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Kidney Function Impairment Amongst Industrial Agricultural Workers in Central America

Ellon Hart- [redacted]

Supervisor: Dr Rebekah Lucas

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Abstract

In several regions of Central America an epidemic of chronic kidney disease exists that is unrelated to conventional risk factors such as hypertension and diabetes. The impact of this disease, now termed chronic kidney disease of non-traditional origin (CKDnt), is devastating, accounting for tens of thousands of fatalities. The exact aetiology of CKDnt is still unknown, although there is strong evidence to favour the involvement of high occupational workloads and heat stress, due to prolonged strenuous work in hot climates. It is believed that frequent exposure to strenuous work under heat stress can cause repeat episodes of acute kidney injury or kidney strain, which can potentially lead to a chronic functional and structural damage, especially when recovery time between work shifts is minimal.

There is therefore an urgent need to understand more about CKDnt pathophysiology, in order to develop effective strategies to mitigate the risk. In addition, more research is warranted to help improve the diagnosis and monitoring of at-risk workers, through more consistent and standardised field-based testing protocols, and the potential implementation of more sensitive biomarkers. This thesis will aim to address some of these issues. Firstly, a protocol chapter (Chapter 2) is presented that assesses methods of measuring kidney function/injury, their advantages, and disadvantages, as well as some recommendations on how to best use these measures in field-based occupational studies. The latter part of this thesis presents a data chapter (Chapter 3) comparing the recovery of blood and urine biomarkers at the end of a working week (17 hours recovery) to that at the beginning of a working week, after a day off (41 hours recovery). This chapter also examines how these two different recovery periods affect cross-shift changes in blood and urine biomarkers. The study presented in Chapter 3 was conducted at the sugarcane mill Ingenio San Antonio (ISA) in Chinandega, Nicaragua, over the course of 4 days during the mid-harvest season3 (including three workdays (Friday, Saturday, and Monday), and one rest day (Sunday). Biomarkers of kidney function, hydration status, inflammation and muscle damage were collected in 20 male burned sugarcane cutters (aged 33 ± 7 years) at 5 time points across the course of the study (End of work week: Friday post-shift, Saturday pre- and post-shift; and Beginning of work week: Monday pre- and post-shift). Our results showed that recovery of blood and urine biomarkers was not significantly different following 41 hours (Saturday post- to Monday pre-shift), compared to 17 hours, (Friday post- to Saturday pre-shift). In addition, cross-shift reductions in kidney function were significantly greater after this longer 41-hour recovery period, compared to a 17-hour recovery

period. However, it is possible that any significant benefits from a longer recovery period may be dampened by the high degree of individual variability in our results.

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Abbreviations

% HR_{max}	Percentage age predicted maximum heart rate
ACR	Albumin: Creatine ratio
AKI	Acute kidney injury
AUC	Area under the curve
BSA	body surface area
CKDnt	Chronic kidney disease of non-traditional origin
CK	Creatine kinase
CRP	C- Reactive protein
eGFR	Estimated glomerular filtration rate
$eGFR_{cys}$	Estimated glomerular filtration rate for serum cystatin C
$eGFR_{creat}$	Estimated glomerular filtration rate for serum creatinine
HR	Heart rate
IGFBP-7	Insulin growth factor binding protein 7
IL-18	Interleukin 18
KIM-1	Kidney injury molecule 1
L-FABP	Liver- fatty acid binding protein
NGAL	Neutrophil gelatinase-associated lipocalin
sCr	Serum creatinine

T_{GI}	Gastrointestinal temperature
WBGT	Wet-bulb globe temperature

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1. Introduction

1.1. Overview: CKDnt epidemiology, and characteristics

Over the last 20 years there has been a growing epidemic of chronic kidney disease of non-traditional origin (CKDnt) in several regions of Central America that is unrelated to conventional risk factors such as hypertension and diabetes (Johnson 2019; Torres, 2010; Peraza, 2012; Wesseling, 2014). CKDnt was first recognised by clinicians in the late 1990's in pacific coastal regions of El Salvador, Costa Rica, and Nicaragua (Wesseling, 2015), giving rise to the term 'Mesoamerican Nephropathy' (MeN). However, this disease was likely prevalent as early as the 1970's with anecdotal evidence showing higher CKD mortality rates in the hottest province of Costa Rica, Guanacaste, compared to the rest of the country (Wesseling, 2015). Recognition came after overwhelming numbers of young, male sugarcane workers were being admitted to hospital with end stage renal disease (ESRD) (Garcia-Trabanino, 2002; Cerdas, 2005), and increasing CKD mortality rates were being recorded in these populations, in the absence of typical risk factors such as hypertension and diabetes (Brooks, 2009; Ordunez, 2018).

CKDnt hotspots are localised in, but not restricted to, lowland coastal regions of Central America, with hotter, humid climates (Glaser, 2016; Torres, 2010; Peraza, 2012). The disease disproportionately affects young males (aged 30-50), who work in physically demanding agricultural occupations (particularly sugarcane cutters) (Correa-rotter, 2014; Glaser, 2016; Wesseling, 2015; 2020). However, more recent evidence has identified impaired kidney function in other physically laborious agriculture occupations, (i.e., cotton, rice, corn, and banana plantation workers), as well as in construction workers, miners, fishermen, and artisan manufacturing (Torres, 2010; O'Donnel, 2011; Peraza, 2012). Furthermore, CKDnt is now emerging in other geographical locations including Guatemala, Honduras, Panama, and parts of India, Sri Lanka Africa, North America, and the Middle East (Glaser, 2016; Abraham, 2015; El Minshawy, 2011; Moyce, 2016). Despite an obvious male predominance, female labour workers are also affected (Torres, 2010) and share similar occupational risk factors and geographical trends (Peraza, 2012), albeit with lower prevalence and fewer fatalities (Torres, 2010; Peraza, 2012). This male predominance is likely a reflection of different occupational roles, rather than differences in gender disease susceptibility (Peraza, 2012). Finally, there appears to be a socioeconomic influence on the populations affected by CKDnt. Low-income countries and more deprived communities have higher CKDnt mortality rates (Nerbass, 2017;

Kjellstrom, 2009; 2014). This may be because manual labour is often the only available work in these regions, access to affordable healthcare is limited, there are poor education opportunities, and at-risk working populations live in substandard housing (Verite, 2017; Donoghue, 2004k; Wesseling, 2014).

Individuals with CKDnt present with an asymptomatic reduction glomerular filtration rate (GFR); an elevated serum creatinine; low-grade or absent proteinuria (unlike typical CKD); and in some cases, microhematuria, hyperuricemia, and hypokalemia (Schlader, 2019; O'Donnell, 2011). However, not all of these symptoms need to be present, and there is often heterogeneity between individuals (Johnson, 2019). While few renal biopsies have been carried out, tubular atrophy, glomerulosclerosis, mild vascular lesions, and interstitial fibrosis are consistent findings (Wijkstrom, 2013). The early stages of CKDnt are largely asymptomatic, so it can go undetected for many years, allowing the disease to silently progress (Torres, 2010; Glaser, 2016). When symptoms do begin to develop, CKDnt has often already progressed into ESRD, at which point treatment is too late. Consequently, fatalities are high, and it is estimated that between 1997 and 2012 CKDnt has accounted for tens of thousands of deaths in pacific coastal regions of Central America (Ordunez, 2018; Ramirez-Rubio, 2013). This is compounded by the fact that affected populations are primarily from low-income, rural communities, with little access to treatment such as kidney dialysis or transplantation (Ramirez-Rubio, 2013). Since it was first identified, rates of CKD related mortality have drastically increased in certain areas of Central America, in comparison to nearby countries (e.g., U.S.A.). In El Salvador alone, mortality rates increased by 17.6% between 1997 and 2012, primarily in young, male agricultural workers (Ordunez, 2018). Therefore, there is a growing need to better understand the mechanisms of CKDnt to help diagnose (especially early diagnosis), develop treatment and prevention strategies, and ultimately prevent these fatalities.

1.2. Risk factors and pathophysiology

As mentioned above, CKDnt is not strongly associated with typical CKD risk factors such as hypertension, obesity, and diabetes. If anything, hypertension can be a consequence, rather than a cause of kidney disease in CKDnt hotspots, and is not unusual in at-risk populations (Peraza, 2012) as advanced impairments in renal function eventually hampers the kidneys' ability to regulate blood pressure (O'Donnel, 2011; Torres, 2010).

Numerous hypotheses have been proposed to explain the cause of CKDnt, including, exposure

to toxic chemicals, pesticides, consumption of contaminated water, infectious disease, NSAID overuse, high workloads, and heat stress (Correa-Rotter, 2014; Lameire, 2013; Ramirez-Rubio, 2013; Wesseling, 2014; 2016; Glaser, 2016). However, supporting evidence for many of these risk factors are limited, and there are often inconsistencies between studies (Gonzalez-Quiroz, 2018; Schlader, 2019; Correa-Rotter, 2014), therefore, the underlying aetiology of CKDnt, and associated risk factors are still inconclusive. That being said, the leading hypothesis currently favours a form of heat stress nephropathy, associated with repetitive manual labour in hot, humid conditions. The consensus is that frequent and prolonged exposure to heat stress, dehydration, and high workloads puts significant physiological strain on the kidney, enough to causes recurrent episodes of acute kidney injury (AKI) (Schlader, 2019; Hansson, 2019; Wesseling, 2016; 2020; Roncal-Jimenez, 2015; 2017; Glaser, 2016). While AKI is typically reversible and benign, there is accumulating evidence to suggest repeated injury, with minimal recovery time, can cause long term deterioration of kidney structure and function (Schlader, 2019; Hansson, 2019). This is likely to be exacerbated by organisational factors such as piece rate work (which enforces higher work intensities), few safety protocols, long hours, few breaks, and limited access to water and shade (Nerbass, 2017; Verite, 2017).

There is a growing body of epidemiological, laboratory and field-based evidence supporting the heat stress nephropathy hypothesis. Firstly, there is a clear geographical pattern to CKDnt prevalence, with hotter, coastal regions predominating (Correa-rotter, 2014; Glaser, 2016; Peraza, 2012; Torres, 2010; Wesseling, 2015). Additionally, several cross-sectional occupational field-studies show a correlation between the severity of cross-shift AKI, and both workload (Hansson, 2019) and heat stress (Butler-Dawson, 2019; Paula-Santos, 2015; Mix, 2018; Garcia-trabanino, 2015). This link between acute kidney strain and heat stress/workload is further supported by laboratory-based studies, showing that the magnitude of hyperthermia and dehydration induced by exercise in the heat is strongly associated with the severity of AKI (Schlader et al, 2017), and alleviating each of these factors minimises this injury (Chapman et al, 2020). The possibility for such sublethal AKI to cause chronic kidney damage in occupational settings was first identified in 1970 when several cases of heat stroke and AKI were reported in Bantu goldminers (Kew, 1970). Notably, while these cases of AKI seemed reversible in the short term, injured workers later presented with CKD and chronic interstitial fibrosis (Kew, 1970). More recently, longitudinal field studies have shown associations between acute kidney strain and long-term kidney dysfunction in sugarcane cutters in Central

America. Kupferman (2018) found that 30% of sugarcane workers who developed AKI during harvest, had CKD one year later, while Wesseling et al (2016) demonstrated that cross-harvest declines in kidney function were associated with cross-shift increases in serum creatinine in 29 male sugarcane cutters in Nicaragua. This is further supported by a water-rest-shade intervention study, implemented amongst cohorts of sugarcane workers, two months into a 5-month harvest in El Salvador. During the first two months, all workers experienced a decline in kidney function (eGFR), however, in the intervention group, this decline stabilised for the remainder of the harvest after undertaking the work-rest-shade intervention (Wegman, 2018). Finally, several pioneering animal studies have consistently demonstrated that daily heat exposure elicits nephropathy in just 4-5 weeks, with fluid replenishment during this heat exposure almost abolishing kidney injury during this time (Roncal Jimenez, 2014; 2017; 2018).

The exact mechanism underlying the link between exertional heat stress, and acute kidney injury is not clear. However, it has been proposed that the mechanism likely involves a mismatch between renal energy demand and supply, in conjunction with systemic inflammation, muscle damage, and changes in fructose metabolism (Hansson, 2020; Schlader, 2019). Exercise, hyperthermia, and dehydration independently cause reductions in renal blood flow and oxygen delivery. This is due to the redistribution of blood to the periphery and muscles, primarily regulated via increased sympathetic nerve activity and RAAS/vasopressin activation (Smith, 1952; Hansson, 2020; Volianitis, 2016; Low, 2011; Johnson, 2014). However, there's also a simultaneous increase in energy demand in the kidney, with renal tubular cells working harder to maintain fluid balance by actively increasing sodium reabsorption and potassium excretion. In addition, changes to renal fructose metabolism occur that require additional energy, specifically, activation of the polyol-fructokinase pathway. Collectively, this leads to ATP depletion, oxidative stress, and inflammation within the kidney that may precede tubular epithelial and endothelial cell dysfunction (Bellomo, 2012; Devarajan, 2006; Hansson, 2020; Bragadottir, 2009; Basile, 2012). An increase systemic inflammation worsens this pro-inflammatory state. This occurs primarily due to an increase in gastrointestinal permeability following ischemia, allowing endotoxins to enter the circulation, however, systemic energy depletion, and muscle breakdown may also contribute (Hansson, 2019; Leon, 2010a; Leon, 2010b; Suzuki, 2018; Hennigar, 2020). There's also a risk of renal toxicity, and tubular obstructions following exertional heat stress, which further aggravates the inflammatory state in the kidney. This occurs due to an increased filtered load of; myoglobin

(caused by muscle breakdown), uric acid (a by-product of the polyol-fructokinase activation), and endotoxins, (Hansson, 2020; Patel, 2009; Spencer, 1976; Sanchez-Lozada, 2008; Roncal-Jimenez, 2015; Patel, 2009).

Due to the immediate activation of repair pathways, most AKI cases are easily resolved, causing little cause for concern (Basile, 2012). AKI becomes problematic when recovery time between episodes is limited, as a result of frequent exposure to the injurious stimuli. This prevents cells from fully repairing, which can cause chronic inflammation and growth arrest, inhibiting cell differentiation. Furthermore, repeated injury can cause the repair process to become maladaptive, as excessive cell proliferation can lead to the accumulation of scar tissue (fibrosis). Collectively, these processes can render regions of the kidney non-functional (Basile, 2012; Hansson, 2019; Venkatachalam, 2015). However, it is undetermined whether bouts of repeated AKI need to be of a certain severity to cause long term CKD, or whether mild kidney strain is a sufficient stimulus.

1.3. Future research

Much is still unknown about the pathophysiology of CKDnt, and the link between acute kidney strain and CKD is still relatively novel in this context. It is clear that more research is needed to better understand the factors contributing to this CKDnt epidemic in order to develop practical strategies to mitigate the risk.
There is also a need to improve the testing, and diagnosis of CKDnt. Earlier detection is vital, which is made difficult by the asymptomatic nature of CKDnt in the early stages. Furthermore, current diagnostic tools have numerous methodological limitations, and few non-clinical guidelines exist for field-based kidney function measures.

Therefore, this thesis will firstly review key measures of kidney function and injury and consider their advantages and disadvantages for use in field-based occupational studies (Chapter 2). Following this review, kidney function assessment recommendations are proposed, with a particular focus on field-based studies. Finally, a data chapter (chapter 3) is presented examining recovery of blood and urine biomarkers in sugarcane workers following one night (17 hours; at the end of a working week), compared to two nights and one day's recovery (41 hours; at the beginning of a working week, after a day off)

2. Protocol Paper

2.1 Background

Despite the global threat that CKDnt poses to numerous occupational populations (Ordunez, 2018; Ramirez-Rubio, 2013), many cases of CKDnt tend to go undetected until treatment is too late, and ESRD has progressed. This is partly due to the nature of chronic kidney disease, which is asymptomatic in the early stages (Torres, 2010; Glaser, 2016). However, it may also be attributable to the insensitivity of current diagnostic tools, and the improper or infrequent testing protocols used in occupational field settings (Wesseling et al, 2014; Caplin et al, 2017; Schlader, 2019). Indeed, the measurement of kidney function in field-based occupational settings is challenging, especially in workers frequently exposed to manual labour in hot conditions. Under such conditions, it is difficult to control extraneous variables, such as diet, drug/medication usage and physiological changes associated with exertional heat stress, e.g., muscle damage, or fluctuations in hydration status (Delanaye, 2012, 2017; Poortmans, 1984; Thomas, 2017; Heymsfield, 1983). Furthermore, the majority of CKD research to date has been based on clinical populations in hospital settings. This determines that reference values, and measurement timings for renal function are difficult or not applicable to occupational populations in field settings.

Therefore, there is a need to advance the assessment of acute and chronic kidney function in populations at risk for CKDnt, with a more comprehensive protocol that is specific to occupational populations. This will promote earlier detection, enable a better understanding of CKDnt aetiology and inform what measures can be implemented to prevent CKDnt. This paper will describe some of the key measures of kidney function and injury, and consider their advantages, and disadvantages for use in field-based occupational studies. Some recommendations will also be highlighted regarding measurement timing, and additional measures to record/control/minimise the influence of confounding variables.

