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Research Article

Water Rehydration Blunted Vasopressin, Angiotensin II And HSP70 Responses To High Environmental Temperature Without Altering Plasma Osmolality In Male Sprague-Dawleys Rats

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ABSTRACT

Chronic exposure to high environmental temperature (HET) is a reno-cardiovascular risk factor. Studies have shown that HET alters body fluid balance and elevates plasma osmolality, with the consequent release of angiotensin II and arginine vasopressin (AVP), which play key roles in the pathophysiology of some forms of hypertension. This study investigated the effect of water rehydration on plasma osmolality, angiotensin II, arginine AVP and heat shock protein (HSP70) during chronic exposure to HET. Eighteen (18) male Sprague-Dawley rats (n = 6/group, 8 weeks old, weight: 90-100g) were either kept in an environmental chamber maintained at a HET (38.5±0.5ºC) 4hrs daily with (RH) or without access to water (H) compared to control rats (C), maintained at a room temperature of 25 ± 0.5ºC ºC. The experiment lasted 8 weeks. There was a significant increase in plasma osmolality ($P<0.05$), fluid balance ($P<0.001$), angiotensin II ($P<0.01$), AVP ($P<0.001$) and HSP 70 ($P<0.05$) in rats (H) exposed to HET compared to control. However, with water rehydration in rats (RH) exposed to similar HET, increases were only noticed in plasma osmolality ($P<0.01$) and fluid balance ($P<0.001$), with no significant rise in AVP, angiotensin II and HSP70 compared to control. Meanwhile, AVP was significantly lower ($P<0.01$) in RH compared to H rats, whereas plasma osmolality, fluid balance, angiotensin II and HSP70 were not significantly different in H and RH rats. The result suggests that water rehydration during prolong exposure to hot environment blunts AVP, angiotensin II and HSP 70 responses to persistent hyperosmolality.

Keywords: *Angiotensin II, HSP70, arginine vasopressin, plasma osmolality, high environmental temperature, rehydration.*
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INTRODUCTION

High environmental temperature has been identified as a potential source of Physiological stress with the capability to disrupt body fluid and electrolytes balance. The fluid and electrolytes imbalance are apparently occasioned by heat–induced enhanced sweating and subsequent evaporative cooling (Horowitz, 2016; Périard *et al*., 2015). Profuse sweating during exposure to high environmental temperature has been suggested to result in the loss of large amount of sodium ions alongside body fluid (Allan & Wilson, 1971). This in turn has been reported to elicits acclimatization responses which ultimately facilitate sodium reabsorption via the sweat glands and the kidneys, a process aimed at offering protection to body fluid volume (Quinton, 2007).According to the work of Sawka and Coyle (1999), fluid volume expansion following acute exposure to high environmental temperature could take 3-4 days to occur.

Recent study has shown that the population of workers exposed to hot environmental working condition is on the rise globally (Nerbass *et al*., 2017) and by extension in tropical countries, where there is limited access to clean water (Johnson et al., 2014). Examples of workers exposed to high environmental temperature include indoor and outdoor workers namely miners, transporters, traders, firefighter, bakery worker, military personnel, construction worker, factory worker, boiler room worker, landscaper, some athletes and agricultural worker. (Lucas *et al.,* 2014; Meshi *et al*., 2018).

Generally, prolonged exposure of this population of workers to environmental heat usually leads to dehydration (Akerman et al., 2016).Therefore, adequate hydration with

water is a strategy that is advocated for the prevention and the amelioration of the harmful effect caused by prolonged exposure to high environmental temperature (Webber *et al*., 2003, Maté and Siegel, 2016). Sadly, individuals exposed to hot environment are poorly hydrated with water as explained by the delay rehydration hypothesis postulated by Rothstein *et al*., (1947). Furthermore, it has been reported that individuals working in hot environments starts the day with fluid deficit and become further dehydrated on the work (Horn et al., 2012; Hunt *et al*.,2014).

