UNIVERSITY OF HARTFORD
COLLEGE OF EDUCATION, NURSING AND HEALTH PROFESSIONS

Differentiating cellular osmotic stress in healthy well-hydrated and under-hydrated young adults

Colleen X. Muñoz[1] – Michael F. Bergeron FACSM[1,2] – Beata Abramek[1] – Kaitlyn D'Andrea[1] – Kyle Murphy[1] – Mark C. Nwakamma[1] – Kieu-My Nguyen[1] – Jenna M Bartley[3]

[1]University of Hartford, West Hartford, CT, USA – [2]WTA Women's Tennis Association, St. Petersburg, FL, USA – [3]University of Connecticut Health, Farmington, CT, USA

ABSTRACT

Whereas a linkage is evident between hydration practices and chronic health outcomes, viable reported mechanisms and predictive circulating protein biomarkers are limited. Circulating biomarkers of osmotic stress representing tissue level cellular osmotic stress in well- (WH) vs. under-hydrated (UH) individuals could denote allostatic overload and undue negative chronic health risk. **PURPOSE:** Clarify genomic changes in peripheral blood mononuclear cells (PBMCs) in those who are WH and UH. **METHODS:** PBMCs from 11 WH and 8 UH healthy, young (21±1y) men (WH n=3; UH n=3) and women (WH n=8; UH n=5) were obtained from fasted, morning blood samples. RNA was isolated and analyzed for differential expression of 82 osmotic stress genes. Hydration status was assigned by the collective of morning plasma osmolality (Posm: WH=288±5; UH=295±5), morning plasma copeptin (Pcop: WH=4.7±1.6; UH=11.9±5.3), first morning urine osmolality (FMUosm: WH=597±218; UH=971±120), and previous 24h (24Uosm: WH=393±148; UH=891±219) and 5d average Uosm (5dUosm: WH=408±157; UH=898±109). ANOVA with Tukey *post hoc* tests determined significance ($p<0.05$). **RESULTS:** Genes for transcription factor ATF4 and cell adhesion molecule CTGF were upregulated in UH, whereas genes for select channel and transporters (AQP9, MLC1, ABCB1), molecular chaperone HSPA1A, DNA damage and repair/translation regulator MAPK1, apoptosis VEGFA, cell adhesion molecule CCN2, mitogenic TGFA, plasminogen activator PLAT, and transcript factor NFAT5 were downregulated in UH PBMCs. **CONCLUSION:** These data demonstrate differential responses in circulating immune cells stratified by hydration status. Downregulation of specific osmotic stress genes in UH contradicted our assumption that target tissue cells would increase osmotic stress expression in PBMCs. This paradox could be explained by chronic UH downregulating osmotic stress pathways or circulating immune cells possibly being unaffected by or resistant to osmotic stress inherent to other cells/tissues. Further, these biomarkers may not have captured relevant differences in protein translation. Nonetheless, our findings may be instrumental in contributing to profiling chronic health risk related to routine hydration practices.

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INTRODUCTION

Elevations in plasma osmolality disrupt cellular processes and induce compensatory signaling. Debate exists over whether those who are habitually under-hydrated (UH) vs. those who are well-hydrated (WH) experience transiently distinctive elevated plasma osmolality and subsequent excessive cellular osmotic stress. Biomarkers of osmotic stress in circulating peripheral blood mononuclear cells (PBMCs) in WH vs. UH individuals could denote allostatic overload and undue negative chronic health risk.

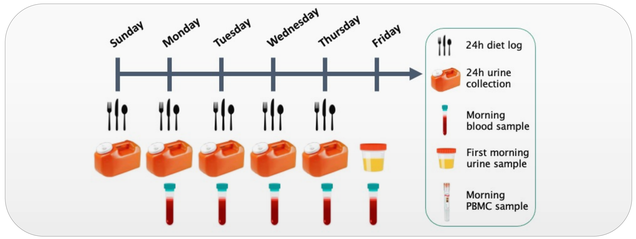
PURPOSE

Determine genomic changes in circulating PBMCS in those who are WH and UH.

