HAIR CORTISOL CONCENTRATION ANALYSIS IN THE STUDY OF THE DEAD AND DYING

STRESS, DYING, AND DISEASE: HAIR CORTISOL CONCENTRATION IN THE STUDY OF STRESS AT THE END OF LIFE IN THE PAST AND PRESENT

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LAY ABSTRACT

Today, death is often regarded with uncertainty and even fear, yet little is known about the experience of dying, especially in the past. Dying is difficult to study in modern people because of communication challenges and the number of complex factors at play while studies of the past are limited because human remains do not reveal how individuals felt. A better understanding of the dying experience can help bioarchaeologists clarify the relationship between dying, death, and skeletal remains and could help improve the care of dying people today.

This research evaluated a new method of stress assessment to study experiences in the last months of life in archaeological, historic, and modern samples. Hair cortisol concentration (HCC) analysis measures stress hormones in human hair to reconstruct stress experience at the time the hair was growing. High levels of HCC in dead individuals from 1st century AD Egypt, 19th-20th century Missouri, and 21st century Florida indicate that dying is stressful across time and place. HCC values from the Egyptian sample were higher than most living people but were lower than other archaeological samples which may be a result of cortisol leaching out of the hair shaft after death. Although higher than living people, HCC levels still differed between individuals and across individual hair shafts, indicating that stress experience can be different between individuals and change over an individual's final months. These differences are not a result of biological factors such as duration of disease or the presence of certain preexisting conditions suggesting that stress at the end of life is complex. Despite advancements in medicine, the modern sample displayed similar HCC levels to those from earlier historic periods and for a number of individuals from the historic sample, hospital entry led to a temporary reduction in HCC levels. Together, these findings suggest that, while modern medical advancements have not improved stress levels at the end of life, some aspects of care could reduce stress.

The results of this study indicate that dying is a stressful, complex, and dynamic phenomenon that modern medical treatment alone may not be able to improve. Furthermore, studies of HCC in archaeology must focus on the effects of dying and be wary of leaching. Ultimately, HCC analysis could contribute to a greater knowledge of the dying experience, the understanding of past peoples, and improvement of the experience of dying.

ABSTRACT

Dying produces human remains and is a unique period of human lives that remains poorly understood. The aims of this research were to validate the use of hair cortisol concentration (HCC) analysis in the study of stress at the end of life and to explore the effects of biocultural factors on stress experience in the last months of life. This study examined the dead from the 1st century CE Egypt, 19th-20th century Missouri, and 21st century Florida. A framework of embodiment and the good death was employed to interpret lived experience from HCC and examine the relationships between HCC, death, cause of death, duration of disease, and medical care and treatment.

HCC in the dead is higher than in the living, varies considerably between individuals, and can fluctuate across the last months of life. High HCC at the end of life cannot be easily accounted for by medications, serious disease, or decomposition; are within possible biological ranges; and are dynamic. However, leaching of cortisol from the archaeological samples is likely. Duration of disease or presence of multiple medical conditions does not influence HCC. While modern medical advancements do not improve stress levels in the dead, a reduction in stress is observed following hospital entry in the past.

HCC is a valid measure of stress at the end of life. The last months of life are periods of significant stress but dying is an inherently personal and dynamic experience that varies between individuals and over many months leading up to death due to the interaction of multiple biocultural factors. These findings contribute to the understanding of a unique period of individual lives, suggest that studies of HCC in bioarchaeology must focus on the dying period and be wary of leaching, and highlight the potential of HCC in palliative care research.

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LIST OF ABBREVIATIONS AND SYMBOLS

<	Less than
>	Greater than
AS	Asian
BL	Black
F	Female
HCC	Hair cortisol concentration
IQR	Interquartile range
M	Male
N	Sample size
SD	Standard Deviation
WH	White

DECLARATION OF ACADEMIC ACHIEVEMENT

I declare that the content of the research in this document has been completed by myself, Kaitlin East, with recognition of the contributions of Dr. Megan Brickley, Dr. Tracy Prowse, Dr. Stan Van Uum, and Dr. Lana Williams in both the research process and the completion of the thesis.

CHAPTER 1 - INTRODUCTION

The period preceding an individual's death can encompass unique and complex experiences related to dying and death. A poor understanding of the dying period limits the knowledge of the full breadth of past human lives and could obscure forces that interact to shape assemblages of human remains, while also contributing to fear of the dying period and inadequate care of dying people today. Given the nature of skeletal material, a better understanding of dying is unlikely to be possible from skeletal material alone. In palliative care, investigations of dying are limited by declining health in, and the difficulties of communicating with, dying patients. As a result, there is little objective data regarding the experience of dying, especially from the perspective of the dying person (Bretscher et al. 1999) or in the past. Recent advancements in the field of stress research have enabled new approaches to the investigation of stress from preserved human tissues. Stress acts as a mechanism by which multifaceted experience is embodied and is closely tied to quality of life. Therefore, the study of stress through hair cortisol concentration (HCC) analysis has the potential to shed light on experience at the end of life in ways that human remains often cannot; by reflecting holistic experience in a narrow, but important window of time. HCC analysis has the ability to reveal monthly averages of stress experience but has yet to be validated in studies of the dying and dead. Therefore, the current study will investigate applications of the novel method of HCC in the study of the dying and will explore stress associated with dying in the past and the present. If validated, HCC analysis has the potential to provide a new tool for the study of stress experience in the last months of life and contribute to understanding how the

experience of dying has changed over time; the relationship between dying, death, and assemblages of human remains; and the factors contributing to poor quality of life among the dying.

This dissertation explores stress experience in the last months of life in individuals from three distinct places and times: 21st century Florida, 20th century Missouri, and 1st century CE Egypt. **The aims of this study are two-fold: 1) to validate the use of HCC analysis in the study of stress in the dead and dying and 2) to determine if stress experience may vary between individuals and months leading up to death and what biocultural factors may be the cause.**

1.0. Context

As medical advancements have prolonged the process of dying and long-term care of dying people has become more common, understanding the way people die has taken on new urgency in the field of palliative care (Stewart et al. 1999). However, social, spiritual, and psychological suffering at the end of life remains substantial and objective data and detailed accounts of the experience of dying are limited (Bretscher et al. 1999, Kellehear 2009). Major scientific institutions have sounded the alarm about the inadequacy of care at the end of life and called for more research regarding the experience and needs of dying people (Council on Scientific Affairs 1996; The Institute of Medicine 1997). Researchers have argued that understanding the experiences of dying people and development of more effective tools for evaluating and translating those experiences to healthcare workers are key to improving care for dying people (Emmanuel et al. 2004, Kellehear 2009). However, investigating the dying experience is limited by the factors

intrinsic to the methods available. Common methods used in palliative care such as questionnaires generally do not account for the unique experience of terminal illness or dying, may rely on proxies [i.e., family members or members of the healthcare team], and risk artificially parsing holistic experience into component parts (e.g., Cohen et al. 1995; Emanuel and Emanuel 1998). Other methods include assessing health outcomes such as disease progression, length of survival, and mortality risk, which do not adequately reflect quality of life (e.g., Wenger and Rosenfeld 2001). These methods are applied to limited contexts and causes of death, precluding a complete and nuanced understanding of stress experience at the end of life (Kayser-Jones 2002, Lunney et al. 2003).

The importance of dying and death has received some attention in bioarchaeology with the rise of the biocultural approach and advancements in understanding the forces shaping skeletal assemblages. Dying is an aspect of human lives that is experienced across all places and times; thus, it is of interest in bioarchaeology. Furthermore, Wood and colleagues (1992) have argued in their work on mortality bias that skeletal assemblages are shaped by the lives and deaths of the individuals that comprise them, and Milner and Boldsen (2017) emphasize the importance of forces related to dying and death in shaping skeletal assemblages. Death shapes skeletal assemblages, in part because certain causes of death selectively target people of specific ages or relate to earlier experiences in different ways while the experience of dying and disease may directly affect the condition and appearance of skeletal remains. Developments in paleodemography have shed light on differences in skeletal assemblages produced through different death events (e.g., DeWitte 2012). Despite the potential significance of

better understanding the dying process in bioarchaeology, few methods exist that can assess experience in the last months of life, and those that can, such as stable isotope analysis and HCC, have rarely been examined in relation to dying and death (see D'Ortenzio et al. 2015 for an exception).

Advancements in the study of stress experience provide a promising foundation from which to address the lack of knowledge regarding experiences of dying. Stress is defined here as complex process consisting of exposure to a stressor, a state of disharmony, and a response (Goldstein 1995). Although stress experience itself has received little attention in palliative care, stress reduction has received some attention (Smith et al. 2005); and stress is closely tied to distress, quality of life, and suffering which are key foci of palliative care research (Rummans et al. 2000; Steinhauser et al. 2000; Kellehear 2009). The study of stress has also been a prominent feature of exploring health and wellbeing in the past within broader social, biological, and environmental structures in bioarchaeology (Temple and Goodman 2014). Therefore, the study of stress is well suited to the investigation of the last months of life in the past and present. Yet, little is known about how stress is experienced in the last months of life and how it differs between individuals, across cultures and time periods, or in conjuction with modern medical treatments.

