Dehydration studies at University of Montana:

Based on an IRB-approved protocol, 23 participants were subjected to a dehydration and re-hydration regimen administered at the University of Montana-Missoula during which physiological parameters such as weight, hematocrit, hemoglobin, blood pressure (using a cuff), and the CareTaker parameters were tracked.

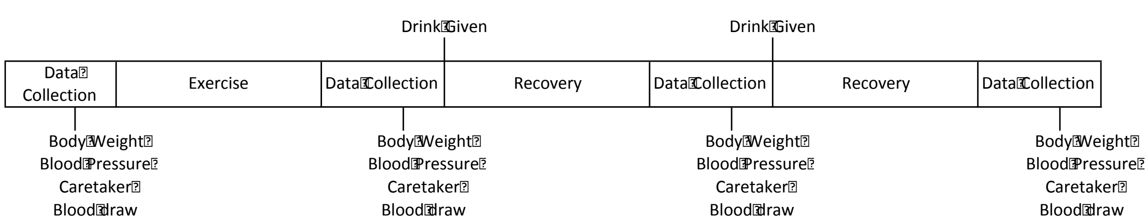
Participants were briefed on the purpose and methods of the study, as well as the requirements for participants as a study participant. Prior to any testing, they completed a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease. Participants were then be asked to complete the following tests: 1) A measure of percent body fat obtained using underwater weighing, 2) A maximal cycle ergometer test to measure aerobic fitness levels, 3) A one-hour exercise session in the heat followed by two hours of recovery. This included the following (consumption of water after exercise, a total of four blood samples and four measures of nude body weight obtained before, right after, and at 1 and 2 hours after exercise. The subjects' descriptive data was as follows: age: 26±1, height (cm): 182±2, weight (kg): 76±6, body fat (%): 15±1, max Watts: 311±12, 60% Watts: 186±7)

Exercise and Recovery protocol

Participants reported to the laboratory after a 12-hour fast. Prior to each trial, an initial measure of nude body weight was obtained. Subjects then rested in a supine (laying down) position for a period of 10-minutes. After this period of rest, a blood sample was taken from an arm vein. Participants then exercised (cycle) for one-hour at 60% of their maximal exercise intensity in a heated room (38°C, 40% humidity). Upon completion of the exercise, participants left the heat chamber, toweled off, and a second measure of nude body weight was obtained. Subjects then put on dry clothes and recovered lying down for the next 2 hours. Blood samples were collected after 10-minutes of recovery and at 60 and 120 minutes of recovery. Two additional measures of nude body weight were obtained at 60 and 120 minutes. All measures of nude body weight were conducted in a private room where the weight result was displayed outside of the room for the researchers to record.

Consumption of Water

Participants were not provided with water during the exercise trial in the heat. However, after exercise, they were given water (immediately after the post-exercise blood sample, approximately 10-minutes post exercise) and again at 60-minutes post exercise. The amount of water they were given depended on the amount of weight loss during the exercise period. At both time points (10-minutes post and 60-minutes post), they were provided with approximately 100% of the total weight lost during exercise (example: 2 lb weight loss during exercise; 2 lb of water (32 oz); provided at both 10-minutes and 60-minutes post exercise). The chart below provides a time line.



Results

We present here overall results of 23 subjects regarding changes in body mass, hematocrit, blood pressure, and hemoglobin at four stages during the de- and rehydration experiments, pre, post, 1 hour later, and 2 hours later.