2.2 Methods: Measures of kidney function and injury

The most common, and practical method of kidney function assessment in both clinical and field settings is dipstick urinalysis (commonly used for glucose, albumin, or urine specific gravity). Dipstick tests provide rapid results, require minimal expertise, and are low-cost. Thus, dipstick urinalysis is beneficial for larger-scale field studies, or as an initial screening measure. However, this measure can be inaccurate, insensitive, and relatively non-specific

(Simmerville, 2005; Delanghe, 2014; Baumgarten, 2011). Therefore, the use of more advanced urinary measures, such as small proteins of tubular damage (NGAL, KIM, IGFBP-7), urinary sediment, or albumin: creatinine ratio, could be used to improve the reliability of kidney function assessment, if there are less constraints on cost, time, and expertise.
Blood biomarkers are also integral to kidney function assessment. Both acute and chronic kidney disease manifest as an asymptomatic build-up of nitrogenous waste products in the blood (such as creatinine), due to a reduction in glomerular filtration rate (GFR) (Mehta, 2007; Lopez Giacoman, 2015). Serum creatinine (sCr) is therefore a principal index of renal function and is used to classify both AKI and CKD (Kellum, 2012; Levin, 2013). However, the use of sCr in kidney function assessment is widely criticised, especially in occupations involving manual labour/heat stress. This is due to the confounding effect of muscle catabolism on sCr levels, fluctuations in sCr (and subsequent GFR) with physical exertion or hydration, as well as the insensitivity of sCr levels to small changes in kidney function (Delanaye, 2012, 2017; Poortmans, 1984; Thomas, 2017; Heymsfield, 1983). Alternative blood biomarkers include cystatin C, or NGAL, which can also be used to indicate GFR, and may provide a more sensitive estimation of GFR compared to sCr (Kar, 2018; Nickolas, 2008). However, cystatin C and NGAL are more novel measures. This may compromise the relevance of results and findings using these markers as there are fewer studies available to compare results, plus there is a lack of standardisation in their reference values.

The preferred method of assessing kidney function will depend on the context of the study, and will involve weighing up the importance of cost, practicality, and effectiveness. Kidney function measures that will be discussed in this paper include estimated glomerular filtration rate (serum creatinine), urinary albumin: creatinine ratio (ACR), dipstick urinalysis (albumin, glucose, urine specific gravity), small proteins of tubular damage, and urinary sediment.

Lastly, there are key differences between traditional CKD and CKDnt that need to be emphasised for the context of this review (Caplin, 2017). Both CKD and CKDnt present with a similar asymptomatic rise in serum creatinine/reduction in GFR (< 60ml/min/1.73m^2), however, there are two important differences in the urinary profile of established CKD vs CKDnt. Firstly, traditional CKD is characterised by chronic abnormally high albuminuria (≥30mg/g), while this is minimal or absent in CKDnt populations. Secondly, CKD is primarily associated with hypertension, diabetes, and obesity, but CKDnt shows no association with these risk factors (Garcia-Trabanino, 2005; Torres, 2010; O'Donnell, 2011;

Wijkstrom, 2013; 2017). Therefore, assessing urinary albumin and glucose helps to discriminate these two subtypes of CKD (Caplin, 2017).

eGFR (Estimated Glomerular Filtration Rate)

Glomerular filtration rate (GFR), is currently regarded as the best overall index of kidney function for both AKI and CKD (Kellum, 2012; Levin, 2013). The most accurate way to measure GFR (mGFR) is via the renal clearance of an exogenous substance, e.g., inulin, however, this requires intravenous infusion and repeat sampling, so can be time consuming, impractical, and costly in field-based research. Instead, GFR is usually estimated, rather than measured, based on endogenous serum creatinine, a by-product of muscle catabolism (Schlader, 2019; Traynor, 2006). Serum creatinine (sCr) is released into the blood at a relatively constant rate, assuming muscle metabolism is stable during the period of assessment. Under such conditions, when GFR declines there will be a concomitant increase in sCr, providing an approximate indicator of kidney function (Schlader, 2019; SBU, 2013; Heymsfield, 1983; Spencer, 1986).

Serum creatinine GFR estimations ($eGFR_{creat}$) are integral to kidney function assessment in occupational populations, as asymptomatic elevations in sCr are widely accepted as a hallmark feature of CKDnt (Wijkstrom, 2013; Torres, 2010; Peraza, 2012; Glaser, 2016). Furthermore, acute cross-shift elevations in sCr are commonly documented in workers following prolonged physical work in the heat (Garcia-Trabanino, 2015; Moyce, 2017; Mix, 2018; Sorensen, 2019; Butler-Dawson, 2019). Thus, $eGFR_{creat}$ calculations can be beneficial in highlighting both chronic declines in renal function, and acute renal stress.

Advantages

- Assessing sCr is relatively inexpensive compared to other biomarkers of renal function, making it more feasible for large-scale studies.
- Measures of sCr allow GFR to be estimated off a single blood sample. This is more practical and less time consuming than alternative clearance methods, which require timed urine samples or infusion of exogenous substances (Beierwaltes, 2013; Delanaye, 2017).
- Blood measures remove the need to correct for urinary concentration or flow rate.

- Numerous estimating equations exist to correct for age, sex, race, and body size, minimising sCr variability from muscle metabolism (Levin, 2013; Beierwaltes, 2013; Delanaye, 2017; Levey, 1999; 2009).
- eGFR is universally accepted as the principal index of renal function. Estimation of $eGFR_{creat}$ can be used to define clinical AKI and CKD, with specific reference standards for disease classification and staging (Schlader, 2019; Kellum, 2012; Levin, 2013; Traynor, 2006). Therefore, $eGFR_{creat}$ remains the most appropriate measure when making comparisons between studies/different populations.

Disadvantages

- Measures of sCr are directly influenced by muscle mass, size, and breakdown, irrespective of any change in kidney function (Perrone, 1992). Therefore, sCr can be an inaccurate measures of GFR in individuals who have experienced a sudden change in muscle mass, those at the extremes of muscle mass (Nyman, 2014; Levey, 2009; Heymfield, 1983;), or in the context of strenuous exercise, especially in the heat, where there is an increased likelihood of muscle breakdown (Khan, 2009; Bagley, 2007).
    - The latter is particularly relevant in occupational settings, where workers perform strenuous manual labour, in hot conditions. Therefore, cross-shift elevations in sCr should be interpreted with caution as elevations may not be solely due to a decline in kidney function but likely reflect some muscle breakdown.
    - It is worth highlighting here that muscle breakdown itself can be an independent cause of AKI during exertional heat stress, which complicates its role as a confounding variable in this context (Junglee, 2013; Vanholder, 2000; Bosch, 2009; Bagley, 2007).
- Some races and ethnicities have been underrepresented in the development of eGFR equations, such as those from Asian, Hispanic, African, or Caribbean decent (Rule, 2004; Caplin, 2017). This may result in inaccurate eGFR calculations for these populations (Kumar, 2018; Jessani, 2014; Eneanya, 2019). Furthermore, the two most common eGFR equations (CKD-epi, MDRD) make binary racial adjustments (black vs white) based solely on a US population that may not be applicable to other nationalities and ethnicities (Rocha, 2020; Deventer, 2008; Eneanya, 2019).
    - This presents challenges in occupational settings, if eGFR comparisons between different ethnic working populations are made. Furthermore, migrant workers from

different nationalities and ethnicities may form part of a working population and confound eGFR measures across a cohort.

- Diet has a direct influence on sCr. The consumption of cooked meat can transiently increase sCr level (for approximately 12 hours), resulting in higher eGFR values, independently of any true changes in kidney function (Nair, 2014; Heymsfield, 1983; Jacobsen, 1979; Williamson, 2014).
- Different sCr calibration methods can reduce the reliability of cross-laboratory comparisons (Myers, 2006; Caplin, 2017; Coresh, 2002).
- GFR estimations assume plasma creatinine is in steady state. Therefore, creatinine may not be a reliable measure of kidney function in those with rapidly changing creatinine concentrations (Munikrishnappa, 2009; Nikolas, 2008). This presents challenges in occupations where workers perform strenuous manual labour in hot conditions, resulting in acute fluctuations in renal haemodynamics and sCr (Garcia-Trabanino, 2015; Moyce, 2017; Mix, 2018; Sorensen, 2019).
- sCr sensitivity is not constant over a wide range of GFRs, such that, at the top end of kidney function, it takes a very large reduction in GFR to produce considerable increases in sCr (Molitoris, 2016; Devarajan, 2010). This is problematic in CKD screening, with the potential for sCr to only reflect significant reductions in GFR once an individual has become seriously ill (Devarajan, 2010).
- GFR is non-specific. It only provides an indicator of functional, not structural injury and provides no information regarding the nature or location of renal injury.
- GFR is an insensitive marker of renal health. The response is often delayed, and secondary to structural damage in the kidney (Rosner, 2009; Devarajan, 2008; 2010).
- GFR can be acutely affected by physiological changes upstream of kidney in response to dehydration, physical exercise, and heat exposure via reductions in renal blood flow, also known as ‘Pre-renal’ AKI (Basile, 2012; Devarajan, 2006). These physiological changes are common in occupational settings that require prolonged manual labour in hot conditions. Thus, GFR may be a reflection of transient, benign reductions in renal blood flow, rather than intrinsic AKI, and should be interpreted with caution in this context. This being said, the significance of pre-renal stress in eliciting long term damage is not known, especially when episodes are recurrent with minimal recovery.
- Renal blood flow and GFR are also impaired by drugs such as NSAIDs (Whelton, 1999; Saker, 2000). This is of particular relevance in occupations with high workloads

and musculoskeletal problems, which has resulted in a reported overuse of NSAIDs (Orantes, 2009; Wegman, 2018; O'Donnell, 2011).

Alternative measures of eGFR

Serum cystatin C is a more accurate measure of eGFR in populations where muscle metabolism can interfere with sCr (Kar, 2018; Kellum, 2012; KDIGO, 2012). Cystatin C is produced from all nucleated cells at a constant rate. Therefore, it is largely unaffected by diet, age, sex, race, or muscle mass, and has demonstrated superior sensitivity, compared to sCr (Newman, 1995; Christensson, 2003). Another benefit of using serum cystatin C in conjunction to sCr, is the potential to provide insight into the mechanism of declining renal function. Creatinine is a smaller protein compared to cystatin C (113 Da vs 13, 300 Da, respectively), therefore, if eGFR values based on sCr are higher than that of cystatin C, it implies that the glomerular pores are reduced in size. This reduction in pore size may result from glomerular hypertrophy, a pathological process that can occur with chronic inflammation and injury (Wijkstrom, 2013; Grubb, 2015). Cystatin C does have some limitations, such as the influence of glucocorticoid use, and thyroid dysfunction (SBU, 2013). Furthermore, it is not as widely used as sCr. Therefore, between study comparisons are currently limited. Although, cystatin C may become more relevant in the future.

Urinary Albumin: Creatinine Ratio (ACR)

Urinary Albumin: Creatinine ratio (ACR) provides a measure of albuminuria, the excretion of abnormal quantities of albumin in the urine (> 30mg/g). ACR gives a more accurate measure of albuminuria compared to albumin alone. This is because urinary creatinine provides a surrogate measure of urine flow rate, allowing urinary concentration to be corrected for (Schlader, 2019; Beierwaltes, 2013; Ellam, 2011). Albuminuria is primarily indicative of glomerular dysfunction and is a well-established consequence of CKD caused by traditional risk factors (Cravedi, 2013; Levin, 2013). However, as previously discussed, albuminuria is absent in CKDnt, potentially indicating a tubular rather than glomerular aetiology. Thus, measures of ACR can be used to discriminate CKD from CKDnt (Garcia-Trabanino, 2005; Torres, 2010; O'Donnell, 2011; Wijkstrom, 2013; 2017; Caplin, 2017).

Albuminuria may also be a useful tool to assess acute kidney injury or stress in occupational settings. This is because tubulointerstitial damage is known to impaired tubular reabsorptive capacity and potentially activate a renal albumin gene, thereby increasing urinary albumin excretion (Ware, 2010; Bolisetty, 2011; Wolyneic, 2016).

Advantages

- ACR corrects for urinary concentration without the need for timed urine samples, which are impractical and time-consuming in field settings.
- ACR correlates well with the 24-hour urine albumin excretion (the gold standard), particularly when assessed from a first morning bladder void (Schwab, 1987; Witte, 2009; heerspink, 2010; Jermendy 2001; KDIGO, 2013).
- ACR is a more accurate predictor of adverse renal outcomes compared to urinary albumin alone (Kestenbaum and Boer, 2010; Bakker, 1999; Ellam, 2011; Ruggenenti, 1998; Heerspink, 2010).
- International clinical guidelines recommend ACR as the most accurate method for CKD diagnoses and classification (Levin, 2013; KDIGO, 2013).
- Albumin and creatinine immunoassays are less susceptible to interference from urinary contaminants compared to dipstick analysis.
- ACR is sensitive at all levels of albuminuria and can accurately detecting small amounts of albumin in urine unlike dipstick tests (Park, 2017; Konta, 2007; Lim, 2014; National Kidney Foundation, 2002).
- ACR can provide additional information on overall health, such as low muscle mass and declining GFR, which both manifest as an attenuation in urinary creatinine secretion and increased ACR (Heerspink, 2010; Ruggenenti, 1998).

Disadvantages

- Creatinine is affected by muscle metabolism, which may cause ACR variability within, and between participants, independent of kidney function (Heerspink 2010; Ellam, 2011).
    - Particularly relevant in CKDnt populations who experience high occupational workloads, and heat stress, both of which increase the risk of developing rhabdomyolysis (Junglee, 2013).
- ACR can be confounded by various physiological variables independent of kidney disease or injury, including prolonged/strenuous exercise, posture, hydration status, and fever. These stressors can cause transient increases in glomerular permeability, and an acute proteinuria response (Mogensen, 1995; Poortmans, 1984; Brandt, 2010; Gurevich, 2018). Urinary creatinine can also be affected by exercise and dehydration, due to transient reductions in GFR.

    - This is particularly relevant in occupational settings, whereby workers perform prolonged bouts of manual labour, increasing the likelihood of benign glomerular proteinuria. This can impact cross-shift ACR measures, or baseline ACR measures if urine samples are given too close to cessation of exercise (i.e., within 24 hours). Furthermore, cross-shift ACR measures may also be affected by workers drinking large quantities of fluid just prior to giving their urine sample (e.g., in-between stropping work and giving sample).
    - Changes in posture (orthostatic proteinuria) causes diurnal variations in albuminuria (Koopman, 1989), which can confound ACR measures taken at different times of the day.
- Certain medications can also influence ACR, such as NSAIDs and antibiotics (Ahmed, 1997; Mogensen, 1995; Simerville, 2005)
    - This is of particular importance given the reported overuse of painkillers, especially NSAIDs in occupational populations at risk for CKDnt (Orantes, 2009; Wegman, 2018; O'Donnell, 2011).
- Urine samples in field settings can be impractical if toilet access is limited or unsanitary.
- Baseline ACR measures usually use first morning bladder voids, which can be poorly adhered to and relies on participants remembering to do so.
- ACR measurement requires lab analysis, so results are not immediately available (in contrast to dipstick analysis).
- There is currently no reference standard for albumin assays and calibration. Therefore, the use of different methods may impact cross-laboratory comparisons (Miller, 2008)
- The use of ACR in large-scale field studies may be limited, due to greater expertise, expense, and resources required for analysis, with dipstick analysis favoured as an initial screening measure (Caplin, 2017).
- The role of albuminuria in AKI/CKDnt is inconclusive and requires further research (Junglee, 2012). Furthermore, cross-shift increases in urinary albumin should be interpreted with caution in occupational populations due to the confounding effect of post-exercise proteinuria, which is likely to occur following prolonged manual labour in hot conditions (Poortmans, 1984; Wolyneic, 2019).

Urinary dipstick tests; albumin, glucose, urine specific gravity

Dipstick urinalysis is primarily used as a low-cost initial screening measure. Urine analytes that are commonly assessed include albumin, glucose, and specific gravity of urine.
In occupational field studies, the assessment of albuminuria and glucose can help identify people with traditional CKD, as previously discussed (Caplin, 2017).
The assessment of urine specific gravity (USG) provides a measure of urine osmolality, and the kidneys urinary concentrating ability (Leehay, 2013; Roberts, 2015; Simervillle, 2005). Assessing USG also improves the reliability of albumin and glucose dipstick analysis, by indicating whether false positives/negatives may have occurred as a result of extremely dilute or concentrated urine. This may be particularly relevant in workers performing prolonged manual labour in hot conditions.

Advantages

- Dipstick tests are inexpensive, require minimal expertise, and they can be easily/quickly carried out without the need for specialised equipment or resources. This is beneficial for large-scale field studies, especially in low-resource communities. Dipstick tests are also suitable as an initial screening measure to detect any overt abnormalities that require further investigation with more specific tests.
- Results are obtained instantly, without any need for laboratory analysis.
- Spot urine samples remove the need for 24-hour or timed urine collections, which are impractical and costly in large-scale field studies, and are often not well adhered to.
- Dipstick tests have demonstrated good accuracy, sensitivity, and low false detection rates, for 'severe' albuminuria ≥300 mg/g (Park, 2017; Lim, 2014; White, 2011).

Disadvantages

- Dipstick urinalysis does not correct for urine concentration. Therefore, highly concentrated or dilute urine can give false dipstick readings (Simmerville, 2005; Delanghe, 2014). For example, extremely concentrated urine, often yields false positives for albuminuria. This is of particular importance when measuring resting albuminuria as this is often measured from first morning bladder voids, when urine is the most concentrated.
- The accuracy of dipstick albuminuria is poor when detecting small amounts of protein in the urine, which makes dipstick testing for low-mild albuminuria less reliable.

