Assessment of urinary 8-OHdG as a potential biomarker of early heat health effects and acclimatization status in Washington tree fruit harvesters

Kristopher Kohlbacher

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Committee:

Sverre Vedal (Chair)

June T. Spector

Paul Faestel

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Department of Environmental & Occupational Health Sciences

University of Washington

Abstract

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Kristopher Kohlbacher

Chair of Supervisory Committee:
Sverre Vedal
Department of Environmental & Occupational Health Sciences

Background: Heat-related injury (HRI) remains a significant public health concern in heat exposed outdoor occupational groups and especially in agricultural workers like tree fruit harvesters. HRI detection is currently training and resource intensive, leading to a potential delay in diagnosis and an increasing severity at detection. Early accurate detection of HRI increases preventive and protective planning options increasing occupational safety. Urinary 8-OHdG, a biomarker of oxidative DNA damage, has shown early promise in detecting early heat health effects and acclimatization status.

Objective: This study aimed to assess the practical usefulness of urinary 8-OHdG to detect early heat health effects and to characterize the relationship between urinary 8-OHdG, heat stress, heat strain, and acclimatization in outdoor tree fruit harvesters in Yakima Valley, Washington.

Methods: A secondary analysis was performed on cross-sectional data collected during August and September of 2015 on 46 pear and apple harvesters from six orchards in Yakima Valley, Washington during a single work shift. The relationship between change in urinary 8-OHdG cross a work shift, heat stress, heat strain, and acclimatization were assessed using the time-weighted average Wet Bulb Globe Temperature (TWA WBGT), the Physiologic Strain Index (PSI), a validated audio computer-assisted self-interview (A-CASI), and linear regression modeling techniques.

Results: There was a statistically significant mean increase in urinary 8-OHdG of 3.27 ng/dL after a work shift within participants (p-value = 1.303E-6). With greater acclimatization a lower change in cross shift urinary 8-OHdG was seen in all models, but the association was not statistically significant (p-value = 0.220). Tree fruit harvesters with higher heat stress (TWA WBGT) showed higher signs of heat strain (PSI) in the crude model (p-value = 0.025). This association was attenuated after adjusting for age (p-value = 0.052). There was no evidence of effect modification by cross shift change in urinary 8-OHdG or acclimatization on the relationship between heat strain (PSI) and heat stress (TWA WBGT) (p-values of interaction term 0.217 and 0.799 respectively). Exploratory analysis showed statistically significant associations between heat strain and acclimatization as well as with a cross shift change in urinary 8-OHdG and heat stress (p-values 0.038 and 0.019).

Conclusions: Yakima valley tree fruit harvesters are working in heat exposed conditions and are at risk for heat-related injury. Urinary 8-OHdG shows promise as a biomarker of heat exposure: it is able to be successfully measured in field conditions, found to be elevated after a heat-exposed work shift, found to be less elevated with acclimatized heat exposure, found not to be associated with heat strain, and found to be associated with heat stress. The full potential of 8-

OHdG to detect early heat health effects requires further study which the results of our study strongly support. Greater heat stress may be associated with greater heat strain, but a more definitive conclusion requires larger studies. Defining and understanding the full relationship between heat stress, heat strain, acclimatization, and urinary 8-OHdG is complex and more research is needed.

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INTRODUCTION

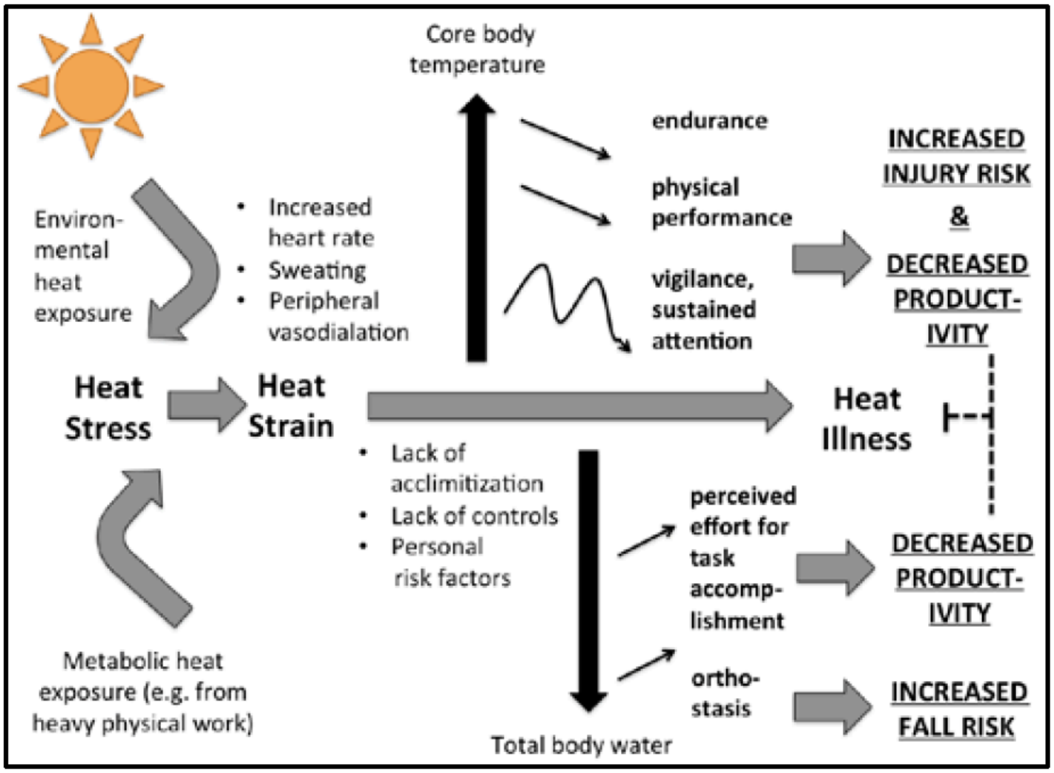
Occupational significance of Exertional Heat Illness (EHI)

Adverse heat health effects have been described in medical and scientific literature for many years but remain a current source of substantial morbidity and mortality (Environmental Protection Agency, 2016). Exertional Heat Illness (EHI) has a very well understood pathophysiology yet still remains a significant source of loss of occupational productivity, hospitalization, and mortality (McInnes et al., 2018). Agricultural workers, athletes, and military personnel are at an especially elevated risk for EHI. Over 400 cases of heat stroke were reported in agricultural workers alone from 1992 to 2006 (Centers for Disease Control and Prevention, USA, 2008). Heat-related deaths in occupational workers were found to surpass 350 between 2000 and 2010 in the United States (Gubernot, Anderson, & Hunting, 2015). Heat-related injury (HRI) in athletes remains a hot topic with young previously healthy football players dying from heat stroke from the grade school to the collegiate level and even in the professional ranks (Jamie Schultz, W. Larry Kenney, Andrew D. Linden, 2014). HRI in the military remains a significant threat to troops despite an increase in training and resources allotted to education and prevention (Kevin Lilley, 2017). In 2017, the Defense Health Agency (DHA) reported 464 cases of heat stroke and 1,699 cases of heat exhaustion; a significant increase from the previous year (Armed Forces Health Surveillance Center (AFHSC), 2018). HRI in this context is defined by injuries directly related to heat exposure and does not include injuries from traumatic events while under heat exposed conditions. Of note, HRI is a term that is often found in military research and reporting tools. Non-military or civilian literature may use HRI to describe heat-related illness. Both definitions of HRI are similar and can be considered interchangeable for discussion purposes.

Environmental Heat Stress, Heat Strain, and Acclimatization

Environmental heat exposure is one of the main sources of heat stress for workers, athletes, and the military (United States Department of Labor, 2018.). Heat strain, as defined, is the individual physiological response to heat stress with acclimatization defined as the individual physiologic adaptation to heat stress that acts to protect against heat strain (de Freitas & Grigorieva, 2015). *Figure 1* outlines a proposed model describing the physiology, pathophysiology, and interrelationship between heat stress, heat strain, and acclimatization. This model outlines the effects of environmental heat on the individual and will be used to further evaluate heat in our study. The complex relationship between heat stress, physiological heat strain, and heat injury has been studied but much can still be learned.

Figure 1: Proposed model of the relationship between heat exposure, heat strain, and heat illness. (June Spector, 1May2018)



Detection of Heat-related Injury (HRI)

Clinical HRI is currently detected after the injury by medical personnel measuring physiological changes in pulse and body core temperature or through medical imaging modalities (Cuddy, Buller, Hailes, & Ruby, 2013; Kobayashi et al., 2011) . These methods, though effective, require medical training, are resource intensive, and potentially delay diagnosis (Holm, Pahler, Thiese, & Handy, 2016). There is currently no established, quick, objective, and accurate way to assess for pre-clinical signs of heat stress and heat strain. Existing prevention strategies are also limited by the lack of methods for quickly and accurately identifying early heat effects and susceptibility (NIOSH Criteria for a Standard - Heat.pdf, n.d.; NIOSH Health Hazard Evaluation Report.pdf, n.d.). The relationship between continuous and intermittent heat exposure over different periods of time and health effects is not fully understood thus further complicating detection and preventions methods (NIOSH Criteria, Section 10.7). Field studies that evaluate these questions could benefit from the use of feasible and practical early markers of heat effects. HRI can be successfully treated and potentially prevented if detection methods were simplified.

Urinary 8-hydroxy-2’-deoxyguanosine (8-OHdG)

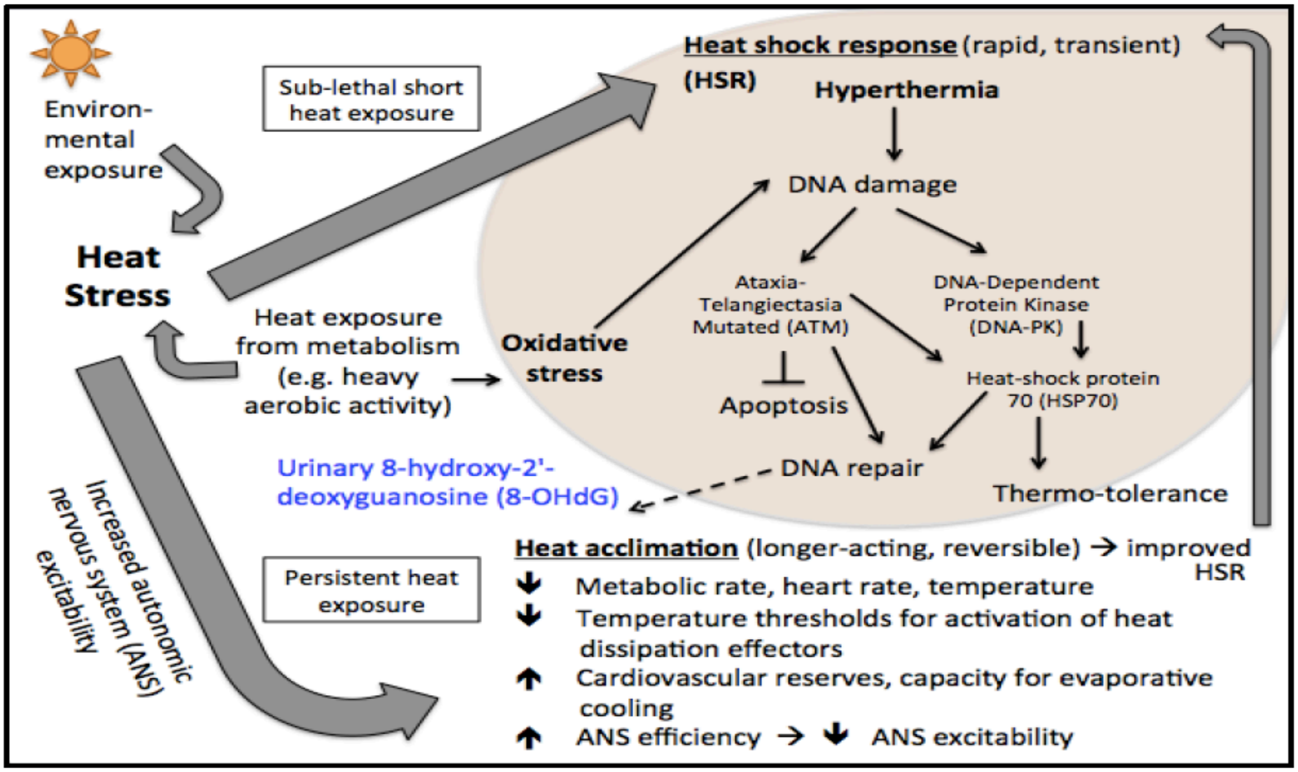
Urinary 8-OHdG is a known biomarker of oxidative DNA damage often used to assess occupational and environmental exposures most notably Cadmium and Arsenic metals (Pilger & Rüdiger, 2006). It was shown in 2012 that heat acclimatized Navy boiler tenders had lower urinary 8-OHdG elevation than the unacclimated comparison group (Huang et al., 2012). In 2015, Zanolin et al. showed that 8-OHdG levels were elevated in individuals within high heat stress environments (Zanolin et al., 2015). These preliminary studies indicate that 8-OHdG may also have promise in identifying the interplay of heat stress, heat strain, and acclimatization.