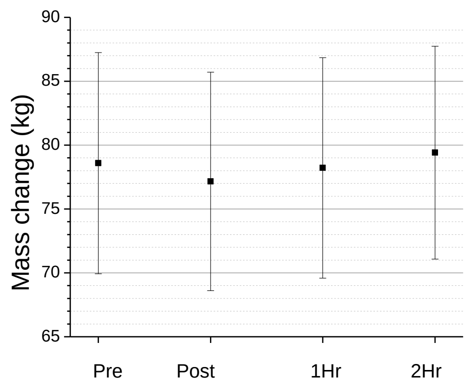


Figure 1

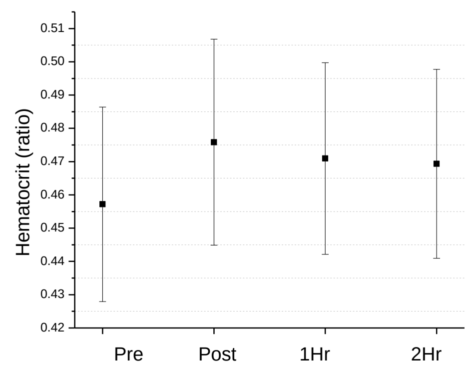


Figure 2

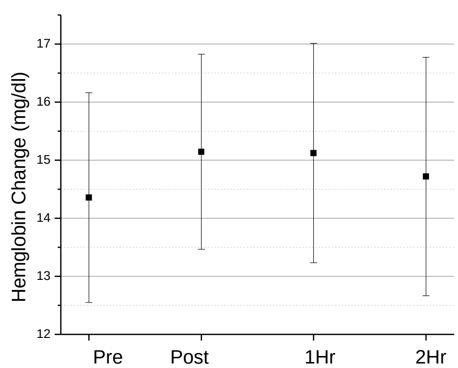


Figure 3

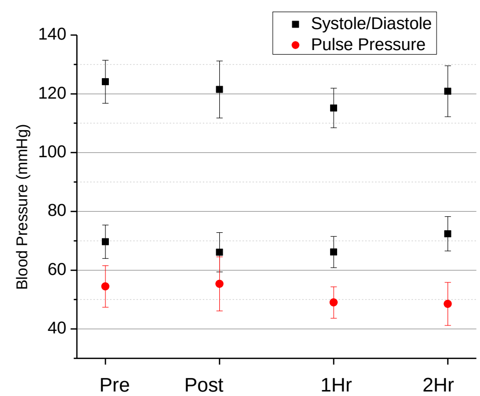
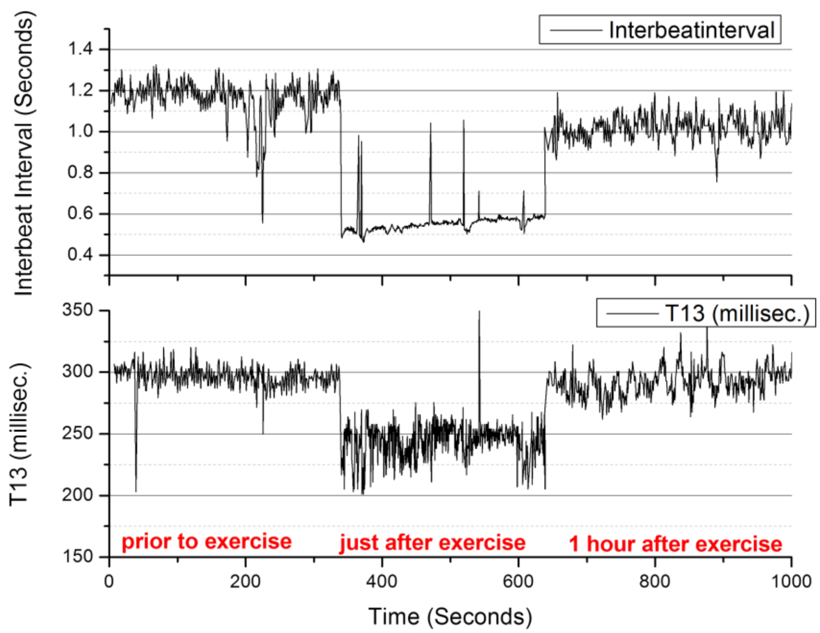


Figure 4

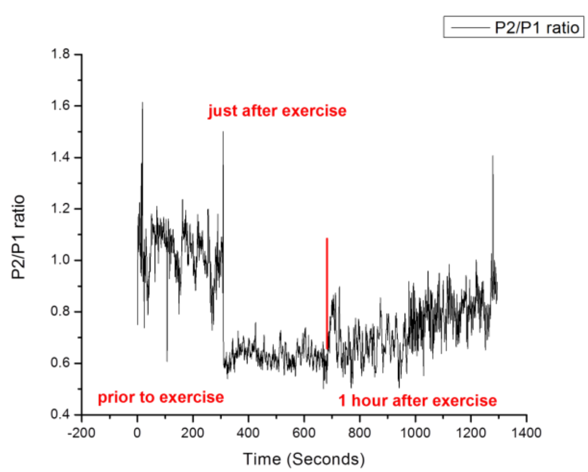
The trends in the four data graphs follow expectations: Dehydration is associated with a distinct mass loss and subsequent recovery. A slight overshoot was recorded in this study with rehydration 2 hours after the end of exercise. The results regarding hemoglobin and hematocrit suggest that rehydration in fact takes longer. Readings after the conclusion of exercise demonstrate significant increases compared to pre-exercise readings, with subsequent decreases as rehydration progresses. The pre-exercise baselines were however not reached after 2 hours of rehydration.