(Park, 2017; Zamanzad, 2009; Baumgarten, 2011; Simerville, 2005; Sheets, 1986; Lim, 2014). This may be significant when screening for CKDnt in occupational settings, where participants typically have low-grade proteinuria (Torres, 2010; O'Donnell, 2011, Cravedi, 2013).

- The chemical reaction on urinary dipstick tests are susceptible to interference from red blood cells, uric acid, bacteria, low pH, and myoglobin. This increases the likelihood of false positives and negatives (Simerville, 2005; Nagrebetsky, 2012; Han, 2013; Delanghe, 2014).
    - Haematuria, urinary tract infections, hyperuricemia, dehydration, acidic urine, and rhabdomyolysis have been reported amongst agricultural workers in Central America and are conditions commonly associated with heat stress and prolonged exercise (Glaser, 2016; Crowe, 2014; Garcia-trabanino, 2015; Brooks, 2009; Borg, 2019).
- There are numerous confounding variables that can acutely affect urinary protein excretion, independent of kidney injury or disease. This reduces the reliability of albuminuria from a single spot sample. Transient proteinuria can be caused by physical activity, dehydration, fever, and posture (Mogensen, 1995; Witte, 2009; Simerville, 2005; Poortmans and Jeanloz, 1968; Poortmans,1984; Gurevich, 2018). This is particularly problematic in occupations that are frequently exposed to heat stress and prolonged physical activity.
- Albuminuria can also be confounded by certain comorbidities that cause persistent proteinuria, such as cardiovascular disease, hypertension, and diabetes. In addition, certain medications such as antibiotics and NSAIDs can also affect urinary albumin excretion (Simmerville, 2005; Baumgarten, 2011; Mogensen, 1995). The latter is particularly relevant in agricultural workers in Central America, where there is a reported overuse of painkillers, especially NSAIDs (Orantes, 2009; Wegman, 2018; O'Donnell, 2011).
- Glycosuria is frequently reported in non-diabetic CKD as a result of impaired tubular function (Lee, 2020; Hall, 2014; Foreman, 2019; Hung, 2016). Therefore, glycosuria may be also consequence of established CKDnt, and not exclusive to diabetes.
- Collection of urine samples in field studies may be difficult if there is a lack of toilet accessibility, and sanitation.

- First morning bladder voids are usually favoured for baseline measures however this is not often well adhered to.

Small Proteins of Tubular Damage

The need for a more specific, sensitive biomarker of kidney injury is growing, especially in occupational populations at risk of CKDnt, where the insensitivity of current diagnostic tools allows many cases of subclinical AKI to go undetected. Such cases of subclinical AKI, or 'kidney stress', may have the potential to cause long term damage if episodes are recurrent and recovery is minimal (Schlader, 2019; Hansson, 2020). Thus, detection of subclinical AKI could identify individuals at an increased risk of developing CKDnt.

Research into the identification of a novel AKI biomarker is constantly evolving (Vaidya, 2008; Rosner, 2009; Ostermann, 2015). Biomarkers that have shown promise in early AKI detection include, NGAL, KIM-1, IGFBP-7, L-FABP, IL-18, β2-Microglobulin, Trefoil Factor-3 (Alge, 2015; Devarajan, 2007; Rosner, 2009; Barrera-Chimal, 2012). These small proteins are predominantly detected in the urine and are expressed by tubular cells upon exposure to injurious stimuli. Alternatively, some novel biomarkers are detected in the blood, secondary to a decline in GFR (Devarajan, 2007; Rosner, 2009).

While tubular damage proteins are primarily indicative of AKI, there is also evidence to suggest they have a role in early CKD detection, specifically in the AKI-CKD transition (Ko, 2010; Devarajan, 2010). This overcomes a significant problem in current CKD screening, in that GFR may not decline significantly until an individual becomes severely ill, at which point intervention/treatment may be too late.

Advantages

- Small tubular damage proteins are more sensitive measures of kidney injury compared to traditional markers of kidney function, with the ability to detect early signs of structural injury prior to any changes in sCr (Ostermann, 2015; 2016; Rosner, 2009; Vaidya, 2008).
    - L-FABP, IGFBP-7, NGAL, IL-18, and KIM-1 have shown to be more sensitive than sCr in hospital settings (Doi, 2011; Negishi, 2009; Kashani, 2013; Arthur, 2014; Vaidya, 2010; Nickolas, 2008), and urinary IGFBP-7/TIMP-2 are currently the only validated biomarkers approved by the FDA for AKI risk identification and have the ability to 'predict' subsequent AKI (Kashani, 2013; Endre, 2014).

- While there is limited evidence to show the superiority of certain biomarkers in occupational settings, or in the context of heat stress/high workloads, numerous studies have demonstrated that increases in urinary and plasma NGAL correspond to eGFR declines, following physical work in the heat. This is evident in laboratory settings (Chapman, 2020; Schlader, 2017; Junglee, 2013) and in occupational field studies, (Wesseling, 2016; Laws, 2016; Sorensen, 2019). Furthermore, IGFBP-7 has shown promise as a sensitive biomarker of kidney strain in the context of exertional heat stress. Chapman et al (2020) demonstrated that IGFBP-7 was the only urinary biomarker of kidney injury to increase after normalising to urine flow rate, suggesting high sensitivity to small changes in renal function.

- Different tubular proteins reflect different pathophysiological processes in AKI, such as tubular injury, cell cycle arrest, pro-inflammatory pathways, oxidative stress etc. Thus, different biomarkers can provide a more mechanistic understanding of AKI (Schaub, 2016; Ostermann, 2015; 2016).
- Tubular proteins often originate from specific regions of the nephron, and can indicate site-specific injury (Schlader, 2019; Schaub, 2016). Therefore, selecting a panel of different biomarkers can allow researchers to create a 'map' of structural damage. For example, IGFBP-7 is primarily secreted from proximal renal tubular cells, whereas TIMP-2 is secreted from distal tubular cells (Emlet, 2017; Chapman, 2020).
- The excretion rates of different tubular proteins can indicate different stages of the injurious process, such as injury risk, established injury, recovery, or transition to CKD (Vaidya, 2008; Ostermann, 2015; 2016).
- Tubular damage proteins have a role in identifying early signs of CKD, even before declines in GFR, with sustained elevations of certain biomarkers potentially indicating the transition from AKI to CKD (Ko, 2010; Devarajan, 2010). Some biomarkers may also have a superior ability to predict CKD progression and mortality, in comparison to traditional markers such as proteinuria and GFR (Fassett, 2011).
- Comparing the response of sCr to that of tubular damage proteins can help determine the nature of AKI (Singer, 2011). For example, an elevation in sCr without tubular proteins would suggest functional, or pre-renal AKI, rather than intrinsic injury.

Disadvantages

- Many small proteins of tubular damage are still undergoing validation as a biomarker of kidney injury/disease. Furthermore, research in this area is constantly evolving with new proteins frequently being discovered. This may limit comparisons between different studies, and compromise the future relevance of research, with uncertainty over the correct tubular protein to use in specific populations and environments. The novelty of many of these small tubular markers also complicates the interpretation of results, as the lack of reference standard (to indicate what is clinically relevant or meaningful) means that different studies can have different cut-offs to establish negative and positive results (Ostermann, 2015).
- Some tubular markers are much more sensitive than traditional AKI measures and can reflect an increased risk of AKI prior to any structural damage. While this can be advantageous in detecting sub-clinical AKI, the long-term implications of subclinical AKI or AKI 'risk' are not well understood (Schlader, 2019).
- The majority of research to date has focussed on hospitalised patients in clinical settings, with variable and limited research demonstrating the successful use of small tubular markers in occupational settings, or in context of heat stress and high workloads.
- AKI is multifactorial, a single tubular marker is unable to reflect all aspects of injury. A panel of biomarkers is considered most favourable; however, this is expensive.
- Analysis of tubular damage markers with specific assays can be expensive and more time consuming than other kidney function measures, especially for large scale studies. This type of analysis also requires laboratory expertise.
- The temporal response of these small tubular proteins in response to injury is not well understood, which complicates the timing of when to take a sample. This can be especially problematic when using a panel of different markers.
- Urinary markers of tubular damage require normalisation to urine concentration, however, there is no single recommended protocol, which limits between study comparisons (Ostermann, 2015). Normalisation can be done via urine osmolality, urinary creatinine, or urine flow rate. However, the use of urinary creatinine is unreliable in exercise contexts, and urine flow rate requires timed urine samples therefore, is time consuming in field settings (Schlader, 2019; Waikar, 2010; Junglee, 2012; 2013).

- The role of tubular damage proteins in early CKD detection/monitoring are new and have variable results (Devarajan, 2010; Fassett, 2011; Lopez-Giacoman, 2015).
- Many tubular damage proteins have non-renal origins (heart, lung, gastrointestinal, liver), with assays unable to distinguish between different origins. Furthermore, some small tubular proteins can be confounded by comorbidities, or physiological changes upstream of the kidney such as hypertension, systemic infections, inflammatory conditions, or anaemia (Ostermann, 2015; Martensson, 2014; Schrezenmeier, 2016; Lin, 2015; Parikh, 2004; Deverajan, 2010).
- Baseline renal function or abnormalities in the urinary tract can affect how small tubular markers respond to injury. Specifically, urinary NGAL has been shown to be a poor indicator of AKI in individuals with compromised baseline renal function (McIlroy, 2010; Devarajan, 2007), and can be affected by urine tract infections or neutrophil count in urine (Delanghe, 2014).

Urinary Sediment

Urinary sediment refers to the presence of microscopic particles in the urine, including, isolated blood cells or renal epithelial cells, crystals, microorganisms, or casts (cylindrical structures primarily consisting of solidified proteins) (Simerville, 2005; Perazella, 2015; Ringsrud, 2001). The urine of healthy, resting individuals should only have few sedimentary particles; thus, urinary sediment can be indicative of renal stress, injury, or disease.
Urinary sediment is more commonly associated with acute renal injury. A previous study using microscopic urinalysis has shown evidence of leukocytes, casts, and crystals in post-shift urine samples of workers exposed to high workloads/heat stress (Garcia-trabanino, 2015). This type of urinary sediment is also frequently seen in response to prolonged bouts of exercise or hyperthermia (Poortmans, 1984; Kew, 1967; Schrier, 1970). While urinary sediment is less common and relatively minimal in CKDnt (Lusco 2018; Wijkstrom, 2017; 2013; O'Donnel, 2011), there are some reports of mild leukocyturia and haematuria in workers with declining renal function (Glaser, 2016; Hansson, 2020; Muiru, 2020).

Advantages

- Sediment is a more specific and mechanistic marker of kidney injury compared to serum creatinine. The type of sediment can indicate the nature of renal injury (pre-renal vs. intrinsic), the cause of renal injury, and the location of injury along the nephron

(tubular vs glomerular) (Simerville, 2005; Perazella, 2015; Devarajan, 2006; Thadhani, 1996).

- The presence of cellular casts, leukocytes, and renal epithelial cells are usually indicative of tubulointerstitial damage and inflammation (Simerville, 2005; Perazella, 2015; Devarajan, 2006). These types of urinary sediments are particularly relevant in CKDnt populations as the lack of significant proteinuria has prompted researchers to believe this disease is largely tubulointerstitial in origin (Peraza, 2012; O'Donnell, 2011). Urinary erythrocytes tend to indicate glomerular damage (Perazella, 2015; Simerville, 2005). This is also relevant in populations at risk for CKDnt, as haematuria and glomerular damage have been reported in individuals with declining renal function and established CKDnt (Hansson, 2020; Muiru, 2020; Wijkstrom, 2017).
- Acellular urinary casts are typically caused by functional or pre-renal injury, due to a reduction in renal blood flow. The formation of acellular urinary casts are further exacerbated by low urine pH (Perazella, 2015). Given that strenuous exercise and heat stress have been shown to cause reductions in renal blood flow (Smith, 1952; Volianitis, 2016; Low, 2011; Johnson, 2014), it is likely that acellular urinary casts may be prevalent in in occupational populations working under such conditions.

- Urinary sediment is not only a useful marker of intrinsic kidney injury but can also indicates the presence of muscle damage and hyperuricemia, which are also risk factors for AKI, especially following exertional heat stress (Devarajan, 2006; Thadhani, 1996; Patel, 2009). Urinary casts containing myoglobin would be indicative of muscle damage, and urinary crystals of hyperuricemia. This may provide an indirect measure of AKI risk.
- Sediment can predict adverse renal outcomes following AKI in hospitalised patients (Chawla, 2008). Therefore, urinary sediment measures may be beneficial in identifying workers at risk of developing CKDnt in the future.

Disadvantages

- Microscopic urinalysis is costly and impractical for large scale studies.
- There is minimal evidence and research involving the use of sediment as a marker of renal injury in occupational populations.

- Urinary sediment can be affected by urinary concentration (correct for urinary flow, urinary concentration, or urinary creatinine). For example, false negatives are likely with very dilute urine. This may an issue if large quantities of fluid are consumed prior to sampling (e.g., at the end of a work shift).
- Urinary sediment analysis is less useful in workers with established CKDnt. Most studies report inactive sediment in those with CKDnt (Lusco 2018; Wijkstrom, 2017; 2013; O'Donnel, 2011).
- Sediment can be indicative of mild renal stress following prolonged exercise/dehydration, rather than intrinsic kidney damage. It is unknown what severity of urinary sediment represents 'significant' kidney injury and increased risk of long-term damage.
- Urinary sediment can occur in the absence of kidney injury. For example, following muscle breakdown, urinary tract infections, drug usage (e.g., loop diuretics, acetazolamide, certain antibiotics and antiviral medications) (Ringsrud, 2001; Matlaga, 2003).
- Identifying microscopic urinary sediment can be difficult, time consuming, and requires a high level of expertise.

2.3 Recommendations

Timing of measures

Baseline or resting measures

- The time of day at which baseline/resting measures of kidney function are taken is of particular importance when assessing albuminuria. First morning bladder voids are considered to be the most reliable for albuminuria (ACR and dipstick) and closer to 24-hour measures of urinary album excretion (considered the 'gold standard'), compared to random spot urine samples at other times of day (Witte, 2009; Koopman, 1989). This is because morning samples are less influenced by orthostatic proteinuria, or activity levels during the day (Hansen, 2002; Mogensen, 1995; Witte, 2009*).* First morning bladder voids are also preferred when assessing urinary sediment, as erythrocytes are best preserved in more acidic and concentrated urine.
- Baseline measures should not be taken within 12-24 hours of strenuous or prolonged exercise:

- sCr is positively influenced by exercised induced reductions in GFR and can potentially be falsely elevated due to muscle breakdown following strenuous exercise, particularly in the heat (Devarajan, 2006; Perrone, 1992).
- Albuminuria will be influenced by a transient post-exercise proteinuria response (Poortmans, 1984; Mogensen, 1995), which can cause elevations in urinary protein excretion for up to 4 hours (Poortmans et al, 1989).
- Strenuous exercise is capable of eliciting intrinsic AKI, which may alter baseline measures of tubular AKI biomarkers in the urine (Hodgson, 2017; Bongers, 2018; Mccullough, 2011). Blood biomarkers of AKI such as NGAL will also be affected by acute reductions in GFR following exercise.

Cross-shift measures

- It is difficult to state definitive recommendations for the timing of cross-shift kidney function biomarkers. This is because there are limited studies assessing their temporal response in the context of heat stress and exercise. Therefore, the exact point at which these biomarkers peak following exercise in the heat is unknown. The response will likely depend on the intensity/length of exercise, and severity of heat/humidity exposure (Schlader, 2017, Chapman, 2020; Bongers, 2018; Sato, 2019). Based on the research done thus far, some recommendations are outlined below:

- For cross-shift measures of sCr, blood samples should be taken close to the cessation of work, within an hour post-shift. Chapman et al, (2020) demonstrated that sCr concentrations following prolonged exercise in the heat, were greater 20-minutes post exercise, compared to 1-hour post exercise, suggesting that sCr peaks somewhere between exercise cessation and an hour post-exercise (Chapman, 2020). This being said, Schlader et al (2017) suggests a slightly more delayed peak, with higher levels of serum sCr at 1-hour post-exercise, vs 20 minutes post-exercise. Taken together, this suggests that measures taken much later than 1 hour may not capture maximum sCr values post-exercise/work .
- For cross-shift assessment of urinary tubular damage proteins, there is evidence to suggest that samples should be taken slightly later than blood biomarkers such as sCr. Chapman et al, (2020), demonstrated higher urinary NGAL and urinary IGFBP-7 concentrations at 1 -hour post-exercise/heat stress, compared to 20 minutes post, where sCr peaked.

- Excessive fluid intake between work cessation and sampling can cause an acute increase in urine flow rate and GFR. Therefore, urine samples for the assessment of post-shift AKI should be taken before fluid consumption.

Other considerations regarding measurement timings:

- Blood samples for sCr measurements should ideally be taken approximately 12 hours after consumption of cooked meat, as this can positively affect sCr values (Jacobsen, 1979; Nair, 2014). Assessment of a populations' diet habits can help inform if and when sCr may be affected by consumption of cooked meat.
- Repeat measures should be carried out at the same time of day, due to the influence of diurnal variations on numerous kidney function biomarkers, such as sCr, albuminuria, and tubular AKI biomarkers (Jacobsen, 1979; Koopman, 1989; Helmersson-Karlqvist, 2013).
- Measures of baseline kidney function should ideally be repeated, on 2-3 occasions over a period of 3 months (Martin, 2011; Levin, 2013). This is to ensure a spot sample is representative of an individual's true kidney function and to confirm that kidney function measures are consistent, and not falsely increased by confounding variables such as acute hydration/fever/exercise at the time of sampling,
- Additional tests should be performed if a participant is unwell or dehydrated at the time of sampling (Baumgarten, 2011; Athuraliya, 2011).