Osmolality level, normally maintained within a physiological range of 275 and 295 mOsm/kg (Gennari *et al*.,1984), increases in the face of dehydration (Keller *et al*.,2003). It is defines as the concentration or number of osmole of solute or fluid particles per kilogram of solvent (Knepper *et al*.,2015 ; Rasouli *et al*.,2016). Osmolality determines the direction of fluid movement within the body system. Specifically, fluid flows from a compartment of low osmolality to that of high osmolality (Shah & Mandiga, 2020). The alteration in plasma osmolality is sensed by specialized neurons within the hypothalamic circumventricular organs, which lack a complete blood-brain barrier (Kinsman *et al*., 2017; Stocker *et al*., 2003). In turn, arginine vasopressin is released from the magnocellular cells of supraoptic nuclei and paraventricular nuclei (PVN) in the hypothalamus (Birnbaumer, 2000). Together, the hypothalamic response to change in osmolality initiates thirst response, enhances arginine vasopressin (AVP) release and increases renal responsiveness to vasopressin (Verbalis, 2007). Both the peripheral and central angiotensin II play important role in mediating these thirst response and vasopressin release. Plasma vasopressin is reported to be elevated in some forms of hypertension (Matsuhisa *et al.*,2000), and this elevation is suggested to correlates with the severity of hypertension (Johnston,1985). Besides, its adverse role has also been documented in congestive heart failure (Matsuhisa *et al*.,2000). Furthermore, abnormally high plasma osmolality has been shown to increase sympathetic nerve activity in animals (Weiss *et al.*,1996; Shi *et al*.,2007).

Prolonged exposure to high environmental temperature activates widespread induction and the release of heat shock proteins (HSPs) in the body. HSP70 is one example of such proteins (Kampinga *et al*., 2009). The main function of HSPs is the protection of cells against programmed cell death referred to as apoptosis. They achieve this by acting as 'molecular chaperones', which help to stabilize macromolecules, guide protein folding, perform refolding and remove irreversibly denatured proteins in the cells (Beere, 2005; Jiang *et al*., 2001; Jolly & Morimoto, 2000). They also assist in thermotolerance (Taulien &Lindquist, 1993). The proteins are also induced by hyper-osmotic stimuli (Burg et al., 2007) and dehydration (Akerman *et al*., 2016). HSP 70 level is also reported to be high in chronic disorders such as essential hypertension (Srivastava *et al*., 2016). This study is aimed at investigating the effect of water rehydration on high plasma osmolality, plasma angiotensin II, vasopressin and HSP 70 during chronic exposure to high environmental temperature.

MATERIALS AND METHODS

Animals: The approval of the study protocol was obtained from the Health Research Ethics Committee of College of Medicine of the University of Lagos (CMUL/HREC/11/18/471). Eighteen (18) male Sprague-Dawley rats were used for the study. The rats were obtained from College of Medicine of the University of Lagos Animal House and kept in the animal unit of Department of Physiology of College in plastic cages that were well ventilated in 12-hour light and dark cycle at 25 ± 0.5ºC . The rats were acclimatized for one week before the commencement of the experiment. There was free access to rat chow and clean water ad libitum. Animal care was provided in line with the Declaration of Helsinki and Guiding principles for research involving animals.

Study design: The rats (8 weeks, 90-100g) were randomly assigned into three groups. Group I rats were maintained at room temperature (25 ± 0.5ºC); Group II rats were exposed to high environmental temperature of 38.5 ± 0.5ºC, 4 hours daily for a period of 8 weeks without any access to water during the exposure. Group III rats were exposed to the same high environmental temperature for the same period but they had free access to water during the exposure.

Procedure for heat exposure: The rats were exposed to high environmental temperature (HET) using the method described by Barney and Kuhrt (2016) with some slight modification. Briefly, the rats were exposed to 38.5 ± 0.5ºC, 4hrs daily for 8 weeks in a chamber of relative humidity of 65-75% .The chamber was made of plastic with a heat source, which could be regulated manually (Agbaraolorunpo *et al*., 2019). The chamber temperature and the relative humidity of the chamber were monitored by HTC-2 device. Core body temperature was measured with rectal digital thermometer before and after the exposure. Percentage Body.

Measurements of Angiotensin II, Vasopressin and HSP70
Plasma angiotensin II, vasopressin and HSP70 were determined using enzyme linked immunosorbent assay techniques using rat Elisa kits. Blood samples were collected from retro-orbital veins of male Sprague-Dawleys rats into heperinized bottles. The samples were centrifuged at 3000rpm for 15minues to separate plasma from whole blood. Plasma samples were kept in eppendorf tubes and stores at -25ºC before assay for the respective peptides.
Plasma angiotensin II concentrations were determined using Rat Angiotensin II Elisa kit (MyBiosource.com, USA) according to the manufacturer instruction. Plasma vasopressin concentrations were determined using Rat Vasopressin Elisa Kit (BioAim Scientific Inc, Canada) according to the manufacturer instruction. Plasma and HSP-70 concentrations were determined using Rat HSP-70 Elisa Kit (MyBiosource.com, USA) according to the manufacturer instruction.

