CURCUMIN IMPROVES SYSTEMIC RESPONSES TO EXERTIONAL HYPERTHERMIA
BUT DOES NOT ALTER PROTEIN CONTENT OF CIRCULATING LEUKOCYTES

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ABSTRACT
Exertional heat stress increases gastrointestinal (GI) barrier permeability and risk of exertional heatstroke (EHS) via a TLR4-mediated inflammatory pathway. Curcumin has been shown to inhibit the MyD88 & TRIF-dependent pathways of TLR4 signaling in vitro but has not been examined in a human exertional heat stress model. PURPOSE: This work investigated the effect of 3d of 500mg/d dietary curcumin supplementation on the cellular and systemic responses to exertional heat stress in non-heat acclimated humans. METHODS: Subjects (N=6) ran (65%VO2max) for 60min inside an environmental chamber (37°C; 65%RH) twice times (CURCUMIN/PLACEBO). Core temperature (Tc), heart rate (HR), and physiological strain index (PSI) were measured throughout exercise. Peripheral blood mononuclear cells (PBMC) were isolated from blood samples that were taken before (PRE), after each (POST), 1hr after (1POST), and 4hr after (4POST) exercise. From these samples, Western Blot was used to analyze the protein content of markers along the TLR4 signaling pathway (TLR4, MyD88, pIkB, IκB, pNFKB, NFKB), as well as indicators of cellular energy status (SIRT1 & AMPK) and mediators of the heat shock response (pHSF-1, HSF-1, HSP70). Group differences were determined with 2-Way (Condition x Time) RM ANOVA. RESULTS: Tc rose less (23±1:8; 15°C) under CURCUMIN (p<0.01) and both HR and PSI were lower (HR: 9±3:2 bpm; PSI: 12±8:1%) from 45-60min of exercise (p<0.05). In PBMC, the ratio of pNFKB to NFκB at 1-POST was increased by 64% in PLACEBO and 51% in CURCUMIN (p<0.05). There was no difference between groups and the level of p-NFKB/NFKB at 4-POST had returned to baseline in both groups. There was a trend towards increased HSP70 in CURCUMIN, but this did not reach statistical significance (p<0.11). p-AMPK was reduced by 62% in PLACEBO and by 57% in CURCUMIN at 1POST (p<0.05), again with no difference between groups. CONCLUSION: Despite robust improvements in systems physiology responses to exertional heat stress under CURCUMIN (reported here) and improvements in circulating inflammatory markers (reported elsewhere), we did not detect any significant differences in the protein content of PBMCs collected under PLACEBO and CURCUMIN conditions. With regard to exercise, we did note a significant elevation in pNFKB/NFKB at 1-POST, which (ironically) coincided with a significant reduction in TLR4 (but not MyD88). (Figure 4)

RESULTS

Figure 1: Physiological Responses to Exertional Heat Stress under Placebo and Curcumin Supplementation. (A) Core Temperature; (B) Heart Rate and (C) Physiological Strain Index (PSI) measured throughout exercise. (D) Paradoxically, this coincided with a significant reduction in TLR4 (but not MyD88). *indicates significant difference from PRE; p<0.05 for N=6.

Figure 2: Exertional heat stress alters the metabolic status of PBMC. (A) p-AMPK and (B) SIRT 1 responses to 60 min exercise (65% VO2max) performed under hot (37°C) dry (25%RH) ambient conditions. *indicates significant difference from PRE; p<0.05 for N=6.

Figure 3: Protein markers of the heat shock response in PBMC. (A) p-PHSF-1, HSF-1, HSP70. *indicates significant difference from PRE; p<0.05.

Figure 4: Exertional heat stress alters the TLR4 mediated signaling cascade in PBMC. (A) TLR4, (B) MyD88, (C) IκBα, (D) pNFKB, (E) NFKB (F) NFκB responses to 60 min exercise (65% VO2max) performed under hot (37°C) dry (25%RH) ambient conditions. *indicates significant difference from PRE; p<0.05.

Figure 5: Summary Figures. [A] System-level physiological responses were improved following the 3-day curcumin supplementation protocol. [B] We suspect AMPK was downregulated in an effort to maintain pro-inflammatory capacity of PBMC during the “open window”, as reductions in pAMPK have been shown to potentiate LPS-induced activation of leukocytes in select animal models. (1)

CONCLUSIONS
• GI perfusion is reduced during exertional heat stress, resulting in ATP depletion in enterocytes. The tight junctions between enterocytes are also damaged, resulting in endotoxin translocation into circulation and systemic inflammation by way of a TLR4-mediated pathway. [1][2]
• This increased ratio of pro-inflammatory (TNF-α, IL-6, MCP-1) to anti-inflammatory (IL-1RA, IL-10) cytokines contributes to the disseminated intravascular coagulation and multiple organ failure that accompany exertional heatstroke. [2]
• Curcumin is a polyphenol supplement that affords antioxidant and anti-inflammatory properties. It has also been shown to suppress the activity of the pro-inflammatory NF-kB pathway in both cell culture and human exercise models. [1][4]
• This double blind, placebo controlled research protocol investigated the effect of 3 days of 500mg/d dietary curcumin supplementation on human subjects responses to exertional heat stress. Systems-level physiological responses were assessed. Changes in the protein content of markers along the TLR-4 signaling pathway were assessed in peripheral blood mononuclear cells (PBMC), which were isolated from whole blood samples collected before (PRE), after each (POST), 1hr after (1POST) and 4hrs after (4POST) exercise.

METHODS
Subjects (N=6) ran (65%VO2max) for 60min inside an environmental chamber (37°C; 65%RH) twice times (CURCUMIN/PLACEBO). Core temperature (Tc), heart rate (HR), and physiological strain index (PSI) were measured throughout exercise. Peripheral blood mononuclear cells (PBMC) were isolated from blood samples that were taken before (PRE), after each (POST), 1hr after (1POST), and 4hr after (4POST) exercise.


REFERENCES