Cortisol is a hormone produced by the adrenal glands; it is an end product of the hypothalamic-pituitary-adrenal axis, which is activated in response to stressors (Miller et al. 2007). Cortisol in one centimeter of hair reflects roughly monthly averages of systemic cortisol (Meyer and Novak 2012). A large body of research investigating various factors

affecting living people have proven HCC to be a valid and robust measure of chronic stress (e.g., Staufenbiel et al. 2013; Vives et al. 2015; Stalder et al. 2017), but the effects of dying and factors related to death on HCC have not yet been investigated. In bioarchaeology, analysis of HCC has produced nuanced depictions of individual experiences in relation to mobility and dietary changes (e.g., Webb et al. 2014; 2015), but no studies have explicitly considered the effects of dying and factors related to death on HCC. Without accounting for such variables, the use of HCC in investigations of the dead remains limited.

This study is the first to investigate the novel method of HCC analysis for stress assessment within the context of dying and death. By exploring the effects of dying and taphonomic factors following death on HCC in three distinct (i.e., modern, historic, and archaeological) samples, this study will determine if HCC can be productively applied to the study of stress in contexts other than modern living populations. By determining the utility of a new methodology in studying experience at the end of life, the findings of this study could have implications for understanding experiences of dying and stress at the end of life.

1.1. Research Questions

To validate the use of HCC in the study of stress in the dying and dead, I will determine if HCC is a useful measure of stress in the face of factors that are unique to the dying period or related to the process of death. I will investigate the effects of pharmaceutical interventions, metabolic disturbances, and decomposition on HCC values

and will evaluate whether HCC continues to be responsive to changes in stress experience as death nears. I will also address the following question:

1. How does stress at the end of life relate to the experience of dying?

To evaluate if stress experience at the end of life varies between individuals or across the months leading up to death as a result of biocultural factors, I will assess the following questions:

1. Are there differences in stress experience in the last months of life between individuals as indicated by HCC?
    1.1. Can these differences in HCC be explained by medical interventions, cause of death, preexisting disease, or broader temporal and social contexts?
2. Does stress experience change across the months leading up to death in an individual, as indicated by changes in HCC?
    2.1. How do these fluctuations compare to expected disease trajectories, hospital entry, or disease diagnosis?

1.2. Organization of thesis

This thesis is divided into eight chapters. Following this introductory chapter, Chapter 2 provides necessary background information regarding studies of stress and dying, HCC analysis, and the theoretical frameworks employed in this study. Chapter 3 outlines details regarding the composition of each sample and their historical

backgrounds, including causes of death and availability of medical treatments. Chapter 4 outlines the definitions and classification systems employed in the analysis and summarizes the methods used in the HCC, quantitative, and anthropological analyses. The first half of the results of the investigation, which relied only on comparisons within the study sample is provided in Chapter 5, while the second half of the results incorporates information from previous literature and is presented in Chapter 6. The results are interpreted and discussed in Chapter 7. Chapter 8 summarizes the significant findings and contributions of this research to the fields of bioarchaeology and palliative care.

1.3. Implications

The results of this investigation will determine the utility of a novel method to study a historically difficult-to-assess period of human life in both the past and present. The combination of methods from the clinical sciences with approaches and frameworks from bioarchaeology makes the most of diverse data sets to explore the effects of various factors that cannot be controlled for in a clinical setting such as access to care or cause of death. This innovative approach is key to establishing the utility of HCC analysis in the study of the dying and dead and providing unique data about the experience of stress at the end of life across time and space.

As the first investigation of stress in the dead across time and space using HCC analysis, the results will provide a foundation for a more comprehensive understanding of the range of experiences of dying people and could have significant implications for theory and practice in the fields most concerned with the study of the dead and dying:

bioarchaeology and palliative care. This study of stress will investigate whether HCC analysis can be usefully applied to the study of stress in the dead. Such findings could have significant implications for understanding the relationship between dying in the past and skeletal remains. The current study will also investigate contributions to the model of a ‘good death’ and the utility of HCC analysis in providing an innovative, holistic, and objective tool for evaluating quality of life at the end of life. Ultimately, a better understanding of dying and death in the past and the present has the potential to improve the care and experiences of dying people, which could benefit all who will face a prolonged period of dying or fear they might.

CHAPTER 2 − BACKGROUND

2.0. Introduction

Traditional bioarchaeological methods of studying stress in the dead offer little insight into the dying experience, while common methods of studying stress in the living and dying, such as questionnaires and assessments of health outcomes in palliative care are not applicable to the dead. The novel method of hair cortisol concentration (HCC) analysis has been fruitfully applied to the study of stress in living people from varied contexts yet has received little attention in the study of dying and death. Therefore, the interpretation of HCC in the dead must be carefully contextualized within the current research of HCC in the living and hypothalamic-pituitary-adrenal (HPA) axis activity in the dying and those with disease.

This chapter will situate the current study within the literature on stress analysis in bioarchaeology and palliative care as well as the study of dying and death in these fields. The development of HCC analysis and its potential applications will be reviewed as will the current literature on HPA axis activity in disease, dying, and death. The chapter will end with an introduction to the concepts of embodiment and the 'good death' which provide the theoretical framework for the current study.

2.1. Stress

The modern-day stress concept can be traced back to advancements in the understanding of living systems in the mid to late 1800s, including the work of Claude Bernard on the *milieu intérieur*, published in 1859, and Walter B. Cannon on homeostasis, published in 1932 (Kopin 1995; Goldstein and Kopin 2007; Ice and James

2012, Fink 2016). Hans Selye produced dramatic advancements in the study of stress as early as the 1930s through his research on the physiological aspects of the stress response (Selye 1956; Mason 1975; Cooper and Dewe 2004). Selye was the first to use the term stress in biomedicine and defined it as a "state manifested by a specific syndrome which consists of all the non-specifically induced changes within a biologic system" (Selye 1956:54). This syndrome was known as the General Adaptation Syndrome (GAS) and referred to the stages of alarm, resistance, and exhaustion that characterize exposure to a stressor (Selye 1956; Fink 2016). Selye's work, especially his characterization of the role of the adrenal glands in stress, was instrumental in illustrating the biologic components of the stress response system (Selye 1956; Fink 2016). However, Selye argued that stress response was non-specific and that some factors, such as stomach ulcers, were always indicative of stress; these claims have been refuted (Chrousos and Gold 1992; Goldstein 2010; Fink 2016). The Selyean stress concept is rarely used in the biomedical sciences today (Kopin 1995; Carlson and Chamberlain 2005).

Developments in the understanding of homeostasis have produced new insight into the concept of stress in biomedicine. It is now generally accepted that, while some body systems require very specific parameters to operate and are consistent with the concept of homeostasis, most systems operate within a range of conditions. Allostasis refers to this maintenance of stable internal conditions through changes in acceptable ranges over time (McEwen and Stellar 1993; Sterling and Eyer 1988). Employing a model of allostasis, stress is best conceived of as a broad process beginning with the identification of a threat (Goldstein and McEwen 2002) or as a state of discrepancy

between reality and expectations, which can be both biological and psychological (Goldstein and Kopin 2007). Routine fluctuations as part of survival are part of the stress process, and when exposure to a threat requires adaptation, or set points must become altered, an allostatic state arises (McEwen 2004; Schulkin 2004; Carlson and Chamberlain 2005). Over time, "wear and tear on the body and brain" (McEwen and Stellar 1993: 37) otherwise known as allostatic load, can occur as a result of chronic stress or normal, repeated readjustments of the allostatic system over the life course (McEwen 1998; 2004; Carlson and Chamberlain 2005).

A primary component of the stress response is the HPA axis (Figure 2.1). The hypothalamus is activated when a threat is perceived or anticipated (biologically or consciously) and releases corticotrophin releasing hormone (CRH) which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH), which in turn, signals the adrenal cortex to secrete cortisol (Gow et al. 2010; Staufenbiel et al. 2013; Lee et al. 2015). Cortisol stimulates glucose production by the liver, dampens immune and inflammatory responses, and limits metabolic activities not essential for survival in order to increase available energy to respond to the threat (Greendale et al. 1999; Norbury et al. 2008; Russell et al. 2012; Lee et al. 2015). Under normal conditions, the axis will return to baseline through negative feedback loops once the threat passes; if dysregulation occurs allostatic load may result (McEwen and Stellar 1993; Miller et al. 2007).

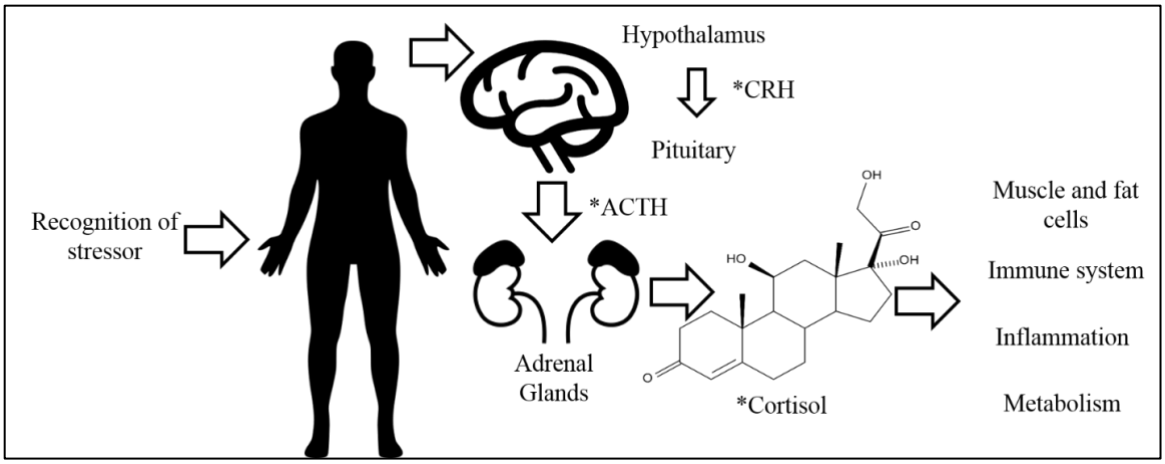

Figure 2.1. A simplified diagram of the HPA axis. Abbreviations: CRH = corticotrophin releasing hormone, ACTH = adrenocorticotropic hormone.* indicates hormones.

