participant was informed of the risks and discomforts associated with this investigation and signed an informed consent document before the onset of the experiments. The study was approved by the Camilo Jose Cela Ethics Committee in accordance with the latest version of the Declaration of Helsinki. Participants' rights and confidentiality were protected during the whole experiment, and the genetic information was only used for the purposes included in this investigation.

Experimental design

A descriptive and comparative study was used for this investigation. All the participants underwent the same testing under the same experimental conditions. After a preliminary analysis, four

Table 1. Age, anthropometric characteristics, running experience, and training status of marathoners with different CFTR genotypes

Variable (units)	All	7T/7T	7T/9T	5T/7T	9T/9T (p.L206W)
n	51	38	11	1	1
Frequency (%)	100.0	74.5	21.6	2.0	2.0
Age (year)	43.1 ± 9.4	42.5 ± 9.1	41.9 ± 7.3	60	53
Body mass (kg)	74.5 ± 10.8	73.7 ± 10.8	75.0 ± 10.6	91.7	81.1
Height (m)	175 ± 8	175 ± 8	176 ± 9	185	171
Running experience (year)	12.3 ± 10.2	12.8 ± 10.9	9.7 ± 7.9	10	16
Completed marathons (number)	9.1 ± 11.2	9.5 ± 11.7	7.1 ± 10.3	5	16
Average training distance/week (km)	75.7 ± 29.1	76.6 ± 30.0	69.0 ± 27.9	100	80
Training sessions/week (number)	4.3 ± 1.1	4.4 ± 1.0	3.8 ± 1.2	5	6

Data are mean ± standard deviation.

groups of participants were established according to their genetic profile in the CFTR gene (see below). All the participants completed the 2014 edition of the Rock 'n' Roll Madrid Marathon with no indications about running pace or fluid and food strategies. The marathon race was held in April 2014 under a clear sky with a mean dry temperature of 24.4 ± 3.6 °C (range from 18.3 to 29.1 °C, temperature readings at 30-min intervals from 0 to 5 h after the race onset) and a mean relative humidity of 27.7% ± 4.8% (range from 23% to 40%). The race started at 09:00 h and the race course varied from 600 to 720 m above sea level.

Experimental protocol

Twenty-four hours before the race, a 10-mL venous blood sample was obtained from an antecubital vein after 10 min of supine resting. From the total, 3 mL was introduced into a tube with ethylenediaminetetraacetic acid while the remaining blood was allowed to clot and serum was later obtained by centrifugation at 5000 *g*. Participants were instructed to avoid pain-relieving strategies (e.g., analgesic medications), caffeine, and alcohol 24 h before the onset of the race. The day of the race, participants had their pre-competition meal at least 3 h before the race and it was not standardized among participants to avoid affecting participants' pre-competition routines. Participants' pre-competition meals were later recorded and analyzed (PCN software, Cesnid, Spain) and their contents in carbohydrates, proteins, and salt were similar in all groups of participants. Moreover, there were no differences between groups in the amount of energy, carbohydrates, and salt ingested during the 24 h before the race. Runners were encouraged to ingest 500 mL of plain water 2 h before the start of the race to increase the likelihood of being euhydrated at the start line. Thirty minutes before the race, participants were weighed (± 50 g scale; Radwag, Radom, Poland) in their competition clothes and after they had emptied their bladders. At this time, two sweat patches (Tegaderm + Pad, 3M, Minnesota, USA) were placed on the forearm to collect sweat samples during the race, as previously described (Del Coso et al., 2015). For this measurement, the forearm skin was gently cleaned with distilled water and alcohol and dried with clean gauze to eliminate any remains of previous sweat/electrolytes from the skin. After this, the sweat patch was firmly adhered to the skin and fastened with an elastic tubular net bandage (Elastofix, Insfarma, Germany).

During the race, participants wore a race bib with a time chip to calculate the actual amount of time it took them from the starting line of the race to the finish line (net time). Participants completed the race at their own pace and drank *ad libitum* at the hydration stations placed at 5-km intervals. In the hydration stations, spring water, sports drinks, and fruits (oranges, apples, and bananas) were available in excess for all runners. Participants were encouraged to memorize the amount and types of drinks and food ingested during the race, in addition to the personal nutrition supplements used in the race.

Within 2 min of the end of the marathon race, participants went to a finish area where body mass was immediately measured using the same apparatus previously described. Participants were instructed to avoid drinking from the finish line until the post-race weighing place where a researcher assured compliance. Participants then rested for 5 min and a venous blood sample and post-race blood oxygen saturation were obtained. During this resting period, the sweat patches were removed using clean tweezers and placed in a sterile 10-mL tube. Sweat patches that were detached from the skin or presented a leak were discarded. The sweat from the patches was extracted by centrifugation at 5000 *g*. The ratings of perceived exertion after the race were assessed using the Borg scale (from 6 to 20 arbitrary units) while lower limb muscle soreness (from 0 to 10 arbitrary units) was self-rated using a visual analog scale. Participants also filled out a detailed questionnaire about fluid and food intake during the race. Data on this questionnaire were used to calculate fluid and electrolyte intake during the race using the nutritional facts of the products consumed. Participants were also asked about stoppages during the race to urinate or defecate, but none of the participants reported any of these types of stoppages. After that, participants were provided with fluid (water and sports drinks) and finished their participation in the study.