Controlling Confounding variables

Independently of any changes in kidney function, biomarkers of kidney function and injury can be influenced by numerous variables, which should be recorded when measuring kidney function to improve the reliability of measures.

- Physical activity: A cohort's activity 24-48 hours prior to sampling should be recorded and controlled for where possible due to the acute influence of strenuous exercise on GFR, glomerular permeability, and muscle damage. Not controlling for physical activity can confound sCr, blood NGAL, and albuminuria measures.
- Dietary habits: Recording and understanding the dietary habits of your study population can be an important consideration, particularly the consumption of meat as

this acutely causes elevations in sCr, which can take 12 hours to normalise (Jacobsen, 1979; Nair, 2014).

- Hydration status (e.g., via urine osmolality) should be assessed alongside kidney function measures. Dehydration can cause AKI via pre-renal reductions in GFR (Smith, 1952; Nadal, 1941), which will cause elevations in blood markers such as serum creatinine and NGAL. Therefore, it is important for workers to be in a euhydrated state when sampling for baseline kidney function measures. Furthermore, unless urine concentration is corrected for, hydration status can result in false positives and negatives when assessing urinary markers if the urine is overly concentrated or dilute, respectively.
- Drug and medication usage: Habitual use of medication and drugs should be recorded in working populations. This is particularly noteworthy in occupations performing strenuous manual labour, due to a higher prevalence of musculoskeletal problems, and reported overuse of NSAIDs, which are known to cause reductions in renal blood flow and GFR.
- Underlying medical conditions such as diabetes, and hypertension should be recorded in a population, as previously discussed. These are both risk factors for typical CKD and will allow cases of CKDnt to be discriminated (Caplin, 2017).
- Overall health should be recorded. Fever and UTIs can influence albuminuria, and other illnesses involving vomiting/diarrhoea could potentially skew GFR measures via dehydration.
- Anthropometric data such as body composition, or body mass is important to assess in a cohort. This provides an indicator of muscle mass, which influences muscle catabolism and thus, measures of kidney function involving creatinine (sCr, ACR) (Caplin, 2017).
- Assay calibration method: Different methods of serum creatinine assay calibration can affect the result, so assays should be calibrated with a standardised method, traceable to an isotope dilution mass spectrometry (IDMS) reference. This will improve the reliability of measures reported within a study as well as the comparisons between laboratories/studies (Levin, 2013; Caplin, 2017; Myers, 2006; Coresh, 2002).
- eGFR estimating equations: As previously discussed, to minimise the variable influence of muscle metabolism on sCr levels within and between participants, GFR should not be estimated based on sCr alone. Clinical practice guidelines recommend

using the CKD-epi and MDRD equations to estimate GFR (Levin, 2013). The more recent CKD-epi equation is preferred as it demonstrates less bias than the MDRD equation, especially at higher GFR's (>60 ml/min/1.73 m2) (Levey, 2009; Levin, 2013). However, equations should be used with caution as they are not valid in all ages/body types/ethnicities. Alternative equations can be used that exhibit less bias in certain subgroups, however, the type of equation should be recorded to allow for reliable cross-study comparisons.

General considerations for urinary measures

- Normalisation of urinary biomarkers to urine concentration should be considered as extremely dilute or concentrated urine can lead to false negatives or positives, respectively.
  - Normalisation to urinary creatinine is the most common method currently used in practice (Glassford, 2013; Helmersson-Karlqvist, 2013; Mishra, 2005; Parikh, 2004). However, urinary creatinine excretion can vary with exercise induces reductions GFR, and muscle metabolism (Schlader, 2019; Junglee, 2012; Ellam, 2011). Therefore, when measuring urinary biomarkers in exercise/heat stress contexts, urine concentration should be corrected for using a measure of urine osmolality. Timed urine samples are also an option (Waikar, 2010; Chapman, 2020; Schlader, 2019), although they are time consuming and impractical in field settings.
  - The recommended protocol for urinary concentration normalisation is uncertain due to the lack of standardised procedures for many biomarkers.
- General protocols for urinary sample collection and storage:
  - Midstream urine sample collection is usually advised to reduce the risk of contamination from bacteria (Simerville, 2005).
  - Collection of an adequate volume to prevent any sample point losses (5- 12 mL) (Miller, 2008; Martin, 2011; Delanghe, 2014).
  - Urine samples that are extremely cloudy or have residual precipitate (urinary sediment), should be centrifuged prior to analysis (Matin, 2011; Miller, 2008)
  - Many biomarkers are unstable and vulnerable to significant degradation if urine samples are left too long at room temperature. Therefore, samples should be refrigerated or frozen if unable to be analysed within 24 hours (Schuh et al, 2016; Delange, 2014; Simerville, 2005). Preservative/stabilizer should also be used

(Simerville, 2005; Delanghe, 2014; Martin, 2011), and multiple freeze-thaw cycles should be minimised.

- Dipsticks should not be submerged in the urine for too long (Delanghe, 2014), or exposed to air for prolonged periods (Cohen, 1991), to minimise false positives.

General considerations for blood measures

- Post-shift blood samples should ideally be taken after a period of seated or supine rest for 15-20minutes following cessation of physical activity, to allow for postural changes in plasma volume to normalise (Schlader, 2017; Hagan, 1978).
- General protocols for serum creatinine sample collection and storage:
  - Ideally samples should be analysed as soon as possible following collection. At room temperature, significant changes can be seen 10-24 hours following collection (shepard, 2007).
  - If samples cannot be analysed within 24 hours, they should be refrigerated, as they are more stable and resilient to protein degradation at temperatures between +2°C to +8°C. (CDC, 2004).
  - If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed (CDC, 2004). Freezing samples for greater than 10 days can also cause increases in creatinine values (Vernekar, 2017).
- General protocol for Plasma NGAL:
  - In plasma, NGAL is susceptible to significant degradation at room temperature (Wang, 2015). Thus, to improve the stability of NGAL measures from plasma, samples should be stored at 4 °C for the short-term, as plasma samples have been shown to be stable at this temperature for 48 hours (Pederson, 2010).
  - For longer term storage, plasma NGAL should be frozen. Plasma samples have been shown to be stable at -80°C for 11 months, however, freeze-thaw cycles should be limited to 3 (Wang, 2015; Pederson, 2010).

3. Recovery Study

3.1 Background

The importance of recovery in the possible pathogenesis of CKDnt has recently been outlined by Hansson et al (2020) and shares many parallels with the well-understood 'overtraining syndrome' in athletes (Kreher and Schwartz, 2012). In the 'overtraining syndrome', a maladaptive inflammatory response develops, due to frequent exposure to physiological stressors associated with intense exercise and insufficient recovery. It is possible that the development of CKDnt has a similar aetiology, with the physiological stressors primarily hypothesised to be heat stress, high workloads, and dehydration (Schlader, 2019, Wesseling, 2016; Correa-Rotter, 2014; Glaser, 2016). Acutely, this can cause episodes of renal inflammation, oxidative stress, and energy depletion, increasing the risk of acute kidney injury or strain (Bellomo, 2012; Devarajan, 2006; Chapman, 2020; Schlader, 2017; 2019). Due to the immediate activation of repair pathways, such episodes are often transient in nature (Basile, 2012). However, AKI can become problematic when experienced repeatedly, with insufficient recovery time. This prevents renal cells from fully repairing, inducing chronic inflammation and a maladaptive repair process, characterised by excessive cell proliferation and renal fibrosis. Collectively, these processes can render regions of the kidney non-functional (Basile, 2012; Hansson, 2019; Venkatachalam, 2015), which is consistent with findings of interstitial fibrosis amongst renal biopsies of CKDnt patients (Wijkstrom, 2013).

Industrial agricultural workers in Central America also experience unfavourable work-rest schedules, characterised by long, strenuous work shifts in hot conditions, and short rest breaks, resulting in workers spending the majority of their work shift, including breaks, above 50% HR_{max} (Hansson, 2019; Lucas, 2015). Furthermore, workers are often required to work 6 days a week, meaning recovery time is limited between work shifts during a harvest season. Organisational factors within industrial agriculture are likely exacerbating this issue, as there are few organisations protecting the human rights of workers. In addition, the piece-rate nature of the work incentivises workers to adopt higher workloads and take fewer, shorter breaks during their work shift (Wesseling et al, 2014; 2015; Nerbass, 2017; Verite, 2017). Therefore, recovery time may be a crucial, risk factor in the development of CKDnt development amongst agricultural communities (Hansson et al, 2020). Consequently, recovery between work-shifts may be an important factor to consider modifying, or investigating further, to present long term kidney injury.

3.2. Aims and Hypotheses

The aim of this study was to assess the recovery of blood and urine biomarkers after one night's recovery from a work shift (approximately 17 hours) at the end of a working week and compare this to recovery of blood and urine biomarkers after two nights and one day's recovery (approximately 41 hours), at the beginning of a working week, after a day off. We also aimed to compare how these two different recovery periods affected cross-shift changes in blood and urine biomarkers.

Blood and urine biomarkers of kidney function (serum creatinine, cystatin C, urea, uric acid) , hydration status (urine pH, urine specific gravity, serum sodium, potassium, albumin) systemic inflammation (serum CRP), and muscle damage (serum creatine kinase) were assessed. The study also examined internal heat stress, work intensity, fluid intake and environmental heat exposure during a work shift, as well as workers' physical activity, sleep patterns, and ambient temperatures away from work, in order to assess how these parameters may affect blood and urine biomarkers.

It was hypothesised that, recovery of blood and urine biomarkers would be greater after a longer recovery duration of 41 hours (two nights and one day recovery), compared to 17 hours (one night's recovery). We anticipated recovery to be smaller after just 17 hours at the end of the working week, due to the cumulative impact of high workloads and heat stress on kidney function throughout the week, compared to that at the start of the week after a day off (41 hours recovery). In addition, we hypothesised that cross-shift changes in blood and urine biomarkers, particularly kidney function, would be improved following a longer recovery period of 41 hours, compared to just 17 hours.

3.3. Methods

This study was conducted at sugarcane mill Ingenio San Antonio (ISA) in Chinandega, Nicaragua. Data collection occurred during the mid-harvest season, in February 2020, over the course of 4 days: Friday 21st-Monday 24th. Friday, Saturday, and Monday were working days, while Sunday was a rest day.

Participants were recruited from workers already participating in a larger cross-harvest study (see Hansson et al., 2019), and included 20 male burned cane cutters, age 33 ± 7 years, body mass 68.3 ± 11.2 kg, and height 164 ± 5 cm. A Water. Rest. Shade (W.R.S) intervention programme formed part of this larger cross-harvest study. This W.R.S. intervention included: a 2-week acclimatisation period, access to water (Personal 5 L thermos as well as 40 L thermos),

access to 300mL electrolyte solution (containing 7g of sugar, 50mg sodium chloride and 20mg potassium monophosphate per 100mL); access to tents for rest in shade during mandated breaks (≤50 m from every cutter); movable tents made of a netted fabric that open on two sides, thus giving shade while also being ventilated; stools for seated, shaded rest, mandatory 10-20-min rest breaks each hour from 8am onwards.
Ethical approval for this study was given by the Comité de Ética para Investigaciones Biomédicas (CEIB), Facultad de Ciencias Médicas, Universidad Nacional Autónoma de Nicaragua (UNAN - León (FWA00004523/IRB00003342).

Study Design

Data collection on Working days

At the start of each day, all workers were equipped with a heart rate monitor immediately before they started work. Heart rate (HR) was then measured continuously throughout each work shift (Friday, Saturday, Monday), for assessment of individual work intensity. Gastro-intestinal temperature (T_{GI}) was measured continuously throughout the work shift, to assess internal heat stress. T_{GI} was only collected in nine participants (Group 1, worker IDs: 3, 5, 6, 8, 14, 15, 18, 19, 20) on Friday and another nine participants (Group 2, worker IDs: 1, 2, 4, 7, 9, 10, 11, 12, 13) on the Monday, due to limited number of T_{GI} receivers.
Blood and urine measures were taken, pre- and post-shift, for the assessment of kidney function, hydration status, systemic inflammation, and muscle damage. During work shifts, qualitative work observations were also performed by occupational hygienists and senior occupational physicians.
At the end of each working day, workers completed a brief questionnaire, including ratings of perceived exertion (RPE) using a 1-10 scale where 1=’at rest’ and 10 =’maximal effort’, and litres of fluid consumed throughout the shift.

Rest/home measures

Environmental heat stress at home was assessed using ibutton temperature sensors. These ibuttons were attached to key fobs and worn by workers on their belt loops or attached to lanyards and worn around their neck. Workers were given their ibuttons on the Friday and asked to wear these until the end of their Saturday work shift (Fri 1pm-Sat 6am). Participants were given a full charged ibutton on Saturday post-shift and asked to wear them until the end of Monday (Sat 1pm – Mon 6am). Physical activity outside work and sleep activity was

assessed during two workdays (Friday & Saturday) and one rest day (Sunday), using wrist-based accelerometers and HR monitors.

Instrumentation and measurements

For the assessment of blood biomarkers, 10-20mL serum was collected at five time points across the 4-day protocol: Friday post-shift, Saturday pre- and post- shift, and Monday pre- and post-shift. Serum samples were analysed for creatinine, cystatin-C, C-reactive protein (CRP), creatinine kinase (CK), sodium, potassium, urea, and uric acid. Serum samples were separated at a laboratory in ISA. Samples were then frozen (−77°C), before being transported to Skåne University Hospital in Lund, Sweden, for analysis, using a Cobas 701 instrument (Roche Diagnostics, Basel, Switzerland). Urine samples were collected at five time points across the 4-day protocol: Friday post-shift, Saturday pre- and post- shift, and Monday pre- and post-shift. Urine samples were analysed in the field using dipstick tests for specific gravity, pH, leukocytes, and blood (Siemens Multistix Reagent Strips). Heart rate (HR) was continuously recorded from a sensor fitted on a chest strap (RC3X or Polar Team Pro Sensor, Polar Electro, Kempele, Finland), to assess work intensity. HR data was also used to calculate predicted core temperature (Buller et al., 2013). Gastrointestinal temperature (T_{GI}) was continuously measured using a gastrointestinal temperature telemetry pill (BodyCap medical, eCelsius Performance capsule, Hérouville Saint-Clair, France), to assess internal heat stress at work.

Environmental heat stress at work was recorded via portable weather stations in field alongside the workers (QuesTemp® 34, 3M; Kestrel 5400 Heat Stress Tracker, Nielsen-Kellerman). Workers also wore individual ibuttons (DS-1921 H model, Thermochron resolution: 0.125°C; Maxim Integrated, San Jose, CA, USA) on key fobs (IMAC electronic solutions, UK) to measure environmental heat stress outside of work. Physical activity at home was recorded using wrist-based accelerometer (Polar A370), which calculated time spend standing/sitting/lying/walking/exercising, total active time, number of steps. Sleep activity was also measured, including, total sleep duration, interruptions, wake time, bedtime, and average sleeping HR.

Data and statistical analysis

All statistical analysis was performed on GraphPad Prism (Version 9.1.2, La Jolla California USA). Statistical tests used to compare HR, T_{GI}, Blood and Urine biomarkers, were calculated

using one-way repeated measures ANOVAs, or mixed-effects models, as appropriate. Post hoc Tukey's test for multiple comparisons, were performed to calculate significant differences between each time point. P-value < 0.05 was considered statistically significant. Correlational analysis was performed using Pearson's correlation test. Data are given as means ± SD, however, individual data has also been presented in graphs for renal function, and correlations.

Work intensity (HR): HR data are expressed as percentage of maximal HR (%HR_{max}), with a regression equation used to predict HR_{max} (208 - 0.7 x age) (Tanaka, Monahan, & Seals, 2001). Work intensity was categorized based on % HR_{max} zones: Maximal (91-100%); Very Hard (81-90%); Hard (71-80%); Moderate (61-70%); Light Moderate (51-60%); Light (≥50%). AUC was also calculated for % HR_{max}, to provide a better gauge of overall cardiac strain (Pruessner et al., 2003).

Environmental heat exposure: Climate data (WBGT, and air temperature) during the work shift were recorded every 30 minutes. Data has been expressed as mean and standard deviations (averaging data over each work shift). Due to some missing data averages only include values between 8:30 and 12:00. Missing data was as follows: Friday (6:30, 9:00), Saturday (6:30-8:00), and Monday (10:30). Absolute maximum WBGT values have also been reported. Estimated time spent above Occupational Safety and Health Administration (OSHA) threshold limits are presented as these threshold limits evaluate an individual's risk of developing heat related illness based off WBGT, workload and work/rest regimen (OSHA, 2017).

Internal heat exposure: Gastrointestinal temperatures (T_{GI}), are expressed as absolute values. Maximum gastrointestinal temperature (T_{GI} max), and AUC for gastrointestinal temperature (T_{GI} AUC) were calculated and used in statistical analysis. T_{GI} was only collected in nine participants on Friday and another nine participants on the Monday, due to limited number of T_{GI} receivers. Correlational analysis using T_{GI} max is therefore limited to Friday and Monday.