2.1.1. Definitions of stress

Despite the long history of stress research and its near ubiquitous presence in human life, no consensus exists about definitions of the term. Stress is variously defined as a threat (e.g., Mason 1975; Kopin 1995), a process (e.g., Goldstein 1995; Ridner 2004), a state (e.g., Toates 1995), a relationship (e.g., Mason 1975; Lazarus and Folkman 1984; Jones and Bright 2001), or a mediating variable (e.g., Ice and James 2007). Researchers agree however, that the relationship between stress and the environment is a complex one shaped by social factors, genetics, development, and experience (McEwen and Stellar 1993; Goldstein and Kopin 2007). In general, biomedical approaches to stress provide a foundation for understanding the biological components of the stress process. However, biomedical definitions of stress tend to disregard the intertwined nature of human bodies and their environment or the connections between social and biological entities (Young 1980; Schoenberg et al. 2005).

In bioarchaeology, most studies of stress apply the definition of Huss-Ashmore and colleagues (1982) - or a modified version thereof - which states that stress is a "physiological disruption of an organism resulting from environmental perturbation" (Huss-Ashmore et al. 1982:396). Building from the definition of Huss-Ashmore and colleagues (1982), Goodman and Armelagos (1989) developed the most commonly employed model to interpret stress in human remains from archaeological contexts. In this model, the environment is the main source of stressors and if buffering and adaptation fail, disruption to the living system, or stress, occurs. Disruptions include infection, nutritional inadequacy, and trauma, which can result in skeletal changes (Goodman et al. 1984; 1988). The perspective offered by Huss-Ashmore and colleagues (1982) and Goodman and colleagues (1984) is founded on the Selyean stress concept (Zuckerman and Armelagos 2011). Alternative conceptions of stress in bioarchaeology define it as a response (Goodman et al. 1988), deviation (Temple and Goodman 2014), or perturbation (Klaus 2014). Oftentimes studies of stress in bioarchaeology are limited by a reliance on a Selyean stress concept and the difficulty of applying the plethora of stress research from biomedicine to the study of the dead.

2.1.2. The study of stress

Studies of stress in living people, dying people, and the dead each face unique challenges. Many of the approaches to stress in biomedicine are not applicable to bioarchaeology, and oftentimes the approaches to stress in bioarchaeology are not of interest to biomedicine.

2.1.2.1. Clinical science

The measurement of stress is of significant interest to biomedicine because of the consequences for human health and wellbeing. Popular approaches to the analysis of stress in biomedicine is through the measurement of primary mediators of stress, the function of key physiological systems, and neurobehavioral characteristics. McEwen (2004) defined primary mediators as factors (such as cortisol) that lead to changes at the cellular level which may accumulate within a tissue or organ and lead to disease (McEwen and Seeman 1999). Circulating hormones including glucocorticoids, dihydroepiandrosterone (DHEA), catecholamines, and cytokines are commonly measured primary mediators because of the ease of collection and their well-established relationship to secondary and tertiary health outcomes (McEwen 2004). In the measurement of allostatic load, studies generally evaluate physiological activity across cardiovascular, metabolic, and immune categories, although no standard list or method for determining the relative weight of each biomarker exists (McEwen 2004; Duong et al. 2017; Mazgelytė et al 2019). Common biomarkers include blood pressure (cardiovascular category), risk for diabetes (metabolic category), and C-reactive protein (CRP) (immune function; Duong et al. 2017). Other studies have explored heart rate, waist to hip ratio, cholesterol, bone mineral density, body mass index, muscle mass, immune response, and memory (e.g., McEwen and Seeman 1999, McEwen 2004, Olstad et al. 2016). To investigate perception and subjective experience of stress researchers turn to interviews and questionnaires such as the Perceived Stress Scale, which is designed to investigate an

individual's subjective appraisal of stressful experiences using questions directed at assessing feelings of control, predictability, and overloading (Cohen et al. 1983).

2.1.2.2. Palliative care

Palliative care is closely related to biomedical approaches (Section 2.2), although the field takes a more pronounced interest in the role of emotional, social, and existential factors in shaping experience. Measurement of stress experience has received little attention in palliative care although stress reduction and related concepts of distress, suffering, and quality of life have been investigated in depth (see Section 2.2.1.1). Currently, the primary means by which researchers explore concepts related to stress at the end of life include surveys and interviews with patients and family members that could be prospective or retrospective in nature (SUPPORT 1995, Kellehear 2009). Such studies may investigate distress and suffering in general, or explore these experiences before and after interventions, such as hospice entry (Shega et al. 2008).

A number of studies have examined the effects of mindfulness-based stress reduction techniques to reduce stress in cancer patients receiving palliative care using questionnaires as part of randomized controlled clinical trials and uncontrolled clinical trials (Smith et al. 2005). The Profile of Mood States (POMS) questionnaire measures 65 items across six dimensions to assess mood changes (McNair et al. 1971; Carlson et al. 2004), while the Symptoms of Stress Inventory (SOSI) measures responses to stressful situations by investigating how often individuals experience stress-related symptoms in the prior week (Leckie and Thompson 1979, Carlson et al. 2004). Other questionnaires investigate psychological and physical symptoms, health behaviors, depression, and

fatigue, as well as emotional, physical, and general wellbeing (Smith et al. 2005; Shennan et al. 2011). Less commonly, biological factors are measured, such as heart and respiratory rate or blood and saliva levels of cortisol, Dehydroepiandrosterone sulfate (DHEAS), and melatonin (Carlson et al. 2004). Many studies examining mindfulness-based stress reduction do not explicitly define stress.

2.1.2.3. Bioarchaeology

Interest in stress grew in anthropology in the mid-1900s and by the 1970s, stress, specifically a general stress approach, became fundamental to the development of modern bioarchaeology (Temple and Goodman 2014). A relationship between skeletal changes and stress experience is the foundation of a general stress approach in bioarchaeology and has facilitated the advancement in bioarchaeology from descriptive case studies to hypothesis testing (Goodman et al. 1984; Temple and Goodman 2014). A problem-oriented approach has been applied to explore health, wellbeing, and the consequences of adaptation in the past within broader social, biological, and environmental structures (Hutchinson and Larsen 1988; Goodman and Martin 2002). However, the interpretation of skeletal indicators of stress and the general stress approach have received much criticism. These criticisms tend to focus on the unknown relationship between stress and skeletal changes, the flawed and inconsistent assumptions made about this relationship, and simplified methods used for interpreting skeletal changes. The reliance on a Selyean, non-specific concept of stress, which has been largely abandoned in biomedical research, is also a major drawback of the general stress approach (Weston 2012; Klaus 2014).

Mays (2018) argued that a primary limitation in the use of skeletal indicators of stress is a reliance on comparison to reference samples. This comparison requires an assumption that the same skeletal changes, even in less extreme manifestations, result from the same underlying condition, which, without an understanding of the pathophysiology of disease, can be problematic. Similarly, Klaus (2014; 2017) stated that the use of skeletal indicators of stress is limited by a lack of understanding of bone biology and its response to stress and called for a more rigorous approach to differential diagnosis that incorporates advancements from anatomy and biology (Klaus 2017).

Without an understanding of pathophysiology, interpretations about the relationship between skeletal changes and stress can be inconsistent or flawed. For example, skeletal features are variably referred to as 'stress indicators' (Larsen 2015), 'skeletal markers of stress' (Reitsema and McIlvaine 2014), 'stress markers' (DeWitte and Stojanowski 2015), or 'skeletal indicators of nutrition' (Danforth 1999), and have been defined as representing stress (Goodman and Armelagos 1989), episodes of stress (Cohen and Crane Kramer 2007; Reitsema and McIlvaine 2014; Larsen 2015), physiological stress (DeWitte and Stojanowski 2015; Larsen 2015), malnutrition (Danforth 1999), or health (Steckel et al. 2002). A misunderstanding of pathophysiology in the case of periosteal new bone formation led researchers to assume a relationship to systemic stress or non-specific infection, but more recent studies have suggested that localized causes, including those unrelated to infection or stress may also be at play (Weston 2012).

The methods used in the general stress approach can also lead to over-simplification in the interpretation of skeletal changes (Weston 2012). The common use of crude prevalence, for example, does not take into account that skeletal indicators in the dead do not reflect the health or stress experience of the living population (Wood et al.1992). Similar approaches are unable to consider the effects of individual differences in susceptibility or remodeling over the life course (Powell 1988; Klaus 2014). More advanced epidemiological and statistical methods can partially compensate for these challenges, but the underlying problems remain (Klaus 2014).