Fluid balance and Plasma osmolality: 12-hour urine output (V) in ml was determined at the end of 8th week of the experiment, from 7 p.m to 7 a.m in a metabolic cage. The metabolic cage comprised of three compartments, with each

section containing a bottle for urine collection and the second for water intake. 100ml water was provided for each rat in the bottle at the start of the assessment. Water intake was determined from the difference between the initial volume of the water provided in the bottle at the start of the study and the final volume of water left in the bottle at the end of the 12-hour urine collection. Net fluid gain was calculated by subtracting total urine loss from water intake, with the assumption that respiratory water loss and sweat loss at rest were negligible (Nose *et al.*, 1994). Plasma Na^+ in mmol/L was determined with ion selective electrode method (ISE 6000 analyzer, France). Plasma osmolality was calculated from plasma Na^+, plasma glucose and urea as 2Na + 1.15 (Glu/18) + (Urea/6) by a method validated by (Martín-Calderón *et al*., 2015). Plasma urea (mg/dl) was determined calorimetrically with Spectrophotometer. Plasma glucose was determined by glucose oxidation principle with glucometer (Togashi *et al*., 2016).

Statistical Analysis: Results are presented as Mean ± standard error of mean (SEM) in tables and bar charts. The test for significance was carried out using One-way ANOVA followed by Tukey post hoc test. Differences were considered statistically significant at P<0.05. The analysis was done with GraphPad Prism 5.0 software.

RESULTS

Plasma Osmolality, Fluid balance, fluid intake and urine volume: Plasma osmolality (pOsmol) and fluid balance were significantly higher in rats exposed to HET alone compared to control rats (pOsmol: 289.4 ± 3.9 vs 274.0 ± 2.6 Osmol/kg, P<0.05); (fluid balance:19.0 ± 1.1 vs 5.3 ± 0.7ml, P<0.001).This was similar for rats exposed to environmental heat plus water rehydration compared to control rats (296.8 ± 3.1vs 274.0 ± 2.6 Osmol/kg, P<0.01); (19.1 ± 3.2 vs 5.3 ± 0.7ml, P< 0.001) (Table 1). Similarly, fluid intake was higher in rats exposed to chronic exposure to environmental heat alone compared to control rats (21.5 ± 0.8 vs 9.3 ± 0.7 ml, P<0.01) and was also higher in rats exposed to environmental heat plus water rehydration compared to control (24.0 ± 2.3v s 9.3 ± 0.7, P<0.001) (Table 1). Urine volume was lower in rats exposed to environmental heat alone compared to control (2.5 ± 0.4 vs 4.0 ± 0.7ml, P>0.05) but this difference was not significant statistically. Likewise, there was no significant difference in the urine volume of rat group exposed to environmental heat plus water rehydration compared to control rats (5.4 ± 1.0 vs 4.0 ± 0.7ml, P>0.05). However, urine volume was significantly lower in rats exposed to environmental heat alone compared to rats exposed to environmental heat plus water rehydration (2.5 ± 0.4 vs 5.4 ± 1.0ml, P<0.05).

Plasma Angiotensin II

Plasma angiotensin II was significantly higher in the rats exposed to environmental heat alone compared to control rats (675.0 ± 11.0 vs 615 .8 ± 10, P<0.01), but no significant difference was noticed in the rats exposed to environmental heat plus water rehydration compared to control rats (648.3 ± 8.0 vs 615 .8 ± 10 pg/ml) (Figure 1).

Table 1:
Fluid balance, plasma Na^+ , Urinary output and plasma Osmolality in male Sprague-Dawley rats exposed to HET with and without rehydration

Groups /variables	control	heat	rehy + heat
Fluid intake (ml/12hrs)	9.3±0.7	21.5±0.8**	24.0±2.3***
Urine output (ml/12hrs)	4.0±0.7	2.5±0.4	5.4±1.0$^{\#}$
Fluid balance (ml)	5.3±0.7	19.0±1.1***	19.1±3.2***
Plasma Na+ (mmol/L)	134.9±0.8	141.2±2.2*	144.6±1.7**
Plasma osmolality (osmol/kg)	274.0±2.6	289.4±3.9*	296.8±3.1**$^{\#\#}$

**P<0.05, **P<0.01, ***P<0.001 vs control (significantly higher); $^{\#}$P<0.05,$^{\#\#}$P<0.01 vs Heat (significantly higher). Data presented as Mean ± SEM (n=6), One-way ANOVA followed by Tukey post hoc test.*