Genetic testing

To investigate the potential relationship between the CFTR genotype and sweat and electrolyte losses during the marathon, genomic DNA was isolated from the whole blood obtained before the race. The intron 8 of the CFTR gene contains a tract of polyT (IVS8-Tn) which contains three variants: 5T, 7T, and 9T. All the participants in this investigation were grouped following the gene variants in this intron. Using an allele-specific primer extension assay (ARMS: Amplification Refractory Mutation System, Applied Biosystems 3500DX Genetic Analyzer, Life Technologies, USA), genomic DNA of each individual was tested for 50 of the most common cystic fibrosis mutations in the European population representing 85% of the mutations found in the world population (Cystic Fibrosis Centre at the Hospital for Sick Children in Toronto accessed by 1 July 2014). The mutations tested were: CFTRdele2,3, p.E60X, p.P67L, p.G85E, p.Leu88IlefsX22, p.Ile105SerfsX2, p.R117C, p.R117H, p.Y122X, 621 + 1G > T, 711 + 1G > T, p.L206W, p.Phe316fs, p.R334W, p.R347P, p.R347H, p.A455E, p.I507del, p.F508del, p.Tyr515X, p.V520F, 1717-1G > A, p.G542X, p.S549R, p.S549N, p.G551D, p.R553X, p.R560T, 1811 + 1.6kbA > G, 1898 + 1G > A, p.Leu671X, p.Lys684fs, p.Val739TyrfsX16, p.W846X, 2789 + 5G > A, p.Q890X, 3120 + 1G > A, 3272-26A > G, p.R1066C, p.Y1092X, p.M1101K, p.D1152H, p.R1158X, p.R1162X, p.Lys1177fs, 3849 + 10kbC > T, p.S1251N, p.Leu1258fs, W1282X, N1303K.

Blood samples

A portion of each blood sample was introduced *in situ* into a blood glucose analyzer (Accu-chek, Roche, Spain) to determine glucose concentration. The blood was also analyzed for hemoglobin concentration (Coulter ACT5 Diff CP; Beckman-Coulter Instruments, France), and hematocrit was measured by microcentrifugation within 24 h after the race. The remaining blood was allowed to clot and serum was separated by centrifugation (10 min at 5000 *g*) and frozen at –80 °C until the day of analysis. At a later date, the serum portion was analyzed for osmolality (Osmometer 3320, Advanced Instruments Inc, USA) and Na^+, Cl^-, and K^+ concentrations (Spotlyte, Menarini Diagnostics, USA). In addition, serum myoglobin concentrations were measured as a blood marker of muscle damage by means of an immunoassay system (Access II, Beckman-Coulter Instruments, USA).

Sweat samples

The sweat was separated from the patches by centrifugation (10 min at 5000 *g*), transferred to 5-mL sealed tubes and refrigerated at 4 °C. Within 48 h after the race, sweat osmolality was measured with the same osmometer employed for the serum samples while sweat Na^+, Cl^-, and K^+ concentrations were measured in duplicate using photoelectric flame photometry (Flame Photometer 410, Ciba Corning Diagnostics, UK). Typical values of sweat Na^+, Cl^- concentrations, and sweat K^+ values comparable with serum K^+ concentration provided evidence that electrolyte leaching from the epidermal layer was minimum (Weschler, 2008).

Calculations

Blood volume and plasma volume changes were calculated using the equations outlined by Dill and Costill (1974). Sweat loss volume (in L) was calculated from pre- to post-race body mass change and fluid intake volume. Fluid intake volume (in L) was obtained from the data provided by each participant in the post-race questionnaire. Sweat electrolyte losses (in mmol) were calculated by multiplying sweat loss volume and sweat electrolyte concentration. Electrolyte ingestion (in mmol) during the race was calculated from the information provided in the questionnaire and by the nutritional information included in the label of the products consumed (e.g., supplements). Electrolyte balance was calculated by subtracting the amount of sweat electrolyte losses from the amount of electrolyte ingested. Negative values in fluid and electrolyte balance variables indicate deficits in these variables.

Statistical analysis

The normality of each variable was initially tested with the Shapiro–Wilk test. Post-race myoglobin concentration was the only variable that did not follow a normal distribution and thus, it was analyzed with non-parametric statistics. The remaining variables were analyzed with parametric statistics. For the variables obtained once during the experiment (e.g., total race time, body mass change, and rate of perceived exertion), the comparison between groups (7T/7T homozygotes vs 7T/9T heterozygotes) was performed using Student's *t*-test for independent samples. For the variables obtained twice or more during the experiment (blood variables), the comparison between groups was performed using a two-way analysis of variance (time × treatment). The data were analyzed with the statistical package SPSS version 19.0 (SPSS Inc., Chicago, Illinois, USA). The significance level was set at $P < 0.05$. Data are presented as mean ± standard deviation.