Kidney Function: Cross shift changes in serum cystatin C, and serum creatinine are expressed as both absolute values, and % changes from pre- to post-shift ((post-shift – pre-shift/ pre shift) x 100). As there was no pre-shift data collection on Friday, a mean pre-shift value (averaging Saturday and Monday's pre-shift value) was used in % cross-shift calculations. Recovery of kidney function (serum cystatin C and serum creatinine) was calculated following a 17-hour recovery period at the end of a working week (Friday post-shift to Saturday pre-shift) and compared to that after a 41-hour recovery period at the beginning of a working week, after a day off (Saturday post-shift, to Monday pre-shift). Recovery of kidney function

has been calculated as a percentage change.
Glomerular filtration rate was estimated (eGFR), for both serum creatinine ($eGFR_{creat}$) and serum cystatin C ($eGFR_{cys}$) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for all participants (Levey, 2009; Inker 2011; 2012). Cross-shift changes in eGFR data are expressed as absolute values, and percentage changes. eGFR percentage recovery has also been calculated, as above.
Cross-shift changes in serum urea and serum uric acid are expressed as absolute pre-, and post-shift values.
Hydration status: Cross-shift changes in serum albumin, serum potassium, serum sodium, urine pH, and urine specific gravity, are expressed as absolute, pre-, and post-shift values. Urine data were only available for participants 1-15.
Systemic inflammation and muscle damage: Cross-shift changes in serum C reactive protein (CRP), and serum creatine kinase (CK), are expressed as absolute, pre-, and post-shift values.
Bland-Altman analysis: Used to assess the limits of agreement, and bias when comparing eGFR calculated using creatinine ($eGFR_{creat}$), and eGFR calculated using cystatin C ($eGFR_{cys}$). $eGFR_{cys}$ has been preferentially used for correlational analysis, and assessment of clinical CKD, and AKI classification, primarily due to the acute effects of physical exertion on serum creatinine in this population (KDIGO, 2012; National Kidney Foundation, 2002).

3.4. Results

Study context

***Work duration and shift patterns*:** Work shift duration differed between workdays ($p<0.01$). Post hoc comparisons showed that work shift duration was longer on Friday, compared to Saturday ($p<0.01$) and Monday ($p<0.01$). There were no difference between Saturday and Monday ($p=0.7$; table 1). The number of rest periods also differed between workdays ($p<0.01$). Post hoc comparisons showed that workers took more break periods on Friday compared to Saturday ($p<0.01$) and Monday ($p<0.01$), but there was no difference between Saturday and Monday ($p=0.1$; table 1).

Work intensity (heart rate): Rest periods caused a notable decrease in HR throughout the course of each work shift, as illustrated in figure 1. Average % HRmax across the work shift differed between workdays ($p<0.01$). Post hoc comparisons showed no significant differences

between % HRmax Friday and Saturday, or Friday and Monday, however, %HRmax was higher on Monday compared to Saturday ($p<0.01$; table 1).

Average AUC for % HRmax was also different between workdays ($p<0.01$). Post hoc comparisons showed that AUC % HRmax during the Friday work shift was 10% higher than on Saturday ($p<0.01$) and 4% higher than on Monday ($p=0.04$), while AUC % HRmax during Saturday was 6% lower than on Monday ($p=0.02$).

The time (min), and percentage of each shift spent in % HRmax zones, are shown in table 2, and figure 1. Notably, time spent at 71-80% HRmax, was significantly different between workdays ($p=0.01$). Post hoc comparisons showed that, absolute time at 71-80% HRmax was greater during Friday's work shift compared to Saturday's work shift ($p=0.04$), and greater during Monday's work shift compared to Saturday's work shift ($p=0.03$). There were no significant differences between Friday and Monday ($p=0.7$; fig. 1). Time spent within other %HRmax zones was not different between work shifts.

Table 1. Work duration, work/rest periods, and work intensity (heart rate and RPE) in burned sugarcane workers (n=20) at the end of a work week (Friday, Saturday) and beginning of the work week (Monday). Data presented as average ± SD. * Significant differences from Friday, † significant difference from Saturday ($p<0.05$).

	Friday	**Saturday**	**Monday**
Shift duration (hh:mm)	5:13 ± 00:27	4:25 ± 00:37*	4:32 ± 00:31*
Rest Periods (number, duration, hh:mm)	5 ± 0.5† 14:23 ± 01:29	4 ± 0.6* 15:07 ± 01:32	4 ± 0.4* 13:38 ± 02:11
Work Periods (number, duration, hh:mm)	6 ± 0.5 41:22 ± 04:28† ^	5 ± 0.6* 45:52 ± 04:08*	5 ± 0.4* 44:25 ± 04:06*
% HR max	57 ± 5	55 ± 4	58 ± 5†
AUC % HR max	19683 ± 2032	17709 ± 1708*	18877 ± 2241* †
RPE Score	4 ± 2	5 ± 2	5 ± 2

Table 2: Time (minutes), and % of work shift (in brackets) spent at different work intensities (%HRmax zones), in burned sugarcane workers (n=20) at the end of a work week (Friday, Saturday) and beginning of the work week (Monday). Data presented as average ± SD. * Significant differences from Friday, † significant difference from Saturday ($p<0.05$).

	Friday	**Saturday**	**Monday**
Light	90.57 ± 67.76	109.60 ± 24.89	83.90 ± 34.59
below 50% HRmax	(25 ± 15)	(35 ± 7)	(26 ± 11)
Light – Moderate	104.10 ± 58.55	91.45 ± 48.37	77.85 ± 34.60
51-60% HRmax	(29 ± 13)	(29 ± 14)	(24 ± 11)
Moderate	103.90 ± 63.55	98.10 ± 48.81	105.90 ± 51.05
61-70% HRmax	(30 ± 13)	(30 ± 15)	(32 ± 14)
Hard	46.05 ± 40.98	22.85 ± 29.08 *	48.7 ± 38.14 †
71-80% HRmax	(14 ± 13)	(6 ± 8)	(15 ± 12)
Very Hard	7.15 ± 21.96	0.65 ± 1.87	8.05 ± 21.85
81-90% HRmax	(2 ± 7)	(0 ± 1)	(3 ± 7)
Maximal	0.00 ± 0	0.15 ± 0.67	0.15 ± 0.67
91-100% HRmax	(0 ± 0)	(0 ± 0)	(0 ± 0)

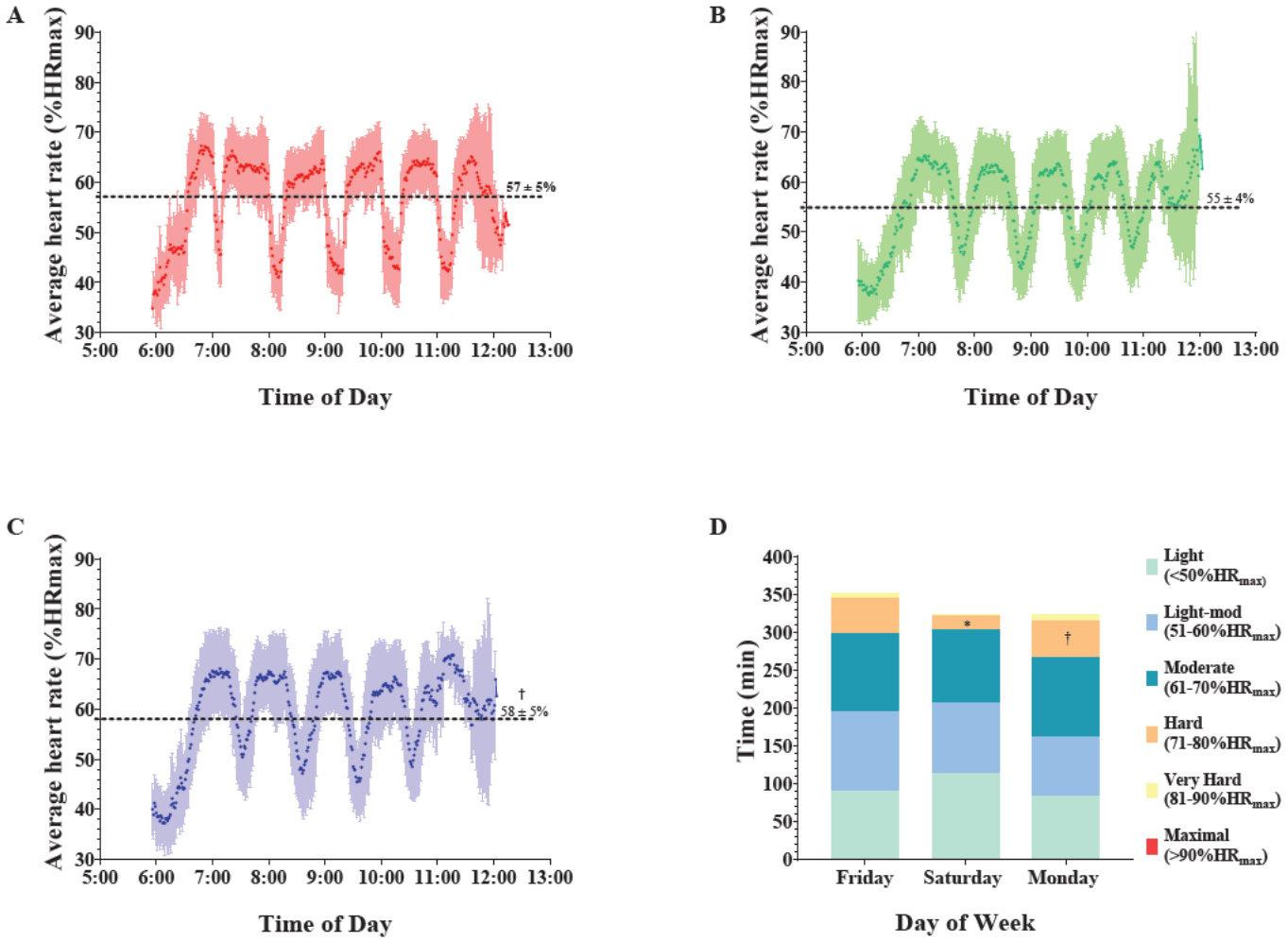

Figure 1: Heart rate (% HR_{max}) in burned sugarcane workers (n=20) across the work shift (including breaks) at the end of a work week (Friday (A), Saturday (B)) and beginning of the work week (Monday (C)). Data presented as average ± SD %. Dotted line is the daily average %HR_{max}. Bar graph (D) shows the average time (min) spent at different work intensities (based on % HR_{max}), throughout each workday. * Significant differences from Friday, † significant difference from Saturday ($p<0.05$).

***Environmental heat exposure*:** Air temperatures in the field reached maximum values of 34.5 °C (Friday), 36.3°C (Saturday), and 33.6 °C (Monday). Average air temperature across the work shift did not differ between days ($p=0.13$). Figure 3 shows the gradual increase in air temperature across the work shift on Friday, Saturday, and Monday.

Wet bulb globe temperature (WBGT) in the field reached maximum values of 27.7 °C (Friday), 29.3 °C (Saturday), and 30.8 °C (Monday). Average WBGT across the work shift differed between workdays ($p<0.01$). Post hoc comparisons showed that WBGT was significantly lower on Friday (26.9 ± 0.79 °C), compared to Monday (29.0 ± 1.1 °C; $p<0.01$). WBGT on Saturday (27.7 ± 0.75 °C), was significantly lower than Monday ($p=0.03$), but not different to Friday ($p=0.2$).

Figure 4 shows the gradual increase in WBGT across the work shift on Friday, Saturday, and Monday, in relation to the OSHA threshold limit value (27.5 °C), denoted by a dashed line. OSHA threshold values are used as a screening tool to evaluate an individual's risk of heat strain based on WBGT, workload and work rest regime (OSHA, 2017). According to these guidelines, when WBGT exceeds a threshold limit of 27.5 °C, during heavy intensity work (i.e., heavy material handling) for 30-45 minutes each hour, there's an increased risk of heat related illness. Estimated time workers spent above this threshold in ours study varied between days: approximately 30 minutes on Friday, 90 minutes on Saturday, and 4 hours on Monday.

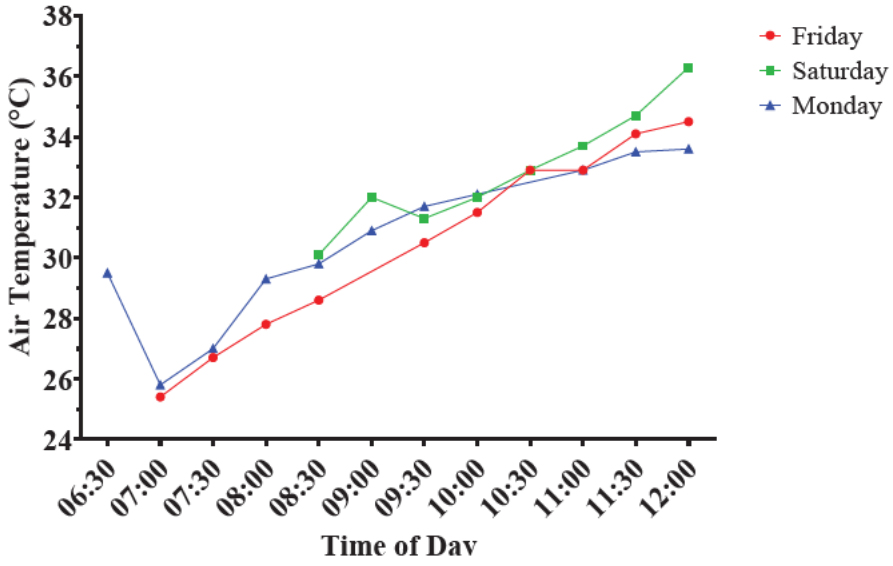

Figure 2: Air temperature (°C) at 30-min intervals at the end of the work week (Friday and Saturday) and beginning of work week (Monday).

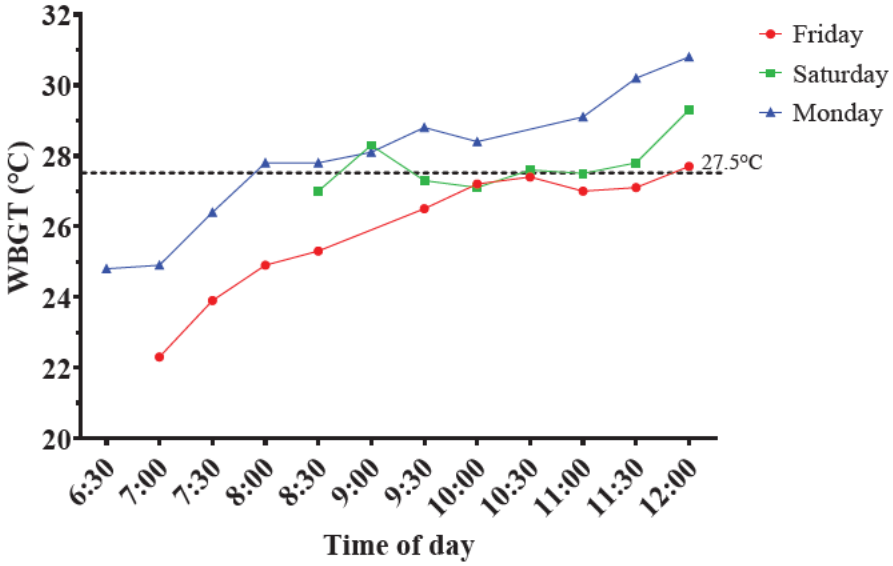

Figure 3: Daily distribution of WGBT at 30-min intervals at the end of the work week (Friday and Saturday) and beginning of work week (Monday). Dashed line represents OSHA threshold (27.5°C) for heat-related illness risk when performing heavy intensity work.

Internal heat exposure: Maximum gastro-intestinal temperature (T_{GI} max) was 0.32 °C higher on Monday compared to Friday (37.79 ± 0.3 vs, 37.47 ± 0.2 °C, p=0.02). T_{GI} AUC was also significantly higher on Monday compared to Friday (24282 ± 1262, vs 25986 ± 1641, p=0.02). As illustrated in figure 4 below, T_{GI} was greatly affected by water intake during rest breaks, causing large troughs in T_{GI} throughout the work shift.

