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Abstract

Purpose: Previous research shows dehydration decreases sweating and skin blood flow during heat stress but it is unknown whether these decrements are from pre-synaptic (i.e. central nervous system) or post-synaptic (i.e. sweat gland/blood vessel) alterations. The purpose of this study was to determine whether dehydration affects post-synaptic sweating and skin blood flow responses. We hypothesized that dehydration will negatively affect post-synaptic sweating and both endothelium-dependent and independent vasodilation. **Methods:** 12 males (age= 25 ± 3 y, height = 178.4 ± 5.1 cm, body mass = 75.9 ± 10.9 kg) participated. 24 h before each trial, subjects were passively heated for ~2 h. In the euhydrated trial, subjects were given water to maintain euhydration and were encouraged to maintain hydration for the remainder of the day. In the dehydrated trial, subjects were not given any fluids during passive heating and were fluid restricted for the remainder of the day, which lead to a 2.2 ± 0.7 % body mass loss. Hydration status was confirmed by urine specific gravity (U_{SG}) and urine osmolality (U_{OSM}) at the time of testing; dehydrated individuals had a $U_{SG} > 1.020$ and a $U_{OSM} > 700$ mOsm/kg (mmol/kg). Seven incremental doses of sodium nitroprusside (SNP; induces endothelium-independent vasodilation; 5×10^{-8} to 5×10^{-2} M at 10-fold increments) and methylcholine (MCh; induces local sweating and endothelium-dependent vasodilation; 1×10^{-7} to 1×10^{-1} M at 10-fold increments) were administered via two subcutaneous fibers in the forearm (i.e. microdialysis). Local sweat rate (LSR) via ventilated capsule and cutaneous vascular conduction (CVC) via Laser Doppler were recorded over the site of drug administration at the last minute of each dose. At the end of the 7th dose, maximal vasodilation was elicited by delivering a maximal dose of SNP for 10 minutes followed by local heating of the skin (~44°C) for 30 min; all CVC data are expressed as %max CVC. A 2 (hydration) x 8 (dose) repeated measures ANOVA was used to evaluate the effect of dehydration on sweating and CVC responses. Paired t-tests were used to compare hydration biomarkers between trials. An alpha of <0.05 defined significance. **Results:** There were significant differences (all $p < 0.01$) in U_{SG} and U_{OSM} when euhydrated versus dehydrated (U_{SG}: 1.009 ± 0.005 versus 1.028 ± 0.002; U_{OSM}: 337 ± 174 versus 1085 ± 70 mOsm/kg, respectively). Dehydration did not significantly alter post-synaptic sweating or endothelium-dependent or -independent CVC (i.e. SNP and MCh, respectively) responses ($p > 0.05$). **Discussion:** The hypothesis that dehydration negatively affects post-synaptic sweating and both endothelium-dependent and -independent vasodilation was not supported. Dehydration did not affect post-synaptic LSR and endothelium-independent or -dependent CVC. These findings suggest dehydration decreases sweating and cutaneous vasodilation via central mechanisms.

Background

Thermoregulation is necessary for humans to survive in the environment in which they live. The main thermoregulatory mechanisms by the human body to control temperature are through the evaporation of sweat and by increasing skin (cutaneous) blood flow (Nadel, 1988).

Many steps are involved to increase sweating and skin blood flow. Sensory afferent neurons constantly monitor skin and core temperature, sending signals to the central nervous system, specifically the hypothalamus. The hypothalamus then sends signals via efferent neurons to the sweat glands and blood vessels in order for them to sweat and vasodilate, respectively. Once the stimulus is received at the synaptic end bulb of the neuron, neurotransmitters, such as acetylcholine (ACh), are released into the synaptic cleft, between the efferent neurons and sweat glands/blood vessels (effectors) and once ACh binds to the effector, sweat secretion and vasodilation occurs, respectively (Shibasaki et al., 2002).