Results

CFTR gene variants

From the 51 participants in this investigation, 38 (74.5% of the total) showed a 7T homozygous genotype in intron 8 of the CFTR gen and 11 participants (21.6% of the total) showed a heterozygous genotype 7T/9T (Table 1). One participant (2.0% of the total) carried the rare 5T/7T genotype while another participant (2.0% of the total) harbored a 9T/9T genotype and the p.L206W mutation.

Race time, perceived fatigue, and muscle pain

In the whole group, marathon race time was 230 ± 37 min. Marathon race time was similar between 7T/7T (232 ± 39 min) and 7T/9T participants (215 ± 21 min; $P = 0.08$). The runner with the 5T/7T genotype completed the race in 303 min and the runner with 9T/9T genotype and the p.L206W mutation completed the race in 243 min. Perceived fatigue (16 ± 2 and 16 ± 2 A.U., $P = 0.56$) and muscle pain during the marathon (6 ± 2 and 5 ± 1 A.U., $P = 0.58$) were similar between 7T/7T and 7T/9T groups. The 5T/7T runner (13 and 3 A.U., respectively) and the 9T/9T p.L206W runner (15 and 5 A.U., respectively) reported perceived exertion and muscle pain values similar to the remaining marathoners. The energy (377 ± 233 kcal) and carbohydrate intakes (86.7 ± 54.8 g) during the race were very similar for all the participants.

Sweat osmolality and electrolyte concentration

Sweat osmolality was higher in runners with the 7T/7T genotype (144 ± 37 mOsm/kg H_2O) compared with 7T/9T runners (121 ± 39 mOsm/kg H_2O; $P = 0.04$). The participant with the 5T/7T genotype (79 mOsm/kg H_2O), and the participant with the 9T/9T p.L206W genotype (110 mOsm/kg H_2O) showed typical sweat osmolality values. Figure 1 depicts sweat Na^+, Cl^-, and K^+ concentrations according to the CFTR genotype. 7T/7T runners showed an increased sweat Na^+ concentration when compared with 7T/9T runners (42.2 ± 21.6 vs 29.0 ± 24.7 mmol/L; $P = 0.04$) while the 5T/9T (10.2 mmol/L) and 9T/9T p.L206W participants (20.5 mmol/L) showed low-range sweat Na^+ concentrations. However, 7T/7T runners showed comparable sweat Cl^- concentration to 7T/9T runners (40.2 ± 20.0 and 32.6 ± 24.6 mmol/L; $P = 0.28$) while the 5T/9T (7.2 mmol/L) and 9T/9T p.L206W participants (24.2 mmol/L) showed low-range sweat Cl^- concentrations. Finally, sweat K^+ concentration was very similar for CFTR 7T/7T (6.6 ± 1.4 mmol/L) and 7T/9T runners (6.7 ± 1.3 mmol/L) and comparable with 5T/9T (6.5 mmol/L) and 9T/9T (6.1 mmol/L) individuals (Fig. 1).

Fluid balance

Table 2 depicts fluid balance variables during the marathon. As a whole group, the marathoners reduced their

body mass by 3.1% ± 1.0% ($P < 0.01$), increased their osmolality by 2.4% ± 2.2% ($P < 0.01$), and reduced blood volume (–4.3% ± 3.0%; $P < 0.01$) and plasma volume (–7.8% ± 5.3%; $P < 0.01$) from pre-exercise values. 7T/7T runners showed very similar values of body mass change, blood osmolality change, and blood and plasma volume reductions to those found in participants with the 7T/9T genotype. The negative fluid balance found in both groups of participants was associated to sweat rates three times greater than the fluid intake rates. The 5T/7T runner showed lower values of body mass change, blood osmolality change, and blood and plasma volume reductions according to his good association between sweat rate and fluid intake rate (Table 2). However, the 9T/9T p.L206W participant showed a body mass change of –5.2%, an increase of blood osmolality of 3.6%, while blood volume and plasma volume change were very similar to the mean values observed in the groups. The level of dehydration attained by this participant was due to a sweat rate six times higher than the fluid intake rate (Table 2).

