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Transition duration of ingested deuterium oxide to eccrine sweat during exercise in the heat

Adam Church, Fanny Lee, Michael J. Buono*

San Diego State University, San Diego, CA 92182, United States

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ABSTRACT

The time necessary for the initial appearance of ingested water as sweat during exercise in the heat remains unknown. Based on the current literature, we estimated fluid transition through the body, from ingestion to appearance as sweat, to have a minimum time duration of approximately three minutes. The purpose of this study was to test this prediction and identify the time necessary for the initial enrichment of deuterium oxide (D_2O) in sweat following ingestion during exercise in the heat. Eight participants performed moderate intensity (40% of maximal oxygen uptake) treadmill exercise in an environmental chamber (40 °C, 40% rH) to induce active sweating. After fifteen minutes, while continuing to walk, participants consumed D_2O (0.15 ml kg^{-1}) in a final volume of 50 ml water. Scapular sweat samples were collected one minute prior to and ten minutes post-ingestion. Samples were analyzed for sweat D_2O concentration using isotope ratio mass spectrometry and compared to baseline. Mean ± SD Δ sweat D_2O concentration at minutes one and two post-ingestion were not significantly higher than baseline (0 min). Minutes three (9 ± 3 ppm) through ten (23 ± 11 ppm) post-ingestion had Δ sweat D_2O concentrations significantly ($P < 0.05$) higher than baseline. Such results suggest that ingested water rapidly transports across the mucosal membrane of the alimentary canal into the vasculature space, enters the extravascular fluid, and is actively secreted by the eccrine sweat glands onto the surface of the skin for potential evaporation in as little as three minutes during exercise in the heat.

1. Introduction

Ingestion of water during prolonged sub-maximal exercise in the heat is beneficial for at least two important reasons. First, water intake has been shown to attenuate the reduction seen in plasma volume, thus maintaining cardiovascular stability (Gonzalez-Alonso et al.,1997, 2008; Mountain and Coyle, 1992). Second, water intake maintains sweat production at levels sufficient to support evaporative cooling, thus attenuating hyperthermia (Sawka et al., 1985; Buono and Wall, 2000). In support of these two statements, failure to consume adequate water to prevent dehydration during exercise results in hypovolemia, hemoconcentration, decreased sweat production, and hyperthermia (Schlader et al., 2015).

It is well documented, that following ingestion, water is rapidly absorbed by the gastrointestinal mucosa and within 2 min starts to appear in the plasma, thus maintaining blood volume (Davis et al., 1985; Lambert et al., 1999). However, it is currently unknown how long it takes following ingestion for water to start to appear as sweat on the skin surface, which would be available for evaporative cooling.

Only three studies (Armstrong, et al., 2010; Davis et al., 1985; Koulmann, et al., 1997) in the scientific literature have addressed this topic, and none were specifically designed to determine how rapidly ingested water starts to appear as sweat. For example, the seminal study by Armstrong et al. (2010) determined fluid kinetics from ingestion to sweat secretion using deuterium oxide (D_2O) enrichment during an hour of exercise in the heat. Their protocol limited sampling resolution to 10 min intervals and thus could not identify initial D_2O enrichment in sweat as significant appearance was already apparent in the first sample at 10 min following ingestion. Furthermore, the subjects were not actively sweating at the point of D_2O ingestion. This is problematic if trying to determine the initial appearance of sweat D_2O from ingested fluid because eccrine secretory coil activation is delayed for several minutes at the onset of exercise (Roberts et al., 1977; Johnson and Paik, 1981).

Due to these issues, the precise initial appearance of ingested water as eccrine sweat during exercise in the heat remains unknown. Using previously published time estimates for fluid movement through various tissues in the body, we estimated that the minimum time

Abbreviations: ANOVA, Analysis of variance; D_2O, Deuterium oxide; [D_2O], Concentration of deuterium oxide; ppm, Parts per million; USG, Urine specific gravity; VO_{2max}, Maximal oxygen uptake

* Correspondence to: San Diego State University, MC-7251, San Diego, CA 92182, United States.
E-mail address: mbuono@mail.sdsu.edu (M.J. Buono).

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duration required for ingested fluid to appear as sweat to be approximately three minutes. This is considerably less than the 10 min period currently reported in the literature (Armstrong, et al., 2010). Therefore, the purpose of this study was to test our theorized time estimate for water movement by identifying the initial enrichment of D_2O in eccrine sweat following fluid ingestion during continuous exercise in the heat. Evidence supporting our time estimation would support recommendations for continual fluid consumption during exercise in the heat to sustain thermoregulation.

2. Materials and methods

2.1. Participants and ethical approval

Four male and four female participants (n =8) with a mean ± SD age of 28 ± 11 years, height of 1.70 ± 0.10 m, body mass of 64.3 ± 11.4 kg, and maximal oxygen uptake of 45.1 ± 3.2 ml kg^{-1}·min^{-1}, volunteered for the current investigation. Maximal oxygen uptake was measured during a graded exercise test to volitional exhaustion using a calibrated metabolic cart (True One, Parvomedics, Sandy, UT) prior to the D_2O test day. All volunteers initially provided a urine sample on the day of testing and hydration status was assessed for urine specific gravity (USG) using a clinical refractometer (Model 5711-2021; Schuco®, Williston Park, NY). USG values below 1.018 were taken to indicate euhydration (Stuempfle and Drury, 2003). Pregnant females and any volunteer with a history of heat exhaustion were excluded from the study. All volunteers fasted a minimum of two hours prior to participation. Volunteers also refrained from physical exercise for 24 h prior to participation. The study was conducted in accordance with the ethical standards of the San Diego State University Institutional Review Board for the protection of human subjects and with the 1964 Helsinki declaration and its later amendments. Informed written and oral consent were obtained from all participants in the study prior to data collection.