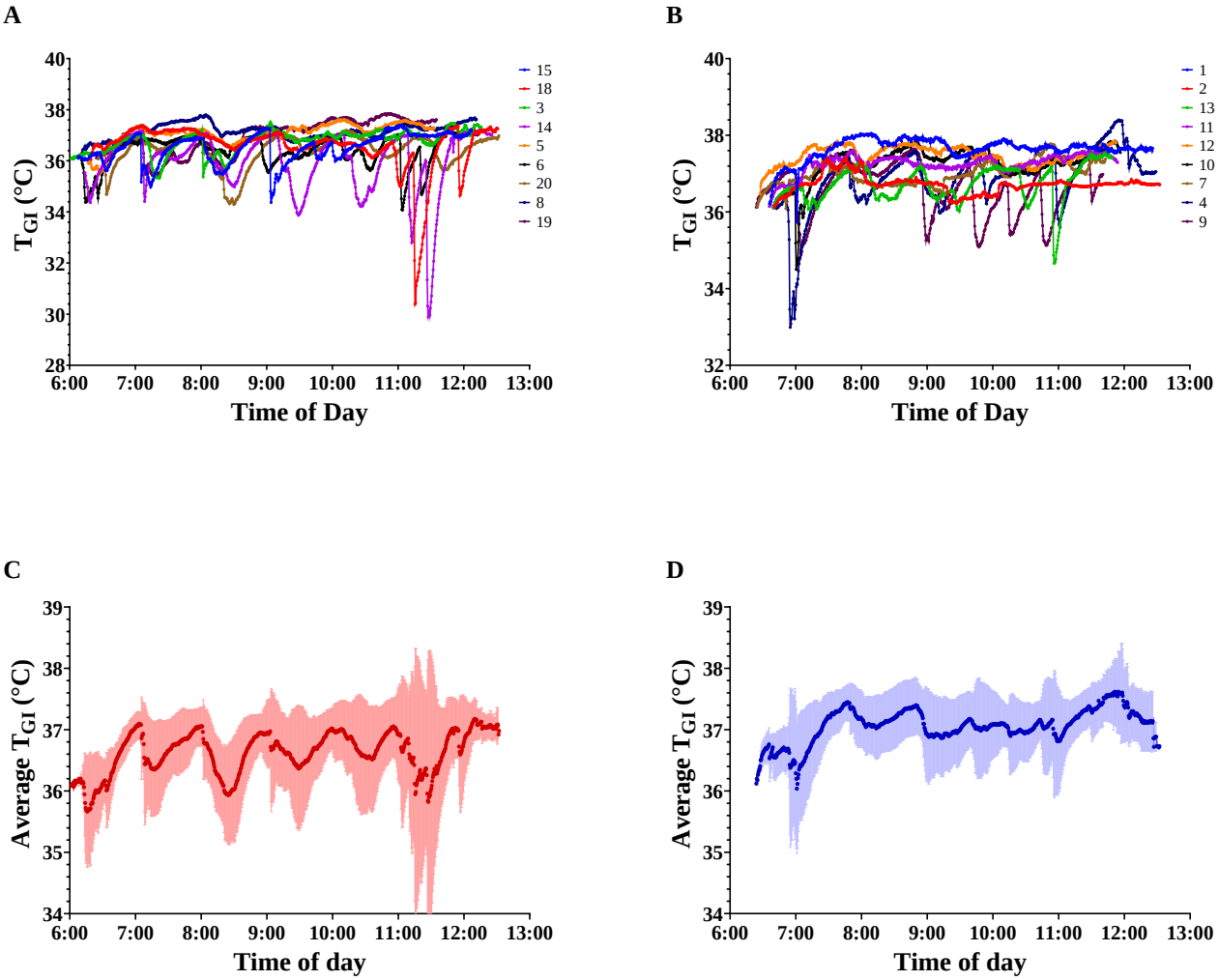

Figure 4: Individual gastrointestinal temperature (T_{GI}) across the work shift on Friday (A), and Monday (B). Average T_{GI} across work shift on Friday (C), and Monday (D). N=9.

Fluid intake: On average, self-reported water intake (L) during Friday, Saturday and Monday work shifts was 8 ± 1.7, 8.2 ± 2.4, and 7.6 ± 1.4 L, respectively.

Sleep activity away from work: Participants slept the most on Saturday night, the night prior to their day off (table 3). On a workday participant woke at approximately 02:40 am. On their day off they slept until ~05:22 am.

Table 3: Sleep activity as measured from a wrist-based accelerometer for burned sugarcane workers during two workdays (Friday & Saturday) and one rest day (Sunday). Some sleep data was not collected as participants took their monitor off, n=18 on Friday and Saturday, n=20 on Sunday. *Sleep interruptions was not consistently calculated for all participants

	Friday	**Saturday**	**Sunday**
Sleep duration (h:min)	5:14 ± 1:05	6:41 ± 2:02	5:01 ± 1:05
Sleep interruptions (h:min) *	0:38 ± 0:11 *(n=15)*	53 ± 28 *(n=15)*	0:37 ± 0:09 *(n=12)*
Bedtime (24-hr time)	21:13 ± 1:06	22:40 ± 2:24	21:28 ± 1:53
Wake time (24-hr time)	02:47 ± 0:39	05:22 ± 1:50	02:34 ± 0:58
Sleep resting HR (bpm)	44 ± 5	44 ± 5	47 ±5

Biomarkers of kidney function

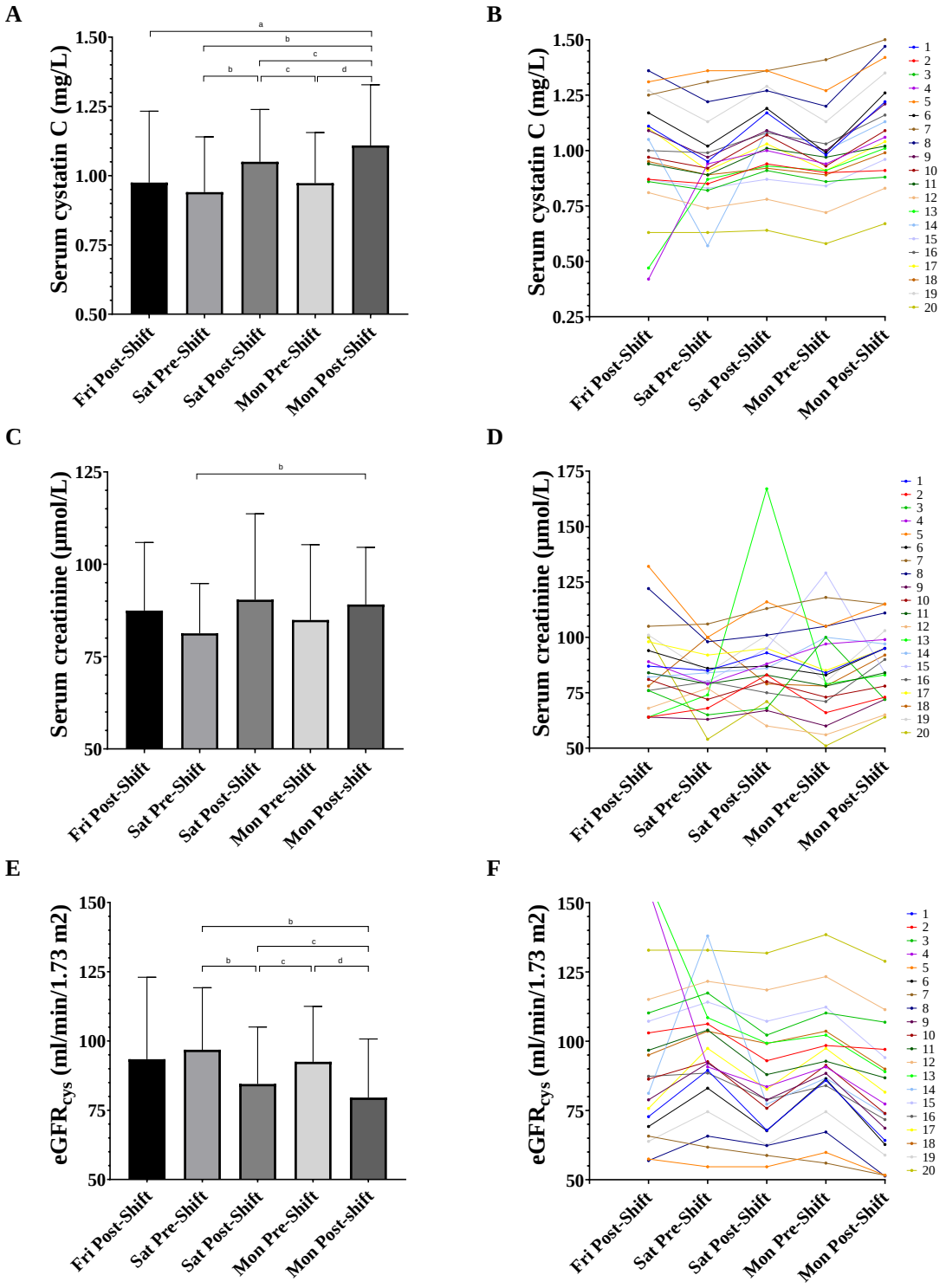

Figure 5: Serum biomarkers of kidney function, cystatin-C (A-B), creatinine (C-D) and $eGFR_{cys}$ (E-F), at: Friday (post shift), Saturday (pre and post shift), Monday (pre- and post-shift). Graphs A, C, and E express data as mean ± SD. Significant differences from Friday Post-Shift [a]; Sat Pre-Shift [b]; Sat Post-Shift [c]; Mon Pre-Shift [d] ($P<0.05$). Graphs B, D, F, express data as individual values.

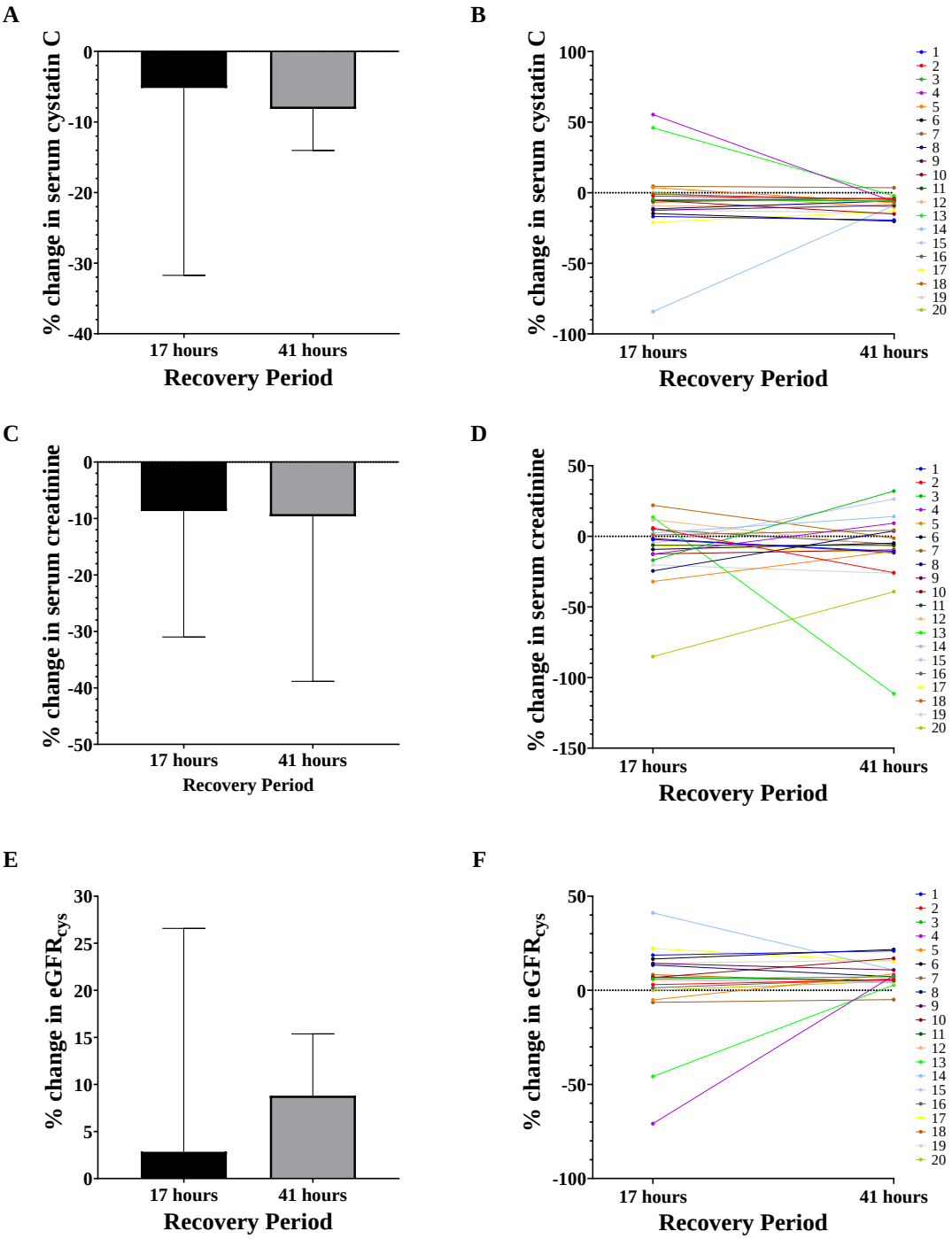

Figure 6: Recovery of kidney function. Percentage change in Serum cystatin-C (A-B), serum creatinine (C-D) and $eGFR_{cys}$ (E-F), after 17 hours (Friday post-shift, to Saturday pre-shift), vs two 41 hours (Saturday post-shift, to Monday pre-shift). Graphs A, C, E express data as mean ± SD Graphs B, D, F, express data as individual values.

Table 4: Biomarkers of kidney function, hydration status, inflammation, and muscle damage. Data presented as mean ± SD. [a] Significant difference from Friday Post-Shift, [b] significant difference from Sat Pre-Shift, [c] significant difference from Sat Post-Shift, [d] significant difference from Mon Pre-Shift (P<0.05). CRP- C reactive protein, CK- creatine kinase. For cross shift data: * significant difference from Friday, † significant difference from Saturday (p<0.05).

Parameter	Fri post	Sat pre	Sat post	Mon pre	Mon Post
Kidney Function					
Serum Cystatin C (mg/L)	0.98 ± 0.26	0.94 ± 0.20	1.10 ± 0.19 [b]	0.97 ± 0.18 [c]	1.10 ± 0.22 [abcd]
Cross-shift (%)	2 ± 21		10 ± 9		16 ± 9 [*†]
Serum Creatinine (µmol/L)	87 ± 18	81 ± 13	90 ± 23	85 ± 20	89 ± 15 [b]
Cross-shift (%)	7 ± 24		10 ± 29		8 ± 11
$eGFR_{cys}$ (ml/min/1.73 m^2)	93 ± 30	97 ± 22	84 ± 21 [b]	92 ± 20 [c]	80 ± 21 [bcd]
Cross-shift (%)	-1 ± 23		-11 ± 8		-16 ±8 [*†]
$eGFR_{creat}$ (ml/min/1.73 m^2)	101 ± 21	107 ± 16	99 ± 21	103 ± 22	98 ± 19 [b]
Cross-shift (%)	-4 ± 15		-6 ± 16		-6 ± 10
Serum Urea (mmol/L)	5.7 ± 3.4	5.2 ± 1.6	5.1 ± 1.4	5.2 ± 1.5	4.8 ± 1.5
Serum Uric acid (µmol/L)	280 ± 59	270 ± 59	270 ± 75	280 ± 56	280 ± 56
Hydration status					
Serum Albumin (g/L)	44 ± 3	44 ± 3	44 ± 3	43 ± 2	46 ± 3 [ad]
Serum Sodium (mmol/L)	140 ± 3	140 ± 1	140 ± 3	140 ± 2	140 ± 3
Serum Potassium (mmol/L)	4.0 ± 0.4	4.3 ± 0.4 [a]	4.0 ± 0.4 [b]	4.4 ± 0.4 [ac]	3.8 ± 0.3 [bd]
Urine pH	6.3 ± 0.5	6.4 ± 0.7	6.2 ± 0.7	6.4 ± 0.8	6.3 ± 0.4
Urine Specific Gravity	1015 ± 4.6	1016 ± 4.3 [d]	1016 ± 5.3	1012 ± 4	1015 ± 5.5 [b]
Inflammation and muscle damage					
Serum CRP (mg/L)	1.1 ± 1.4	2.2 ± 4	2.6 ± 5.4	2 ± 4.4	1.4 ± 2.7
Serum CK (µkat/L)	3.6 ± 1.3	2.8 ± 1.1 [a]	3.6 ± 1.6 [b]	2.8 ± 2	4.1 ± 4

Table 5: Recovery of kidney function biomarkers. Percentage change in serum cystatin C, $eGFR_{cys}$, serum creatinine and $eGFR_{creat}$, after one night recovery/17 hours (Friday post to Saturday pre-shift), vs two nights, and one day recovery/41 hours (Saturday post to Monday pre-shift, 41 hours). Data presented as mean ± SD (%).

Recovery Period	Serum cystatin C (%)	$eGFR_{cys}$ (%)	Serum Creatinine (%)	$eGFR_{creat}$ (%)
17 hours	-5 ± 27	3 ± 24	-9 ±22	6 ± 15
41 hours	-8 ± 6	9 ± 7	-10 ± 29	2 ± 22

***Cross-shift changes in kidney function*:** Serum cystatin C concentration (mg/L) differed between work shifts ($p<0.01$). Post-hoc analysis showed a significant increase in serum cystatin C between pre- and post-shift measures on Monday ($p<0.01$), and Saturday ($p<0.01$; fig. 5, table 4). Cross-shift change in serum cystatin C (%) also differed between workdays ($p<0.01$). Post-hoc comparisons show a greater cross-shift increase in serum cystatin C on Monday (16 ± 9), compared to Saturday (10 ± 9, $p<0.01$), and Friday (2 ± 21, $p<0.01$; , table 4).

$eGFR_{cys}$ (ml/min/1.73 m^2) differed between work shifts ($p<0.01$). Post-hoc analysis showed a significant decline in $eGFR_{cys}$ between pre- and post-shift measures on Monday ($p<0.01$) and Saturday ($p<0.01$; fig. 5, table 4). Cross-shift change in $eGFR_{cys}$ (%) also differed between workdays ($p=0.01$). Post-hoc analysis showed a greater cross-shift decline in $eGFR_{cyst}$ on Monday (-16 ± 8), compared to Saturday (-11 ± 8, $p<0.01$), and Friday (-1 ± 23, $p=0.02$; table 4).

According to clinical practice guidelines, on Saturday, baseline pre-shift $eGFR_{cys}$ was 'mildly decreased' (60-89 ml/min/1.73 m^2) in 5 workers (25%), and moderately decreased (<60 ml/min/1.73 m2) in 1 worker (5%). On Monday, pre -shift $eGFR_{cys}$ was 'mildly decreased' in 6 workers (30%), and moderately decreased in 2 workers (10%) (National Kidney Foundation, 2002; KDIGO, 2012).