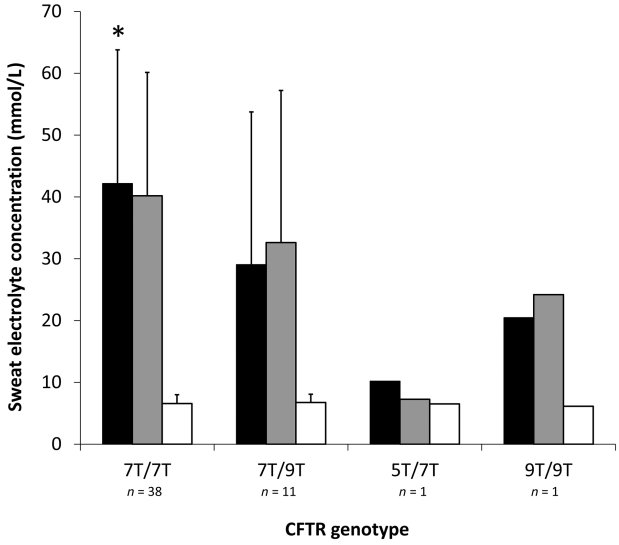

Fig. 1. Sweat electrolyte concentration in marathon runners with different CFTR genotypes. Data correspond to 38 runners with the CFTR-7T/7T genotype, 11 runners with the CFTR-7T/9T genotype, one runner with the CFTR-5T/7T genotype, and one runner with the CFTR-9T/9T-p. L206W genotype. *Different from 7T/9T at $P < 0.05$. ■, Na^+; ▒, Cl^-; □, K^+.

Electrolyte balance

By subtracting the amount of electrolytes lost by sweat during the race from the electrolytes ingested, we calculated electrolyte balance for Na^+, Cl^-, and K^+ (Fig. 2). As a whole group, the marathon runners showed deficits in all the electrolytes measured in this study ($P < 0.01$). Participants with the 7T/7T genotype had higher sodium lost by sweat ($P = 0.03$) and a higher sodium imbalance ($P = 0.04$) during the race than participants with 7T/9T genotype. However, there were no between-group differences in the values of Cl^- and K^+ lost by sweat, the amounts ingested of these electrolytes, and the resultant electrolyte balances. The participants with 5T/7T and 9T/9T p.L206W genotypes reported normal values for all the electrolyte balances (Fig. 2).

Serum responses

Serum concentrations of Na^+, Cl^-, and K^+ increased from pre-race to post-race ($P < 0.01$ for all the comparisons). There were no differences in the pre or post-race serum electrolyte concentrations between 7T/7T and 7T/9T groups. The marathoner with the 5T/7T genotype and the marathoner with the 9T/9T p.L206W genotype showed normal serum electrolyte concentrations before and after the race, according to the mild dehydration induced by the race. Serum glucose concentration was well maintained during the race while serum myoglobin concentration increased from pre-race values ($P < 0.01$ for all the comparisons) in all the participants.

Discussion

The aim of this study was to determine the association between CFTR genotype and body water and electrolyte balance during a real marathon competition. Previous studies investigated the influence of the CFTR gene on

Table 2. Fluid balance variables during a marathon competition in runners with different CFTR genotypes

Variable (units)		All	7T/7T	7T/9T	5T/7T	9T/9T (p.L206W)
n		51	38	11	1	1
Body mass (kg)	Pre	74.5 ± 10.8	73.7 ± 10.8	75.0 ± 10.6	91.7	81.1
	Post	72.2 ± 10.5	71.4 ± 10.4	72.8 ± 10.2	91.2	76.9
	Change (%)	–3.1 ± 1.0	–3.2 ± 0.9	–3.0 ± 0.7	–0.6	–5.2
Blood osmolality (mOsm/kgH_2O)	Pre	292 ± 5	293 ± 5	292 ± 5	284	292
	Post	299 ± 5	299 ± 5	299 ± 6	298	306
Blood volume change (%)		–4.3 ± 3.0	–4.9 ± 3.1	–3.1 ± 2.7	–1.3	–4.2
Plasma volume change (%)		–7.8 ± 5.3	–8.4 ± 5.6	–5.5 ± 4.5	–2.8	–8.1
Sweat rate (L/h)		0.9 ± 0.3	0.9 ± 0.3	1.0 ± 0.2	0.6	1.3
Fluid intake rate (L/h)		0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.5	0.2

Data are mean ± standard deviation.

sweat sodium wasting during exercise; however, the small sample used (Brown et al., 2011a) and the absence of sweat sodium concentration measurements (Lewis et al., 2014) have precluded the obtaining of clear-cut conclusions on this issue. In the present study we analyzed 50 of the most typical mutations and the three variants in a tract of polyT (IVS8-Tn) in intron 8 of the CFTR in healthy and experienced marathon runners while measuring physiological variables to determine the amount of Na^+ and Cl^- lost during exercise. The main outcomes were (a) 74.5% of the athletes' samples tested in this investigation showed a 7T homozygous genotype in intron 8 of the CFTR (7T/7T) gene, 21.6% were heterozygous for 7T/9T, 2.0% showed the rare 5T/7T genotype while the remaining 2.0% exhibited a 9T/9T genotype with the p.L206W mutation; (b) 7T/7T runners had increased sweat loss of Na^+ during the marathon, due to increased sweat electrolyte concentrations, as compared with 7T/9T runners. This higher Na^+ wasting was not present for Cl^- or K^+ balances (Fig. 2); (c) the 5T/7T genotype and the 9T/9T p.L206W genotype did not show abnormal sweat electrolyte losses during the race; and (d) unrelated to the CFTR genotype, all the athletes maintained regular serum electrolyte concentrations after the race (Table 3).