Once collection and detection assays are optimized for urinary 8-OHdG, measurement could serve as an early pre-clinical risk biomarker of HRI. Further, earlier identification could allow for alteration of heat exposures to ultimately prevent advanced HRI. A better understanding of 8-OHdG as a potential early marker of heat effects and susceptibility is needed to evaluate the appropriateness of 8-OHdG's potential application in: 1) studies of adverse heat health effects; and 2) the development of prevention strategies.

Biochemical Detection of Heat Exposure

Heat exposure, in short or prolonged periods, from any source is a known contributor to oxidative stress and DNA damage. Heat exposure detection methods, at the biochemical level, historically have been focused on the measurement of serum Heat Shock Protein (HSP) (Horowitz, Michael, Sharon D.M. Robinson, 2007). Using the current knowledge of the established cellular protective response mechanism to heat stress further supports the potential promise of urinary 8-OHdG. *Figure 2* outlines a proposed model showing how urinary 8-OHdG detection relates to degrees of heat stress, heat strain, and acclimatization. This proposed model will be used to further evaluate urinary 8-OHdG in our study.

Figure 2. Model of cellular and physiological responses to heat stress and their relationship with Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG)(June Spector, 1May2018)



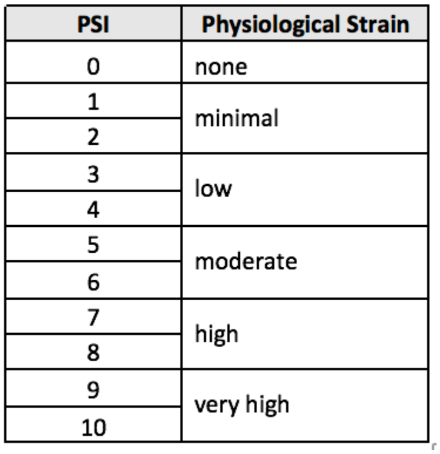
Wet Bulb Globe Temperature (WBGT)

Identifying all sources of heat contributing to heat stress can be cumbersome but the Wet Bulb Globe Temperature (WBGT) has been shown to effectively capture the major contributors (*NIOSH Criteria for a Standard - Heat.pdf*, n.d.). The WBGT is a well validated heat index that incorporates measurements of radiant heat, humidity, and dry air temperature to define heat stress. WBGT index determination can be done reliably and quickly using handheld calibrated electronic units making quantification of heat stress effective in experimental conditions (Cooper et al., 2017).

Heat Strain and the Physiologic Strain Index (PSI)

Physiological heat strain measurement and severity classification is a major barrier to the study, effective treatment, and prevention of HRI. Moran et al. recognized this and created the physiologic strain index (PSI) in response. The PSI is a well-established mechanism to evaluate heat strain using change in baseline heart rate and core temperature values as compared a point measurement or average over an interval. As seen in *Figure 3,* PSI severity is expressed categorically on the 0 to 10 scale and is used to estimate physiologic strain (Moran, Shitzer, & Pandolf, 1998).

Figure 3. Calculated Physiological Strain Index



PSI	Physiological Strain
0	none
1	minimal
2	
3	low
4	
5	moderate
6	
7	high
8	
9	very high
10	

Heat Exposure and Urinary 8-OHdG

Despite separate studies showing 8-OHdG may be elevated with heat stress and show relatively less elevation with acclimatization no study to our knowledge has looked at this together. Further, no known study has sought to measure 8-OHdG levels in individuals showing the effects of heat stress using the physiologic strain index (PSI). Through study a better understanding of the utility of measuring urinary 8-OHdG biomarker levels may become clearer.

STUDY GOALS, SPECIFIC AIMS, AND HYPOTHESES

The purpose of this project was to better understand whether 8-OHdG may be a useful biomarker of early exertional heat effects and acclimatization status for research and practical applications in heat exposed outdoor workers in Washington State. This study attempts to assess urinary 8-OHdG as an accurate biomarker which could serve to aid in earlier classification of heat strain and allow acclimatization to be more thoroughly assessed.

The specific aims of this study were to:

1. Determine if there is a cross shift change in urinary 8-OHdG levels in our cohort of summer outdoor tree fruit harvesters.

   Hypothesis 1: Urinary 8-OHdG levels will be elevated post-shift relative to baseline.

1B. Determine if cumulative acclimatization in the 7 days prior is associated with a cross shift change in urinary 8-OHdG levels in our cohort of summer outdoor tree fruit harvesters.

   Hypothesis 2: Acclimatized harvesters will have a lower cross shift change in urinary 8-OHdG levels.

2. Determine if there is an association between heat stress and heat strain in our cohort of summer outdoor tree fruit harvesters.

   Hypothesis 3: Elevated heat stress will lead to elevated heat strain

2B. Determine if the relationship between heat stress and heat strain is modified by cross shift change in 8-OHdG.

   Hypothesis 4: Harvesters with large cross shift change in urinary 8-OHdG will show increased heat strain

2C. Determine if the relationship between heat stress and heat strain is modified by cumulative acclimatization.

Hypothesis 5: Acclimatized harvesters will show decreased heat strain

The results of this study are expected to advance the understanding of heat health effects with an ultimate goal to reduce the rate of adverse heat health effects through more effective identification and prevention of heat related injury.

METHODS

Study Population and Design

A secondary analysis of observational cross-sectional data collected from five pear and one apple orchard in Yakima Valley, Washington during August and September 2015 was performed in this study. The preliminary analysis was conducted by Quiller et al. in 2016 with the main aim to assess heat stress, heat strain, and their role in occupational productivity in Washington tree fruit harvesters (Quiller, 2016). Prior to initial study being carried out or data collected the procedures were reviewed and approved by the University of Washington Institutional Review Board. No new datum were collected as part of the secondary analysis, and all analyses took place at the University of Washington.

Study participants were selected via convenience sampling methods at the selected six orchards. Adults (age 18 or older) who reported compensation only through piece-rate payment were recruited through the University of Washington Pacific Northwest Agriculture Safety and Health Center (PNASH). Participants provided informed consent prior to selection and agreed to study parameters and data collection methods. A maximum of four consented tree fruit harvesters per day were monitored for a total of 46 total participants in the study, of which 34 participants were monitored in August during pear picking season and 12 in September during apple picking season. August and September were chosen as they are historically high heat stress months with August being historically slightly hotter than September. In 2014, the year prior to data collection, the months of August and September had average high temperatures of 90.5°F (32.5°C) and 80.4°F (26.9°C) respectively (usclimatedata.com). Upon successful completion of the study, participants were compensated $50 for their time and participation. Further details organized by specific aim and exploratory aim appear below.

Specific Aim 1: Determine if there is a cross shift change in urinary 8-OHdG levels in our cohort of summer outdoor tree fruit harvesters.

Hypothesis 1: Urinary 8-OHdG levels will be elevated post-shift relative to baseline.

A. Participant Characteristics

A baseline characterization of 46 eligible participants was assessed using a validated audio computer-assisted self-interview survey instrument (A-CASI) on tablet computers. Personal factors including demographics of age and sex, self-reported health problems, and self-reported being overweight were collected. Age was calculated from self-reported year of birth at the time of A-CASI survey completion. Self-reported health problems included categories of diabetes, high blood pressure, heart disease, lung disease (including asthma), prior heat-related illness, kidney disease, liver disease, cancer, or stroke.

B. Urinary 8-OHdG measurement

Urine was successfully collected from 45 participants using aseptic technique with instructions for clean catch specimen collection. Instructions were adapted from the University of California San Francisco laboratory manual (See Supplemental Section A for link to instructions). Urine specific gravity was measured at the site and samples were stored on dry ice and away from ultraviolet light until frozen to - 20°C. Samples were processed in Professor Chris Simpson's laboratory at the University of Washington using high performance liquid chromatography and adjusted to measured specific gravity (See Supplemental Section B for Full Processing and Analysis Techniques).

Specific Aim 1 analysis

Age, sex, self-reported health problems, and self-reported being overweight were summarized using descriptive statistics. Difference between measured specific gravity adjusted post-shift urinary 8-OHdG and measured specific gravity adjusted baseline urinary 8-OHdG were examined using RStudio statistical analysis software. The difference cross shift within all pairs was calculated and a mean difference within participants (m) and standard deviation (s) was determined for the number of participants (n). As seen in *Figure 4,* a paired t test was conducted on the 45 eligible participants using a significance level of 0.05. Minimal detectable difference was determined to be 2.31 ng/dL. All statistical analyses were carried out using RStudio Version 1.0.153 - © 2009-2017 RStudio, Inc.

Figure 4. Paired t-test formula

$$t = \frac{m}{s/\sqrt{n}}$$

Specific Aim 1B: Determine if cumulative acclimatization in the 7 days prior is associated with cross shift change in urinary 8-OHdG levels in our cohort of summer outdoor tree fruit harvesters.

Hypothesis 2: Acclimatized harvesters will have a lower cross shift change in urinary 8-OHdG levels.

A. Participant Characteristics

Data was collected for 45 eligible participants using A-CASI as described in Aim 1. Personal factors including demographics of age, sex, self-reported health problems, and self-reported being overweight were collected.

B. Predictor: Acclimatization Assessment

Data collected through self-reporting using A-CASI as described in Aim 1 was used to define cumulative acclimatization over the previous 7 days. Participants chose from pre-selected ranges of shift start time and stop times. Number of shifts worked in the previous 7 days was also reported. The midpoint for starting and stopping time was used for daily hours calculation, and this number was multiplied by number of days worked to get cumulative acclimatized hours. Acclimatization was defined using only occupational sources as captured in A-CASI.