With regards to acute changes in kidney function across work shifts, no workers developed acute kidney injury sufficient to meet RIFLE and AKIN criteria (eGFR decrease >50%)

(Bellomo, 2004; Mehta, 2007), while 3 workers overall developed a decrease in $eGFR_{cys}$ >25% over the course of the study, satisfying RIFLE and AKIN criteria for 'acute kidney risk'. Specifically, participant 14 developed acute kidney risk on Friday, Saturday, and Monday, and participants 1 and 6, met this criteria on Monday (fig. 5).

Serum creatinine concentration (µmol/L) did not differ between work shifts (p=0.2). $eGFR_{creat}$ (ml/min/1.73 m^2) also showed no significant difference between work shifts (p=0.2; fig. 5, table 4). Cross-shift change in serum creatinine concentration (%) did not differ between work shifts (p=0.8). Cross-shift change in $eGFR_{creat}$ (%) also showed no significant difference between work shifts (p=0.7; table 4).

Serum urea concentration (mmol/L) did not differ between work shifts (p=0.5), and serum uric acid concentration (µmol/L) did not differ between work shifts (p=0.7; table 4).

Recovery of kidney function: Recovery of serum cystatin C (%) after 17 hours (one night recovery), was not significantly different to that after 41 hours (two nights/ one day recovery) (p= 0.61). There were also no significant differences between 17 hours vs. 41 hours recovery of $eGFR_{cys}$ (%) (p=0.24), serum creatinine (%) (p=0.91), or $eGFR_{create}$ (%) (p=0.55; fig. 6, table 5).

***Correlational analysis of kidney function ($eGFR_{cys}$), HR, and T_{GI}*:** A correlation of r = -0.02 exists between cross-shift change in $eGFR_{cys}$ (%), and HR AUC on Friday, Saturday, and Monday (fig. 7, graph A). A correlation of r = -0.26 exists between cross-shift change in eGFRcys (%) and T_{GI} max (°C) on Friday and Monday (fig. 7, graph B). Recovery of $eGFR_{cys}$ (%), between Friday post-shift and Saturday pre-shift (17 hours), had a correlation of r=0.18 with Friday's HR AUC, and r= -0.17 with Friday's T_{GI} max (Fig. 7, graph C and D). We were unable to calculate any correlations between kidney function and HR/ T_{GI} max for the 41 hour recovery period, due to only having T_{GI} max data on Friday and Monday.

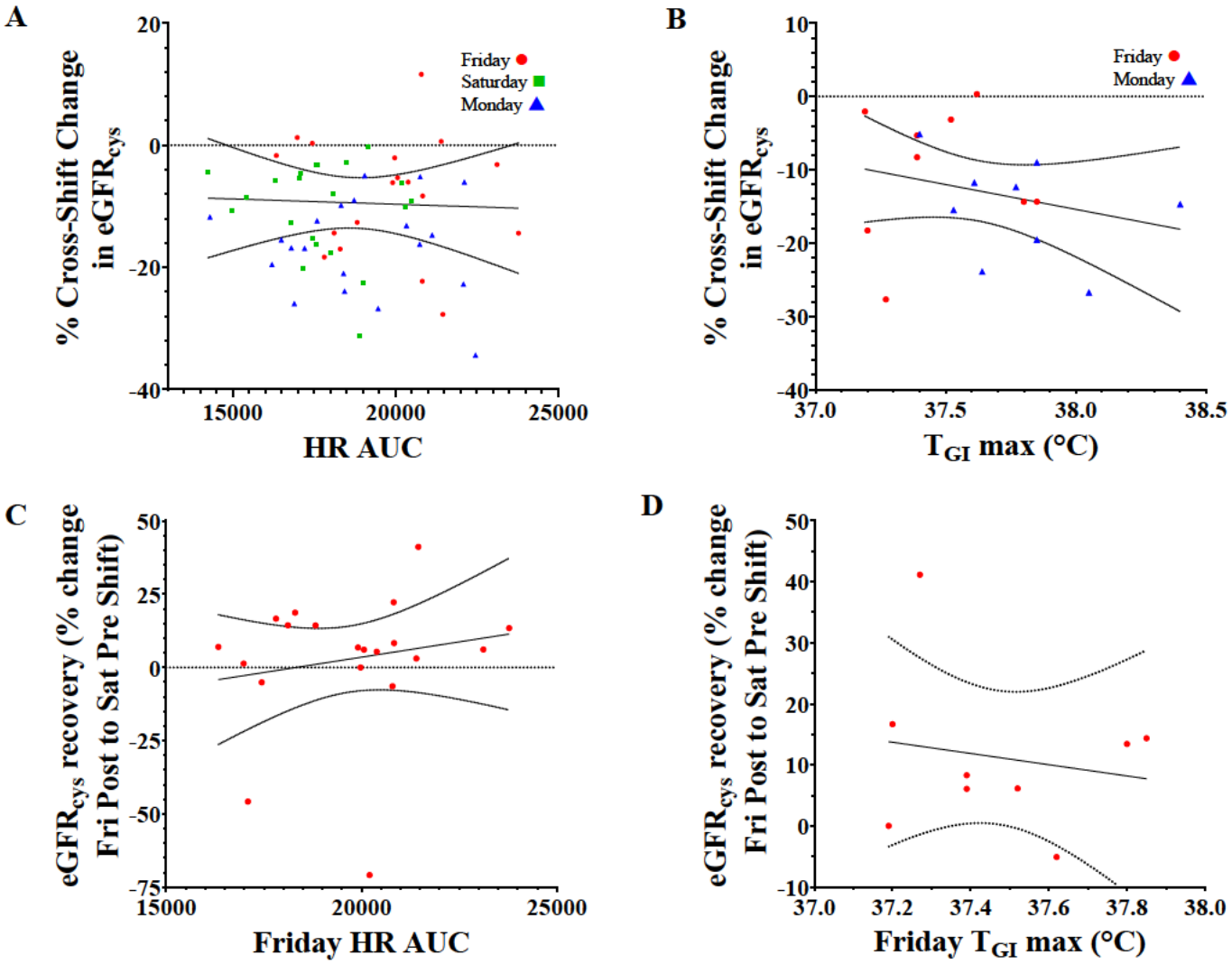

Figure 7: Correlational analysis between kidney function, T_{GI} max and heart rate. Graphs A, and B show the correlation between % cross-shift change in $eGFR_{cys}$, and heart rate AUC (A), and T_{GI} max (B). Graphs C and D, show the correlation between Friday's kidney function recovery (percentage change in $eGFR_{cys}$ from Friday post-shift to Saturday pre-shift), and HR AUC (C), and T_{GI} max (D), on Friday. Data shown are individual values.

Bland-Altman analysis of eGFR: A Bland Altman plot assessing the degree of agreement between $eGFR_{cyst}$ and $eGFR_{creat}$ is shown in figure 8. There's a mean bias of -12 ± 20, and 95% CI limits of agreement are -50-26 ml/min/1.73 m^2. Therefore, despite a strong positive correlation (r=0.6), $eGFR_{creat}$ overestimates $eGFR_{cyst}$ in this population.

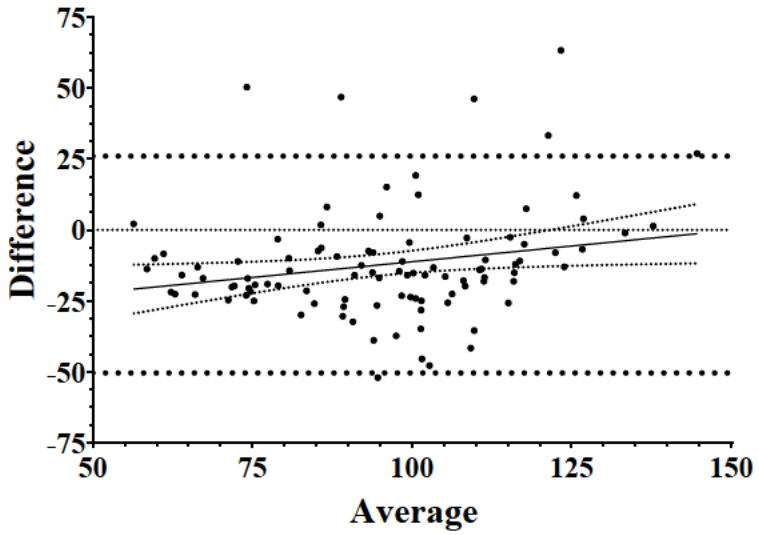

Figure 8: Bland Altman plot showing the degree of agreement between $eGFR_{cyst}$ (a) and $eGFR_{creat}$ (b). The Y axis represents the differences between the two methods (a-b), and the x axis shows the average of the two methods (a+b/2). Dotted lines represent the 95% limits of agreement.

Biomarkers of hydration status

Serum albumin: Serum albumin concentration (g/L), differed between work shifts ($p<0.01$). Post-hoc analysis showed higher serum albumin levels on Monday post-shift compared to Monday pre-shift ($p<0.01$), and Friday post-shift ($p=0.03$; table 4). Cross shift changes in serum albumin were positively correlated (r=0.3) with cross shift changes in serum cystatin C (fig. 9, graph A). A positive correlation (r=0.3) was also found between T_{GI} max and cross shift changes in serum albumin (fig. 9, graph B).

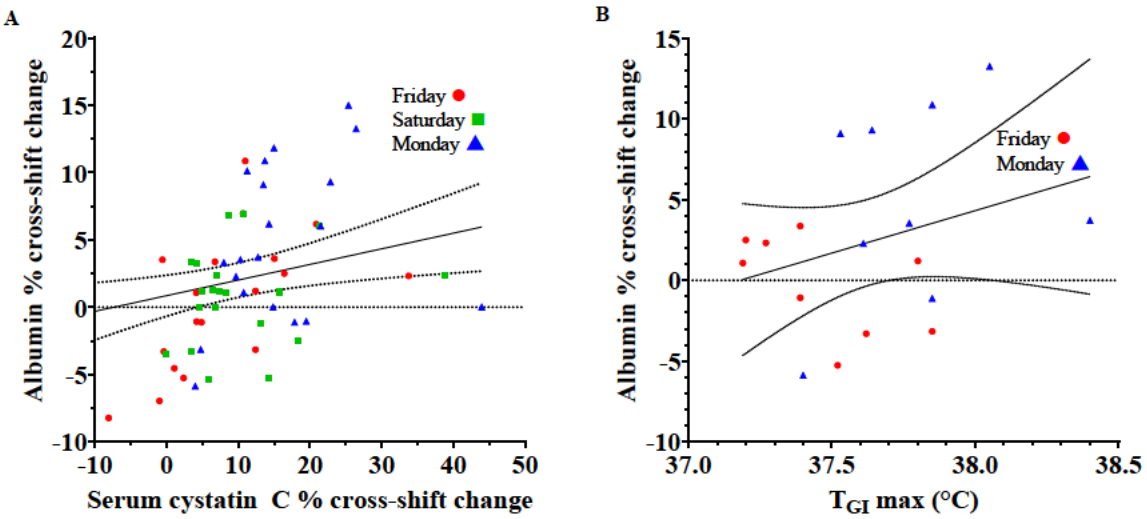

Figure 9: Correlational analysis of serum albumin. Graph A shows a correlation between cross-shift change in serum cystatin c (%), and cross-shift change in albumin (%), on Friday, Saturday, and Monday. Graph B shows a correlation between T_{GI} max, and % cross-shift change in albumin, on Friday, and Monday. Data shown are individual values.

***Serum Potassium*:** Serum potassium concentration (mmol/L) differed between work shifts ($p<0.01$). Post-hoc analysis showed a significant decline in serum potassium between pre- and post-shift measures on Monday ($p<0.01$) and Saturday ($p=0.01$; table 4).

Serum Sodium: Serum sodium concentration (mmol/L) did not differ between work shifts ($p=0.09$; table 4).

Urine pH: Urine pH did not differ between work shifts ($p=0.8$), (table 4).

Urine Specific Gravity: Urine specific gravity differed between work shifts ($p=0.02$). Post-hoc analysis showed higher average values on Saturday pre-shift compared to Monday pre-shift ($p=0.02$; table 4).

Biomarkers of inflammation and muscle damage

Serum creatine Kinase: Serum creatine kinase concentration (µkat/L) did not differ between work shifts ($p=0.2$; table 4).

Serum CRP: Serum CRP concentration (mg/L) did not differ between work shifts ($p=0.2$; table 4).

3.5. Discussion

Recovery of blood and urine biomarkers at the beginning of a working week, following a day off (41 hours) was similar to recovery at the end of a working week, following no days off (17 hours). Furthermore, cross-shift changes in blood/urine biomarkers, showed no significant improvements following 41 hours vs 17 hours. Instead, cross-shift reductions in kidney function ($eGFR_{cys}$) were significantly greater on Monday (after 41 hours recovery), compared to Friday or Saturday (after 17 hours recovery). These results do not support our hypothesis, as we anticipated a smaller recovery of these biomarkers, and a greater cross-shift decline in kidney function, hydration, muscle damage and inflammation following just 17 hours, particularly given the impact of cumulative exposure to heat stress and high workloads throughout the week.

Recovery of blood and urine biomarkers:

In this cohort of workers, we were unable to demonstrate any significant improvement in kidney function following a more prolonged recovery period away from work (41 hours), compared to a shorter recovery period (17 hours; table 5). To our knowledge, no previous field-based study has investigated kidney recovery in an industrial agricultural population. However, several lab-based studies have measured kidney function recovery over the course of 24 hours following prolonged exercise in the heat, from which some comparisons can be drawn (Chapman, 2019; 2020; Schlader, 2017; Junglee, 2012). In a study by Schlader et al. (2017), 19 healthy adults (23 ± 4 y), performed 60 min of exercise in a heat chamber at 38°C, 50% RH, wearing firefighter protective clothing aiming to increase internal heat load. Plasma NGAL, a marker of glomerular filtration rate (Schlader, 2019; Testani and Brisco, 2016), peaked immediately (20-min) post-exercise, then began to decline throughout recovery, with values appearing to return to pre-exercise values at 24 hours post-exercise. This positive effect of recovery time on kidney function is also seen in a similar study by Chapman et al. (2020), whereby a cohort of 13 healthy adults (23 ± 2 y) performed treadmill walking for 2 hours at 55% HR max, in hot environmental chamber (39.7 ±0.6°C, 32 ±3% relative humidity). Results showed an increase in serum creatinine by 0.29 mg/dL immediately after exercise, and 0.19 mg/dL at 1-hour post-exercise, with values appearing to return to pre-exercise values at 24 hours post-exercise. Unfortunately, in both these previous studies, the statistical significance of improvements during recovery were not reported. However, these data do suggest a positive effect of recovery time on kidney function following exertional heat stress, and that a 24-hour

recovery period is sufficient to recover from a 1-hour bout of exercise in the heat. Given that the shorter recovery period in our own study (approximately 17 hours) was less than 24 hours, we anticipated that some indication of kidney strain would remain after this shorter recovery period, particularly given the cumulative kidney strain caused by the preceding 4-days of work, and more prolonged bouts of physical work in our own study. Therefore, we expected that the longer recovery period (approximately 41 hours) after completing a work week, would allow for a more complete recovery of kidney function. While our results did not show any significant benefit of a longer recovery, it is important to note that Chapman et al. (2020) observed a greater post-exercise increases in serum creatinine (0.29 mg/dL) than we saw from pre-to-post-shift (0.1 mg/dL on Saturday, and 0.05 mg/dL on Monday). This may be due to Chapman et al. (2020) participants being less acclimated to heat stress compared to sugarcane cutters in our own study, or the ability to have greater control over extraneous variables such as diet, hydration, and work intensity in a lab-based study, which may influence the rate of recovery. It should also be noted that our results do show a trend towards improved kidney function ($eGFR_{cys}$) recovery after the longer recovery period of 41 hours (8.8 ± 6.6 %) vs. 17 hours (2.8 ± 24 %), suggesting that a longer recovery duration may have some positive effect on kidney function, albeit non-significant in the current study. The absence of any significance in the magnitude of kidney function recovery may be attributable to the considerable individual variability in renal responses observed within and between workers. Such variability is potentially related to inconsistencies in workload (fig. 1), and internal heat stress (fig. 4) over the course of the work shift, and between individuals, owing to the field-based, free-living nature of this study. To our knowledge, very few studies have examined kidney recovery to date, and none have examined kidney recovery in a agricultural cohort that are at risk of CKDnt. Therefore, comparing our recovery data with other studies, and gauging whether literature in this field supports our data, is difficult.