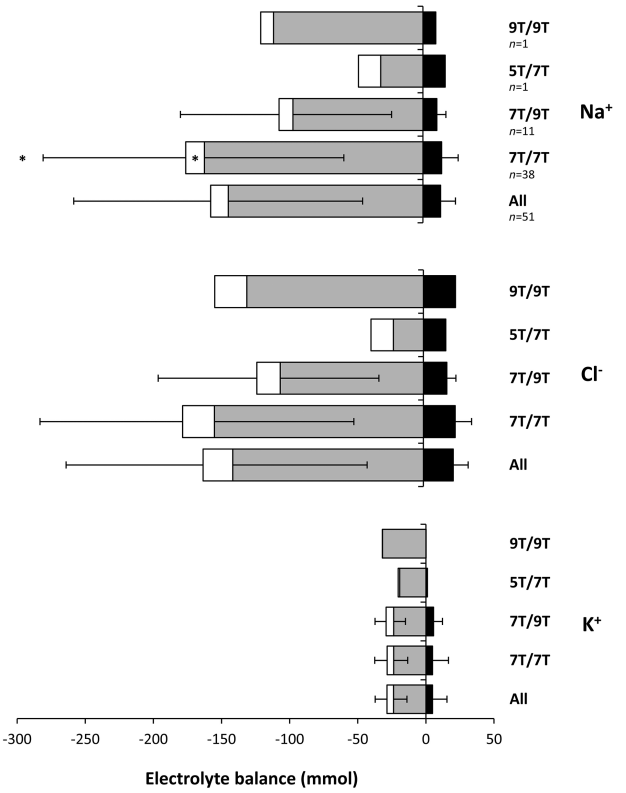

Fig. 2. Electrolyte balance variables during a marathon competition in runners with different CFTR genotypes. Data correspond to 38 runners with the CFTR-7T/7T genotype, 11 runners with the CFTR-7T/9T genotype, one runner with the CFTR-5T/7T genotype, and one runner with the CFTR-9T/9T-p.L206W genotype. *Different from 7T/9T at $P < 0.05$. □, sweated; ■, ingested; ▒, balance.

The CFTR protein is responsible for the absorption of Cl^- in the sweat duct while it simultaneously activates ENaC channels to reabsorb Na^+ (Eichner, 2008). Thus, a malfunctioning CFTR protein or a decreased presence of CFTR channels in the sweat duct should be related to increased sweat electrolyte concentration during exercise, as happens in patients with cystic fibrosis (Brown et al., 2011b). This association has been recently verified in healthy individuals with extraordinary sweat electrolyte concentrations (Brown et al., 2011a); the higher loss of electrolytes found in non-cystic fibrosis salty sweaters was related to a reduced abundance of CFTR in the sweat gland's reabsorptive ducts. However, the causes of this reduced CFTR occurrence in the sweat glands are unclear. Salty sweaters were not heterozygous carriers and they did not show CFTR mutations when compared with healthy individuals with typical sweat (Brown et al., 2011a). In addition, participants with EAH during an ultra endurance competition were also free of CFTR mutations that could explain the prevalence of this electrolyte imbalance (Lewis et al., 2014).

Table 3. Serum electrolyte concentration and myoglobin concentration before (Pre) and after (Post) a marathon competition in runners with different CFTR genotypes

Variable (units)		All	7T/7T	7T/9T	5T/7T	9T/9T (p.L206W)
n		51	38	11	1	1
Serum Na^+ concentration (mmol/L)	Pre	140.2 ± 1.1	140.3 ± 1.1	140.1 ± 0.9	139	140
	Post	142.1 ± 1.4	142.1 ± 1.3	142.4 ± 1.6	140	143
Serum Cl^- concentration (mmol/L)	Pre	104.1 ± 1.5	104.2 ± 1.3	103.5 ± 1.6	101	106
	Post	106.3 ± 1.8	106.1 ± 1.9	106.7 ± 1.5	107	107
Serum K^+ concentration (mmol/L)	Pre	4.3 ± 0.2	4.3 ± 0.2	4.3 ± 0.2	4.0	4.4
	Post	4.7 ± 0.3	4.7 ± 0.3	4.8 ± 0.4	4.7	4.7
Serum glucose concentration (mg/dL)	Pre	104 ± 13	104 ± 14	104 ± 10	98	104
	Post	107 ± 24	104 ± 22	118 ± 30	95	100
Serum myoglobin concentration (μg/mL)	Pre	29.3 ± 13	27.2 ± 10.4	30.4 ± 10.7	68.5	19.3
	Post	685.7 ± 483.9	659.5 ± 469.2	530.0 ± 263.3	1112.4	183.3

Data are mean ± standard deviation.