Despite these challenges, stress is an important aspect of the relationship between humans and their biosocial environment and a universal human experience. While Mays (2018) and Klaus (2014) suggested that skeletal markers can become more useful with a greater understanding of their underlying pathophysiology, Weston (2012) argued that skeletal indicators may only be useful in a very broad sense as indicators of overall stress. Therefore, stress is worthy of attention in bioarchaeology and holds promise in understanding human experience across times and places but is difficult to assess in the skeleton. HCC analysis offers a new approach to, and perspective on stress in bioarchaeology, and will be discussed in detail below (Section 2.3).

2.2. Death and dying

Dying and death are experienced by individuals across all cultures and time periods. Palliative care is founded upon concern for improving the experience of dying.

Bioarchaeology exclusively studies those who have died. The approach and methods to study dying and death differ considerably between the fields.

2.2.1. Palliative care and the study of dying

Attempts to alleviate discomfort at the end of life have been common throughout human history (Kellehear 2007). However, death has become an increasingly medical event; patients and doctors seek to postpone death and create tension between cure, alleviating pain, prolonging life, and accepting death (Franklin and Lock 2001; Stephenson 2001; Kaufman and Morgan 2005; Beckstrand et al. 2006; Green 2008). As a result, it was not until the 20th century that the experience of, and care for, the dying started to receive direct attention (Clark 2007).

The experience of dying was first systematically investigated in North America by William Osler between 1900 and 1904, who identified themes of pain, physical and mental distress, fear, and individual variation (Kaufman 2005). As more people began to die of chronic conditions throughout the 1900s, it became clear that hospital care was inadequate for dying people and homes for terminally ill people were established in the 1950s in England followed by the development of the first hospice in 1967 (Clark 2007; Milligan and Potts 2009). At the same time, Glaser and Strauss (1964) were studying experiences of dying people to identify different trajectories of dying (Mak and Clinton 1999) and Kübler-Ross (1969) was categorizing the stages of acceptance for dying people. While the stages have been criticized for being deterministic, Kübler-Ross was one of the few to explore the inner experiences and feelings of dying people and emphasize the importance of patients' autonomy (Kaufman 2005). In 1974, Balfour

Mount, a pioneer of palliative care in North America, coined the term palliative care in Canada and in 1987 palliative care was defined as a subspecialty of general medicine (Clark 2007). In general, palliative care developed to combat the medicalization of the dying process and the lack of autonomy afforded patients and families, while focusing on symptom management and quality of life and providing alternative approaches to dying (Palgi and Abramovitch 1984; Kissane et al. 2000; Timmermans 2005).

Today, the goal of palliative care is to maintain an optimal quality of life through a focus on symptom control and wellbeing when curative treatments are no longer possible or beneficial (Mount 2003). Hospice care is generally seen as a subfield of palliative care and is defined as "a predominantly community-based program that provides interprofessional multidimensional care for patients with terminal illness (i.e., expected survival <6 months) and their families" (Hui et al. 2013: 6). According to the WHO, palliative care consists of six key features: "(1) affirms life by regarding dying as a normal part of life, (2) neither hastens nor postpones death, (3) provides relief from pain and other distressing symptoms, (4) integrates psychological and spiritual aspects of care, (5) offers support systems to help patients remain physically and mentally active as long as possible, and (6) offers support systems to help caregivers cope during the patient's final days and during the bereavement period" (Rummans et al. 2000: 1308).

Despite the significant advancements in the field, concerns persist regarding the quality of care for dying people. The SUPPORT working group (1995) called for a better understanding of the effects of life sustaining treatment on quality of life. The Council on Scientific Affairs (1996) requested more research into the needs of dying patients and

better ways to assess care outcomes. The Institute of Medicine appealed for the development of a more substantial knowledge base about the end of life, end-of-life care, and better tools for evaluating quality of life (Field and Cassel 1997, Institute of Medicine 1997). In 2001, another report from the Institute of Medicine highlighted the progress since the earlier report and emphasized ongoing challenges, which include the focus on curative research (Foley and Gelband 2001). In 2004, the National Institutes of Health joined the call for improvements in end-of-life care (Grady 2005).

Researchers have echoed these concerns, arguing that techniques for the evaluation of hospice care effectiveness, impact of management strategies, and quality of life are seriously lacking (Sprangers and Aaronson 1992; Brody et al. 1997; Cohen et al. 1997; Mak and Clinton 1999; Hanson et al. 2002). Other areas of inadequacy include the evaluation of changes over time, the lack of validated outcome measures, and minimal research focusing on patients' perspectives (Sprangers and Aaronson 1992; McCormick and Conley 1995; Mak and Clinton 1999). Although freedom from pain has been cited as the greatest concern of dying people, research has found that symptoms experienced by dying people, especially pain, are inadequately controlled in hospital and hospice settings (Emanuel and Emanuel 1998; Lo and et al. 1999; Vig et al. 2002). Studies have also found that the shift to palliative care is often delayed in favor of prolonged, aggressive treatments, leading to greater patient suffering (Cassel 1998; Tilden 1999). Research is especially lacking for vulnerable groups such as those with dementia, in a coma or persistent vegetative states, or who do not speak the language of the healthcare workers (Kayser-Jones 2002).

2.2.1.1. Stress, Distress, Suffering, and Quality of Life in Palliative Care

Stress is generally not an area of focus in palliative care research. However, the concept of stress is closely related to concepts that have received substantial attention in palliative care research: distress, suffering, and quality of life. Distress and suffering are common themes in the study of dying people and the alleviation of suffering and maintenance or improvement of quality of life are seen as key goals of palliative care (SUPPORT 1995; Rummans et al. 2000; Terry et al. 2006; Kellehear 2009).

Suffering has received substantial attention in palliative care research although definitions vary (Kellehear 2009). Krikorian and colleagues defined suffering as ‘‘a multidimensional and dynamic experience of severe stress that occurs when there is a significant threat to the whole person and regulatory processes are insufficient, leading to exhaustion’’ (2012: 45). According to Cherny (2011) suffering is an “aversive experience characterized by the perception of personal distress that is generated by adverse factors that undermine quality of life” (35). “Suffering is a specific distress caused by a person’s perception of his or her imminent destruction” according to Krikorian and colleagues (2012: 42). Suffering may result from social context, disease, cognitive state, perceptions, uncontrolled symptoms, function, mental conditions, lack of social or family support, and existential issues (Kissane et al. 2010; Cherny 2011). According to Cassel (1998) suffering is experienced by the whole person, not just the body; while Kellehear (2009), argued that not all dying people suffer. In general, definitions tie stress and distress to suffering and agree that suffering is subjective and multidimensional, involving social, spiritual, and psychological components (Cassel 1998; Kellehear 2009).

Distress has been a theme among studies of the dying since William Osler conducted his early investigations in the United States and distinguished between physical distress, which he equated with pain, and mental distress (Kaufman 2005; Kellehear 2009). Distress can refer to emotional phenomena (SUPPORT 1995) or responses to physical symptoms (McCarthy et al. 1996) but is often undefined. Armstrong-Coster (2004) drew an explicit connection between suffering and distress, saying "Patients suffered; their pain, whether real or imagined, physical or psychological, became manifest in distress" (136). Selye (1974) initially made a distinction between stress which could be either good or bad; eustress, which was positive; and distress, which was negative. The concept of eustress has been found to be minimally useful in the clinical sciences, and studies instead focus on stress and distress (Bienertova-Vasku et al. 2020). Distress is generally associated with poorer quality of life (Campbell et al. 2009).

Quality of life is another major focus of palliative care research. Testa and Simonson defined quality of life as the "physical, psychological, social, and spiritual domains of health that are influenced by a person's experiences, beliefs, expectations, and perceptions" (1996: 1306). The multidimensional nature of quality of life is a common component of definitions (Testa and Simonson 1996; Bretscher et al. 1999, Stewart et al. 1999). Quality of life is a subjective experience that can only be evaluated and given meaning by the patient (Testa and Simonson 1996; Stewart et al. 1999). As such, individuals with the same disease and disease trajectory will experience a unique quality of life, and, even in the face of declining physical health, quality of life may remain stable (Testa and Simonson 1996; Rummans et al. 2000).

Suffering, stress, and distress are often used interchangeably in palliative care research or are only vaguely defined. Their relationship to each other and quality of life is also often taken for granted. Krikorian and colleagues (2012) envisioned eustress, distress, and suffering as separate responses to stressors. Eustress is an adequate response to challenge, distress is an inadequate response to a threat, and suffering is an inadequate response to a threat coupled with exhaustion (Krikorian et al. 2012). However, instead of viewing suffering, distress, and stress as separate experiences, they are best understood as overlapping phenomena that can all be experienced at once (Figure 2.2). Suffering is a specific and severe form of distress, which is itself a specific and negative form of stress. Suffering, distress, and excessive stress are associated with a reduction in quality of life, which encompasses all of these factors and more (Campbell et al. 2009; Cherny 2011). In sum, suffering, distress, stress, and quality of life are all subjective, multidimensional experiences that relate in complex ways to one another and to an individual's experiences, beliefs, and perceptions. Thus, quality of life is the broadest, most holistic level of experience and assessment of each of a component parts (suffering, distress, stress) comes one step closer to a holistic understanding of quality of life.