Blood pressures did not follow a correlative trend with the de- and rehydration trends of any of the other three physiological variables. The only changes in any of the primary blood pressure components to reach statistical significance set at 0.05 were the increase in systole of 7.8 mmHg from the post-exercise mark to the 1 hour post-exercise mark and the increase in diastole by 6.1 mmHg from the 1 hour post-exercise mark to the 2 hour post exercise mark. Except for the transition from the post exercise to the 1 hour post-exercise mark, pulse pressure did not change



significantly (7.25 mmHg).

The response of the CareTaker parameters P2P1 and T13 in response to the exercise-induced dehydration and subsequent rehydration cycles was uniform across all participants. T13, starting from different baselines, would exhibit a statistically significant decline in the pre-exercise to post-exercise transition. At the 1 hour post-exercise mark T13 would recover to the pre-exercise baseline, with the mean difference for the cohort having no statistical difference. That baseline was maintained for the final 1 hour recovery time interval. Figure 5, bottom graph, displays an example of this response for subject 16.The data displayed in the figure are not continuous, as indicated by the red-formatted text at the bottom of the graph.



P2P1 would exhibit a significant decline during the exercise period, with a gradual recovery through the 1 hour and 2 hour post-exercise marks. However, P2P1 was the only parameter that would not recover to its pre-exercise baseline. As an example, Figure 6 displays the real-time response of P2P1 and the inter-beat interval for subject 21 As in Figure 5, the data displayed are not continuous but offset by the indicated time offsets.

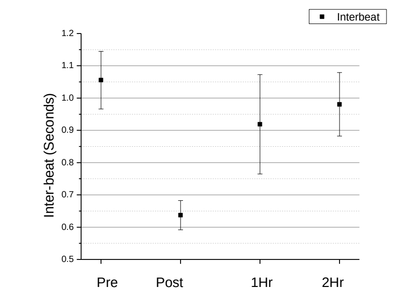


Figure 7

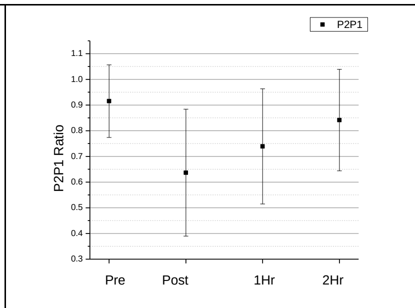


Figure 8

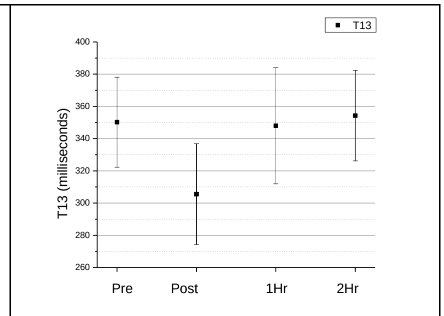


Figure 9

In Figures 7 to 9 we present the overall results of the study for the inter-beat interval, P2P1 and T13, respectively. Comparison with Figures 2 & 3 of the evolution of hematocrit and hemoglobin content, respectively, suggest that the correlation with P2P1 and the inter-beat interval has an inverse relationship. In Figures 10 & 11 we present correlations of the P2P1 response with hematocrit and hemoglobin, respectively, while Figure 12 presents the correlation between hemoglobin and the inter-beat interval.

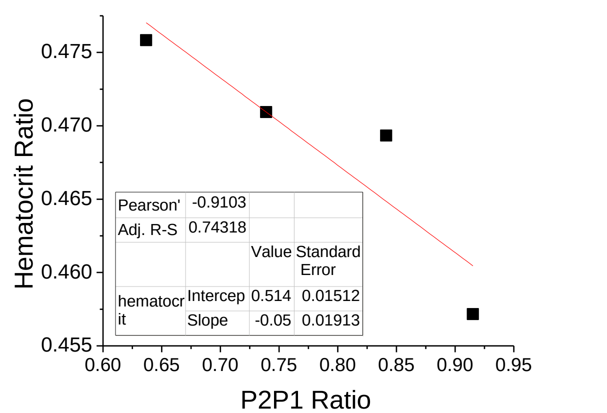


Figure 10: Hematocrit vs. P2P1 ratio response for the four dehydration and rehydration stages.