Our study provides novel information regarding the relationship between the CFTR genotype and the concentration of Na^+ and Cl^- in thermoregulatory sweating. First, our study sample only included a participant with a CFTR mutation (p.L206W mutation) despite it would have been expected a slightly higher proportion of mutation carriers, according to the ratio of CFTR mutations found in the European population (e.g., one in each 25 individuals; Cuthbert et al., 1995). The proportion of mutation carriers present in this investigation (1:52) was probably biased by the target population (e.g., experienced marathoners) that discarded unhealthy participants unable to complete a marathon. The individual information obtained for the participant with the 9T/9T genotype and the p.L206W mutation deserves special attention. This is a class II mutation that has been previously found in patients with cystic fibrosis, although the phenotypic expression of this mutation is highly variable (Clain et al., 2005). In fact, this type of mutation has been observed in patients with cystic fibrosis that contain normal or borderline sweat Cl^- levels (Feldmann et al., 2003), suggesting that p.L206W individuals might possess functional CFTR proteins in the sweat ducts. In agreement with this previous information, the participant with this mutation was asymptomatic for cystic fibrosis or other related pathologies. Besides, the participant with the p.L206W mutation showed low-range sweat electrolyte concentrations likely linked with his training status (Table 1). It is worthy of mention that this individual was the athlete with the highest body mass decrease reached during the race (e.g., −5.2%) according to the low fluid intake during the race (e.g., 0.2 L/h; Table 2). However, our data do not include a plausible explanation for the reduced voluntary rehydration rate found in this subject.

In addition to the analysis of CFTR mutations, we also characterized polymorphic variants in intron 8 of the CFTR gene that contains a tract of polyT with allelic 3 variants: 5T, 7T, and 9T. These three variants are not considered an indicative of a malfunctioning CFTR protein except for homozygous individuals for 5T or heterozygous for 5T plus a mutation (Dumur et al., 1996). According to the polyT variants, we established four different clusters (Table 1). Interestingly, 7T/7T runners had increased Na^+ sweat loss during the marathon (Fig. 2) when compared with 7T/9T counterparts. The higher sweat Na^+ loss during the race was related to a 45.1% increased sweat Na^+ concentration because there were no differences in the sweat rates obtained during the race (Table 2). This information modifies previous conclusions made about the association between the CFTR genotype and sweat Na^+ concentration; athletes with 7T/7T genotype in the CFTR gene showed slightly higher sweat electrolyte wasting during exercise. These results need additional studies to elucidate if this effect is related to a lower presence of CFTR proteins in the duct of the sweat glands (Brown et al., 2011a).

Despite the physiological relevance of the link between the CFTR genotype and sweat Na^+ losses during exercise, the clinical relevance of this association is minimal. One of the three explanations proposed to describe the hyponatremia associated with prolonged exercise is the presence of large Na^+ losses (Montain et al., 2006; Noakes et al., 2005). Athletes with the CFTR 7T/7T genotype had higher sweat Na^+ losses during exercise, but they presented similar post-race serum Na^+ concentrations to athletes with the 7T/9T genotype (Table 3) according to the mild dehydration induced by the race. Because the amount of Na^+ ingested and the fluid intake during the race was not different between groups, the compensation for the higher sweat Na^+ losses in the 7T/7T group was likely produced by the exchangeable stores of osmotically inactive Na^+ (Noakes et al., 2005). These results suggest that increased sweat Na^+ wasting associated to the 7T/7T genotype did not represent a physiological challenge for the maintenance of normal serum Na^+ concentration during the marathon. Nonetheless, the effects of the 7T/7T CFTR genotype should be investigated in longer endurance events such as 100-km races and Ironman triathlons.

Our experimental design presents some limitations that should be discussed to understand the scope and practical applications of the outcomes. First, we tested the 50 most common mutations of the CFTR gen while there are a total of 1993 mutations described in the most complete database of cystic fibrosis (Cystic Fibrosis Centre at the Hospital for Sick Children in Toronto accesed 1 July 2014). Thus, the high sweat sodium concentrations found in some athletes could be related to a mutation not tested in our study. A second limitation of this study is the lack of data on urinary volume and urinary electrolyte concentrations because it was impossible to collect urine samples during the race. Although participants did not report any stopping to urinate during the race, it is possible that the calculation of sweat electrolyte balances can be affected by the urine that remained in the bladder. A third limitation is the use of self-reported questionnaires to calculate the amount of fluid and salt ingested during the race. Although participants were encouraged to memorize all the products (including amounts and trademark) and the questionnaires were filled out just at the end of the race, it is possible that participants presented some inaccuracies in their responses. Despite these limitations, this ecological experiment shows relevant information to understand the causes for the variability in the amounts of electrolytes contained in thermoregulatory sweat.