C. Outcome: Change in Urinary 8-OHdG Across Measured Shift Assessment

Urinary 8-OHdG was collected as described in Specific Aim 1. Change in Urinary 8-OHdG was calculated for each individual using the specific gravity adjusted measurements using the following equation:

Change in Urinary 8-OHdG (COH) = Post-shift 8-OHdG – Baseline 8-OHdG

Specific Aim 1B Analyses

Age, sex, self-reported health problems, self-reported being overweight, cross shift change in urinary 8-OHdG, and acclimatization were summarized using descriptive statistics. Participant characteristics were summarized using a table, scatter plots, and box plots. The association of cross shift change in specific gravity adjusted urinary 8-OHdG with cumulative acclimatization in 7 days prior to measured shift was modeled using linear regression. Age, sex, self-reported being overweight, and self-reported health problems were identified as potential confounders a priori, as they were hypothesized to be related to both predictor and outcome. Age was coded as a continuous variable by whole year on date of measured shift. Sex was coded as binary (0 = Female, 1= Male). Self-reported being overweight and self-reported health problems were coded as binary (0 = No, 1 = Yes).

Multiple models were developed sequentially ranging from the crude unadjusted model to fully adjusted model initially as seen in *Table 1.* Covariates that showed evidence of confounding, associate with both outcome and predictor using p-value < 0.20, or that were associated with the outcome variable using p-value < 0.20 were included in final models sequentially. Sensitivity analysis included repeating the analysis after removal of outliers identified using deletion diagnostics.

Table 1. Hypothesized Aim 1B Models

Specific Aim 1b Models	Model
1. 8-OHdG Association with Acclimatization - Unadjusted	$E[(COH)\|X] = \beta_0 + \beta_{accl}*accl$

2. 8-OHdG Association with Acclimatization - Age adjusted	$E[l(COH)\|X] = \beta_0 + \beta_{accl}*accl + \beta_{age}*age$
3. 8-OHdG Association with Acclimatization - Sex adjusted	$E[l(COH)\|X] = \beta_0 + \beta_{accl}*accl + \beta_{sex}*sex$
4. 8-OHdG Association with Acclimatization - Overweight adjusted	$E[l(COH)\|X] = \beta_0 + \beta_{accl}*accl + \beta wt*wt$
5. 8-OHdG Association with Acclimatization - Health Problems Adjusted	$E[l(COH)\|X] = \beta_0 + \beta_{accl}*accl + \beta pm*pm$
6. 8-OHdG Association with Acclimatization - Fully adjusted	$E[l(COH)\|X] = \beta_0 + \beta_{accl}*accl + \beta_{age}*age + \beta_{sex}*sex + \beta wt*wt + \beta pm*pm$

Specific Aim 2: Determine if there is an association between heat strain and heat stress in our cohort of summer outdoor tree fruit harvesters.

Hypothesis 3: Elevated heat stress will lead to elevated heat strain

A. Participant Characteristics

Data was collected for 46 eligible participants using A-CASI as described in Specific Aim 1. Personal factors including demographics of age, sex, self-reported health problems, and self-reported being overweight were collected.

B. Predictor: Heat Stress Assessment

Heat stress was assessed using Wet Bulb Globe Temperature (WBGT) measurements on monitored days at the selected sites. WBGT measurement accounts for temperature, humidity, wind speed, sun angle, and solar radiation and was used to estimate heat stress as felt by participant tree fruit harvesters in direct sunlight. WBGT was measured during the primary study using a hand-held WBGT monitor (Extech HT30 WBGT Meter, Extech Instruments; Nashua, NH) with measurements taken as close to participant's location as possible. WBGT measurements were taken regularly (about 60-90 minutes) with notation if measurement was taken in direct sunlight. A time-weighted average (TWA) for WBGT was calculated for all eligible participants. TWA WBGT was calculated by weighting each field measurement of WBGT by interval of work time at that measurement then dividing by total time in monitored work shift.

C. Outcome: Heat Strain Assessment

Heat strain was assessed using the Moran et al's Physiological Strain Index (PSI) (1998). PSI was estimated over the monitored shift through the collection of pulse and temperature data. Participant's core body temperature and pulse were monitored using CorTemp™ sensors (HQ Inc; Palmetto, FL) and Polar® chest band monitors (Polar Inc; Lake Success, NY). CorTemp™ sensor systems utilize small FDA registered and cleared (510K, No. 880639) ingestible thermometer 'pills' that allow continuous temperature collection. Continuous collected core temperature via CorTemp™ sensor and heart rate data via Polar® chest band monitors were then wirelessly transmitted to data recorders worn by workers. Heart rate and core temperature were collected together every 20 seconds and data were summarized over the course of the monitored shift. CorTemp™ sensors do not equilibrate immediately therefore morning pre-shift tympanic membrane temperature measurements were used as input for initial core body temperature (Braun; Kronberg, Germany). Core temperature data that measured less than tympanic temperature were excluded, as core temperature is reported to be greater than measured aural temperatures in similar populations (Quiller, 2016). An adjustment factor of +0.27°C was used to account for the difference between tympanic membrane and core temperature (Huggins et al., 2012).

Baseline temperature was calculated using the first 6 measured temperature values that had an accompanying measured pulse. Baseline pulse was calculated using the first 6 measured pulse values that had measured temperature values. The first 6 measurements for temperature and pulse comprised the first 2 minutes of measurement and allowed for stabilization of measurements. Temperature average and pulse average were calculated for the monitored shift. Participants that did not have temperature or pulse data measured were excluded from this portion of the study (n =1). Participants that had baseline or average temperature lower than 35°C or greater than 39.5°C were excluded as these values were outside of normal physiologic range (n=5). After exclusion there were 40 eligible participants from the initial recruited 46 participants.

The Physiologic Strain Index (PSI) allows quantitative assessment of heat strain using pulse and temperature data. Baseline temperature (To), average temperature over shift (Tx), baseline pulse (HRo), and average pulse over shift (HRx) are used to calculate the average PSI over the monitored shift through the use of the following equation (Moran et al., 1998).

$$PSI = 5 (Tx - To) x (39.5 - To)^{-1} + 5 (HRx - HRo) x (180-HRo)^{-1}$$

PSI values can be interpreted according to estimated physiologic strain as shown in *Figure 3***.** With a larger PSI index value being associated with a higher physiological strain or greater change from baseline in heart rate and/or core temperature.

Specific Aim 2 Analyses

Age, sex, self-reported health problems, self-reported being overweight, heat stress using TWA WBGT, and heat strain using PSI were summarized using descriptive statistics. The association of heat stress using TWA WBGT with heat strain using PSI was modeled using linear regression. Age, sex, self-reported being overweight, and self-reported health problems were identified as potential confounders a priori as previously described. Covariate coding remained the same as outlined previously.

Multiple models were developed sequentially ranging from the crude unadjusted model to fully adjusted model initially as seen in *Table 2.* Criteria for determining covariates to be included in models were the same as for Aim 1B. Sensitivity analysis included repeating the analysis after removal of outliers identified using deletion diagnostics as before.

Table 2. Hypothesized Aim 2 Models

Specific Aim 2 Models	Model
1. Association of Heat Strain and Heat Stress- Unadjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT$
2. Association of Heat Strain and Heat Stress - Age adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+ \beta_{age}*age$
3. Association of Heat Strain and Heat Stress - Sex adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+ \beta_{sex}*sex$
4. Association of Heat Strain and Heat Stress - Overweight adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+ \beta wt*wt$
5. Association of Heat Strain and Heat Stress – Health Problems Adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+ \beta pm*pm$
6. Association of Heat Strain and Heat Stress - Fully adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+ \beta_{age}*age + \beta_{sex}*sex+ \beta wt*wt + \beta pm*pm$

Specific Aim 2B: Determine if the relationship between heat stress and heat strain is modified by across shift change in 8-OHdG.

Hypothesis 4: Harvesters with large across shift change in urinary 8-OHdG will show increased heat strain.

A. Participant Characteristics

Data was collected for 40 eligible participants as previously described. Personal factors including demographics of age, sex, self-reported health problems, and self-reported being overweight were collected.

Specific Aim 2B Analyses

Age, sex, self-reported health problems, and self-reported being overweight, heat stress using TWA WBGT, heat strain using PSI, and change in Urinary 8-OHdG cross measured shift were summarized using descriptive statistics. Effect modification by change in Urinary 8-OHdG cross measured shift on the association of heat stress using TWA WBGT with heat strain using PSI was modeled using linear regression. Age, sex, self-reported being overweight, self-reported health problems were identified as potential confounders a priori as previously described. Covariate coding remained the same as outlined previously.

Multiple models were developed sequentially a priori as previously described and as seen in *Table 3***.** Criteria for determining covariates to be included in models were the same as for Aims 1B and 2. Sensitivity analysis included repeating the analysis after removal of outliers identified using deletion diagnostics as before.

Table 3. Hypothesized Aim 2B Models

Specific Aim 2B Models	Model
1. 8-OHdG Effect on Association of Heat Strain and Heat Stress-Unadjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{COH}*COH+ \beta_{WBGT}*WBGT*COH$
2. 8-OHdG Effect on Association of Heat Strain and Heat Stress - Age adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{COH}*COH+ \beta_{WBGT}*WBGT*COH+ \beta_{age}*age$

3. 8-OHdG Effect on Association of Heat Strain and Heat Stress - Sex adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{COH}*COH+ \beta_{WBGT}*WBGT*COH+ \beta_{sex}*sex$
4. 8-OHdG Effect on Association of Heat Strain and Heat Stress - Overweight adjusted	$E[(PSI)\|X] = \beta_0 +\beta_{WBGT}*WBGT+\beta_{COH}*COH+ \beta_{WBGT}*WBGT*COH+ \beta wt*wt$
5. 8-OHdG Effect on Association of Heat Strain and Heat Stress – Health Problem Adjusted	$E[(PSI)\|X] = \beta_0 +\beta_{WBGT}*WBGT+\beta_{COH}*COH+ \beta_{WBGT}*WBGT*COH+ \beta pm*pm$
6. 8-OHdG Effect on Association of Heat Strain and Heat Stress - Fully adjusted	$E[(PSI)\|X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{COH}*COH+ \beta_{WBGT}*WBGT*COH+ \beta_{age}*age + \beta_{sex}*sex+ \beta wt*wt + \beta pm*pm$

Specific Aim 2C: Determine if the relationship between heat stress and heat strain is modified by cumulative acclimatization.

Hypothesis 5: Acclimatized harvesters will show decreased heat strain.

A. Participant Characteristics

Data was collected for 40 eligible participants as previously described. Personal factors including demographics of age, sex, self-reported health problems, self-reported being overweight, and cumulative acclimatization over the previous 7 days were collected.

Specific Aim 2C Analyses

Age, sex, self-reported health problems, self-reported being overweight, heat stress using TWA WBGT, heat strain using PSI, and cumulative acclimatization over the previous 7 days were summarized using descriptive statistics. Effect modification by cumulative acclimatization over the previous 7 days on the association of heat stress using TWA WBGT with heat strain using PSI was modeled using linear regression. Age, sex, self-reported being overweight, self-reported health problems were identified as potential confounders a priori as previously described. Covariate coding remained the same as outlined previously.

Multiple models were developed sequentially a priori as previously described and as seen in *Table 4***.** Criteria for determining covariates to be included in models were the same as for Aims 1 and 2. Sensitivity analysis included repeating the analysis after removal of outliers identified using deletion diagnostics as before.