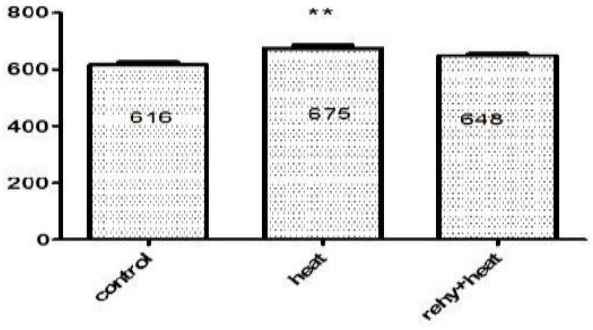

Figure 1:
Effect of water rehydration on plasma Angiotensin II in rats exposed to chronic HET: **P<0.01 vs control (significantly higher); Data presented as Mean ± SEM, One-way ANOVA followed by Tukey post hoc test.

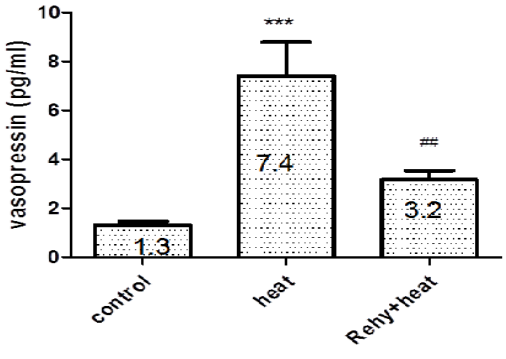

Figure 2: Effect of water rehydration on on plasma arginine vasopressin (AVP) in rats exposed to chronic HET:***P<0.01 vs control , $^{\#\#}$P<0.01 vs heat ; Data presented as Mean ± SEM, One-way ANOVA followed by Tukey post hoc test

Plasma Arginine Vasopressin

Plasma vasopressin level was significantly higher in the rats exposed to environmental heat alone compared to control rats (7.4 ± 1.3 vs 1.3 ± 0.2, P<0.001) but was not significantly

different in the rats exposed to environmental heat plus water rehydration compared to control rats (3.2 ± 0.4 vs 1.3 ± 0.2, P>0.05). However, plasma vasopressin level was significantly higher in the rats exposed to environmental heat alone compared to the rats exposed to environmental heat plus water rehydration (7.4 ± 1.3 vs 3.2 ± 0.4 ,P<0.01) (Figure 2).

Plasma Heat Shock Protein 70 (HSP70)

Plasma HSP70 was significantly higher in rat group exposed to environmental heat alone compared to control rats (1.5 ± 0.2 vs 1.1 ± 0.1, P<0.05), but no difference was noticed in the group exposed to environmental heat plus water rehydration compared to control rats (1.3±0.01 vs, 1.1 ± 0.1,P>0.05) (Figure 3).

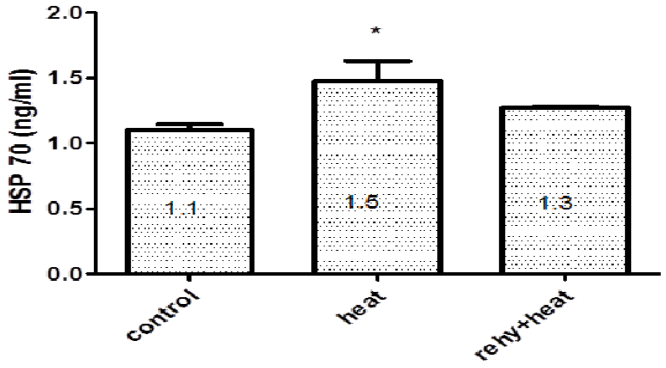

Figure 3: Effect of water rehydration on plasma HSP70in rats exposed to chronic HET :*P<0.05 vs control ; Data presented as Mean ± SEM, One-way ANOVA followed by post hoc test.