METHODS

Eleven WH and eight UH healthy, young (21±1y) men (WH n=3; UH n=3) and women (WH n=8; UH n=5) were observed for five consecutive days.

METHODS (cont.)



WH and UH were characterized via the collective agreement of several supporting indicators of healthy hydration:

- **Plasma osmolality (Posm)**
  - **Fasted morning sampling**
- **Plasma copeptin (Pcop)**
  - **Fasted morning sampling**
  - **Measured via BRAHMS, KRYPTOR**
- **Urine osmolality (Uosm)**
  - **Previous 24h, previous 5d, and first morning (FM)**
- **Total water intake (TWI)**
  - **Previous 5d average derived via 24h diet log**

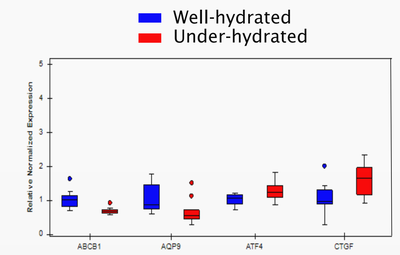
PBMC RNA was extracted via PAXgene Blood RNA System and 82 osmotic stress-related genes were analyzed via RT-qPCR (BioRad, RT² Profiler PCR Array). Data were normalized via modified Pfaffl method with multiple references genes (TBP and GAPDH).

	Well-hydrated X(SD)	Under-hydrated X(SD)
Posm (mOsm·kg^{-1})	288(5)	295(5)[a]
Pcop (pmol·L^{-1})	4.7(1.6)	11.9(5.3)[a]
FMUosm (mOsm·kg^{-1})	597(218)	971(120)[a]
Uosm (24h; mOsm·kg^{-1})	393(148)	891(219)[a]
Uosm (5d; mOsm·kg^{-1})	408(157)	898(109)[a]
TWI (ml)	3237.8(1477.8)	2107.5(639.9)[b]
TWI (ml/kg)	45.2(20.6)	27.4(12.0)[a]

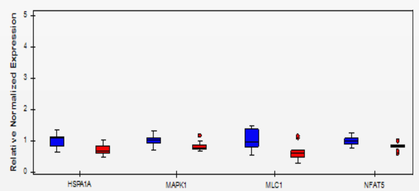
[a] $p < 0.05$; [b] $p = 0.06$

RESULTS

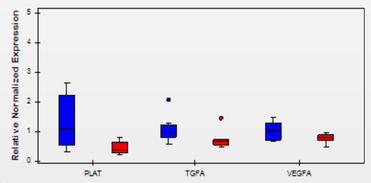
Differential PBMC osmotic stress-related gene expression between WH and UH ($p<0.05$)



Gene	Function
ABCB1	Drug and phospholipid transporter
AQP9	Water transporter
ATF4	Protective gene, regulates stress adaptation
CTGF	Connective tissue growth and remodeling (including new blood vessels)



Gene	Function
HSPA1A	Protection of cellular proteins and cell survival
MAPK1	Regulates cell replication, maturation, and numerous essential processes
MLC1	Water and other molecule transport
NFAT5	Osmotic stress-specific transcription factor



Gene	Function
PLAT	Blood clot breakdown, tissue remodeling, neuron development
TGFA	Regulates cell replication, maturation
VEGFA	Regulates blood vessel cell replication and maturation

These preliminary data indicate modest but differentially experienced osmotic stress exposure of PBMCs between WH and UH, despite only a mild difference in plasma osmolality as measured by fasted morning sampling.

Downregulation of specific osmotic stress genes in UH contradicted our assumption that target tissue cells would increase osmotic stress expression in PBMCs.

This paradox could be explained by chronic UH downregulating osmotic stress pathways or circulating immune cells possibly being unaffected by or resistant to osmotic stress inherent to other cells/tissues.

CONCLUSION

These preliminary data demonstrate differential responses in circulating immune cells stratified by hydration status. Notably, these biomarkers may not have captured relevant differences in protein translation and/or variations in lifespan and osmotic stress responses amongst various PMBC subsets. Nonetheless, our findings demonstrate a need for targeted investigation of hydration practice-induced variation in cellular function and viability, with potential implications of organismal chronic health and aging.