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Extracellular Vesicle Concentration but Not Size Differs Between Men and Women During Military Operational Stress

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Extracellular vesicles (EVs) are known mediators of intercellular communication in states of homeostasis and hormesis. Study of EV heterogeneity may extend our understanding of their physiological impact. However, no study has yet characterized EVs in the context of military operational stress. **PURPOSE:** Characterize EV profiles before and after physical exertion during simulated military operational stress (SMOS) in men and women. **METHODS:** Ten male and 10 female service members between the ages of 19 and 37 with average body fat percentages of 18.8% and 28.2%, respectively, completed a 5-day SMOS protocol comprised of daily cognitive tasks, marksmanship, and physical exertion. Sleep and energy intake were restricted by half (4 h; 50% estimated needs) on days 2 and 3 of the 5-day study. Blood was drawn on day 1 (baseline) and day 3 (peak stress) before and after an occupationally-relevant physical exertion protocol lasting approximately 90 min. EVs were isolated using size exclusion chromatography and characterized for size and concentration with nanoparticle tracking analysis. EV concentration was square root-transformed to satisfy assumptions from linear statistics. Data were analyzed to assess the influence of sex, day, and time with three-way mixed ANOVAs. **RESULTS:** There were no three-way interactions for mean EV concentration (particles/mL) or size (nm) ($p > 0.05$ for both). For EV concentration, there was a two-way interaction between sex*day ($p = 0.026$) and sex*time ($p = 0.029$). EV concentration declined from day 1 to day 3 in men ($p = 0.003$) but not women ($p = 0.115$). There was no change in EV concentration from pre- to post-exertion in men ($p = 0.701$), but women displayed reduced EV concentrations after exertion ($p = 0.003$). There was a main effect of day ($p = 0.002$) and time ($p = 0.001$) on mean EV size, which increased from day 1 to day 3 and from pre- to post-exercise when averaged across time and day, respectively. **CONCLUSION:** We demonstrate that common military operational stressors decrease EV concentrations in men but not women. Mean EV size increases from pre- to post-exertion and across operational stress in both sexes. These findings highlight sex differences in intercellular signaling mechanisms that warrant further investigation of the biological content stored within EVs and their physiological impact.

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