DISCUSSION

The disruption of body fluid balance and elevation of plasma osmolality by high environmental temperature is reported to exert physiological burden on the cardiovascular systems (Crandall & Wilson, 2015) .This ultimately promotes the evolution, progression and outcome of cardiovascular and chronic kidney diseases. (Kaya *et al.*, 2017; Ozsari, 2017; Nerbass *et al*., 2017). Therefore, there is a campaign in favour of adequate hydration with water in order to mitigate the potential harmful effect of prolonged exposure to hot environment (Webber *et al*.,2003, Maté and Siegel, 2016). Our previous work in male Sprague-Dawley rats demonstrated that chronic exposure of the rats to high environmental temperature increased the animals'blood pressure, myocardial workload and oxidative stress, with no amelioration by concomitant rehydration with water during the exposure (Agbaraolorunpo et al.,2018). However, this present study revealed that chronic exposure of similar animal models to high environmental temperature increased plasma osmolality with associated increase in fluid balance caused by an increase in fluid intake and slight reduction in urine output. Similarly, plasma osmolality, fluid consumption and fluid balance increased by chronic exposure to high environmental temperature were sustained in the rehydrated rats. Additionally, urine output was high in the rehydrated rats, hence the sustenance of elevated hyperosmotic state in this group of rats.

First, our result confirms that environmental heat stress increases plasma osmolality (Gagnon *et al*., 2017) with associated activation of thirst and increased water consumption (Barney & Kuhrt, 2016) but reduced urine output (Azahan & Sykes, 1980). Secondly, rehydration did not reverse the heat-induced increased plasma osmolality as well as the increased fluid consumption in rats exposed to chronic exposure to high environmental temperature. This may be due to reduced water reabsorption by the kidney of the rats, as evident in the increased urinary output. This increased renal water loss apparently reduced the fluid gain needed to restore the elevated plasma osmolality back to normal. The explanation for the sustained hyperosmolality could also be that the body fluid of the animals was continuously loss to evaporative cooling despite the available water rehydration during the exposure, similar to the pattern reported by Barney & Kuhrt (2016).

Dehydration is a common cause of body fluid deficit occurring when fluid loss exceeds fluid gain. Exposure to hot environment is a major cause of dehydration resulting from fluid and electrolytes loss from sweating (Allan & Wilson, 1971). As sweating increases, plasma volume falls and blood become more concentrated (Fortney & Miescher, 1994). Therefore, prolong exposure of rats in this current study to high environmental temperature possibly caused compensatory increase in plasma sodium ion in agreement with previous study (Allahverdi *et al*., 2013), and this possibly occurred via enhanced sodium ion retention in the sweat glands and the kidneys (Quinton, 2007).This evidently reflected in the elevation of plasma sodium ion in rats exposed to high environmental temperature, with no moderation by water rehydration. This sodium retention evidently contributed to the increased plasma osmolality observed in the two groups of rats exposed to high environmental temperature, as sodium ion constitutes the mainstay of plasma osmotic status. In turn, the increased plasma osmolality activates neurohumoral signals crucial for the restoration of osmotic balance.

The neurohumoral signals may be mediated by AVP (arginine vasopressin) , angiotensin II and heat shock proteins (HSPs) induction. Specifically, AVP increases water reabsorption in the kidney and reduces urine output (Cuzzo et al., 2020), while angiotensin II stimulates thirst , increases sodium reabsorption and potentiates AVP release (Szczepanska-Sadowska et al., 2018). In this present study, both vasopressin and angiotensin II were significantly increased in the rats exposed to environmental heat alone, but were not significantly increased in the rehydrated rats exposed to hot environmental condition. This observation suggests that water rehydration during exposure to high environmental temperature blunted arginine vasopressin and angiotensin II responses. The increased vasopressin response was associated with slight downward urine output in rats exposed to HET alone. This could be due to the associated increase in water reabsorption mediated by the insertion of water protein channels in renal tubules by AVP (Cuzzo *et al.*, 2020). Conversely, the blunted plasma AVP response was accompanied with increased renal fluid loss and sustained

increased plasma osmolality in the rehydrated rats exposed to the HET. The increased plasma angiotensin II by exposure to HET suggests the involvement of this peptide in enhancing fluid consumption. The sustenance of fluid consumption in the presence of blunted angiotensin II response in the rehydrated rats may be due to the persistent hyperosmotic state.