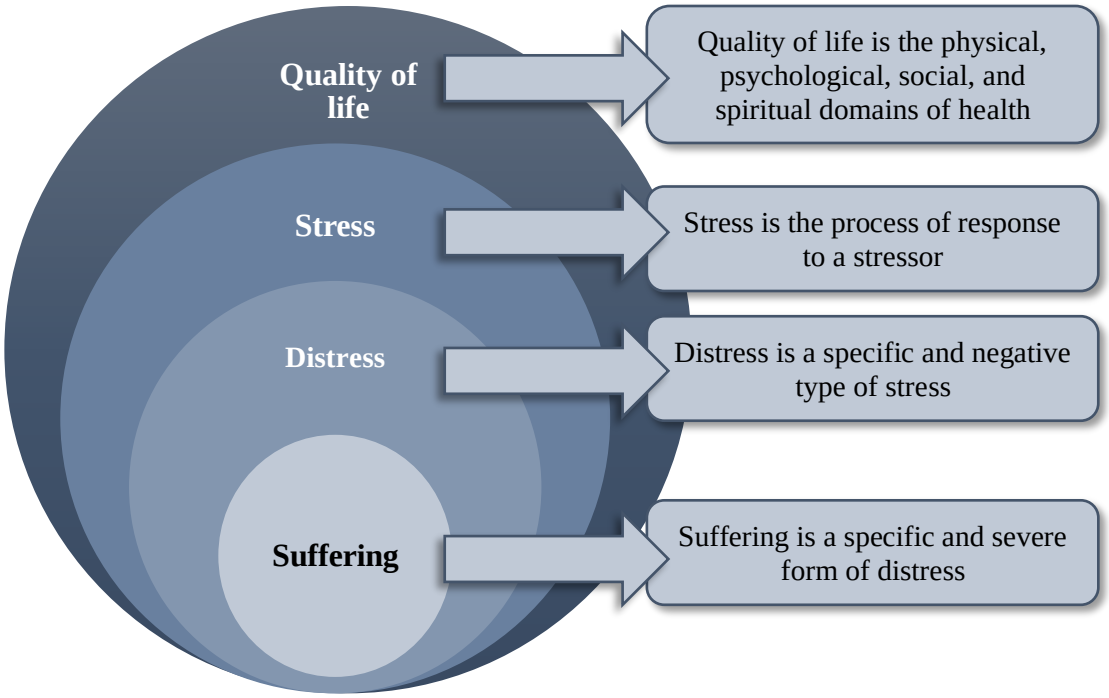

Figure 2.2. Diagram depicting the nested relationship between suffering, distress, stress, and quality of life.

2.2.1.2. Analytical instruments

Current approaches to investigating end-of-life experiences include quantitative and qualitative techniques that are prospective or retrospective in nature. Research designs for evaluating quality of life tend to be large non-randomized longitudinal studies exploring specific predictors of quality of life, smaller randomized studies of specific clinical interventions, or cost- benefit analyses (Testa and Simonson 1996). Quantitative approaches to measuring quality of end-of-life experiences and care generally focus on health outcomes such as length of survival and mortality risk (Wenger and Rosenfeld 2001; Giese-Davis et al. 2011). Qualitative approaches to determining quality of life at the end of life include interviews, surveys, questionnaires, and scales administered to patients or proxies. The “Death Experience Scale”, for example, evaluates physical and psychological symptoms, monetary stability, caregiving access, social relationships,

spiritual needs, and hopes (Emanuel and Emanuel 1998). Questionnaires are generally used for measuring quality of life and focus on depressive and anxious symptoms, functioning, social roles and support, and existential components such as outlook and meaning (Cohen et al. 1995). Similar questionnaires have been developed to assess suffering in dying people, including the Pictorial Representation of Illness and Self Measure questionnaire, although clinical interviews are generally considered the best method (see Krikorian et al. 2013 for a detailed discussion). Questionnaires and interviews are also the most common method of assessing distress in end-of-life care and include the Distress Thermometer in which the degree of distress is ranked (Campbell et al. 2009).

The most effective tools for the investigation of experience at the end of life consider physical, psychological, and social aspects of individual experience and are relevant to the dying period (Sprangers and Aaronson 1992; Steinhauser et al. 2000; Vig et al. 2002; Campbell et al. 2009). Successful approaches also account for pain and symptom management, cultural needs, cognitive ability, and treatment by staff (Kayser-Jones 2002). Testa and Simonson (1996) argued that tools should be reliable and replicable, responsive and sensitive enough to identify true changes in experience, validated in measuring what they claim to, and should measure all variables of concern to patients and influenced by interventions. Similarly, Krikorian and colleagues (2013) suggested that a technique for assessing suffering should be able to evaluate subjective elements, and be simple, fast, non-invasive, and easy to interpret. A summary of key elements of effective tools for evaluating end-of-life experience is provided in Table 2.1.

Table 2.1. Key components of effective methods and weaknesses of current methods to evaluate end-of-life experiences.

How components should be measured	**What should be measured**	**Weaknesses of current methods**
• Reliable • Replicable • Validated • Sensitive/ accurate • Patient centric • Simple to administer and interpret • Time and cost efficient • Non-invasive	• Response to change/ interventions • Aspects specific to dying experience • Subjective elements • Multidimensional/ holistic phenomenon • Pain and symptoms • Cultural needs • Treatment by staff	• Focus on survival and health outcomes • Reliance on proxies • Not relevant to dying period • Invasive • No independent outcome measurement

The methods currently available to study the end of life are limited in regard to these standards in a number of ways. Measuring survival and declines in physical health outcomes does not accurately correlate with quality of life (Cohen et al. 1997; Kellehear 2009). Questionnaires are often lengthy, focus on physical symptoms, or do not account for terminal illness (Cohen et al. 1995; Emanuel and Emanuel 1998). Many patients may not be able to complete the questionnaires or interviews and will rely on a proxy such as a family member or health care worker who tend to misjudge pain and psychological well-being (Sprangers and Aaronson 1992; Cohen et al. 1995; Fowler et al. 1999). Proxies will often judge an individual's quality of life at the end of life with reference to earlier stages of life and consistently rate the quality of life of the patient lower than the patient does (Bretscher et al. 1999). Furthermore, because no independent outcome measurement exists, questionnaires are often validated only by their consistency to others (Cohen et al. 1995; 1997).

Dying is also an inherently difficult process to study. Dying patients pass through a number of different phases and the dying period is often difficult to distinguish (McCormick and Conley 1995; Fowler et al. 1999; Leichtentritt and Rettig 2001; Wenger and Rosenfeld 2001). In general, patients may not be able to communicate towards the end of life and signs of physical decline may complicate assessments of quality of life (Sprangers and Aaronson 1992; Fowler et al. 1999; Terry et al. 2006).

2.2.2. Death and dying in bioarchaeology

The importance of death as a shaping force of skeletal assemblages has long been recognized by bioarchaeologists. The issues of selective mortality and mortality bias were highlighted by Wood and colleagues (1992) and refer to the fact that skeletal samples do not directly reflect the living population from which they are drawn. The distribution of lesions in a skeletal sample does not reflect that of the living population at any point in time, in part because the experiences resulting in skeletal lesions also contribute to death and because the state of a skeleton following death does not adequately represent that individual throughout their life (Wood et al. 1992; Wright and Yodder 2003; DeWitte and Wood 2008). At the same time, the factors that put individuals at risk for entering a skeletal assemblage at a particular age vary across a population and are often not visible in the skeleton (Wood et al. 1992; Wright and Yodder 2003).

Recent work in bioarchaeology has produced important insights by focusing on the factors that impact the composition of skeletal assemblages, such as mortality, and taking into consideration mortality bias (Milner and Boldsen 2017). The study of skeletal

indicators of stress and age at death has shed light on the relationship between stress and mortality. For example, enamel hypoplastic defects, used as a proxy for earlier periods of stress experience, have been correlated with lower mean age at death, lower probability of survival, and shorter life expectancies in some studies, suggesting a relationship between earlier stress and mortality risk (e.g., Palubeckaite et al. 2002; Boldsen 2007). Others have found no relationship between the presence of enamel hypoplastic defects and age at death or an inverse relationship indicating that the relationship between mortality risk and stress may vary (e.g., Saunders and Keenleyside 1999; Lewis and Gowland 2007; Amoroso et al. 2014). Recent studies have applied multistate hazard models to explore the relationships between age, skeletal indicators of stress, morbidity, and mortality (Wright and Yodder 2003; DeWitte and Bekvalac 2010; DeWitte 2014; Wilson 2014). Such investigations have been able to explore effects of environmental change, frailty, and status on mortality (Redfern and DeWitte 2011; DeWitte et al. 2013; DeWitte 2014). DeWitte and Hughes-Morey (2012) found that shorter stature was associated with increased mortality during the Black Death but not in periods without plague. In addition to mortality bias, studies of mortality patterns in the past are challenged by population growth, variability in susceptibility, and multigenerational cemeteries (Goodman et al. 1984; Wood et al. 1992; Roksandic and Armstrong 2011).

Despite receiving little explicit attention in the literature, the relationship between cause of death and mortality bias is significant (Schneidel 2001). Certain conditions resulting in death selectively target people of specific ages or relate to earlier experiences in different ways. Therefore, the age at which an individual enters a skeletal assemblage

and the condition of their skeleton when they do, may be related to cause of death. Some researchers have suggested that catastrophic assemblages, in which individuals died because of natural disaster, famine, epidemic, or war, may provide a less-biased image of a living population because the forces are not selective by age (DeWitte and Wood 2008; Redfern and Chamberlain 2011). Redfern and Chamberlain (2011) found a greater number of young adult males in a cemetery associated with warfare, which they argued, more accurately reflected the living population. DeWitte (2012) found a higher rate of periodontal disease in males in attritional but not catastrophic assemblages indicating that cause of death does play a role in altering the distribution of periodontal disease. However, DeWitte (2010) and DeWitte and Wood (2008) found that the risk of mortality with age and with presence of skeletal lesions was similar in plague and non-plague cemeteries indicating that the disease was only slightly less selective than normal mortality. It is unlikely that any cause of death is completely unbiased in its selectivity.