2.2. Experimental design

After meeting the inclusion criteria, participants donned a chest-strap heart rate monitor (Model FS1; Polar®, Lake Success, NY) to measure heart rate for the entire protocol. A 9 cm^2 area was identified on the right scapula halfway between the superior border and the inferior angle and marked with indelible ink. This site was chosen for ease of measurement on a walking subject. Each participant then began walking on a treadmill at approximately 40% of their maximal oxygen uptake, which elicited an absolute oxygen uptake of approximately 1.2 L min^{-1}, in an environmental chamber set to 40 °C and 40% relative humidity. Ten minutes after beginning exercise VO_2 was measured for one minute using a Douglas bag to quantify the relative work rate. Fifteen minutes after initiating exercise the identified area was blotted dry and a 9 cm^2 absorptive paper was applied to the area identified on the right scapula to collect sweat for one minute and then immediately retained in a sealed 1.5 ml micro-centrifuge tube (Number 89004-304; VWR International, Radnor, PA). A water-retardant foam block maintained the absorptive paper in contact with the skin and prevented sweat evaporation during collection. All subjects were visibly sweating prior to the first sweat collection. Each participant then ingested 0.15 ml kg^{-1} body weight D_2O (Cambridge Isotopes Laboratory, MA, 99.9% D_2O) in a final volume of 50 ml of cold (5 °C) water within a 5 s period. Sweat samples were collected for an additional ten minutes following D_2O ingestion while participants continued to walk. Thus, eleven separate sweat samples were collected per participant; one minute prior to and ten minutes post D_2O ingestion.

2.3. Sweat sample analysis

Each absorptive paper with its respective micro-centrifuge tube was weighted before and after sweat collection using a calibrated and zeroed electronic balance (Libror® Model AEL-40SM; Shimadzu Corporation, Kyoto, Japan) accurate to ± 0.0001 mg. Total mass change of each absorptive paper with its respective micro-centrifuge tube indicated total sweat volume collected for 9 cm^2 min^{-1}.

Each absorptive paper was then agitated with 50 µl of de-ionized water and then centrifuged. Thirty µl of this solution was retained and analyzed in duplicate for D_2O concentration (Morrison et al., 2001) using an isotope ratio mass spectrometer (IsoPrime IRMS; GV Instruments Ltd, Manchester, UK). D_2O concentrations ([D_2O]) from the assay were then adjusted for sweat sample volume, de-ionized water dilution, and naturally occurring D_2O in the body. Delta (Δ) sweat [D_2O] was calculated by subtracting the baseline (min 0) from the calculated value for each one-minute time point.

Test-retest reliability for measuring sweat D_2O was found to be r =0.92 and the coefficient of variation was calculated to be 3.6%.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) with repeated measures was performed to identify Δ sweat [D_2O] means that differed at $p < 0.05$. All data were evaluated for normal distribution using a Shapiro-Wilk test. The assumption of sphericity was assessed using a Mauchly's test, and a Greenhouse-Geisser correction was made to the ANOVA when that assumption was violated. Bonferroni post-hoc tests identified any significant increase in Δ sweat [D_2O] above baseline (0 min) at $p < 0.05$. All statistical analyses were conducted using IBM© SPSS© Statistics, Version 23.

3. Results

Mean relative oxygen uptake and heart rate for all participants after ten minutes of treadmill walking in the heat was 40 ± 2% of VO_{2max} and 128 ± 15 beats per minute, respectively. Shapiro-Wilk tests were non-significant, indicating normally distributed data. Mauchly's Test showed significance ($p < 0.05$) and therefore a Greenhouse-Geisser correction was made. Significantly different Δ sweat [D_2O] were identified by a one-way repeated measures ANOVA for minutes one through ten.

The results for the mean Δ sweat [D_2O] following ingestion are shown in Fig. 1. Bonferroni post-hoc comparisons indicate no significant difference in Δ sweat [D_2O] values between minutes one (p=0.93), and two (p=0.28) versus baseline. In contrast, post-hoc

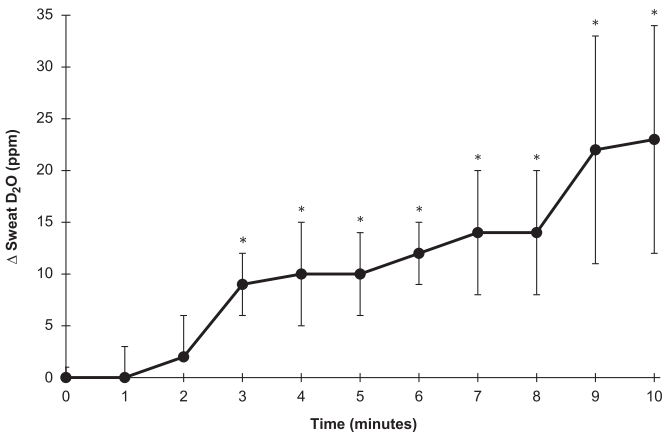

Fig. 1. Sweat deuterium oxide change in concentration in ppm before and after ingestion. Values are means ± SD. * indicates that the value was significantly ($P < 0.05$) different from the pre-D_2O ingestion (minute 0) value.