Increases in skin blood flow (SKBF) during heat stress (i.e., cutaneous vasodilation) occur as a result of similar efferent pathways from the hypothalamus to the blood vessels. ACh is still one of the primary neurotransmitters involved in vasodilation, but the cascade of events leading to vasodilation is a bit more complicated. Simply put ACh begins the cascade of events, but one of the major metabolites downstream causing vasodilation is nitric oxide (NO). NO is produced and released from the endothelium of the blood vessel and then acts directly on the smooth muscle of the blood vessel to cause vasodilation (Shibasaki et al., 2002). In the present study, we used exogenous MCh (a neurotransmitter synonymous with ACh that is not rapidly hydrolyzed by acetylcholinesterase) to stimulate vasodilation, which is called endothelium-dependent vasodilation because the response is dependent on an intact and functioning endothelium to produce NO (Shibasaki & Crandall, 2001). We will also provide a stable form of

NO, sodium nitroprusside (SNP), to stimulate vasodilation. Because this results in vasodilation without needing the endothelium (i.e., SNP directly leads to vasodilation), this is called endothelium-independent vasodilation.

The sweating and skin blood flow response during thermoregulation help control body temperature, but in the absence of adequate fluid intake, sweating leads to dehydration. Dehydration is common in everyday activities and sport. It is well known that dehydration negatively affects whole-body thermoregulation; when dehydrated, there are inadequate increases in SKBF and sweating to maintain thermal equilibrium (Fortney et al., 1981; Sawka et al., 1985; Nadel, 1988; Montain & Coyle, 1992). This leads to a higher core temperature during exercise when dehydrated versus euhydrated (i.e., well-hydrated) (Montain & Coyle, 1992). However, the effect of dehydration on skin blood flow and sweating has only been studied at a whole-body level. The exact mechanism(s) responsible for these changes are largely unknown. The present study, by isolating post-synaptic responses (see below), will be able to provide insight into how dehydration mechanistically affects thermoregulation (i.e. whether central or local modifications occur).

In the present study, we used the exogenous metabolites MCh and SNP to stimulate post-synaptic sweating and vasodilation. This is important to examine because as explained above, there are many aspects to thermoregulation where an error can occur and disrupt the process. For example, sites of possible errors may be where the initial stimulus is detected (afferent feedback), in the hypothalamus, efferent feedback, or in the sweat gland/blood vessel directly. We used a well-established microdialysis (MD) technique that allows us to provide different dosage amounts of MCh and SNP directly underneath the skin to cause a local, post-synaptic change in SKBF and sweating (see Methods for details). In using MD for this study, many of the

potential points where errors can occur were eliminated; this allows for the direct effects of dehydration on sweat glands and cutaneous blood flow to be examined.

Specifically, the purpose of this study was to determine the effect of hydration status on post-synaptic SKBF and sweating. We hypothesized that dehydration will negatively affect post-synaptic sweating and both endothelium-dependent and independent vasodilation.

Methods

We tested 12 healthy males (age= 25 ± 3 y, height = 178.4 ± 5.1 cm, body mass = 75.9 ± 10.9 kg). The University of Arkansas's Institutional Review Board approved this study. Each subject was familiarized with the study before signing a consent form.

In order to control the variables affecting the measures obtained, subjects refrained from food 4 hours before the trial, caffeine 12 hours before the trial, and alcohol and exercise 24 hours before the trial. Subjects underwent two trials, one while dehydrated and one while euhydrated. Twenty-four hours prior to each trial, subjects were passively heated (via a water perfused tube-lined suit) in order to promote sweating. In the dehydration trial, subjects did not receive fluids during the passive heating and also were fluid restricted for the remaining 24 hours, only drinking 250ml of water. This resulted in a dehydration of $2.2 \pm 0.7\%$ body mass loss, which is similar to that observed during exercise in the heat (Montain & Coyle, 1992). In the euhydrated trial, subjects were also passively heated, but they were given fluid throughout the heating to prevent net fluid loss. Subjects were also encouraged to maintain euhydration in the subsequent 24 hours.