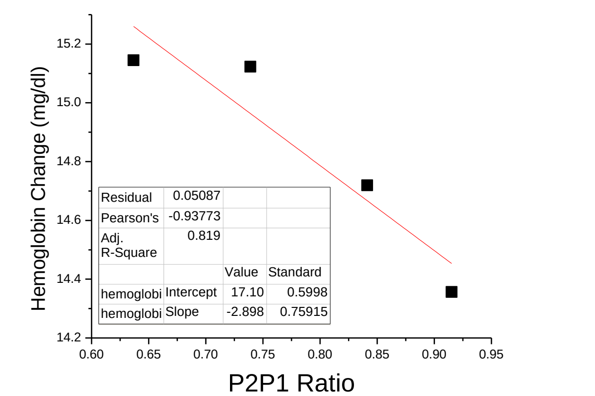


Figure 11: Hemoglobin vs. P2P1 ratio response for the four dehydration and rehydration stages.

Discussion

The correlative results shown in Figures 10 & 11 clearly support the hypothesis that the P2P1 ratio and hematocrit/hemoglobin exhibit an inverse trend. Moreover, the inverse correlation is significantly better than the same correlations with the inter-beat interval. This is probably not surprising as the interbeat interval recovery seems to be dominated by the same quick recovery that characterizes the

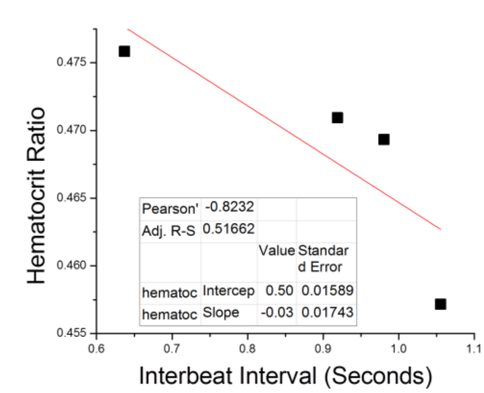


Figure 12: Hemoglobin vs. inter-beat interval response for the four dehydration and rehydration stages

recovery of T13, which clearly does not track the long recovery time constant of rehydration.

It is useful in the context of the discussion of the dehydration results to consider the results of human hemorrhage simulation experiments because of the need for any physiological monitoring system, particularly if it is to be used in a military setting, to distinguish the two. The discussion will therefore frequently make reference to the lower-body-negative pressure (LBNP) performed in Vancouver, CA with the CareTaker, and whose results were published recently, [1] with an accepted follow-on publication that will focus on the modeling of the pressure/velocity response curves of the central arteries. [2]

A number of studies have demonstrated that it is possible to simulate significant internal hemorrhage using LBNP. Negative pressures of 10-20 mmHg correspond to 400 to 550 ml of central blood loss, 20-40 mmHg correspond to 500 to 1000 ml, and negative pressures in excess of -40 mmHg correspond to blood losses exceeding 1000 ml. The subjects were subjected to four stages of negative pressure, -15 mmHg, -30 mmHg, -45 mmHg, and -60 mmHg, each stage lasting typically about 12 minutes. Blood pressure was monitored with an automatic cuff (BP TRU Automated Non-Invasive Blood Pressure Monitor (model BPM-100), VSM MedTech Devices Inc.) set to record blood pressures every three minutes, resulting in typically four readings per LBNP setting as well as an Ohmeda 2300 Finapres, and a pulse oximeter (Ohmeda Biox 3740 Pulse Oximeter, BOC Health Care) monitored oxygen saturation. The CareTaker system collected arterial pulse shapes beat-by-beat via a finger cuff attached to the central phalange of the middle digit.