Some authors have suggested that using occlusive coverings to collect sweat samples of thermoregulatory sweat during exercise produces falsely high sweat electrolyte concentrations (Weschler, 2008). Based on this theory, to cover the skin to obtain sweat creates an artificial restriction for evaporation and sweat remains on

the skin. The sweat remaining on the skin leaches Na^+, Cl^-, and K^+ from the stratum corneum while the stratum corneum absorbs water from the sweat (Armstrong, 2008), ultimately producing an improper rise of sweat electrolyte concentration. The presence of sweat K^+ concentrations similar to the ones found in blood is an easy way to determine the absence of sweat leaching as the sweat K^+ concentration remains constant despite changes in sweat rate and heat acclimatization (Costill, 1977). In our study, we used sweat patches to collect sweat during the marathon but the occurrence of leaching during the sweat collection was minimal, as could be observed from the similar K^+ concentrations in the sweat and serum (Fig. 1 and Table 3).

In summary, athletes with the CFTR 7T/7T genotype presented greater sweat Na^+ losses than athletes with the remaining CFTR genotypes found in this study. Nevertheless, this increased sweat Na^+ wasting was not related to a higher likelihood of suffering EAH during the marathon because post-race serum Na^+ concentration corresponded to athletes with mild body water deficit. One marathoner was a carrier of the rare 5T/7T genotype, but this genetic variant did not contain substantial phenotypic features as compared with other marathoners. Interestingly, the marathoner with the p.L206W mutation was the participant with the highest body mass decrease and the lowest fluid intake rate during the race. According to our results, it appears that CFTR genotypic variants contribute to significant changes in thermoregulatory sweat sodium concentration with minor clinical and medical impact, at least for marathoners.

Perspective

The main aim of rehydration during exercise is to replace the amount of water and electrolytes lost through thermoregulatory sweating. Although medical and sports organizations have formulated practical guidelines for rehydration during exercise (Sawka et al., 2007; Shirreffs et al., 2007), the great inter-individual variability in sweat rate and sweat electrolyte concentration – despite isolating factors such as training status, body mass/area, acclimatization, etc. – advises the use of individualized fluid intake recommendations. Athletes with extraordinarily high values of sweat rate and sweat electrolyte concentration (e.g., salty sweaters) have been the focus of clinical and physiological research because they can be more prone to suffer dehydration and hyponatremia, especially in hot environments. The current investigation is innovative because it determines CFTR genotypic variants as a possible cause of the inter-individual variability in sweat sodium concentration. The current data indicate that marathoners with the CFTR 7T/7T genotype present a slightly higher sodium concentration in thermoregulatory sweating. However, this phenotypic characteristic had minor clinical impact for body water and electrolyte homeostasis during a marathon because their post-race serum electrolyte concentration was ordinary. It is necessary to investigate whether athletes with the CFTR 7T/7T genotype are predisposed to suffer electrolyte imbalances in longer endurance events.

Key words: Sweat, chloride, salt, endurance exercise, hyponatremia, cystic fibrosis.

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Author contributions

J. D. C. formulated the research question, designed and carried out the study, analyzed the data, and wrote the article. B. L. designed and carried out the study, analyzed the data, and revised the article. J. J. S. designed and carried out the study, analyzed the data, and wrote the article. F. A., D. R.-V., C. G.-S., and J. A.-V. designed and carried out the study and revised the article. R. C. formulated the research question, designed the study, and revised the article.

Conflicts of interests

The authors declare that they have no conflict of interest derived from the outcomes of this study.

References

Andrieux J, Audrezet MP, Frachon I, Leroyer C, Roge C, Scotet V, Ferec C. Quantification of CFTR splice variants in adults with disseminated bronchiectasis, using the TaqMan fluorogenic detection system. Clin Genet 2002: 62: 60–67.

Armstrong LE. Commentary on viewpoint: sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. J Appl Physiol (1985) 2008: 105: 1378.

Bennett BL, Hew-Butler T, Hoffman MD, Rogers IR, Rosner MH. Wilderness medical society practice guidelines for treatment of exercise-associated hyponatremia: 2014 update. Wilderness Environ Med 2014: 25: S30–S42.

Brown MB, Haack KK, Pollack BP, Millard-Stafford M, McCarty NA. Low abundance of sweat duct Cl^- channel CFTR in both healthy and cystic fibrosis athletes with exceptionally salty sweat during exercise. Am J Physiol Regul Integr Comp Physiol 2011a: 300: R605–R615.