When the subject arrived 24 hours after the passive heating (i.e. morning of the MD trial), they filled out a 24 hour history form to ensure that they followed the experimental controls.

Subjects provided a urine sample and nude body mass was measured (Health-o-meter digital scale, model 349KLX, Pelstar LLC, Alsip, IL, USA). Urine specific gravity (U_{SG}), quantified on a refractometer (clinical refractometer 30005, SPER Scientific, Scottsdale, AZ, USA), was used to determine hydration status [i.e., dehydration was defined as >1.020; (Sawka et al., 2007)] along with urine osmolality (U_{OSM}) [i.e., dehydration was defined as >700 mOsm/kg; (Sawka et al., 2007)]. A small blood sample was also taken to measure serum osmolality [(S_{OSM}); freezing point depression; Model 3250, Advanced Instruments Inc., Norwood, MA]. Prior to inserting the microdialysis membranes, an ice pack was placed on the individual's arm for 10 minutes to minimize hyperemia (Hodges et al., 2009). Two intradermal MD probes were inserted into the left dorsal forearm by advancing a 23-gauge needle 15 to 20 mm through the dermal layer, and 1 to 2 mm below the skin's surface. The probe was passed through the lumen of the needle and then the needle was withdrawn (Davis et al., 2007). The MD probes were perfused with saline at a rate of 4.0 µl/min by a perfusion pump (IEEM: Harvard Apparatus, Holliston, MA) for 90 minutes to allow hyperemia from insertion to subside. A blood pressure cuff (Tango+; SunTech Medical, Inc., Morrisville, NC, USA) was then placed on the right brachial artery, which was used to measure blood pressure by electrosphygmomanometry. At each time point, two blood pressures were measured and the average was used to calculate mean arterial pressure (MAP). Heart rate (HR) was also taken from 3-lead ECG.

One probe had SNP perfused through it, starting at a rate of 5×10^{-8} M and increasing in 10-fold increments to 5×10^{-2} M. The other membrane had methacholine (MCh) perfused through it, starting at a rate of 1×10^{-7} M and increasing in 10-fold increments to 1×10^{-1} M. Each dose was administered for 1 minute at a rate of 100 µl/min and then switched to 4 µl/min for four additional minutes, so that each dose was administered for at least 5 minutes (Davis et

al., 2007). At the end of the 4th minute the primary outcome measures (i.e. HR, MAP, SKBF, and sweating) were recorded.

Local sweat rate (LSR) and SKBF were measured over the probe perfused with MCh. To record SKBF, an integrated laser-Doppler flowmeter probe (model PF413, Perimed, Sweden) recorded SKBF continuously throughout the protocol. The laser probe was held in place by a capsule that was ventilated with compressed nitrogen gas perfused at a rate of 300 ml/min. LSR was measured using a humidity-temperature capsule (model HMP 35E, Vaisala, Woburn, MA, USA) connected to a humidity data processor [HMT38, Vaisala, Woburn, MA, USA (Davis et al., 2007)]. SKBF was also measured over the probe perfused with SNP using an integrated laser-Doppler flowmeter probe (model PF413, Perimed, Sweden) that was held by a local heater. At the end of the 7th dose, maximal vasodilation was elicited by delivering a maximal dose of SNP for 10 minutes in both MD probes.

Changes in skin blood flow (i.e. cutaneous vasomotor activity), expressed as cutaneous vascular conductance (CVC), were calculated by dividing red blood cell flux by MAP and reported as a percentage of maximal CVC (%max). Maximal CVC at the SNP site was determined after the maximal SNP dose was given by locally heating the skin for at least 30 minutes (until a plateau occurred) at ~44°C (Boignard et al. 2005). Maximal CVC at the MCh site was taken from the highest CVC value during the 40 minutes that the maximal SNP dose was given (see above).