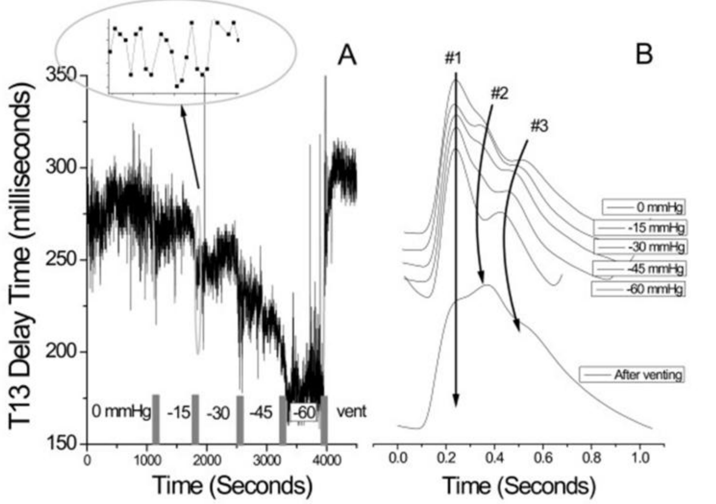


Figure 13: **Evolution of T13 parameter (graph A) and corresponding arterial pressure pulse line shapes (10-pulse averages) for subject #5, 31 y. f, over the course of an entire LBNP session.** The narrowing of the arterial pulse is indicated by the downward (with time) pointing arrows that identify the changing temporal position of component pulses #2 and #3 relative to the primary systolic pulse #1. In this subject the heart rate changed significantly.

The central results in the hemorrhage experiments were that P2P1, and systolic blood pressure, remained essentially constant throughout the 4-stage LBNP runs (see figure 14) while T13, and

[1] Baruch MC, Warburton DE, Bredin SS, Cote A, Gerdt DW, Adkins CM, Pulse Decomposition Analysis of the digital arterial pulse during hemorrhage simulation, Nonlinear Biomed Phys. 2011 Jan 12;5(1):1.

[2] Baruch MC, Warburton DE, Bredin SS, Cote A, Gerdt DW, Adkins CM, Velocity/pressure response curve characterization of the arterial path of the P3 iliac reflection pulse during hemorrhage simulation, accepted for publication: Complex Networks and Their Real-World Interdisciplinary Applications (ed. Dr. Zeraoulia Elhadj)

pulse pressure, diminished progressively with advancing hypovolemia (figure 13). This behavior can be seen in Figure JJ, which shows the evolution of T13 in one subject, along with representative arterial pressure pulse shapes at the various LBNP stages.

The situation in the dehydration and rehydration experiments reported here is reversed. T13, after initially narrowing due to the extreme arterial dilation associated with vigorous exercise, essentially rebounds within an hour to it pre-exercise baseline, despite the fact that the effects of dehydration linger, as evidenced by the blood component measurements. On the other hand it is P2P1 that displays a temporal response that mirrors the blood-based evidence of dehydration.

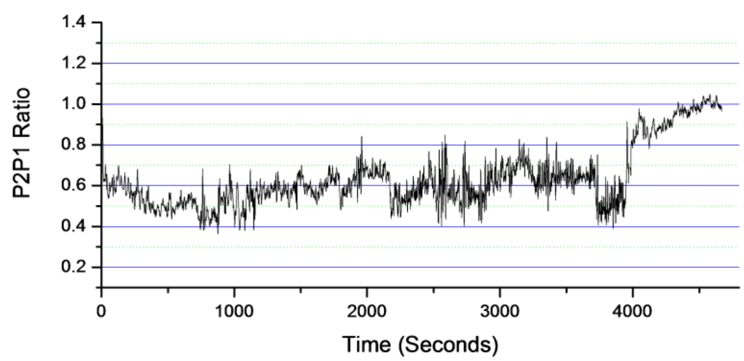


Figure 14: Evolution of P2P1 for subject 5 during the run shown in figure 13. Except for the venting of the chamber, which was always associated with a significant increase in systolic blood pressure, P2P1 remained stable throughout the 1 hour, 20

The T13 response is most likely largely independent of the dehydration effects. 1. The effect rebounds on a time scale much shorter than rehydration and 2. it is an effect readily observed during less strenuous exercise. The most likely reason is that, as tissue oxygenation requirements rise with continued exercise, peripheral arterial tone relaxes to allow better flow. In addition the pressure differential across the artery/arteriole interfaces decreases as the arterioles release to allow better tissue perfusion. With peripheral arterial walls in a lower stress state, arterial pulse pressure propagation velocities slow down. Most affected by the slow-down is the primary left ventricular ejection pulse, P1, as the PDA model predicts, because it travels in a higher arterial wall stress-state due to its higher pressure amplitude. It decelerates relative to the trailing P2 and P3 pulses, narrowing the T13 interval. Cessation of exercise reverses the tissue perfusion requirements and the commensurate arterial wall relaxation, which restores the previous relative pulse propagation velocities and the baseline T13 interval.