Table 4. Hypothesized Aim 2C Models

Specific Aim 2C Models	Model
1. Acclimatization Effect on Association of Heat Strain and Heat Stress- Unadjusted	$E[(PSI)\mid X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{accl}*accl+ \beta_{WBGT}*WBGT*accl$
2. Acclimatization Effect on Association of Heat Strain and Heat Stress - Age adjusted	$E[(PSI)\mid X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{accl}*accl+ \beta_{WBGT}*WBGT*accl+ \beta_{age}*age$
3. Acclimatization Effect on Association of Heat Strain and Heat Stress - Sex adjusted	$E[(PSI)\mid X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{accl}*accl+ \beta_{WBGT}*WBGT*accl+ \beta_{sex}*sex$
4. Acclimatization Effect on Association of Heat Strain and Heat Stress - Overweight adjusted	$E[(PSI)\mid X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{accl}*accl+ \beta_{WBGT}*WBGT*accl+ \beta wt*wt$
5. Acclimatization Effect on Association of Heat Strain and Heat Stress – Health Problem Adjusted	$E[(PSI)\mid X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{accl}*accl+ \beta_{WBGT}*WBGT*accl+ \beta pm*pm$
6. Acclimatization Effect on Association of Heat Strain and Heat Stress - Fully adjusted	$E[(PSI)\mid X] = \beta_0 + \beta_{WBGT}*WBGT+\beta_{accl}*accl+ \beta_{WBGT}*WBGT*accl+ \beta_{age}*age + \beta_{sex}*sex+ \beta wt*wt + \beta pm*pm$

RESULTS

Specific Aim 1

A. Descriptive analyses

Descriptive characteristics for participants are presented in *Table 5***.** Forty-five individuals were eligible for participation in this portion of the study as one individual did not have Urinary 8-OHdG data available. The mean age was 39.1 years (standard deviation of 14.1) years) and median of 36.50 years. 85% of participants were male and 15% female. 63% self-reported being overweight and 35% self-reported current health problems at the time of survey. The mean of differences in urinary 8-OHdG across measured shift was 3.27 ng/dL (standard deviation of 3.92 ng/dL) with median of differences of 2.20 ng/dL. Mean self-reported cumulative acclimatized hours in the 7 days prior was 39.75 hours (standard deviation of 17.97 hours) with median of 45.40 hours. Mean heat stress as calculated using the time-weighted average (TWA) of the wet bulb globe temperature (WBGT) was 20.65°C (standard deviation of 3.64°C) with median of 20.85°C. Mean heat strain as derived using physiologic strain index was 2.98 (standard deviation of 1.16) with median of 3.17.

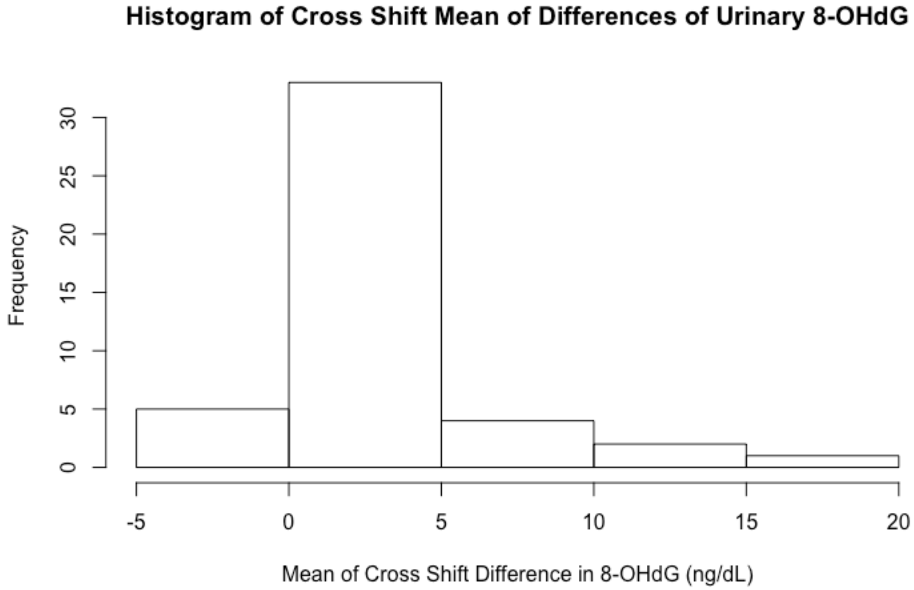
Table 5. Study Participant Descriptive Statistics

Participant Descriptive Statistics				
	Mean	Stdev	Number	Range
Age (years)	39.13	14.11	46	(19, 63)
Sex (M/F)	85% / 15%	N/A	40 / 6	N/A
Overweight (Y/N)	63% / 37%	N/A	29 / 17	N/A
Health Problems (Y/N)	35% / 65%	N/A	16 / 30	N/A
Urinary 8-OHdG Change (ng/dL)	3.27	3.92	45*	(-1.40, 19.60)
Acclimatization (hours)	39.75	17.97	46	(0, 70)
Heat Stress (TWA WBGT °C)	20.65	3.64	46	(14.10, 26.00)
Heat Strain Index (PSI)	2.98	1.16	40**	(-0.06, 4.94)

* Urinary 8-OHdG data was not available for 1 participant
**6 Participants did not have pulse or temperature measured or values were outside physiologic range of (<35 °C, > 39.5°C)

The eligible participants had a strong over representation of males at 85% that supports a suspected higher occupational makeup of males in the tree fruit harvester industry. Participants tended to be relatively healthy population with 65% reporting no current health problems. This is not surprising as tree fruit harvesting can be a physically demanding occupation that requires a certain level of health and fitness to participate. Of the 35% that reported health problems one or more the following were present: diabetes, high blood pressure, heart disease, lung disease (including asthma), heat-related illness, kidney disease, liver disease, cancer, or stroke. As seen in *Figure 5* Urinary 8-OHdG change cross measured shift had a large range and showed evidence of being right-skewed with mean of differences of 3.27 ng/dL being higher than the median of differences of 2.20 ng/dL. Acclimation, heat stress, and heat strain descriptions will be addressed in specific aim sections utilizing these measurements.

Figure 5. Histogram of Across Shift Mean of Differences of Urinary 8-OHdG



B. Inferential analyses

Table 6 shows the results of the paired t-test of across shift change in urinary 8-OHdG in our cohort. There was a statistically significant difference across monitored shift urinary in 8-OHdG within participants in our study (p-value = 1.303 E-6)

Table 6. Association Analysis of Pre and Post Shift Urinary 8-OHdG Within Participant

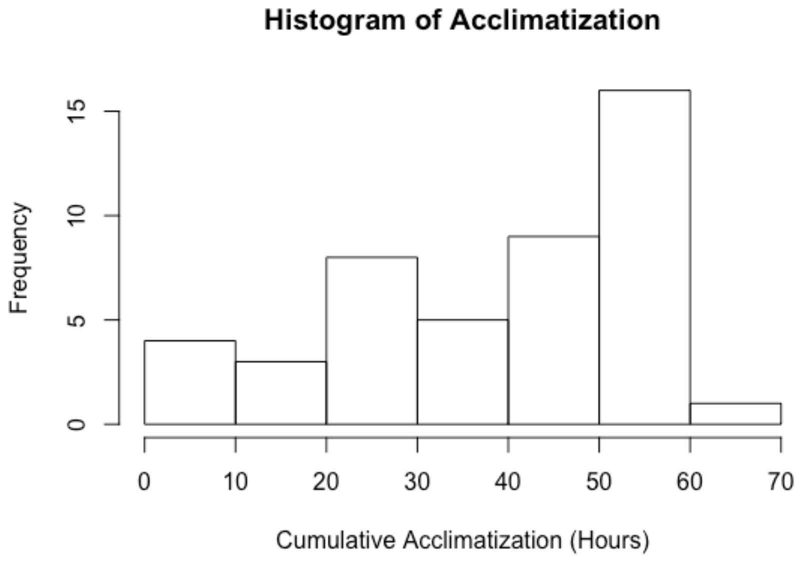
Cross Shift Urinary 8-OHdG Difference (Aim 1A) Paired t-test				
Baseline Mean	Post Shift Mean	Mean of Differences	T statistic	p-value
4.502 ng/dL	7.771 ng/dL	3.269 ng/dL	5.600	1.303E-06

Specific Aim 1B

A. Descriptive analyses

Table 5 describes descriptive statistics for participants. The participant population of 45 tree fruit harvesters was the same as used in *Specific Aim 1* and descriptive statistics apply as previously described. *Figure 6* shows the distribution of cumulative acclimatization. The mean cumulative acclimation was 39.75 hours in previous 7 days (standard deviation of 17.97) was slightly less than the median of 45.50 hours indicating a slightly left-skewed distribution.

Figure 6. Histogram of Cumulative Acclimatization



B. Inferential Analysis: Association between 8-OHdG and Acclimatization

Covariate analyses was conducted to define role of Age, Sex, Reported Overweight, and Reported Health Problems as seen in *Table 7*. Age was found to be a confounder as associated with both 8-OHdG and acclimation. Sex and reported health problems were found to be precision variables as associated with the outcome of 8-OHdG.

Table 7. Variable Analysis Results (Aim 1B)

Outcome Association Variable Analysis Results (Aim 1B)

Outcome Association: Across Shift Change 8-OHdG ~ Covariate				
Variable	Estimate	95% CI (L, H)	p-value (<0.2)	Variable Relationship
Age (yrs)	0.082	(-7.797E-3, 0.172)	0.072	Yes- Confounder
Sex (M:F)	1.926	(0.356, 3.395)	0.017	Yes- Precision
Overweight (Y:N)	-0.524	(-3.158, 2.111)	0.691	No
Health Problems (Y:N)	2.887	(-6.226E-6, 5.780)	0.051	Yes- Precision

Predictor Association Variable Analysis Results (Aim 1B)

Predictor Association: Cumulative Acclimation ~ Covariate				
Variable	Estimate	95% CI (L, H)	p-value (<0.2)	Variable Relationship
Age (yrs)	0.478	(0.121, 0.835)	0.0099	Yes - Confounder
Sex (M:F)	-10.400	(-26.860, 6.050)	0.209	No
Overweight (Y:N)	-0.303	(-11.770, 11.160)	0.958	No
Health Problems (Y:N)	2.540	(-8.295, 13.370)	0.639	No

The relationship of modeled variables age, sex, and reported health problems to outcome of 8-OHdG was explored graphically. As seen in *Figure 7*, there was no clear visual relationship between 8-OHdG and age. *Figure 8* shows that males had a slightly larger mean across shift change in 8-OHdG relative to females. *Figure 9* shows that participants who self-reported medical problems had a slightly larger mean across shift change in 8-OHdG relative to those self-reported no problems.