A 2 (hydration) x 8 (dose) repeated measures ANOVA was used to evaluate the effect of dehydration on LSR and CVC responses. This allowed us to determine the effect of hydration status on changes in post-synaptic cutaneous vasodilation and sudomotor responses. Paired t-

tests were used to compare hydration biomarkers between trials and to compare max CVC values between trials. An alpha of <0.05 defined significance.

Results

U_{SG}, U_{OSM}, and S_{OSM} were significantly greater in the dehydrated trials ($p < 0.01$; see Table 1). There was also a significant difference in body mass (BM) loss between the euhydrated and dehydrated trial ($p < 0.01$; see Table 1).

Heart rate (HR) did not significantly differ between euhydrated and dehydrated trials (grand mean ± SD: euhydrated = 62 ± 1 versus dehydrated = 61 ± 1; p = 0.80). MAP was also not significantly different between euhydrated and dehydrated trials (grand mean ± SD: euhydrated: 86 ± 1 versus dehydrated: 84 ± 0 mmHg; p = 0.44).

Dehydration did not affect the maximum CVC at the MCh site elicited via a maximal dose of SNP (5×10^{-2} M) (114.7 ± 82.6 and 117.6 ± 64.4 AU in euhydrated and dehydrated conditions, respectively; $p > 0.05$). Similarly, dehydration did not affect maximum CVC at the SNP site (101.7 ± 35.5 and 94.5 ± 42.8 AU, in euhydrated and dehydrated conditions, respectively; $p > 0.05$).

CVC (%max) at the MCh and SNP site significantly increased as the dose concentration increased ($p < 0.01$; Figures 1 & 2, respectively). However, dehydration did not significantly affect the increase in post-synaptic endothelium-dependent or -independent CVC (i.e. MCh and SNP, respectively) responses ($p > 0.05$). The main effect of hydration on CVC at the MCh site was p = 0.23, the main effect of dose was $p < 0.01$, and the main effect of hydration*dose, or the interaction of the two, was p = 0.48. The main effect of hydration on CVC at the SNP site was p = 0.33, the main effect of dose was $p < 0.01$, and the main effect of hydration*dose was p = 0.26.

Dehydration did not affect post-synaptic LSR ($p > 0.05$). LSR significantly increased between doses starting at 1×10^{-3} M MCh ($p < 0.01$, see Figure 3). The main effect of hydration on LSR at the MCh site was $p = 0.81$, the main effect of dose was $p < 0.01$, and the main effect of hydration*dose was $p = 0.76$.

Discussion

The purpose of this study was to determine the effects of hydration status on post-synaptic LSR and CVC. The main findings were that hydration status did not affect post-synaptic LSR or endothelium-dependent or -independent CVC, suggesting that dehydration impairs sweating and cutaneous vasodilation via other mechanisms. The hypothesis that dehydration negatively affects post-synaptic sweating and both endothelium-dependent and independent vasodilation was not supported.

As designed, subjects were successfully euhydrated and dehydrated for each trial. When dehydrated, the subjects had a significantly higher U_{SG}, U_{OSM}, S_{OSM}, and percent body mass loss (Table 1). This was important so that we could see the effect on hydration status on post-synaptic CVC and LSR.

We found that hydration status did not have an effect on endothelium-dependent or -independent CVC. Takamata et al. (1997) found that thermoregulatory skin blood flow is impaired by hyperosmolality by increasing the onset of CVC. Since we did not find differences in CVC between trials, it suggests that the decrement in CVC that occurs with dehydration occurs via central mechanisms and not via post-synaptic (local) responses. The nervous system is very complex and the brain is the center that mediates it all. The specific area in the brain responsible for thermoregulation is the preoptic/anterior hypothalamus, which is regulated by a

negative feedback loop that stimulates vasodilation or vasoconstriction when necessary, along with stimulating sweating (Boulant, 2000). Dehydration may affect the afferent signaling, which is responsible for sending information to the brain. Once that information is in the brain, and is processed, efferent signals are sent out. Dehydration could also affect the efferent messages the brain sends out to the blood vessels and sweat glands. However, our data shows that, at a given dose of MCh and SNP, there were similar changes in CVC when euhydrated and dehydrated (Figures 1 and 2). This suggests that the blood vessels *per se* are not affected by dehydration.