Brown MB, McCarty NA, Millard-Stafford M. High-sweat Na+ in

cystic fibrosis and healthy individuals does not diminish thirst during exercise in the heat. Am J Physiol Regul Integr Comp Physiol 2011b: 301: R1177–R1185.
Clain J, Lehmann-Che J, Dugueperoux I, Arous N, Girodon E, Legendre M, Goossens M, Edelman A, de Braekeleer M, Teulon J, Fanen P. Misprocessing of the CFTR protein leads to mild cystic fibrosis phenotype. Hum Mutat 2005: 25: 360–371.
Coso JD, Estevez E, Baquero RA, Mora-Rodriguez R. Anaerobic performance when rehydrating with water or commercially available sports drinks during prolonged exercise in the heat. Appl Physiol Nutr Metab 2008: 33: 290–298.
Costill DL. Sweating: its composition and effects on body fluids. Ann N Y Acad Sci 1977: 301: 160–174.
Cuthbert AW, Halstead J, Ratcliff R, Colledge WH, Evans MJ. The genetic advantage hypothesis in cystic fibrosis heterozygotes: a murine study. J Physiol 1995: 482 (Pt 2): 449–454.
Cystic Fibrosis Centre at the Hospital for Sick Children in Toronto. Cystic fibrosis mutation database. 2014 http://www.genet.sickkids.on.ca/. Accesed by 1 July 2014.
Del Coso J, Gonzalez-Millan C, Salinero JJ, Abian-Vicen J, Areces F, Lledo M, Lara B, Gallo-Salazar C, Ruiz-Vicente D. Effects of oral salt supplementation on physical performance during a half-ironman: a randomized controlled trial. Scand J Med Sci Sports 2015. http://www.ncbi.nlm.nih.gov/pubmed/25683094.
Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974: 37: 247–248.
Dumur V, Gervais R, Rigot JM, Delomel-Vinner E, Decaestecker B, Lafitte JJ, Roussel P. Congenital bilateral absence of the vas deferens (CBAVD) and cystic fibrosis transmembrane regulator (CFTR): correlation between genotype and phenotype. Hum Genet 1996: 97: 7–10.
Eichner ER. Genetic and other determinants of sweat sodium. Curr Sports Med Rep 2008: 7: S36–S40.
Feldmann D, Couderc R, Audrezet MP, Ferec C, Bienvenu T, Desgeorges M, Claustres M, Mittre H, Blayau M, Bozon D, Malinge MC, Monnier N, Bonnefont JP, Iron A, Bieth E, Dumur V, Clavel C, Cazeneuve C, Girodon E. CFTR genotypes in patients with normal or borderline sweat chloride levels. Hum Mutat 2003: 22: 340.
Godek SF, Peduzzi C, Burkholder R, Condon S, Dorshimer G, Bartolozzi AR. Sweat rates, sweat sodium concentrations, and sodium losses in 3 groups of professional football players. J Athl Train 2010: 45: 364–371.
Hamouti N, Del Coso J, Ortega JF, Mora-Rodriguez R. Sweat sodium concentration during exercise in the heat in aerobically trained and untrained humans. Eur J Appl Physiol 2011: 111: 2873–2881.
Horswill CA, Stofan JR, Lacambra M, Toriscelli TA, Eichner ER, Murray R. Sodium balance during U. S. football training in the heat: cramp-prone vs reference players. Int J Sports Med 2009: 30: 789–794.
Lewis DP, Hoffman MD, Stuempfle KJ, Owen BE, Rogers IR, Verbalis JG, Hew-Butler TD. The need for salt: does a relationship exist between cystic fibrosis and exercise-associated hyponatremia? J Strength Cond Res 2014: 28: 807–813.
Montain SJ, Cheuvront SN, Sawka MN. Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. Br J Sports Med 2006: 40: 98–105.
Montain SJ, Sawka MN, Wenger CB. Hyponatremia associated with exercise: risk factors and pathogenesis. Exerc Sport Sci Rev 2001: 29: 113–117.
Muhammad E, Leventhal N, Parvari G, Hanukoglu A, Hanukoglu I, Chalifa-Caspi V, Feinstein Y, Weinbrand J, Jacoby H, Manor E, Nagar T, Beck JC, Sheffield VC, Hershkovitz E, Parvari R. Autosomal recessive hyponatremia due to isolated salt wasting in sweat associated with a mutation in the active site of carbonic anhydrase 12. Hum Genet 2011: 129: 397–405.
Noakes TD, Sharwood K, Speedy D, Hew T, Reid S, Dugas J, Almond C, Wharam P, Weschler L. Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. Proc Natl Acad Sci U S A 2005: 102: 18550–18555.
Rosner MH. Exercise-associated hyponatremia. Semin Nephrol 2009: 29: 271–281.
Sato K, Kang W, Saga K, Sato K. Biology of sweat glands and their disorders. I. Normal sweat gland function. J Am Acad Dermatol 1989: 20: 537–563.
Sawka MN, Burke LM, Eichner ER, Maughan RJ, Montain SJ, Stachenfeld NS. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc 2007: 39: 377–390.
Shirreffs SM, Casa DJ, Carter R 3rd. Fluid needs for training and competition in athletics. J Sports Sci 2007: 25 (Suppl. 1): S83–S91.
Weschler LB. Sweat electrolyte concentrations obtained from within occlusive coverings are falsely high because sweat itself leaches skin electrolytes. J Appl Physiol (1985) 2008: 105: 1376–1377.
Wheatley CM, Wilkins BW, Snyder EM. Exercise is medicine in cystic fibrosis. Exerc Sport Sci Rev 2011: 39: 155–160.