Figure 7. Scatterplot of Across Shift Change in 8-OHdG by Age (Confounding Variable)

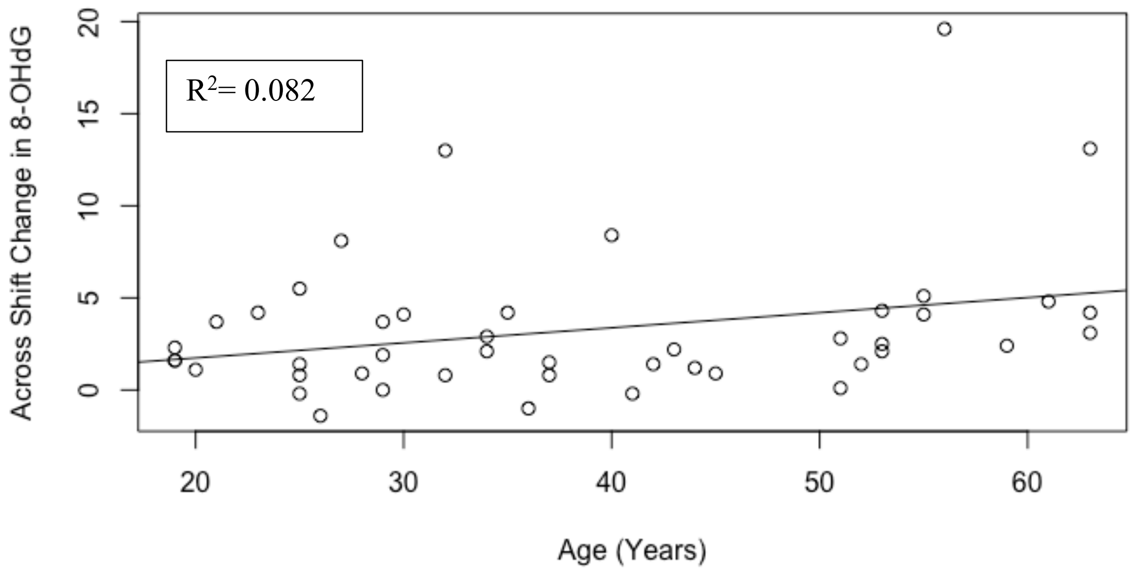


Figure 8. Boxplot of Cross Shift Change in 8-OHdG by Sex (Precision Variable)

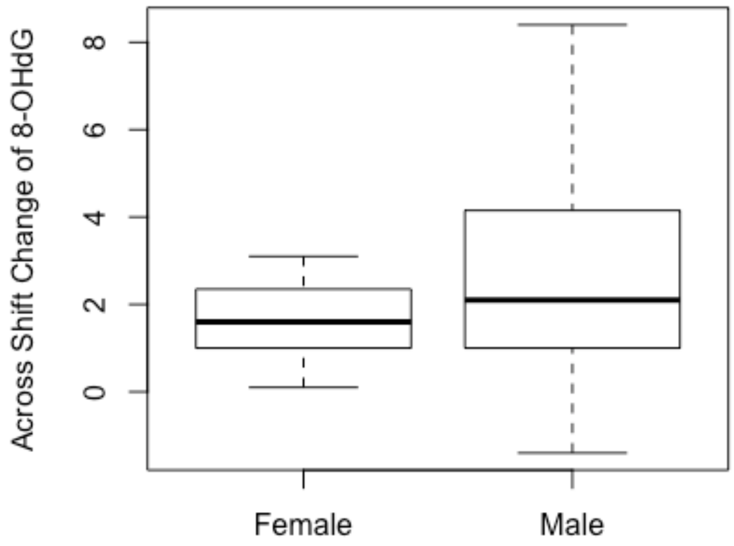


Figure 9. Boxplot of Cross Shift Change in 8-OHdG by Medical Problems (Precision Variable)

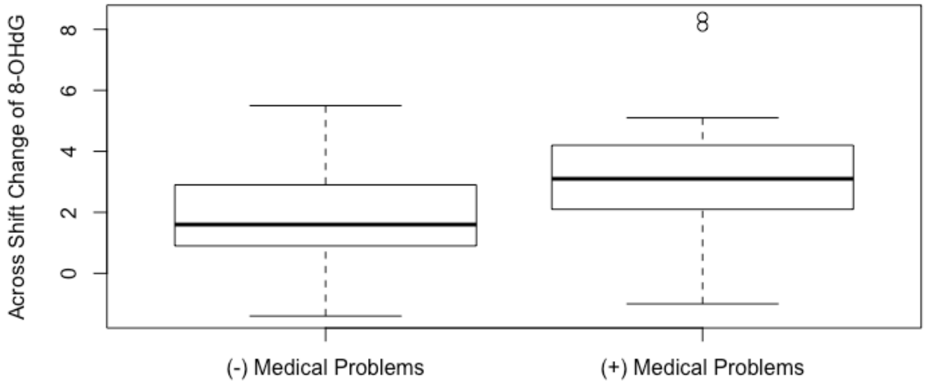


Table 8 shows linear regression model estimates for cross shift change of 8-OHdG on acclimatization. The unadjusted model showed the trend that as cumulative acclimatization was higher across shift change of 8-OHdG was lower; however, this relationship was not significant (coefficient for acclimatization -0.011, 95% confidence interval -0.058, 0.036, p-value 0.634). After adjustment for age (confounder) the effect was larger, but still not statistically significant (coefficient for acclimatization -0.044, 95% confidence interval -0.090, 2.670E-3, p-value 0.064). The fully adjusted model, which included precision variables, showed a similar estimate of effect without statistical significance (coefficient for acclimatization -0.029, 95% confidence interval -0.078, 0.018, multiple R^2 0.082, p-value 0.220)

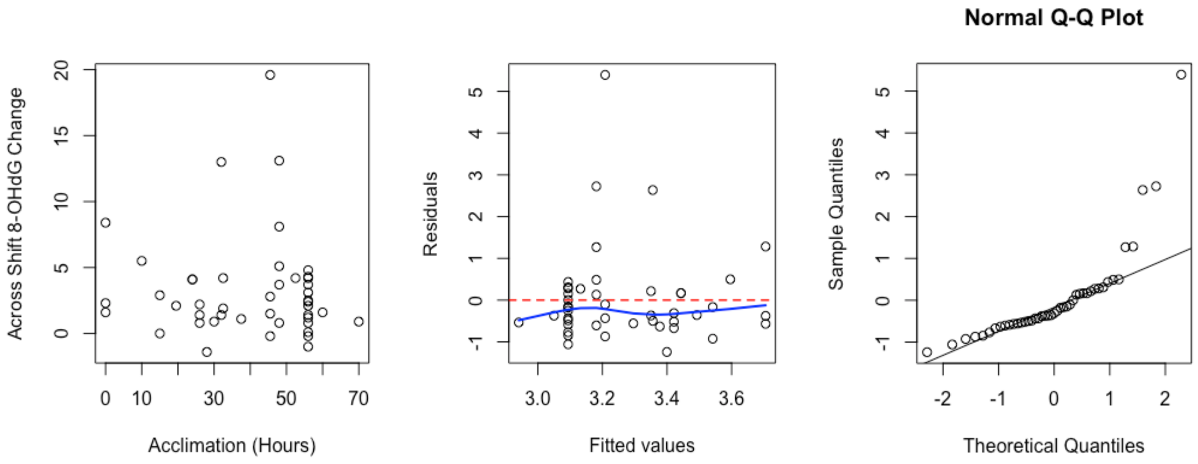
Table 8. Linear Regression Results COH ~ Acclimatization (Aim 1B)

Table 8: Regression Results Across Shift Change 8-OHdG ~ Cumulative Acclimatization (Aim 1B)					
Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	-0.011	0.023	(-0.058, 0.036)	-0.021	0.634
Age Adjusted	-0.044	0.023	(-0.090, 2.670E-3)	0.077	0.064
Sex Adjusted	-0.003	0.024	(-0.052, 0.046)	-0.013	0.902
Health Adjusted	-0.018	0.019	(-0.057, 0.021)	0.089	0.366
Age + Sex + Health Adjusted	-0.029	0.026	(-0.078, 0.018)	0.107	0.220

Model diagnostics were performed, and sensitivity analysis was conducted using the age, sex, and health adjusted model. As seen in *Figure 10*, the top left plot showed no clear association. Residuals were calculated and plotted against fitted predicted values as seen in *Figure 10* the top middle plot. Three possible outliers were identified with the largest residual error (Participants 20, 21, and 24) with no obvious violations of equal variance. The sample quantile versus theoretical quantile plot (Normal QQ-Plot) for the fully adjusted model seen in *Figure 10* top right plot further identified 3 possible outliers (Participants 20, 21, and 24) and no further major violations of normality. The 3 identified possible outliers (Participants 20, 21, and 24) were removed and diagnostics repeated as seen in *Figure 10* lower 3 plots. Removal of 3 participants showed improved model diagnostics.

Figure 10. Regression Model Diagnostic Plots of COH ~ Acclimatization

All Participants Included



Potential Outlier Participants 20, 21, 24 Removed

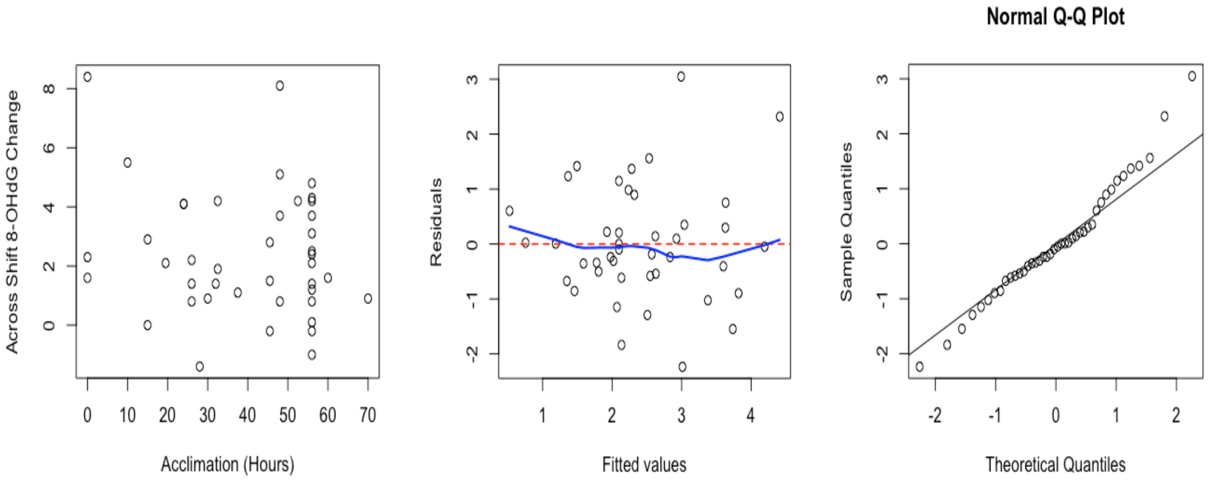


Table 9 shows the shows linear regression model estimates for cross shift change of 8-OHdG association with acclimatization with the 3 identified possible outliers removed. A comparison of the linear effect models was done for all participants included (*Table 8***)** versus the 3 identified potential outliers removed (*Table 9***)**. The effect estimate was similar and in the same direction for the crude and fully adjusted linear models and there was no effective change in statistical significance with removal of 3 possible outliers.