Finally, the prolong exposure of our experimental rats to HET induced increased HSP70 release, but this effect was blunted by water rehydration during the heat exposure .This is in line with similar study in human in which fluid replacement attenuated HSP70 and DNA damage (Roh *et al*., 2016). Most importantly, HSP70 induction offers a level of protections to cells undergoing stress (Jolly & Morimoto, 2000), with the level reported to be high under some disease conditions such as in essential hypertension and chronic kidney diseases where it also acts as marker of inflammation (Sreedharan & Van Why, 2016; Srivastava et al., 2016). Therefore our observation that HSP70 level was not significantly increase in the rehydrated rats exposed to HET could implied that the harmful effect of the high temperature on internal organs might have been ameliorated in this group of rats even though their plasma osmolality was still high. Furthermore, the blunted induction of HSP70 by rehydration in the rats exposed to HET may be related to the blunted response of angiotensin II and vasopressin suggested to be involved with the induction of HSP70 in peripheral and renal cells (Xu *et al*., 1996) respectively (Ishizaka *et al*., 2002). Ultimately, these observations suggest plausible role for HSP70 in recurrent dehydrated state (Akerman *et al*., 2016) especially under prolong exposure to hot environment. Some of these roles may include thermo-tolerance and osmo-tolerance (Sreedharan & Van Why, 2016) .

In conclusion, this study demonstrated that chronic exposure to high environmental temperature in rats resulted in increased plasma osmolality, fluid retention, angiotensin II, vasopressin and HSP70. However, these responses were blunted in rats that had water rehydration during the exposure even though plasma osmolality and fluid retention remained unaltered. These findings indicate that water intake during environmental heat exposure may ameliorate the adverse effects of chronic exposure of the body to high environmental temperature, hence the need to drink adequate water during exposure to hot environment.

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REFERENCES

Agbaraolorunpo, F. M., Oloyo, A. K., Anigbogu, C. N., & Sofola, O. A. (2019): Chronic exposure to high environmental temperature exacerbates sodium retention and worsens the severity of salt-induced hypertension in experimental rats via angiotensin receptor activation. Journal of African Association of Physiological Sciences, 7(2), 109–118.

Agbaraolorunpo, F.M., Oloyo,A.K., Doherty A.A , Said Z.O. and Phillip, O. (2020): Water Intake Attenuates the Hyperglycemic Effect of Chronic Exposure to High Environmental Temperature without Improving Oxidative Stress and Cardiovascular Outcome in Animal Models. Quarterly Journal of Hospital Medicine . Nigerian Quarterly Journal of Hospital Medicine Vol.28(1) (in press)

Akerman, A. P., Tipton, M., Minson, C. T., & Cotter, J. D. (2016): Heat stress and dehydration in adapting for performance: Good, bad, both, or neither? Temperature: Multidisciplinary Biomedical Journal, 3(3), 412–436.

Allan, J. R., & Wilson, C. G. (1971): Influence of acclimatization on sweat sodium concentration. Journal of Applied Physiology, 30(5), 708–712.

Azahan, E., & Sykes, A. H. (1980): The effects of ambient temperature on urinary flow and composition in the fowl. The Journal of Physiology, 304, 389–396. https://doi.org/10.1113/jphysiol.1980.sp013330

Barney, C. C., & Kuhrt, D. M. (2016): Intermittent heat exposure and thirst in rats. Physiological Reports, 4(8).

Birnbaumer M 2000. Vasopressin receptors. Trends Endocrinol Metab 11:406–410.

Beere, H. M. (2005): Death versus survival: Functional interaction between the apoptotic and stress-inducible heat shock protein pathways. Journal of Clinical Investigation, 115(10), 2633–2639.

Burg, M. B., Ferraris, J. D., & Dmitrieva, N. I. (2007): Cellular Response to Hyperosmotic Stresses. Physiological Reviews, 87(4), 1441–1474.

Crandall, C. G., & Wilson, T. E. (2015). Human cardiovascular responses to passive heat stress. Comprehensive Physiology, 5(1), 17–43.

Cuzzo, B., Padala, S. A., & Lappin, S. L. (2020): Vasopressin (Antidiuretic Hormone, ADH). In StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK526069/

Fortney S.M, & Miescher E. (1994): Changes in Plasma Volume During Heat Exposure in Young and Older Men. In Fluid Replacement and Heat Stress. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK231117/

Gagnon, D., Romero, S. A., Ngo, H., Poh, P. Y. S., & Crandall, C. G. (2017): Plasma hyperosmolality improves tolerance to combined heat stress and central hypovolemia in humans. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 312(3), R273–R280.

Gennari, F.J. (1984): Current concepts. Serum osmolality. Uses and limitations. N. Engl. J. Med, 12, 310(2):102-5.