Previous research has shown that sweat rate is reduced with dehydration (Sawka et al., 1985). Montain et al. (1995) also found that the threshold (i.e. onset) for sweating is at a higher core temperature when dehydrated. Fortney et al. (1981) demonstrated that the reduction in sweat rate is related to hypovolemia. However, it is believed that hyperosmolality is more likely the culprit of reduced sweat rate than hypovolemia (Sawka et al., 1985). In this study, the methods of dehydration used made our subjects hyperosmotic (i.e., increased plasma osmolality) as well as hypovolemic (i.e., reduction in body mass) and this led us to test whether LSR would be impaired by alterations post-synaptically in the sweat gland. We found that post-synaptic LSR was not significantly affected by dehydration (Figure 3). This means that individual sweat glands are not impaired by dehydration. So this further leads us to believe that dehydration decreases sweat rate through central mechanisms. The sweat gland's sensitivity to the neurotransmitters is not altered by dehydration but since the preoptic/anterior hypothalamus is also responsible for stimulating thermoregulatory sweating, this also could be implicated as an area responsible for the impairments in sweating with dehydration.

Limitations

There are many vasodilators that are responsible for cutaneous vasodilation (Charkoudian, 2010). Since we only tested two of these, it is possible that the mechanisms involved with SNP and MCh role in vasodilation are not impaired with dehydration but that another vasodilator is. Stanhewicz et al. (2014) found regional differences in cutaneous vasodilation, so this could also have some implication for the results we obtained. Also, our responses also can only be applied to males.

Conclusion

The purpose of this study was to determine if dehydration impairs post-synaptic LSR and CVC. We found that dehydration had no affect on post-synaptic LSR and CVC. It is known that dehydration impairs LSR and CVC but the exact impairment is unknown. This study helped to determine that the sweat glands and cutaneous blood vessels are not directly impaired by dehydration. The impairments seen in LSR and CVC due to dehydration are likely located in central mechanisms of thermoregulation. It is likely that the brain interprets dehydration from afferent inputs and responds to this by altering LSR and CVC in order to preserve bodily fluid.

Acknowledgments

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Table 1. Mean ± SD Hydration Biomarkers For Euhydrated and Dehydrated Trials		
	Euhydrated	Dehydrated
U_{SG}	1.009 ± 0.005	1.028 ± 0.002*
U_{OSM} **(mOsm/kg)**	337 ± 174	1085 ± 70*
S_{OSM} **(mOsm/kg)**	289 ± 6	297 ± 3*
BM change (%)	-0.1 ± 0.7	-2.2 ± 0.7*