Table 9: Linear Regression Results COH ~ Acclimatization (AIM 1B) 3 Outliers Removed

Table 9: Regression Results Across Shift Change 8-OHdG ~ Cumulative Acclimatization (Aim 1B)					
Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	-0.018	0.941	(-0.059, 0.023)	-4.78E-5	0.388
Age Adjusted	-0.031	0.023	(-0.077, 0.016)	0.045	0.189
Sex Adjusted	-0.014	0.021	(-0.057, 0.028)	-6.84E-3	0.492
Health Adjusted	-0.020	0.018	(-0.058, 0.017)	0.102	0.282
Age + Sex + Health Adjusted	-0.023	0.023	(-0.070, 0.023)	0.085	0.317

Specific Aim 2

A. Descriptive analyses

Table 5 describes the descriptive statistics for study participants. The participant population for this aim is 40 tree fruit harvesters as previously described. *Figure 11* shows the distribution of heat stress as estimated by time-weighted average (TWA) wet bulb globe temperature (WBGT) in °C. The mean TWA WBGT of 20.65°C (standard deviation of 3.64°C) was similar to the median TWA WBGT of 20.85°C. *Figure 12* shows the distribution of heat strain as estimated by Physiologic Strain Index (PSI). The mean PSI of 2.98 (standard deviation of 1.16) was similar to the median PSI of 3.166. The mean and median values for PSI show a low physiologic strain as defined in *Figure 3*.

Figure 11. Histogram of TWA WBGT

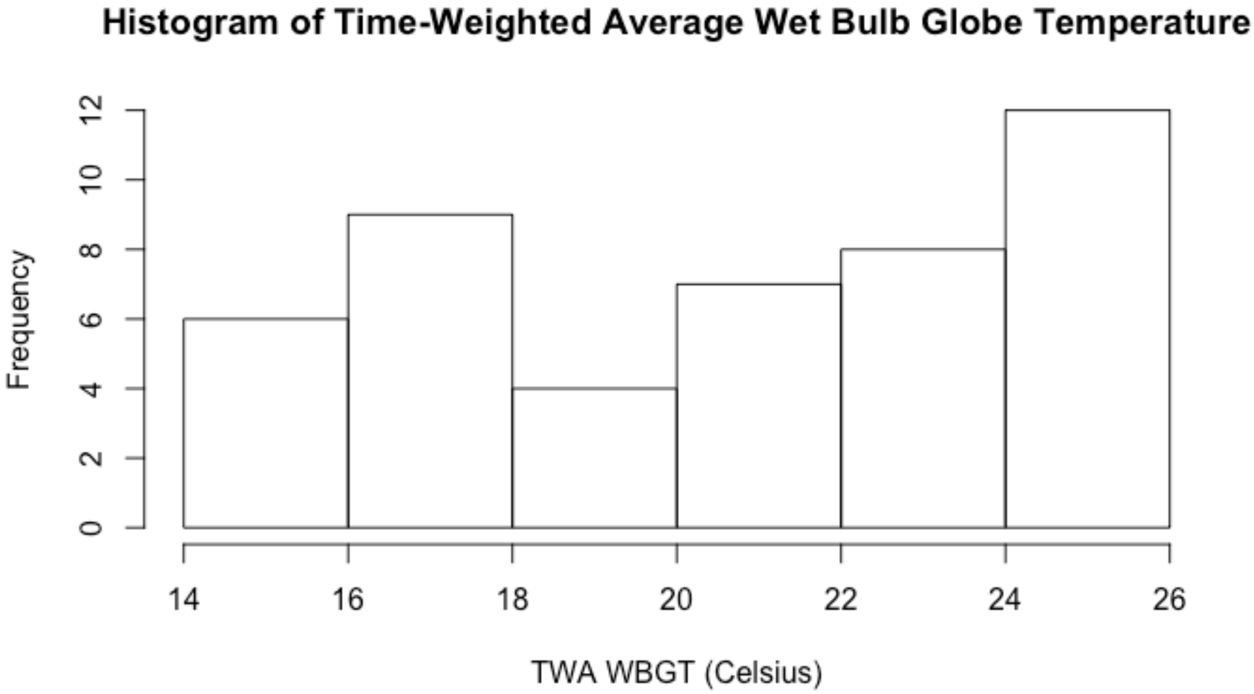
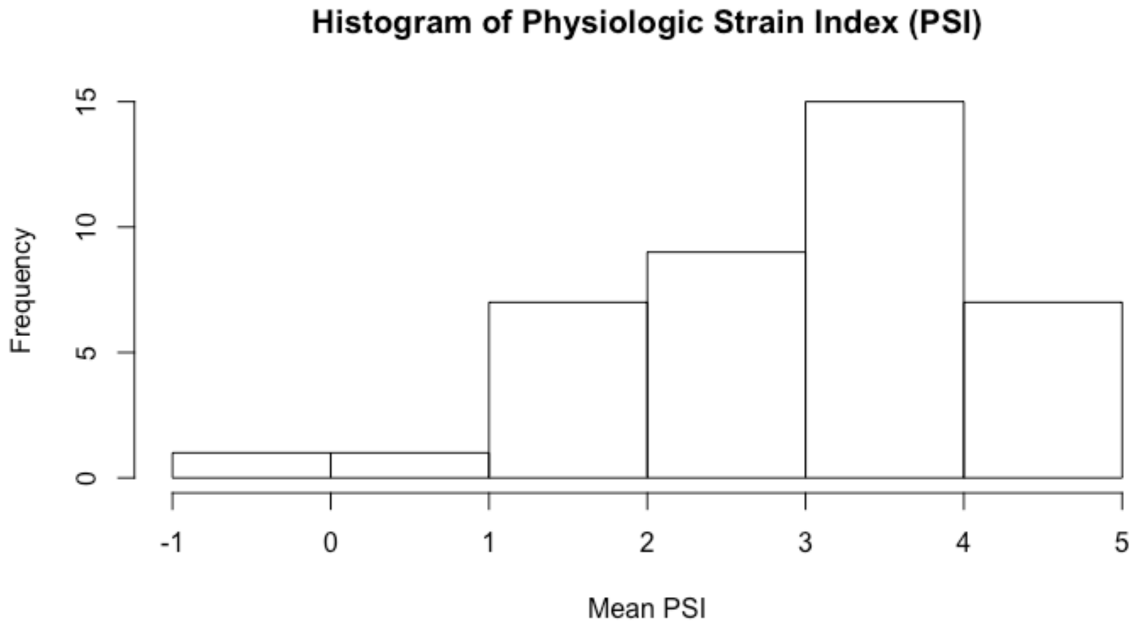


Figure 12. Histogram of PSI



B. Inferential analyses: Association between Heat Strain (PSI) and Heat Stress (TWA WBGT)

Covariate analyses was conducted to define role of age, sex, reported overweight, and reported health problems as seen in *Table 10*. Age was found to be a confounder as associated with TWA WBGT and PSI.

Table 10. Variable Analysis Results (Aim 2)

Outcome Association Variable Analysis Results (Aim 2)

Outcome Association: Heat Strain ~ Covariate				
Variable	Estimate	95% CI (L, H)	p-value (<0.2)	Variable Relationship
Age (yrs)	-0.0214	(-0.0518, 8.974E-3)	0.162	Yes-Confounder
Sex (M:F)	0.761	(-0.500, 2.021)	0.229	No
Overweight (Y:N)	0.337	(-0.542, 1.215)	0.443	No
Health Problems (Y:N)	-0.257	(-1.176, 0.661)	0.574	No

Predictor Association Variable Analysis Results (Aim 2)

Predictor Association: Heat Stress ~ Covariate				
Variable	Estimate	95% CI (L, H)	p-value (<0.2)	Variable Relationship
Age (yrs)	-0.060	(-0.145, 0.025)	0.162	Yes-Confounder
Sex (M:F)	3.946	(1.866, 6.026)	4.100E-4	Yes-None
Overweight (Y:N)	1.043	(-1.101, 3.186)	0.332	No
Health Problems (Y:N)	-0.281	(-2.841, 2.279)	0.826	No

Once covariate analysis was completed the relationship of modeled variable of age to outcome of PSI was explored. *Figure 13* shows the highest PSI was seen with participants near age 60 years. The relationship of age to outcome PSI was explored graphically. As seen in *Figure 17*, there was no clear visual relationship between PSI and age.

Figure 13. Scatterplot of Heat Strain (PSI) by Age (Confounding Variable)

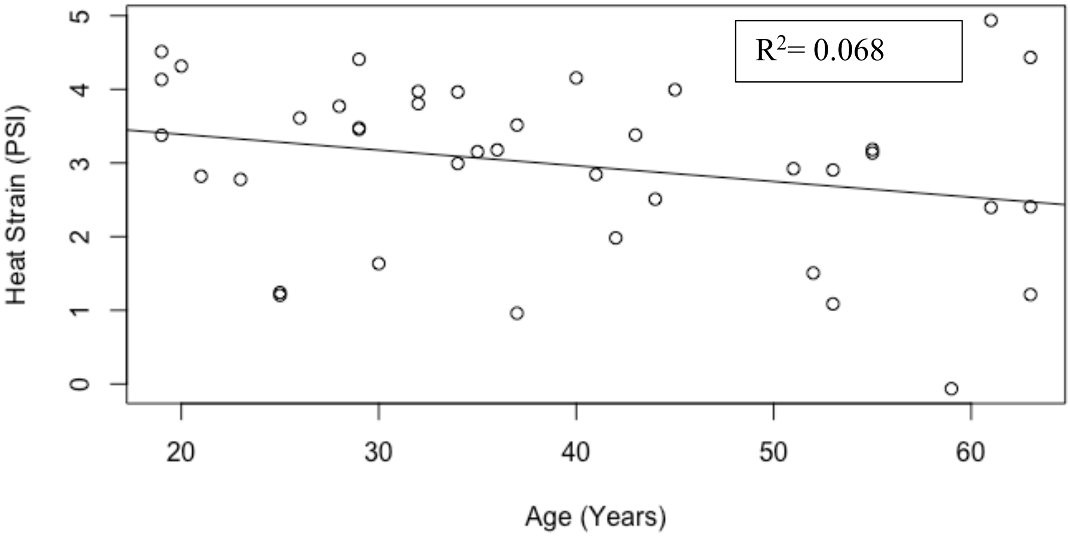


Table 11 shows the linear regression model estimates for PSI association with TWA WBGT. The unadjusted model showed higher PSI for higher TWA WBGT that was statistically significant (coefficient for TWA WBGT 0.123, 95% confidence interval 0.016, 0.230, Adjusted

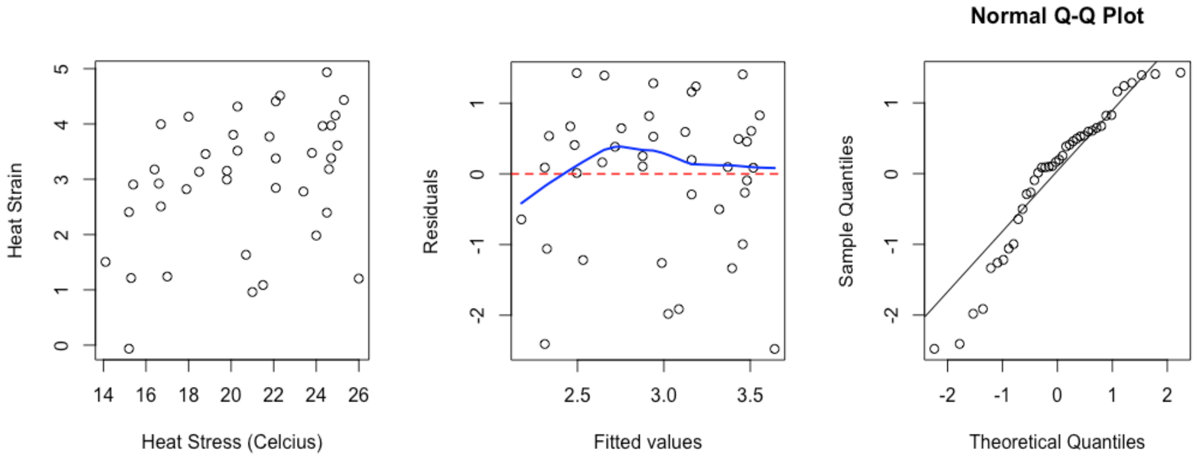
R^2 0.115, p-value 0.025). After adjustment for age (confounder) the effect was attenuated (coefficient for TWA WBGT 0.110, 95% confidence interval -1.630E-3, 0.221), Adjusted R^2 0.128, 0.221, p-value 0.052).