One would expect the recovery of P2P1 to follow the same time scale as the T13 recovery, but in fact it appears to match that of the rehydration. The P2 pressure peak is due to the diameter misalignment between thoracic and abdominal aorta, which is usually highly systole-dependent. In this case, however, the parameter tracks the central vascular changes, specifically the dilation of the abdominal aorta relative to the thoracic aorta. This consideration is based on the fact that the blood pressure changes measured with the automatic cuff are very small and follow no discernible trend as far as the dehydration/rehydration cycle is concerned. Consequently the changes in P2P1 have to be due primarily to vascular changes, which are due to the recovery from exercise and rehydration. The significantly faster recovery of T13 suggests that the recovery in P2P1 after T13 has returned to baseline may be due primarily to rehydration.

The fact that the state of hydration affects vascular changes is well known, but it is highly dependent on the degree of dehydration. Milder dehydration is associated with vasodilation while more severe forms can trigger the release of vasopressors, which can lead to an increase in blood pressure as peripheral arteries contract. The diameter determination of human vessels is the basis of a current approach to assess severe dehydration in children. [3] Here the diameters of interior vena cava and thoracic aorta are measured ultrasonically. Because the IVC diameter shrinks with increasing dehydration, while the thoracic aorta's does not, the ratio of the aorta to IVC correlates with dehydration at a statistically significant level.

In view of the fact that blood pressure decreased slightly in the runs presented here it seems unlikely that dehydration reached a severe enough level to trigger vaso-constriction. More likely is the opposite scenario of vasodilation associated with mild dehydration, further supporting the importance and relevance of the P2P1 response.

Conclusions

The observed responses in the CareTaker parameters appear to be physiologically plausible within the context of mildly dehydrated individuals recovering after vigorous exercise. Very encouraging is the fact that the response of the P2P1/T13 parameters is distinctly different from that observed in the hemorrhage experiments, making it likely that the CareTaker technology will be able to distinguish these two distinct physical distress states that are of great significance in the military context.

Further work in this area will focus on separating the overlap of vigorous exercise and dehydration effects. In this context further experiments are planned, as part of an option, at the University of Montana.

The working environment of the wild land firefighter offers a unique, operationally relevant field condition in the regard to the separation of exercise and dehydration effects. The study would involve 15 healthy wild land firefighters (Type I Interagency Hot Shot crew members) as subjects. The hydration and total energy demands are similar for this occupation in comparison to Marines, Navy Seals, and Army Rangers during field operations/exercises. These tests would be typically done in August because it is the hottest month in Montana and also the most active in terms of forest fires.

The above-mentioned methodology for baseline blood sampling would be adhered to (pre-work shift). The research question would be broken down into hydration concerns over a single day (typically a 12-14 hour work shift) and after 3-days of extended operations. On day 1, measures of nude body weight, above-mentioned blood markers and measurements obtained from the

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[3] Levine AC, Shah SP, Umulisa I, Munyaneza RB, Dushimiyimana JM, Stegmann K, Musavuli J, Ngabitsinze P, Stulac S, Epino HM, Noble VE, Ultrasound assessment of severe dehydration in children with diarrhea and Vomiting, Acad Emerg Med. 2010 Oct;17(10):1035-41.

CareTaker would be obtained pre-work shift (in a fasted state). Following the completion of this work shift, post values would also be obtained as indicated above (10-min supine rest, blood samples, nude body weight, etc).

In addition, sample collections would also occur on day 3 (both pre and post shift) to determine state of hydration stability over multiple days of arduous wildfire work. The additional data collection strategy would include the use of Deuterium Oxide (heavy water) for measures of total body water and water turnover during the measurement periods (both 1-day and 3-days of work). This measurement has been used in the past with this population to describe the hydration demands of the job (averaging approximately 7-liters/24 hours during 5-days of wildfire suppression work).

While some measurements would be taken after recent strenuous work, others would be after several hours of exercise-related recuperation. The evolution of dehydration, however, would continue to progress as a completely unrelated time scale. Specifically, most firemen become progressively dehydrated over the course of their 3-day shift, irrespective of rehydration efforts. In view of the results presented it is likely that the separate vascular effects due to physical strain and dehydration should be resolvable.