Although dying and death shape the composition of skeletal assemblages, investigations into dying and cause of death are rare in bioarchaeology. Cause of death data has been employed in bioarchaeological studies, but such data can be inaccurate and is often only used to test methods or confirm disease diagnosis (e.g., Santos and Roberts 2006; Martin and Harrod 2015). Attempts to determine cause of death are limited to case studies of specific disease or traumatic injury (Martin and Harrod 2015). More rarely, general cause of death for an entire cemetery is the subject of investigation. For example, Castex and colleagues (2011) determined that the mortality profile of St. Benedict's Cemetery in Prague was not consistent with famine or plague as it contained a high

number of 20–29-year-old males in comparison to other cemeteries associated with famine or plague. Other discussions of cause of death are broad and vague. Researchers may claim that juveniles more often die of acute rather than chronic conditions (Johnston 1962; Lovejoy et al. 1990). Similarly, Lewis and Gowland (2007) compare "endogenous" and "exogenous" causes of death, which refer to prenatal and postnatal conditions respectively, to argue an increase in "exogenous" causes of death indicates a more dangerous postnatal period related to early weaning. None of these studies discuss the experience of dying from different conditions.

Ultimately, the effects of death and cause of death in shaping skeletal assemblages has received attention through the study of mortality patterns and mortality bias but the experience of dying in the past is largely inaccessible to bioarchaeologists from human remains alone. Death produces human remains which enter an assemblage through the action of living humans. The human remains themselves, however, are a product of lived experience, dying experience, and death. Therefore, understanding more about how people die could benefit bioarchaeology. However, given the nature of skeletal material, a better understanding of dying experience is unlikely to be possible from skeletal material alone.

2.3. Hair Cortisol Concentration Analysis

Cirimele and colleagues (2000) were the first to identify glucocorticoids in human hair when they examined hair from deceased people receiving steroid treatments prior to death. Endogenous cortisol was first extracted from hair by Koren and colleagues (2002)

who did so from the hair of wild hyraxes and found that quantities correlated with social ranking. Next, Raul and colleagues (2004) extracted endogenous cortisol and cortisone from 44 human subjects. Davenport and colleagues (2006) applied the new method to the study of stress in non-human primates (rhesus macaques). They determined that HCC responds to long-term activity of the HPA axis, which they define as "weeks or months" (Davenport et al. 2006). Sauvé and colleagues (2007) were the first to use an enzyme linked immunosorbent assay (ELISA) protocol on hair from humans. This advancement established the use of a modified salivary ELISA protocol for reliable HCC quantification, making HCC analysis more cost effective and accessible for use in large studies like the current one.

Peripheral production of cortisol within hair follicles was identified as a potential source of HCC in one case (Sharpley et al. 2009), but the study has been largely discounted due to the use of sheep's wool, small sample sizes, and comparison to few samples of salivary cortisol (Salaberger et al. 2016). Furthermore, studies have determined that HCC are correlated with hydrocortisone dose (Gow et al. 2011), and the clinical course of Cushing's syndrome (Thomson et al. 2010) and Addison's disease (Ibrahim and Van Uum 2014). These studies and others have validated HCC as a measure of systemic cortisol concentrations and confirmed the reliability and accuracy of HCC analysis in humans (Kirschbaum et al. 2009; Stalder et al. 2010).

While early studies established the relationship between HCC and HPA axis activity in non-human primates and humans (Davenport et al. 2006; Sauvé et al. 2007; Fairbanks et al. 2011); other studies verified the relationship between chronic stress and

HCC in humans (Yamada et al. 2007; Kalra et al. 2007; Dettenborn et al. 2010). Follow up studies of HCC have firmly established a significant correlation between HCC and chronic stress in humans (D'Anna-Hernandez et al. 2011; Laudenslager et al. 2011; Stalder et al. 2012a; Stalder and Kirschbaum 2012; Vives et al. 2015). These authors generally define chronic stress as stress persisting for weeks or months (Davenport et al. 2006). Stalder and colleagues (2012b) found strong stability of HCC in individuals over time, except when perceived stress levels changed.

HCC has been widely applied in studies of chronic psychosocial stress because it is well suited to long-term studies and can easily be explored before and after stressful events. Studies have identified a relationship between HCC and stressful life events such as the Wenchuan earthquake or other traumatic events (Gao et al. 2014; Simmons et al. 2016). Elevated HCC has also been tied to ongoing stressful experiences such as duration of unemployment and caregiving of individuals living with dementia (Dettenborn et al. 2010; Stalder et al. 2014) and variables that result in chronic stress including low family income, more children, ethnicity (Rippe et al. 2016), and low parental education (Vaghri et al. 2013; Vliegenthart et al. 2016). HCC was elevated among members of the Walpole Island First Nations who were exposed to chronic stressors including disease, socioeconomic marginalization, environmental uncertainty, and discrimination (Henley et al. 2013). Elevated HCC was also identified in a Kenyan population living in slum settlements and was especially elevated in individuals who were female, divorced, low income, and reported feeling unsafe (Henley et al. 2014).

Many authors and multiple meta-analyses have confirmed that HCC is a relatively robust measure of stress. In a systematic review of 19 articles, Staufenbiel and colleagues (2013) determined that chronic stressors led to elevated HCC with a medium to large effect size. A similar review of 66 studies determined that elevated HCC was associated with chronic stress and was especially elevated when stress was experienced at the time of hair sample growth (Stalder et al. 2017). Adversity was found to have a small but significant effect on HCC that was moderated by type, timing, and severity of adversity; and sample characteristics in a 28 study meta-analysis (Khoury et al. 2019). However, the authors point out a small but important number of samples measuring childhood mistreatment that identified a significant negative effect of adverse experiences on HPA activity later in life. Kalliokoski and colleagues (2019) carried out a systematic review that confirmed the elevation of HCC with experiences of stress, especially when the stressor is present at the time of hair growth.

More recent research has started to interrogate the relationship between stressful life experience, stress response, perceived stress, mental health, and HCC. In some cases, the relationship between traumatic events and HCC may be complicated by the duration, timing, and severity of stressor exposure as well as previous experience of the individual (Steudte et al. 2013; Schaliniski et al. 2015; Steudte-Schmiedgen et al. 2015; 2016). The impact of these factors can be difficult to predict, as in the case of childhood trauma, which may lead to lower HCC or higher HCC in response to stressors as an adult (Schalinski et al. 2015; Groer et al. 2016). Some research has found higher HCC values in individuals with depression (Dettenborn et al. 2012a). A meta-review indicated a

reduction in HCC in anxiety disorders and PTSD and inconsistent relationships with mood disorders and depression (Steudte-Schmiedgen et al. 2015; 2016; Stalder et al. 2017). Another systematic review suggested that HCC tends to be elevated in late onset bipolar disorder and depleted in anxiety disorders; those with post-traumatic stress disorder were found to have an initial increase, with an overall decrease in HCC (Staufenbiel et al. 2013). Coello and colleagues (2019) found elevated HCC in individuals newly diagnosed with bipolar disorder. Staufenbiel and colleagues (2013) found that, overall, psychopathological conditions do impact HCC and responses to stressors, but such effects tend to be small to medium, while the effects of chronic stressors are medium to large.

2.3.2. Demographic and biological characteristics

Conflicting results regarding the effects of non-stress related factors on HCC have been reported. Demographic factors such as age, sex, and ancestry as well as biological factors such as genetics, hair growth rate, and hair color have received substantial attention. Lifestyle factors have received less attention including smoking, which shows no relationship with HCC (Stalder et al. 2017), and alcoholism, which was associated with elevated HCC during active phases (Stalder et al. 2010).

A meta- analysis carried out by Stalder and colleagues (2017) found that studies consistently indicated higher HCC among men as well as a positive relationship between age and HCC in correlation studies although non-linear testing revealed no significant effect. They argued that the correlation coefficient increased by 0.09 for each 10-year

increase in the standard deviation of age. These findings contrast the earlier review by Dettenborn and colleagues (2012a) that suggested no relationship between age or sex and HCC. The latter study was able to analyze a larger number of studies and did so more thoroughly. Both sets of authors agreed that more studies with wider age ranges will be necessary to verify the relationship between age and HCC (Dettenborn et al. 2012a; Stalder et al. 2017). In contrast to the findings of Stalder and colleagues (2017), Binz and colleagues (2018) found higher HCC in a toddler group (7 months to 3 years) than an adolescent group, which displayed lower HCC than the adult group. They also found higher HCC among men (Binz et al. 2018). Similarly, Gray and colleagues (2018) carried out a qualitative synthesis and found conflicting results regarding the relationship between HCC and age in children and adolescents, but higher HCC in males. De Kruijff and colleagues (2020) found a strong relationship between HCC and age with children under two years displaying particularly high HCC.