Nose, H., Gary W., Mack G.Z, Shi X., and Nadel. E.R.(1994): Role of Osmolality and Plasma Volume During Rehydration in Humans. National Academies Press (US). https://www.ncbi.nlm.nih.gov/books/NBK231127/

Horowitz, M. (2016): Epigenetics and cytoprotection with heat acclimation. Journal of Applied Physiology, 120(6), 702–710. https://doi.org/10.1152/japplphysiol.00552.2015

Ishizaka, N., Aizawa, T., Ohno, M., Usui, S., Mori, I., Tang, S.-S., Ingelfinger, J. R., Kimura, S., & Nagai, R. (2002): Regulation and Localization of HSP70 and HSP25 in the Kidney of Rats Undergoing Long-Term Administration of Angiotensin II. Hypertension, 39(1), 122–128.

Jiang, B. H., Jiang, G., Zheng, J. Z., Lu, Z., Hunter, T., & Vogt, P. K. (2001): Phosphatidylinositol 3-kinase signaling controls levels of hypoxia-inducible factor 1. Cell Growth & Differentiation: The Molecular Biology Journal of the American Association for Cancer Research, 12(7), 363–369.

Johnson, R. J., Rodriguez-Iturbe, B., Roncal-Jimenez, C., Lanaspa, M. A., Ishimoto, T., Nakagawa, T., Correa-Rotter, R., Wesseling, C., Bankir, L., & Sanchez-Lozada, L. G. (2014): Hyperosmolarity drives hypertension and CKD -water and salt revisited. Nature Reviews. Nephrology, 10(7), 415–420.

Johnston, C.I.(1985): Vasopressin in circulatory control and hypertension. J Hypertens .3,557–569.

Jolly, C., & Morimoto, R. I. (2000): Role of the heat shock response and molecular chaperones in oncogenesis and cell death. Journal of the National Cancer Institute, 92(19), 1564–1572.

Kampinga, H. H., Hageman, J., Vos, M. J., Kubota, H., Tanguay, R. M., Bruford, E. A., Cheetham, M. E., Chen, B., & Hightower, L. E. (2009): Guidelines for the nomenclature of the human heat shock proteins. Cell Stress & Chaperones, 14(1), 105–111.

Kaya, H., Yücel, O., Ege, M. R., Zorlu, A., Yücel, H., Güneş, H., Ekmekçi, A., & Yılmaz, M. B. (2017): Plasma osmolality predicts mortality in patients with heart failure with reduced ejection fraction. Kardiologia Polska, 75(4), 316–322. 13.

Keller, U., Szinnai, G., Bilz, S., & Berneis, K. (2003): Effects of changes in hydration on protein, glucose and lipid metabolism in man: Impact on health. European Journal of Clinical Nutrition, 57 Suppl 2, S69-74.

Knepper, M.A., Kwon, T.H., Nielsen (2015): Molecular physiology of water balance. N Engl J Med. 372: 1349-58.

Kinsman, B. J., Simmonds, S. S., Browning, K. N., & Stocker, S. D. (2017): Organum Vasculosum of the Lamina Terminalis Detects NaCl to Elevate Sympathetic Nerve Activity and Blood Pressure. Hypertension, 69(1), 163–170.

Lucas, R. A. I., Epstein, Y., & Kjellstrom, T. (2014): Excessive occupational heat exposure: A significant ergonomic challenge and health risk for current and future workers. Extreme Physiology & Medicine, 3(1), 1–8.

Martín-Calderón, J. L., Bustos, F., Tuesta-Reina, L. R., Varona, J. M., Caballero, L., & Solano, F. (2015): Choice of the best equation for plasma osmolality calculation: Comparison of fourteen formulae. Clinical Biochemistry, 48(7–8), 529–533.

Meshi, E. B., Kishinhi, S. S., Mamuya, S. H., & Rusibamayila, M. G. (2018): Thermal Exposure and Heat Illness Symptoms among Workers in Mara Gold Mine, Tanzania. Annals of Global Health, 84(3), 360–368.

Maté J and Siegel R 2016): Effect of liquid versus ice slurry ingestion on core temperature during simulated mining conditions. Open J Prev Med.6(6): 21–30.

Matsuhisa A, Taniguchi N, Koshio H, Yatsu T, Tanaka A (2000): Nonpeptide arginine vasopressin antagonists for both V1A and V2 receptors: synthesis and pharmacological properties of 4-(1,4,5,6-tetrahydroimidazo [4,5-d][1]benzoazepine-6-carbonyl) benzanili de derivatives and 4'-(5,6-dihydro-4H-thiazolo[5,4-d][1]benzoazepine-6- carbonyl)benzanilide derivatives. Chem Pharm Bull (Tokyo) 48:21–31.