Table 11. Linear Regression Results Heat Strain ~ Heat Stress (Aim 2)

Regression Results Heat Strain ~ Heat Stress (Aim 2)					
Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	0.123	0.053	(0.016, 0.230)	0.115	0.025
Age Adjusted	0.110	0.055	(-1.630E-3, 0.221)	0.128	0.052

Model diagnostics were performed, and sensitivity analysis was conducted on the age adjusted model. As seen in *Figure 14*, the left plot of the crude model shows a positive association. Residuals were calculated and plotted against fitted predicted values as seen in *Figure 14* the middle plot. No obvious violations of equal variance or readily apparent outliers were seen. The sample quantile versus theoretical quantile plot (Normal QQ-Plot) for the crude model as seen in *Figure 14* the right plot found no major violations of normality.

Figure 14. Regression Model Diagnostic Plots of Heat Strain ~ Heat Stress



Specific Aim 2B

A. Descriptive analyses

Table 5 describes descriptive statistics for participants. The participant population for this aim is 40 tree fruit harvesters as previously described.

B. Inferential analyses: Effect Modification by cross shift change in 8-OHdG on the association between heat strain (PSI) and heat stress (TWA WBGT)

Covariate analyses were conducted as seen previously in *Table 10* with age being found to be a confounder. *Table 12* shows the effect of a cross shift change in 8-OHdG on the association between PSI and TWA WBGT for the unadjusted and age adjusted linear regression models. There was no evidence of statistically significant effect modification in either the crude or age adjusted model (p-values of 0.104 and 0.217).

Table 12. Interaction Check: Heat Strain ~ Heat Stress x Cross Shift Change 8-OHdG (Aim 2B)

Model	Interaction Term p-value
8-OHdG Interaction	0.104
8-OHdG Interaction with Age Adjustment	0.217

Specific Aim 2C

A. Descriptive analyses

Table 5 describes descriptive statistics for participants. The participant population for this aim is 40 tree fruit harvesters as previously described.

B. Inferential analyses: Effect Modification by acclimatization on the association between heat strain (PSI) and heat stress (TWA WBGT)

Covariate analyses were conducted as seen previously in *Table 10* with age being found to be a confounder. *Table 13* shows the effect of acclimatization on the association between PSI and TWA WBGT for the unadjusted and age adjusted linear regression models. There was no evidence of statistically significant effect modification for either the crude or age adjusted model (p-values of 0.562 and 0.799).

Table 13. Interaction Check: Heat Strain ~ Heat Stress x Cumulative Acclimatization (Aim 2C)

Model	Interaction Term p-value
Acclimatization Interaction	0.562
Acclimatization Interaction with Age Adjusted	0.799

Exploratory Aims

The results of *Specific Aims 1 and 2* prompted further questions about the relationships between TWA WBGT, PSI, acclimatization, and cross shift change in urinary 8-OHdG. The relationship was further explored using the 4 exploratory aims below.

Exploratory Aim 1

A. Inferential Analyses: Association of PSI and Cross Shift Change in Urinary 8-OHdG

Table 14 shows the linear regression model estimates for PSI association with cross shift change in urinary 8-OHdG for the 40 eligible participants. The unadjusted model showed a trend of higher PSI for higher across shift change in urinary 8-OHdG that was not statistically significant (coefficient for 8-OHdG 0.072, 95% confidence interval 0.011, 0.155, p-value 0.087). After adjustment for TWA WBGT (potential confounder) the effect was attenuated (coefficient for 8-OHdG 0.038, 95% confidence interval -0.0490, 0.125, p-value 0.380).

Table 14. Linear Regression Exploratory 1 Results: Heat Strain ~ Cross Shift Change 8-OHdG

Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	0.072	0.041	(0.011, 0.155)	0.012	0.087
Heat Stress Adjusted	0.038	0.057	(-0.490, 0.125)	0.032	0.380

Exploratory Aim 2

A. Inferential Analyses: Association of PSI and Acclimatization

Table 15 shows the linear regression model estimates for PSI association with acclimatization. The unadjusted model showed a trend of lower PSI for higher cumulative acclimatization that was statistically significant (coefficient for acclimatization -0.019, 95% confidence interval -0.037, -1.129E-3, p-value 0.038). After adjustment for TWA WBGT (potential confounder) the effect was attenuated and lost a statistically significant strength of association (coefficient for acclimatization -0.010, 95% confidence interval -0.031, 0.011, p-value 0.337).

Table 15. Linear Regression Exploratory 2 Results: Heat Strain ~ Acclimatization

Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	-0.019	8.931E-3	(-0.037, -1.129E-3)	0.067	0.038
Heat Stress Adjusted	-0.010	0.0103	(-0.031, 0.011)	0.111	0.337

Exploratory Aim 3

A. Inferential Analyses: Association Cross Shift Change in Urinary 8-OHdG and TWA WBGT

Table 16 shows the linear regression model estimates for Cross Shift Change in Urinary 8-OHdG with TWA WBGT. The model showed a trend of higher cross shift change in urinary 8-OHdG for higher TWA WBGT that was statistically significant (coefficient for TWA WBGT 0.393, 95% confidence interval 0.068, 0.718, p-value = 0.019).

Table 16. Linear Regression Exploratory 3 Results: Cross Shift Change 8-OHdG ~ Heat Stress

Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	0.393	0.161	(0.068, 0.718)	0.113	0.019

Exploratory Aim 4

A. Inferential Analyses: Association Cross Shift Change in Urinary 8-OHdG and PSI

Table 17 shows the linear regression model estimates for cross shift change in urinary 8-OHdG with PSI. The model showed a trend of higher cross shift change in urinary 8-OHdG for higher PSI that was statistically significant (coefficient for PSI 0.527, 95% confidence interval -0.384, 1.437, p-value = 0.249).

Table 17. Linear Regression Exploratory 4 Results: Cross Shift Change 8-OHdG ~ Heat Strain

Model	Estimate	Robust SE	95% CI (L, H)	Adjusted R^2	p-value
Crude	0.527	0.449	(-0.384, 1.437)	0.012	0.249

DISCUSSION

Summary of Results

We found a statistically significant cross shift difference in urinary 8-OHdG within each participant after heat exposure further supporting the potential utility of urinary 8-OHdG as an early detector of heat health effects (Zanolin et al., 2015). A higher level of acclimatization in the 7 days prior to monitored shift showed an estimated lower mean cross shift difference in 8-

OHdG supporting the proposed effect of acclimatization on oxidative DNA damage in our population of Washington tree fruit harvesters (Huang et al., 2012) and further supporting the utility of urinary 8-OHdG measurement (Zanolin et al., 2015). Heat stress using TWA WBGT and heat strain using PSI showed a statistically significant association in our study with higher heat stress showing higher heat strain, thus supporting the utility of using TWA WBGT and PSI to estimate heat exposure and resultant heat health effects in field studies like orchards (Cooper et al., 2017). Exploratory analysis showed statistically significant associations between heat strain and acclimatization as well as with a cross shift change in urinary 8-OHdG and heat stress (p-values 0.038 and 0.019).

Data Measurement and Interpretation of Results

A. Urinary 8-OHdG

In our study we used specific gravity adjusted urine to standardize our measurement of 8-OHdG to account for variance in concentration and hydration status of participants. An alternative approach would have been to collect 24-hour urine, but this was not done as we were interested in change cross heat exposed work shift, not absolute quantity, but more importantly for ease of study implementation and to aid in recruitment.

As described previously, 8-OHdG has been used as biomarker of exposure in occupational medicine with elevation linked to an increase in cadmium or chromates (Li et al., 2014). The mean of the differences cross shift within workers was significant and unlikely to be from an exposure other than heat, but this cannot be completely excluded based upon information gathered in our study from the participant survey.

Urinary 8-OHdG has been shown in some studies to show circadian variance but without a definitive pattern and with an effect demonstrated in a very small sample size (Kanabrocki EL, 2006). With a secondary analysis of cross-sectional data, we cannot completely account for a possible circadian effect. A repeat study using multiple measurements per subject at varying timepoints would be needed to address this, but with little documented circadian variance effect the utility of this study is questionable.

As seen in *Figures 1 and 2*, total heat stress can be from a couple identified heat sources. Environmental and metabolic heat can both contribute to heat stress and likely did in our study. This study only quantifies the environmental heat contribution to total heat stress through measurement of TWA WBGT. Total heat stress contributes directly to heat strain. The PSI calculation quantifies heat strain from all sources, within the limits of heart rate and core temperature measurement and would account for both environmental and metabolic heat stress. It would have been interesting to measure the metabolic heat contribution via exertion to total heat stress, but the fact that the PSI calculation accounts for heat strain from total heat stress the measurement is not crucial.

B. Heat Stress (TWA WBGT)

This study successfully used handheld WBGT monitors in a field experiment environment to estimate heat stress in our study participants. Using handheld WBGT monitors allows for a better estimation of individual heat stress relative to using area data. Individual measurement is especially beneficial in the agricultural and outdoor industries as conditions may vary frequently throughout the day and between workers. We were able calculate time-weighted averages to account for shift heat stress variation which allowed for more accuracy than a single or an average of measurements.

TWA WBGTs for our study were lower than initially expected for the time of year with a mean of 20.65°C (69.2°F) for all participants. Heat strain can occur at any level of heat stress exposure, but a higher heat stress may have allowed a more thorough study of the desired relationships. A lower mean heat stress did likely contribute to safer working environment as possibly evidenced by no reported signs of HRI in our study.

C. Heat Strain (PSI)

We defined heat strain in our study using the Physiologic Strain Index (PSI) with measured heart rate and temperature data (Moran et al., 1998). PSI was calculated as an average for the entire measured shift using the difference between the average value and baseline as defined over the first 6 measurements or 2 minutes. This 2-minute period was felt to be enough time for stabilization of measurements as well as not too long to delay shift start or have initial measurements be part of the working cycle. We feel the strategy was effective but ideally may have been longer to allow increased precision and normalization of baseline measurements. Average PSI cross shift allows for the effects of outliers to play a larger role relative to the median. However, the shift maximum or median value for PSI corresponds to a single measurement of an instant during the measured shift which could allow for a potential confusion of full shift environment. Point measurements do not reveal any information about duration which is an essential component in heat strain study. Using the average PSI cross shift gives an accurate full shift heat strain picture to compare to the cross shift measured change in urinary 8-OHdG.

PSI values were lower than initially expected with a mean value of 2.98. This is not surprising based upon similarly low heat stress values and the proposed relationship between heat stress and heat strain. Similar to heat stress, a higher PSI may have allowed a more thorough study of the desired relationships but also offered a potentially safer environment to our study participants.