The literature examining the relationship between ancestral groups and HCC is complicated. Although an effect of ancestry was identified by some, most failed to interrogate the reasons for such differences (Slominski et al. 2015; Abell et al. 2016; Rippe et al. 2016). Palmer-Bacon and colleagues (2020) suggested that the differences in HCC between ancestral groups was related to histories of physical abuse and acts of micro-aggression. The role of psychosocial stressors in explaining differences in HCC between ancestral groups requires more investigation (Wosu et al. 2015). O'Brien and colleagues (2013) identified a relationship between ancestry and HCC, but also found that high and low socioeconomic status among minority groups was associated with

particularly high HCC suggesting that perceived discrimination and social identity may affect HCC. It is clear that the relationship between HCC or chronic stress, and ancestry, skin color and social identify requires further research with a more thorough investigation of social structures, culture, and discrimination.

Multiple studies have explored the effects of physical and biological factors on HCC values including, genetics, hair growth rate, and hair color. A study by Rietschel and colleagues (2017) found that genetics can strongly influence HCC values. Studies have convincingly established that growth rates of hair differ between regions of the scalp and between ancestral groups, which could influence the incorporation of cortisol into hair (Sauvé et al. 2007; Focker et al. 2016; Thom 2016). Lastly, while many studies have not found a relationship between hair color and HCC (Raul et al. 2004; Sauvé et al. 2007; Kirschbaum et al. 2009; Manenschijn et al. 2011), those that did found higher HCC in black hair (Neuman et al. 2017; Binz et al. 2018).

2.3.1. HCC and other measures of stress

HCC analysis is a relatively novel technique for stress assessment. Well established measurements of stress have been discussed previously (Section 2.1.2) and include the assessment of cortisol from saliva, blood serum, or urine as well as anthropometry and perceived stress measures. Preliminary studies of ear wax also point to its potential utility as a measure of long-term cortisol secretion (Herane-Vives et al. 2020). The correlation between these variables and HCC further confirms the relationship between HCC and chronic stress.

Cortisol concentrations in saliva reflect bioavailable cortisol and short-term periods of stress (Vanaelst et al. 2012; Ockenburg et al. 2016; Short et al. 2016). Despite promising findings in animal studies (e.g., Davenport et al. 2006; Ockenburg et al. 2016), many studies of humans, have found a weak to moderate correlation between cortisol concentrations in saliva and hair (e.g., Sauvé 2007; D'Anna Hernandez 2011; Meyer and Novak 2012; Ouellette 2015; Schalinski 2015; Steudte-Schmiedgen 2015; Vanaelst 2012; Ockenburg et al. 2016). However, in their systematic reviews, Stalder and colleagues (2017) and Kalliokoski and colleagues (2019) found that there was a positive association overall between HCC and single point salivary cortisol measure and mean diurnal salivary cortisol. Vanaelst and colleagues (2012) argue that the lack of strong correlation in some studies is a result of salivary cortisol being a better measure of short-term stressors whereas HCC is better applied to longer term stress experiences. In a specifically designed validation study, 30 day integrated salivary cortisol measurements correlated significantly with HCC after three weeks (Short et al. 2016).

Serum and plasma cortisol reflect total cortisol and free, or unbound, cortisol can be calculated from total cortisol and cortisol binding protein levels (Beishuizen et al. 2001; Ho et al. 2006). Some authors have reported no correlation between single point measurements of serum cortisol or free serum cortisol and HCC (e.g., Sauvé et al. 2007), and logistical obstacles to blood sampling have precluded many long-term studies. However, Kalliokoski and colleagues (2019) found a relationship between serum cortisol and HCC in their review of the literature, and Hodes and colleagues (2017) found that midnight serum cortisol was correlated with HCC.

Cortisol excretion measured from urine is not generally correlated with saliva or plasma, likely due to metabolism in the kidneys and liver (Barton et al. 1993). Twenty-four-hour free urinary cortisol was strongly correlated with HCC in some studies (e.g., Sauvé et al. 2007; Hodes et al. 2017; Wester et al. 2017) but not others (e.g., Chan et al. 2014) and 24-hr urinary cortisol over 63 or 30 days did not significantly correlate with HCC (Ockenburg et al. 2016; Short et al. 2016). The summary of the relationship between HCC and other measures of cortisol is presented in Table 2.2.

Table 2.2. Comparison of properties of HCC with measures of cortisol from other sources (Russell et al. 2015; Greff et al. 2019).

Source	**Free or total cortisol**	**Invasiveness**	**Retrospective**	**Clinical reference ranges**	**Time assessed**
Serum	Total cortisol	Highly (Blood sample)	No	Yes	Minutes
Saliva	Free cortisol	Minimal (Saliva collection)	No	No	Minutes
Urine	Free cortisol	Moderate (24 hour urine collection)	No	Yes	12−48 hours
Hair	Free cortisol	Minimal (Hair sample)	Yes	No	Weeks to years

In addition to various measures of cortisol, HCC has been compared to anthropometric measures of stress. Stalder and colleagues (2017) reviewed a number of studies to reveal a positive association with body mass index and waist to-hip ratio that have been used as measures of lon- term stress in some studies, as well as systolic blood pressure, which has also been used as a measure of stress. In a meta-analysis of 28 HCC studies in children (11,510 children total), Ling and colleagues (2020) found a positive correlation between HCC and BMI, waist circumference, and body fat. Another review of

36 studies found that HCC was correlated with BMI and waist circumference in children (Gray et al. 2018).

The relationship between HCC and perceived stress as measured through questionnaires and surveys has been inconsistent. Many studies have found no correlation (e.g., Henley et al. 2013; Sumra and Schillaci 2015; Gidlow et al. 2016; Olstad et al. 2016; Prado- Gascó et al. 2019) while others have found a positive correlation (e.g., Kalra et al. 2007; Wells et al. 2014; Abell et al. 2016; Van Manen et al. 2019). The complex relationship is likely related to the limitations of momentary measurements of perceived stress and the monthly average of stress experience as well as the complex relationship between psychological and physiological experiences of stress (Olstad et al. 2016).

Some studies have also argued that elevated HCC may act as a measure of allostatic load. Researchers have argued that higher HCC among older people could be accounted for by the accumulation of allostatic load across the life course (McEwen 1998; 2004; Carlson and Chamberlain 2005; Feller et al 2014). This would suggest that in addition to being a reflection of substantial and chronic stress, elevated HCC could also reflect normal wear and tear over the life course. A significant positive relationship between HCC and allostatic load, as measured via a number of biomarkers, was observed in at least one study (Mazgelytė et al. 2019).

2.3.2. Taphonomic factors

A number of factors may influence HCC values after deposition in the hair shaft during or after life; these will be referred to as taphonomic factors. HCC may be elevated by deposition of cortisol-containing sweat and sebaceous secretions on the outer surface of hair (Wester et al. 2016; Greff et al. 2019). No studies have investigated the impact of decomposition fluids on cortisol contamination of the external hair shaft, but it is potentially significant. An isopropyl wash is generally sufficient to control for external contamination (Greff et al. 2019). Other factors may result in loss of HCC and include waning, leaching, and UV radiation. Leaching refers to the loss of HCC following death due to decomposition of the hair shaft and subsequent water activity (Webb et al. 2010). Previous research has discussed the risk that leaching presents in archaeological samples, but no method for assessing the potential or degree of leaching has been developed (Webb et al. 2010).

Some authors have identified a washout or waning effect in humans and wild chimpanzees in which systemic cortisol decreases along the hair shaft especially beyond the proximal 6 centimeters of hair (e.g., Kirschbaum 2009; Li et al. 2012; Carlitz et al. 2015; Staufenbiel et al. 2015b). Cortisol could be washed out of hair from repeated exposure to water (although cortisol itself is hydrophobic) especially following damage to the hair cuticle (Dettenborn et al. 2012b). An experimental study showed that washing of the hair of rhesus macaques with water or shampoo resulted in a decrease in HCC (Hamel et al. 2011). However, other studies have identified no relationship between hair washing and HCC or no waning effect (Thomson et al. 2010; Dowlati et al. 2010; Manenschijn et

al. 2011; Dettenborn et al. 2012a; Stalder et al. 201a). In their systematic review, Stalder and colleagues (2017) found a waning effect in most studies. Carlitz and colleagues (2015) argue that the lack of identifiable washout effect is due to the use of insufficiently strong statistical methods, but also suggests that the effect could be controlled for by analyzing the same length of hair from all study subjects. Harsh chemical hair treatments such as bleach have been associated with significant loss of HCC in some studies although the presence of harsh chemicals can be easily recognized during the extraction procedure when the methanol changes color (Hoffman et al. 2014). In general, other forms of hair treatment show no impact on HCC (Kristensen et al. 2017; Stalder et al. 2017).

The impact of UV radiation on HCC has been a subject of debate. Some authors argue that UV radiation is responsible for the degradation of HCC during storage prior to analysis while others claim UV radiation during life can impact HCC (Abell et al. 2016; Wester et al. 2016). In in-vitro studies, UV radiation resulted in significant HCC loss likely due to the degradation of steroid molecules (Li et al. 2012; Grass et al. 2016; Wester et al. 2016). No in-vivo studies have identified a correlation between the amount of sun exposure and HCC loss (Staufenbiel et al. 2015b; Abell et al. 2016; Grass et al. 2016; Rippe et al. 2016).