Nerbass, F. B., Pecoits-Filho, R., Clark, W. F., Sontrop, J. M., McIntyre, C. W., & Moist, L. (2017): Occupational Heat Stress and Kidney Health: From Farms to Factories. Kidney International Reports, 2(6), 998–1008.

Ozsari, S. (2017): Association between Serum Osmolarity and Coronary Artery Stenosis Grade. Journal of Family Medicine, 4(6). https://doi.org/10.26420/jfammed.2017.1128.

Périard, J. D., Racinais, S., & Sawka, M. N. (2015): Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports: Adaptations and mechanisms of heat acclimation. Scandinavian Journal of Medicine & Science in Sports, 25, 20–38.

Quinton, P. M. (2007). Cystic Fibrosis: Lessons from the Sweat Gland. Physiology, 22(3), 212–225.

Rauchman, M. I., Nigam, S. K., Delpire, E., & Gullans, S. R. (1993): An osmotically tolerant inner medullary collecting duct cell line from an SV40 transgenic mouse. American Journal of Physiology-Renal Physiology, 265(3), F416–F424.

Rasouli M (2016): Basic concepts and practical equations on osmolality:biochemical approach. Clin Biochem,49(12), 936-41.

Roh, H.-T., Cho, S.-Y., So, W.-Y., Paik, I.-Y., & Suh, S.-H. (2016): Effects of different fluid replacements on serum HSP70 and lymphocyte DNA damage in college athletes during exercise at high ambient temperatures. Journal of Sport and Health Science, 5(4), 448–455.

Rothstein, A., E.F. Adolph, and J.H. Wills. (1947): Voluntary dehydration. in Physiology of Man in the Desert, E.F. Adolph and Associates, eds. Interscience Publishers, New York. Pp. 254-270

Sawka M.N.,& Coyle E.F.(1999): Influence of body water and blood volume on thermoregulatory and exericse performance in the heat. Exerc. Sport Sci, Rev., 27 ,167-218.

Szczepanska-Sadowska, E., Czarzasta, K., & Cudnoch-Jedrzejewska, A. (2018): Dysregulation of the Renin-Angiotensin System and the Vasopressinergic System Interactions in Cardiovascular Disorders. Current Hypertension Reports, 20(3), 19.

Shah, M. M., & Mandiga, P. (2020): Physiology, Plasma Osmolality and Oncotic Pressure. In .StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK544365/

Shi P, Stocker S.D, Toney G.M.(2007): Organum vasculosum laminae terminalis contributes to increased sympathetic nerve activity induced by central hyperosmolality. Am J Physiol Regul Integr Comp Physiol 293: R2279–R2289.

Sreedharan, R., & Van Why, S. K. (2016): Heat shock proteins in the kidney. Pediatric Nephrology (Berlin, Germany), 31(10), 1561–1570.

Srivastava, K., Narang, R., Bhatia, J., & Saluja, D. (2016): Expression of Heat Shock Protein 70 Gene and Its Correlation with Inflammatory Markers in Essential Hypertension. PLOS ONE, 11(3), e0151060.

Stocker, S. D., Smith, C. A., Kimbrough, C. M., Stricker, E. M., & Sved, A. F. (2003): Elevated dietary salt suppresses renin secretion but not thirst evoked by arterial hypotension in rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 284(6), R1521-1528.

Togashi, Y., Shirakawa, J., Okuyama, T., Yamazaki, S., Kyohara, M., Miyazawa, A., Suzuki, T., Hamada, M., & Terauchi, Y. (2016): Evaluation of the appropriateness of using glucometers for measuring the blood glucose levels in mice. Scientific Reports, 6. https://doi.org/10.1038/srep25465.

Verbalis, J. G. (2007): How Does the Brain Sense Osmolality? Journal of the American Society of Nephrology, 18(12), 3056–3059.

Webber R, Franz R & Marx W: A review of local and international heat stress indices, standards and limits with reference to ultra-deep mining. J South African Inst Min Metall. ; 313–324.

Weiss ML, Claassen DE, Hirai T, Kenney MJ: Nonuniform sympathetic nerve responses to intravenous hypertonic saline infusion. J Auton Nerv Syst 57: 109–115, 1996.

Xu, Q., Ganju, L., Fawcett, T. W., & Holbrook, N. J. (1996): Vasopressin-induced heat shock protein expression in renal tubular cells. Laboratory Investigation; a Journal of Technical Methods and Pathology, 74(1), 178–187.