Measurement of heart rate and temperature was done effectively in a field environment but results of measurements on 6 participants made reliable PSI determination not possible. One participant did not have recorded pulse data, which made PSI calculation impossible, and was therefore excluded. Five participants had average temperature values that were below 35°C or above 39.5°C and were excluded for being outside of the physiologic range and leading to doubt of measurement accuracy. It was felt that the reduction in eligible participants for aims requiring PSI at the expense of power was necessary to ensure validity of results.

D. Acclimatization

We found the 39.75 hour mean cumulative acclimatization of the previous 7 days prior to measured work shift to be evidence of the high occupational demand and increased risk for HRI in workers within the outdoor harvesting industry. Acclimatization was a calculated variable from self-reported survey results therefore a potential for reporting error exists. However, this error is likely to be low as the survey was administered at the time of the measured shift and asked about only the 7 days prior. Cumulative acclimatization was then calculated using reported average start and stop time and days worked to get cumulative product. This method of calculation makes the assumption that there was not great variation in start and stop times between worked shifts. This assumption may add error to estimated acclimatization, however this error is felt to be small as shift times don't vary greatly over the course of a normal work week within the same season.

Our cumulative acclimatization value of 39.75 hours accounts only for occupational acclimatization sources. The effect of using only occupational acclimatization sources cannot be completely known but we found no accurate way with collected data to define extra-occupational acclimatization sources. A fully detailed activity list for the previous 7 days would have aided in a better inclusive definition of cumulative acclimatization.

E. Covariate Identification and Treatment

Literature review was used in the preliminary study design period to capture variables that were primarily related to heat stress and worker productivity aims. The identified variables were then formulated into questions in the participant survey in an attempt to define their relationships. The variables identified were helpful at defining their relationship to heat stress and heat strain but could have been defined differently for acclimatization and urinary 8-OHdG. Urinary 8-OHdG may have some diurnal variability and has been shown to be elevated in some medical conditions like, multiple sclerosis (Kanabrocki EL, Ryan MD, Murray D, Jacobs RW, Wang J, Hurder A, Friedman NC, Siegel G, Eladasari B, Nemchausky BA, Cornelissen G, Halberg F., 2006). The noted medical conditions are rare and diurnal variability is not agreed upon, but a more complete survey would have been helpful.

Variable selection in the multiple regression models was based either on evidence of being a potential confounder or a potential precision variable, using associations with the outcome and predictor versus outcome only respectfully, in our study. A larger study might have conceivably identified other covariates for inclusion in the analyses.

F. Association between Cross Shift Change 8-OHdG and Acclimatization

Our study did not find a statistically significant relationship between urinary 8-OHdG and acclimatization, however the effect estimate was in line with the hypothesized direction. Our relatively limited sample size may have limited our ability to detect an association.

Sensitivity analysis was conducted (*Table 7)* and 3 participants (20, 21, and 24) were removed after being identified as outliers. Removing the potential outliers did improve the fit of the linear model *(Figure 10)* but did not substantially change the estimate of effect in all models *(Tables 8 and 9).* This lack of sensitivity enhances the validity of our modeling the association between urinary 8-OHdG and acclimatization.

G. Association between Heat Strain and Heat Stress

Our study found a statistically significant relationship between heat strain (PSI) and heat stress (TWA WBGT) with higher heat stress having higher heat strain (*Table 11)*. When adjusting for age, using results from covariate analysis (*Table 10)* we found the effect was slightly attenuated and lost statistical significance. We cannot explain the full etiology of this but suspect our relatively limited sample size may have been the main contributor. Sensitivity analysis (*Figure 14)* did not show evidence of outliers so results include all eligible participants.

Effect modification seemed highly plausible by acclimatization or cross shift change in urinary 8-OHdG on the relationship between heat strain and heat stress using proposed models (*Figures 1 and 2)*, but we did not find evidence of interaction throughout our study (*Tables 12 and 13)*. Our relatively limited sample size made assessment for interaction an ambitious endeavor that potentially would have been more informative with a larger range of values for our interaction term (*Table 5)*.

Exploratory Analysis

The analysis results of our pre-identified specific aims led to more questions on how heat stress, heat strain, acclimatization, and urinary 8-OHdG interact, prompting creation of the exploratory aims and analyses. After conducting the study and upon further thought, the exploratory aims offer greater insight into the studied relationships and in hindsight may have been better included as part of the *Specific Aims*.

A. Association of PSI and Cross Shift Change in Urinary 8-OHdG *(Table 14)*

We found no statistically significant association between PSI and 8-OHdG change with and without adjustment for TWA WBGT. This finding questions the utility of urinary 8-OHdG measurement. However, with a limited sample size we may lack the power to detect an association. The full relationship may benefit from further study.

B. Association between PSI and Acclimatization *(Table 15)*

The association of PSI and acclimatization was found to be statistically significant and in a direction of association supporting the theorized inverse relationship. This finding supports what we know about the relationship between heat strain and acclimatization. However, when adjusting for heat stress the association was not statistically significant. This once again could be from diminished power but also could show that heat stress has no relationship with the association between heat strain and acclimatization despite *Specific Aim 2* showing an association between heat strain and heat stress.

C. Association between Cross Shift Change in Urinary 8-OHdG and TWA WBGT (*Table 16)*

The association of urinary 8-OHdG change and TWA WBGT was found to be statistically significant and in support of the theorized direction. An increase in urinary 8-OHdG change was found in those with higher heat stress. This finding is interesting in that it supports a potential pre-clinical role for urinary 8-OHdG elevation before the physiological effects of heat stress are expressed as heat strain. However, urinary 8-OHdG, as theorized in *Figure 2* represents a measure of heat strain. This and the results of exploratory aim 1 showing a lack of association between PSI and 8-OHdG are seemingly inconsistent. This again could be a result of poor power. More study is needed to determine the complete relationship of these variables.

D. Association between Cross Shift Change in Urinary 8-OHdG and PSI (*Table 17)*

We found no statistically significant association between urinary 8-OHdG change and PSI. This finding again raises questions as to the utility of urinary 8-OHdG to identify heat strain. Again, limited power may not have allowed us to detect a difference. The full relationship may benefit from further study.

Study Strengths

Our study served to further define the utility of urinary 8-OHdG in assessing heat health effects in outdoor tree fruit harvesters, a highly heat-exposed and difficult to measure population. Secondly, further supported the possibility of reliable field measurement of WBGT, urinary 8-

OHdG, pulse, and core temperature. Thirdly, the results of our numerous regression models proved to validate the relationships between heat stress, heat strain, acclimatization, and urinary 8-OHdG as theorized in our heat pathophysiology model. Fourthly, we were able to provide further support to the benefits of future study of urinary 8-OHdG, as it shows promise and has potential for improved detection of heat related injury.

Study Limitations

First, a secondary analysis of observational cross-sectional data gathered via convenience sampling on 46 participants limits the generalizability as well as causal relationship determination between variables. A large experimental study encompassing numerous heat exposed occupations in multiple regions would allow for a broader determination to be made and is an opportunity for future study.

Second, misclassification of participant characteristics and acclimatization is also a possibility when using a self-reported survey like the A-CASI. As mentioned previously, this error is thought to be small, but a more controlled and complete definition of acclimatization would be ideal and an improvement in future studies.

Third, heat strain calculation using the PSI required heart rate and core temperature data that in 6 participants was not accurate. This loss of 6 participants led to a decrease in study sample for a study where recruitment was difficult. Using a more accurate core temperature monitor or allowing for complete calibration with a function check would have been ideal. Further, changing study protocol to include a back-up core temperature value to be collected should an initial value be outside physiologic range could also prevent the loss of valuable participants.

Fourth, heat stress data using WBGT was done over intervals requiring a time-weighted average to be calculated. Using continuous measurements and using values coinciding with the heart rate and core temperature would have allowed for a more accurate comparison. Using continuous monitoring in future studies would be recommended and ideal if able to do so accurately in a field environment.

Fifth, linear regression modeling throughout our study showed universally low adjusted R^2 values indicating that a large portion of outcome variability was due to factors not explained by our models. This fact would be much more detrimental in prediction modeling rather than estimation of association as in our case, but still merits mention.

Lastly, our small sample size of 46 participants overall and fewer eligible participants in some aims had a major effect on power and our ability to identify associations if they were in fact present. A larger study would have reduced the possibility of Type I and II errors.

Conclusion

Yakima valley tree fruit harvesters are working in heat exposed conditions and are at risk for heat-related injury. Urinary 8-OHdG shows promise as a biomarker of heat exposure: it is able to be successfully measured in field conditions, found to be elevated after a heat-exposed work shift, found to be less elevated with acclimatized heat exposure, found not to be associated with heat strain, and found to be associated with heat stress. The full potential of 8-OHdG to detect early heat health effects requires further study which the results of our study strongly support. Greater heat stress may be associated with greater heat strain, but a more definitive conclusion requires larger studies. Defining and understanding the full relationship between heat stress, heat strain, acclimatization, and urinary 8-OHdG is complex and more research is needed.

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SUPPLEMENTAL INFORMATION

A. University of California San Francisco laboratory manual: collection instructions for aseptic urine. (http://labmed.ucsf.edu/labmanual/mftlng-mtzn/test/pt_instructions.html)

B. 8-OHdG Method for HEAT Project Urine Analyzed July 2016 by Mike Paulson

Urine was analyzed for 8-hydroxy-2'-deoxyguanosine (8-OHdG) using high performance liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS) using a method based on that reported by Hosozumi et al. with modifications (Hosozumi et al., 2012). Prior to analysis, samples were stored at -80˚C. One mL of thawed urine was diluted with an equal volume of purified water and then spiked with internal standard (^{13}C- and $^{15}N_2$-labelled 8-OHdG). Samples were pretreated using solid phase extraction (SPE, Oasis HLB Plus LP, Waters Corp.). SPE cartridges were preconditioned with 5 mL methanol followed by 10 mL water. After loading cartridges with urine samples, they were washed with 10 mL water, vacuum dried for 30 minutes and then eluted with 2 mL acetonitrile:water (90:10).

Sample extracts were analyzed on an Agilent 6410 HPLC-MS/MS. The HPLC column was a Hypersil Gold HILIC, 2.1 x 100 mm, 3 µm particle (Thermo Scientific). HPLC mobile phase was isocratic with 93% 20 mM ammonium acetate containing 0.1% formic acid and 7% acetonitrile. HPLC settings included a flow rate of 0.5 mL/minute, column temperature of 35˚C, and injection volume of 10 µL. The mass spectrometer was equipped with an electrospray ionization source and operated in positive ion selected reaction monitoring mode with the molecular transition of *m/z* 284 → 168 for 8-OHdG and 287 → 171 for the internal standard. Nitrogen drying gas was 6 L/minute at 350˚C and nebulizer pressure was 20 psi. Voltages were 5000, 80 and 8 volts for capillary, fragmentor, and collision energy, respectively.

Quality control included lab blanks, lab spikes, and replicate analysis of a composite urine control sample. Calibration was linear over the range of 0.5 to 200 ng/mL urine and the lower limit of quantification (LOQ) was defined as the lowest calibration standard (0.5 ng/mL). All lab blank values (average 0.36; SD 0.02 ng/mL) were below the LOQ while all samples had detectable 8-OHdG above the LOQ. Assay precision was determined by analyzing six replicates of a composite urine control sample. Average, standard deviation and coefficient of variation for the control urine were 6.2 ng/mL, 0.37 ng/mL, and 6%, respectively. Recoveries from spiked lab water averaged 102% with a coefficient of variation of 4%.

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