U_{SG}=urine specific gravity; U_{OSM}=Urine Osmolality; S_{OSM}=Serum Osmolality; BM Loss=percent body mass loss was calculated from the weight recorded before the passive heating. *Significantly different from euhydrated (all $p < 0.01$).
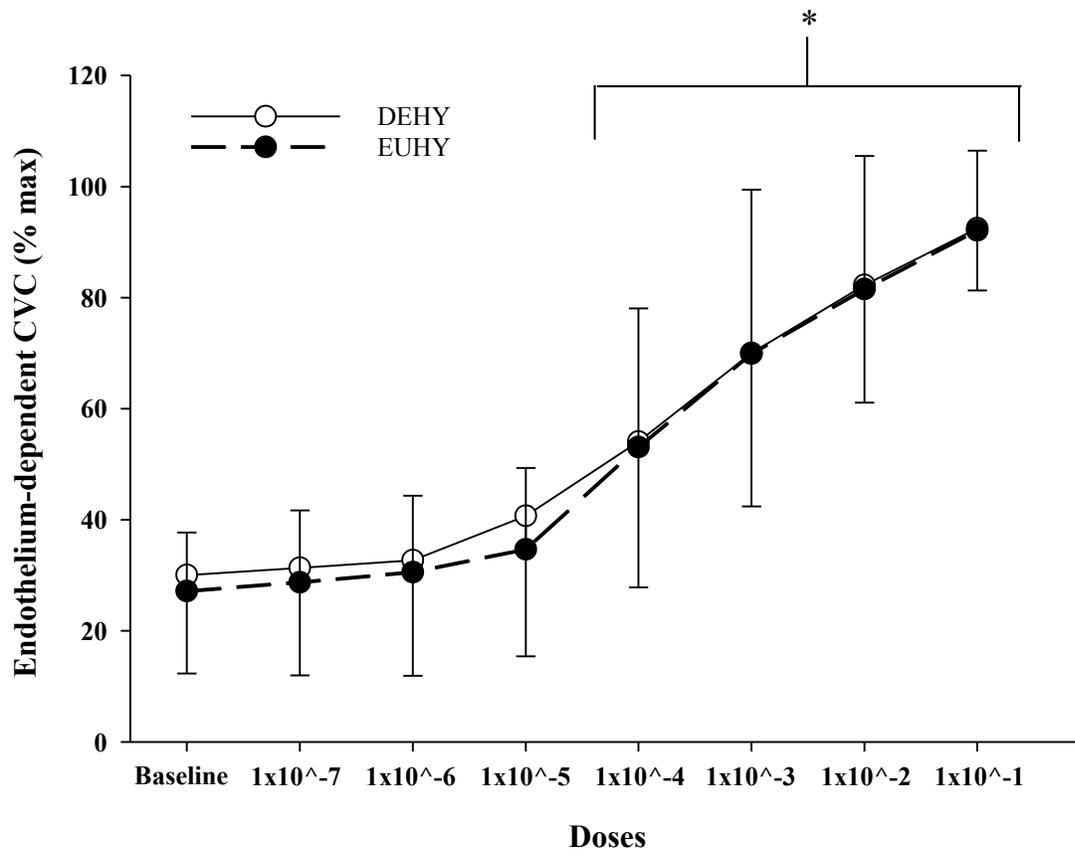
Figure 1. Effects of hydration on CVC at MCh Site
*Significantly different from baseline, independent of hydration status (p < .05)