Cross-shift changes in blood and urine biomarkers:

Overall, our study demonstrated a decline in cross-shift kidney function, which agrees with previous field-based studies assessing sugarcane workers in Central America. In a previous study by Paula-Santos et al (2015), the average cross-shift decline in $eGFR_{creat}$ of 14.6 ml/min/1.73m^2 amongst 23 cane cutters who did not develop AKI,. Similarly, Wegman et al (2018) observed an average cross-shift decline in $eGFR_{creat}$ of 10.5 mL/min/1.73m^2 amongst

80 sugarcane workers in EL Salvador. However, the magnitude of our average cross-shift decline in $eGFR_{creat}$ over the course of our study (6.5 ml/min/1.73m^2), was smaller than these previous studies and was non-significant, which may be attributable to the lower $WBGT_{max}$ values, and/or the shorter shift durations in the current study compared to Wegman (2018) and Paula-Santos (2015), respectively. This being said, our study did find significant cross-shift declines in serum cystatin C/$eGFR_{cys}$. On average, over the course of the study, serum cystatin C increased by 0.15 mg/L and $eGFR_{cys}$ decreased by 10.5 ml/min/1.73m^2 (table 4). Cystatin C provides a more accurate measure of kidney function as it is not affected by changes in muscle metabolism to the same extent as creatinine (which is especially poignant in the context of heat stress/exercise) and has demonstrated superior sensitivity in detecting smaller changes in GFR (Newman, 1995; Christensson, 2003; Kar, 2018; Levin, 2013). Despite these advantages, to our knowledge no previous studies have examined cross-shift changes using cystatin C in sugarcane workers, as it is a relatively new renal biomarker. However, changes in cystatin C have been investigated in the context of exercise, particularly marathon running, which may place a similar degree of strain on renal function as sugarcane cutting in the heat, via renal blood flow reductions, systemic inflammation, and muscle damage (Smith, 51; Hansson, 2020; Leon, 2010a; 2010b; Hodgson, 2017). In a study by Mingels et al (2009), 70 male marathon runners aged 47 years (30-68, 95% CI), demonstrated an acute increase in serum cystatin C of 0.24 mg/L, immediately after running for an average of 3.71 hours (2.87-4.69, 95% CI), with maximum ambient temperatures of 23.4 °C. Similarly, Bongers et al (2018) observed an $eGFR_{cys}$ decline of 15 ml/min/1.73m^2 in 35 male subjects (23 ± 3 years) following 150 minutes of high intensity cycling at 80% of maximum heart rate, at an ambient temperature of 25°C. While these values are comparable to the cross-shift changes in cystatin C measured in the current study, it is difficult to make direct comparisons, due to our study involving considerably greater environmental heat exposure (maximum WBGT values > 30°C), and more prolonged work durations (4-5hours), with variable exercise intensities.

The cause of these transient reductions in renal function seen in our own study, particularly in cystatin C and $eGFR_{cys}$ (fig5, table 4), are not clear, and are thought to be multifactorial. However, the leading hypothesis suggests a form of heat stress nephropathy caused by occupational heat stress, dehydration, and high workloads, as previously discussed in chapter 1 (Wesseling, 2016; Glaser, 2016; Correa-Rotter, 2014; Johnson, 2019; Roncal-Jimenez, 2015; 2016). The findings from our own study somewhat support this hypothesis, with cross-shift increases in serum cystatin C, positively correlating (r=0.3) with cross-shift increases in serum

albumin, a marker of hydration status, and cross-shift declines in $eGFR_{cys}$ negatively correlating with internal heat exposure (r=-0.26) (Fig. 7, 9), potentially indicating that reductions in kidney function across the work shift are attributable to reductions in hydration and increases in internal heat stress.

While we do not see a strong association between workload (HR AUC) and cross-shift kidney dysfunction ($eGFR_{cys}$) (r=-0.02), it is difficult to delineate the importance of workload vs heat stress, as physical exertion exacerbates hyperthermia/dehydration, and vice versa (Periard, 2011; Sawka, 1983; Gleeson, 1998). Therefore, the severity of work intensity may still be contributing to these cross-shift declines in kidney function. Indeed, Hansson et al (2019) showed that workers performing jobs with the highest workload (e.g., seed cutters and burned sugarcane cutters) exhibited greater cross-harvest declines in kidney function when compared workers performing jobs with a lighter lower workload. Notably, the participants burned sugarcane cutters in the current mill were from the same mill as participants in Hansson et al., (2019) study.

One proposed mechanism through which these risk factors may induce acute kidney injury or strain, is through a mismatch between renal energy supply and demand. This increased renal energy demand is partly attributable to upregulation of the renin-angiotensin-aldosterone-system (RAAS) causing an increase in sodium reabsorption and potassium excretion, in an attempt to maintain fluid homeostasis. Hypokalaemia (a reduction in serum potassium), is often the consequence of this increased renal activity (Hansson, 2020; Schlader, 2019), and is likely exacerbated by potassium loss experienced through high sweat rates. The findings of our own study also appear to support this theory, as we observed significant cross-shift reductions in potassium on both Monday and Saturday (table 4), which may be an indirect reflection of increased renal metabolism and dehydration. Cross-shift reductions in serum potassium of a similar magnitude have also been observed in cohorts of industrial agricultural workers by Wesseling et al. (2016) and Garcia-Trabanino et al. (2015). Interestingly, hypokalaemia is not only a reflection of increased renal energy demand or dehydration but can also cause acute kidney strain by causing further reductions in renal blood flow and possible long term tubulointerstitial inflammation (Hansson, 2020). Indeed, several studies have shown that workers who consumed a more electrolyte solution, reduced the likelihood of developing AKI (Butler-Dawson, 2019; Laws, 2015).

While there were clear reductions in kidney function across a work shift in this study, it is important to note that no workers developed clinical AKI (an increase in serum creatinine

≥0.3mg/dL, or GFR decrease of >50%) over the course of a work shift, and only 3 workers experienced acute kidney risk (i.e., GFR decrease >25%), according to RIFLE and AKIN criteria (Bellomo, 2004; Mehta, 2007). The absence of widespread clinical AKI amongst sugarcane workers across a work shift is not an unusual finding (Wegman, 2018; Paula-Santos, 2015; Wesseling, 2016), and does not negate the possibility of long-term renal dysfunction and CKDnt development. This is because, the severity of acute kidney strain required to elicit long term kidney damage remains undetermined. However, there does appear to be some heterogeneity in clinical AKI prevalence in the literature, with some cross-shift studies reporting up to 45% of workers developing 'AKI risk' and 51% of workers developing AKI across a work shift (Sorensen, 2019; Butler Dawson, 2019). Differences in workload, heat exposure, diet, and hydration practices, may all contribute to the differing degrees of kidney strain reported in manual agricultural labourers working in the heat (Schlader, 2017; 2019; Chapman, 2020; Junglee, 2013). Notably, environmental heat exposure was considerably higher in the aforementioned studies, with Sorenson et al, (2019) recording average WBGT values of > 30 °C during data collection between February and April, while our average WBGT values did not exceed 30 °C throughout the 4 days of data collection in February. Furthermore, Butler-Dawson et al, (2019) reported average shift durations of 10 hours, almost double that documented in our own study (table 1).

The effect of recovery on cross-shift changes in blood and urine biomarkers

Another important aim of our study, was to assess how different recovery periods, i.e., an additional day off work (41 hours), vs no days off work (17 hours), affected cross-shift changes in blood and urine biomarkers, particularly kidney function. We anticipated that cross-shift declines in kidney function would be greater on Saturday, at the end of the working week (following just 17 hours recovery), compared to Monday at the beginning of the working week and following a day off (41 hours recovery). The reason for this assumption was that at the end of the week workers have been exposed to consecutive days of intense heat exposure and high workloads, with minimal recovery. Subsequently, the acute impact of such repetitive insult (capable of inducing considerable renal inflammation, oxidative stress, and ischemia) in the absence of sufficient recovery and resolution of inflammation/damage, could have a cumulative effect on kidney dysfunction (Hansson, 2020; Schlader, 2019; Smith, 1952; Bellomo, 2012; Devarajan, 2006). However, our results demonstrated the opposite, i.e., there were significantly greater cross-shift declines in kidney function ($eGFR_{cys}$) on Monday (-16 ±

78 %), following 41 hours, compared to than that on Saturday (-11 ± 7.8 %) following just 17 hours of recovery (table 4). Furthermore, the length of recovery period had no significant effect on recovery, or cross-shift changes in serum CRP, a biomarker of systemic inflammation. This is surprising finding, given its proposed involvement in kidney dysfunction with insufficient recovery (Hansson, 2020; Schlader, 2019). To our knowledge, no other study has assessed cross-shift changes in blood and urine biomarkers after consecutive work-shifts (i.e., no days off), and compared this to cross-shift changes after a day off work in populations at risk for CKDnt. Therefore, we are unable to directly compare our data with other studies and discuss whether literature in this field supports our data. However, a field-based study by Lipman et al (2014) may offer some additional insight into this relationship. They investigated whether 6 consecutive days of endurance running in the heat (40 km a day) had any cumulative effect on kidney function, which shares some parallels with the 6 consecutive days of work in hot conditions experienced by participants in our own study. Lipman et al (2014), took blood samples from 30 participants (23 males, aged 39.6 ± 10.6 years) before and after day 1, 3 and 5 of three different ultramarathons. Results showed that, despite a high prevalence of clinical AKI throughout the course of these races, the percentage change in eGFR was non-significant between day 1 and 5, with participants' kidney function (eGFR) appearing to return to their pre-event baseline before the beginning of each successive day. In other words, they showed no apparent cumulative effect of high workload and heat stress on kidney function, suggesting that these athletes would not benefit from an additional day off between each stage of the ultramarathon, as far as eGFR is concerned. This indirectly supports results of our own study, as we demonstrated that cross-shift decline in kidney function was not improved following a day off compared to that after consecutive work shifts, indicating the apparent absence of any cumulative effect, acutely.

The greater cross-shift reductions in kidney function ($eGFR_{cys}$) following a longer recovery period (41 hours vs 17 hours) demonstrated in our study, may even suggest that a more prolonged recovery period could be acutely detrimental to kidney function. There were also significantly higher workloads on Monday compared to Saturday (fig 1, table 1, 2), which raises the question of whether a longer recovery period enables workers to work harder and subsequently experience greater declines in kidney function. This paradox may off-set potential benefits arising from longer recovery periods and could possibly counteract any practical long-term benefit of longer recovery periods in agricultural workers. However, cross-shift declines in $eGFR_{cys}$, did not correlate strongly with workload (HR AUC) in this study (r=-0.02) (fig 7, A), suggesting this greater kidney strain on Monday may not be attributable to

higher work intensities. Alternatively, the greater environmental heat exposures (WBGT) on Monday vs. Friday and Saturday (fig 3), may explain the higher degree of kidney strain seen on Monday.

3.6. Methodological Considerations

Firstly, there was the marked variation in kidney function dynamics within and between workers, which may be decreasing the statistical power of the results (fig 5, 6). However, such individual variability in renal responses amongst agricultural workers is not unusual and is consistent with the variable decline in eGFR seen in those who develop CKDnt (Garcia-Trabanino, 2015; Paula-Santos, 2015; Laws, 2015, Wijkstrom, 2017). In other words, there appears to be an inherent variability in how the kidney responds to injurious stimuli. This being said, variability in renal responses, may also be attributable to several extraneous, non-renal factors that we were unable to control due to the field-based nature of this study. These factors included the self-paced nature of this work involving variable work intensities across a shift (fig. 1), the variable T_{GI} responses (fig 4), and the free-living home environment where we were unable to control for physical activity, sleep, diet, and ambient temperature. While this may have been a source of variability, it could also be considered a strength of this study as it captured a more ecologically and valid setting and strengthens the practical application of the results. T_{GI} in particular was likely driving a considerable degree of variability in renal responses seen in our results, with cross-shift $eGFR_{cys}$ (%), being more strongly correlated with internal heat exposure (T_{GI} max), compared to workload (HR AUC) (r = -0.26 vs -0.02, respectively) (fig. 7). However, it is important to note that T_{GI} measures in our own study, appear to be affected by drinking patterns, which we were unable to control. While cold water will likely have an indirect cooling effect on internal core temperature, the extent to which this directly influences telemetry pill recordings is uncertain and likely variable (Wilkinson, 2008). Furthermore, T_{GI} recordings using a telemetry pill can be highly variable within and between individuals due to anatomical differences in how the pill passes through the GI tract and different temperature gradients along the GI tract (Byrne and Lim, 2007; Kolka, 1993), thereby complicating this relationship between internal temperature and kidney function in our study.

A second limitation of this study was the relatively low sample size (n=20). Similar cross-shift field studies in agricultural populations have had larger sample sizes (i.e., n > 80) (Garcia-Trabanino et al, 2015; Sorensen et al, 2019; Butler-Dawson et al, 2019; Wegman et al, 2018).

Furthermore, the sample size was further reduced when analysing certain key parameters. Notably, T_{GI} was only collected in nine participants on Friday and separate nine participants on the Monday, due to limited number of T_{GI} receivers. Thirdly, a 'complete set' of cross-shift blood and urine biomarkers, i.e., both pre- *and* post-shift samples, were only obtained on Saturday and Monday. On Friday, only post-shift samples were collected. This meant that comparing cross-shift changes in kidney function on each day was more limited. However, a 'mean pre-shift' value for each parameter was calculated, averaging pre-shift samples from Saturday and Monday, to overcome this challenge. Furthermore, we did not have individual baseline parameters for this cohort, therefore, we were only able to use cross-shift changes in kidney function, and % change in kidney function recovery from post-pre shift, as markers of 'recovery', rather than a percentage change from a true baseline. Finally, there was some missing data in our results. Importantly, no core temperature (T_{GI}) data was collected on Saturday, hydration data was missing for participants 16-20, and Friday's sleep data was not recorded in two participants who removed their monitor (table 3).

3.7. Perspectives and future directions

Any beneficial effect of increased recovery time on kidney function in the current study may have occurred on a microscopic, cellular level, beyond the sensitivity and specificity of serum creatinine or cystatin C based eGFR measures. This is because GFR only provides a broad measure of functional injury, not structural injury and is susceptible to non-renal physiological changes upstream of kidney such as dehydration, physical exercise, and heat exposure, via reductions in renal blood flow (Basile, 2012; Devarajan, 2006). Therefore, a reduction in GFR could simply reflect a transient, benign reduction in renal blood flow, rather than intrinsic AKI. It is also possible that intrinsic cellular damage can occur in the kidney without affecting GFR, and if it does, there is likely a latent period before structural AKI is reflected in GFR. There are numerous emerging biomarkers capable of detecting such structural injury including, NGAL, KIM-1, L-FABP, and IL-18 (Vaidya, 2008; Rosner, 2009; Ostermann, 2015). These small proteins are predominantly detected in the urine and are expressed by tubular cells upon exposure to injurious stimuli (Devarajan, 2007; Barrera-Chimal, 2012). Such markers of tubular damage are not only more sensitive to injury (Ostermann, 2015; 2016; Rosner, 2009; Vaidya, 2008), they are also more specific, in terms of the location and nature of the injury (Schaub, 2016; Ostermann, 2015; 2016; Schlader, 2019).
Therefore, using these biomarkers may enable such structural AKI detection, even in the

absence of functional AKI (eGFR) (Devarajan, 2007; Rosner, 2009; Barrera-Chimal, 2012; Chapman, 2020; Kashani, 2013; Doi, 2011; Wagener, 2006), which may be beneficial in future studies, to better elucidate the effect of recovery time on renal function in this context. However, it should be noted that, many of these biomarkers are still undergoing the process of validation and research in the area is constantly evolving. Furthermore, use of these novel biomarkers in large-scale filed studies may be limited, with limited research outside clinical settings and the greater cost, and resources required.

While this study was unable to show any significant benefit of a more prolonged recovery period, it is worth considering that the length of the longer recovery period investigated (41 hours) may not have been sufficient. Instead, a longer recovery period could be required to elicit an improvement in kidney function after a 6-day work week. Furthermore, due to the acute nature of this study, it is impossible to understand the true impact of a longer recovery period on kidney function without examining the effects over a more chronic period of time. Indeed, it is hypothesised that CKDnt manifests over the course of many months-years of potential repetitive sub-lethal injury (Schlader, 2019; Hansson, 2019; Kupferman 2018). Thus, the effect of recovery in attenuating any negative effects of workload/heat stress on renal function may require equally long trajectory, which may explain why we cannot see significant benefits after just several days. Therefore, it would be beneficial for future studies to explore the effects of longer recovery periods over the course of a harvest. However, it is important to note that implementing longer recovery periods into the working week over an extended period would be difficult and potentially unfeasible in these working groups. Sugarcane work in Central America has few worker-organizations protecting human rights, (Wesseling, 2014). Therefore, there could be issues with the practical application of such research in the field.

Significant declines in cross-shift kidney function were only seen in serum cystatin C, not serum creatinine in this study, which indicates some discordance between these two biomarkers of GFR. Indeed, the degree of agreement between $eGFR_{cys}$ and $eGFR_{creat}$ using Bland Altman analysis was such that $eGFR_{creat}$ overestimated $eGFR_{cys}$ in this population (fig. 8). One reason for this could be the greater sensitivity of serum cystatin C over a wide range of GFRs as compared to serum creatinine. Specifically, at the top end of kidney function, it takes a very large reduction in GFR to produce considerable increases in sCr (Molitoris, 2016; Devarajan, 2010). Therefore, future studies in this field should consider the use of serum

cystatin C for GFR estimations, especially in populations where the decrements in GFR may be small and gradual.

3.8. Conclusion

To conclude, we demonstrated that, a longer recovery period of 41 hours (two nights and one day off work) did not significantly improve the magnitude of kidney function recovery, or the cross shift decline in kidney function during the subsequent shift, when compared to a shorter recovery period of 17 hours (one night off work). However, we did observe a non-significant trend towards a greater percentage increase in kidney function following two nights vs one. Therefore, it is possible that the absence of any significant effect from a longer recovery period may be attributable to the individual variability in this working group, the inability to control extraneous variables such as environmental heat exposure, workload, and home-life, the insensitivity of eGFR measures, or insufficient length of recovery. To achieve a more comprehensive understanding of how recovery time effects kidney function in sugarcane workers, future studies should consider these methodological considerations.

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