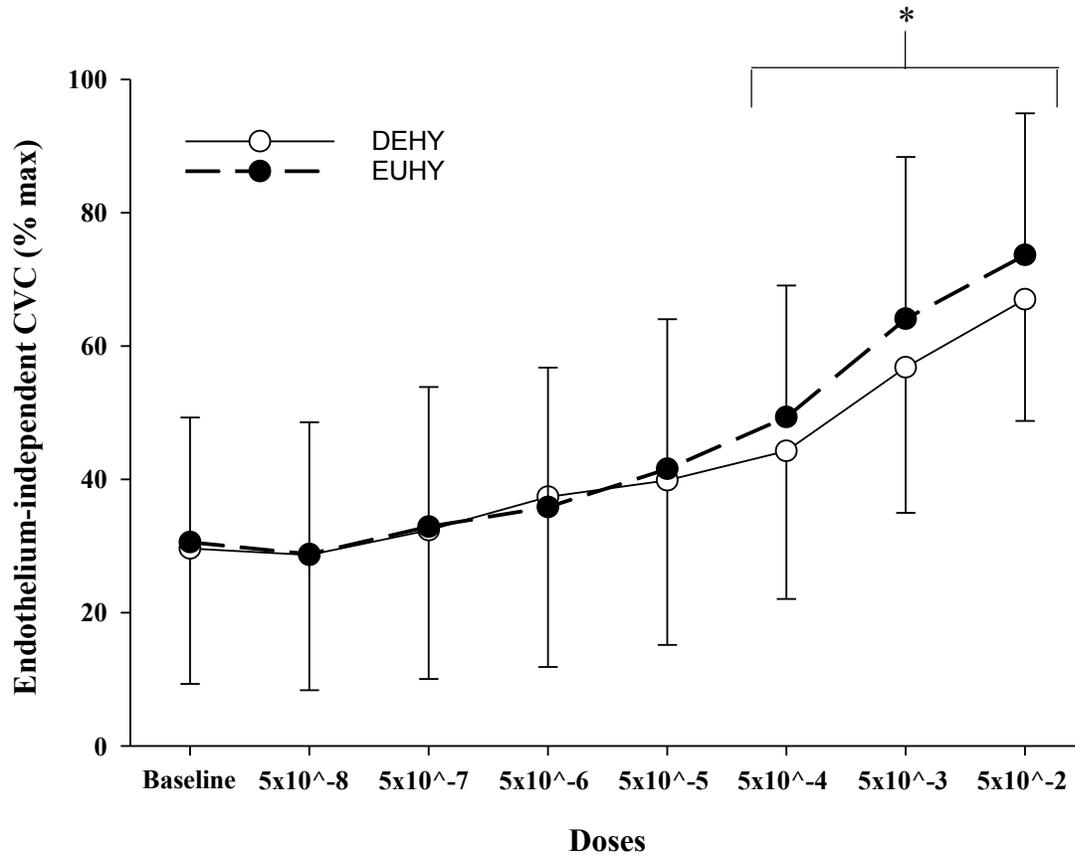
Figure 1. Effects of hydration on CVC at SNP Site
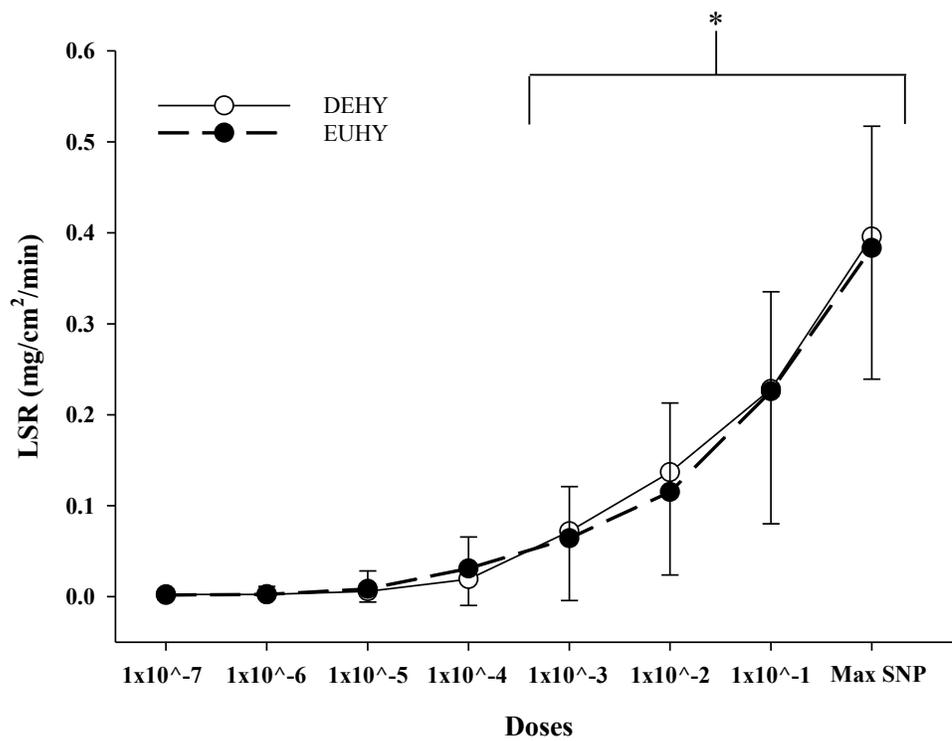*Significantly different from baseline, independent of hydration status (p < .05)

Figure 2. Effects of hydration on LSR
*Significantly different from baseline, independent of hydration status (p < .05)