2.3.4. Ranges for healthy people

Reference ranges for living physically and mentally healthy people have not been standardized in HCC analysis as few studies have carried out controlled, large-scale studies with diverse samples. Furthermore, studies undertaken have shown that the

method of quantification can affect HCC values (Greff et al. 2019). Most studies rely on a control group of healthy individuals to establish a baseline (although the concept of healthy is rarely defined) or have reviewed multiple studies to suggest an appropriate interval. A few studies have reported values for healthy people of relevance to the current study using ELISA (Table 2.3). Thomson and colleagues (2010) suggest a range for healthy individuals of 8−221 ng/g using two standard deviations from the mean they identified (116 ± 54 ng/g; n = 32), which has been referenced in at least one other study (Schaefer 2017). Greff and colleagues (2019) suggest an upper cut off of HCC in healthy people as 75.9 ng/g with a 93−98% chance of avoiding false negatives and a lower boundary of 31.1 ng/g with a 90% chance of avoiding false negatives based on the work of Manenschijn and colleagues (2012) and Wester and colleagues (2017). Albar and colleagues (2013) suggested that despite slightly different procedures, HCC values of different groups of healthy volunteers from four respected laboratories (i.e., Koren laboratory, Van Rossum laboratory, Kirschbaum laboratory, Laudenslager laboratory) tend to be similar (less than or equal to a 2.3-fold difference). Given the possible discrepancies between HCC values from ELISA and liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis, studies employing LC-MS/MS are not reviewed here (Greff et al. 2019).

Table 2.3. Reference ranges of HCC in healthy living people from the literature employing HCC analysis.

Study	**Reference range (ng/g)**	**Sample Size**	**Percent female**	**Age (years)**
Chan et al. 2014	27−200	39	51.2%	20−76
Cieszyński et al. 2019	2−51.63	44	70.5%	63.3 ±10.3
Dettenborn et al. 2012a	18.7±11.5	64	73.4%	39.9 ±12.4
Dettenborn et al. 2012b	16.28 (SD 10.3)	360	48.0%	1−9
Gonzalez et al. 2019	40−128	232	87.0%	18−85
Henley et al 2013	26−204	32	N/A	N/A
Henley et al. 2014	299±110	15	N/A	N/A
Hodes et al. 2017	38.9 ± 25.3	6	83.3%	27.8 ±21.1
Karlén et al. 2011	19.93 (SD 33.35)	95	74.7%	22.1 ± 4.1
Langerak et al. 2015	13.5 ±13.5	181	19.0%	47.3 (SD 11.5)
Manenschijn et al. 2013	16.9−30.5 (IQR)	283	66.0%	70.1–79.6 (IQR)
Noppe et al. 2014a	3.3−6.1	61	N/A	4−7 years
Noppe et al. 2014a	6.9−11.6	67	N/A	8−14 years
Pereg et al. 2011	76.58–949.9	56	N/A	63.7 (SD 12.4)
Prado- Gascó et al. 2019	0.07−9.54	170	52.4%	12−14 years
Sauvé et al. 2007	17.7−153.2	39	51.2%	20−76 years old
Stalder et al. 2013	1.1−77.8	1258	15.2%	16–64
Stalder et al. 2014	20.5±7.3	20	85.0%	72.2 (SD 6.4)
Staufenbiel et al. 2015a	3.52−17.83 (IQR)	195	53.8%	36.17 (SD 12.23)
Thomson et al. 2010	8−221	32	65.0%	20−51
Veldhorst et al. 2014	13−21 (IQR)	20	75.0%	8−12
Wester et al. 2014	6.5−10.9	87	57.0%	20−61
Yamada et al 2007	2.22 (SD 2.11)	22	N/A	38.50 ± 1.23 (weeks)

Abbreviations: SD = standard deviation; IQR = Interquartile Range
Notes: Studies chosen employed ELISA, reported mean and standard deviation or range of control group HCC, and reported raw values. HCC values and ages transcribed exactly as reported in study.

2.3.5. Bioarchaeology

To date, few bioarchaeological studies have applied HCC analysis. An initial study of five different archaeological sites in Peru established that endogenous HCC can remain stable in archaeological remains for up to 1,000 years and be successfully extracted from preserved hair (Webb et al. 2010). This early study also identified high intra-individual variability, higher HCC than modern living populations, and elevated cortisol in the months closer to death (Webb et al. 2010). A later study compared stable carbon isotope ratios ($\delta^{13}C$) and stable nitrogen ratios ($\delta^{15}N$) to HCC in 14 individuals from the Nasca region in Peru (1−1000 CE) to argue that experiences of stress were associated with illness, trauma, mobility, dietary change, and relocation (Webb et al. 2014). The most recent study from Webb and colleagues (2015) compared HCC, $\delta^{13}C$, and $\delta^{15}N$ to identify periods of stress in five children from the Nasca region in Peru (1−1000 CE) associated with weaning, illness, and changes in diet, and were able to distinguish low $\delta^{15}N$ associated with weaning from that of growth on the basis of HCC (Webb et al. 2015).

More recent studies in bioarchaeology have built upon this foundation. One study examined HCC in 19 mummified individuals from San Pedro de Atacama, Chile dated to 400−1400 CE (HCC ranges: 50−125 ng/g) and compared them to 19 modern living people from the same region (HCC ranges: 55−100 ng/g) to argue that ancient individuals did not experience more systemic stress than modern groups because they were well adapted to their local environment (López-Barrales et al. 2015). The authors argue that lower cortisol values and fewer skeletal indicators of stress in the Middle period

(400−1000 CE; 50−70 ng/g) compared to the Late Intermediate period (1000−1400 CE; 80−125 ng/g) indicate differences in quality of life between the two periods (López-Barrales et al. 2015).

An investigation of bulk hair cortisol by Schaefer (2017) from two adults and eight children from the Lambayeque Valley, Peru dating to 1450−1532 CE determined that adult cortisol levels were consistent with clinical data from living people according to Thomson and colleagues (2010). While the author argued, based on the work of Kiess and colleagues (1995) examining salivary cortisol, that lower HCC in subadults compared to adults was related to pubertal development, the mechanism is not clear and the adult and subadult group show some overlap in age estimate (15-25 years vs. 6-17 years respectively). Further evidence is needed to support the assertion of a relationship between HCC and pubertal development in this study. The most recent study of HCC in bioarchaeology examined bulk hair samples from 10 individuals from the Kellis 2 cemetery in the Dakhleh Oasis, Egypt, (100−450 CE) and 10 modern day cadavers, and found cortisol, estradiol, and testosterone were well preserved and fell within clinical values in both samples (Tisdale et al. 2019).

Previous studies of HCC in archaeological human remains have been important in establishing the potential utility of HCC in archaeological samples and have determined that cortisol can survive in archaeological contexts dating back nearly two thousand years. However, the role of dying and death on HCC in bioarchaeological studies has yet to be fully explored. The work of D'Ortenzio and colleagues (2015) elucidates the value

and importance of exploring short-term measures of health and stress in the dead in their study of $\delta^{15}N$ in hair. They explored the relationship between $\delta^{15}N$ in hair and serious disease such as stroke, cancer, and pneumonia in the last months of life of modern dead individuals to show that $\delta^{15}N$ in hair can be influenced by disease. A systematic study of HCC in the dead in the past and present is lacking but necessary for continued application of HCC in bioarchaeology.

2.4. HPA axis in disease, dying, and death

The study of HCC in the dead must consider the effects of dying, disease, and medications on both the HPA axis and HCC. No studies have explored HCC in dying people although a few studies of other cortisol assessment techniques have been carried out. Similarly, while few studies have explored the effects of specific disease on HCC, a substantial amount of research has explored HPA axis activity in disease. Lastly, the effects of medications have received some consideration in the field of HCC analysis, but have received considerably more attention in studies of the HPA axis more broadly.

2.4.1. Cortisol in dying people

In 1956, Sandberg and colleagues found that all patients (n=62) tested 72 hours before to a few minutes after death, displayed plasma cortisol levels two standard deviations or more above normal with the highest levels right before death. Swaab and colleagues (1994) examined cortisol in cerebrospinal fluid (CSF) of 26 patients with Alzheimer's disease and 21 controls who did not have any signs of Alzheimer's disease following a postmortem examination. Postmortem CSF cortisol values in Alzheimer's

patients was 83% higher than in the control group of dead individuals. Specifically, patients under 65 years of age displayed cortisol five times higher than age matched controls while Alzheimer patients and age-matched controls over 65 years of age showed no difference in postmortem cortisol. Controls over the age of 65 displayed cortisol 3.5 times higher than controls under the age of 65. After death Alzheimer patients and controls together displayed postmortem CSF cortisol 13-16 times higher than CSF cortisol levels from living ambulatory patients. The postmortem interval had no effect on cortisol values suggesting the degradation after death is not the cause of elevation, the authors argue that the high levels of cortisol are due to the stress of dying (Swaab et al. 1994).

Erkut and colleagues (2002) measured cortisol in postmortem CSF and blood in patients with and without multiple sclerosis (MS). Postmortem CSF cortisol was significantly higher in MS patients (n=13; median HCC = 381 nmol/l) than controls (n=44; median HCC = 178 nmol/l) after correcting for age because there was a significant difference in age between the two groups. Postmortem CSF cortisol was also higher (although not significantly) in patients with sepsis (n=6; median 354 nmol/l) than in controls (n=44; median HCC = 178 nmol/l) (Erkut et al. 2002). Additionally, the authors discovered that postmortem CSF cortisol was 10−20 times higher than in CSF from living people (5−40 nmol/l vs 250 nmol/l) and found no effect of postmortem interval. They attributed the increases in cortisol to the stress of terminal illness and the process of dying (Erkut et al. 2002).

In a later study, Erkut and colleagues (2004) examined postmortem CSF cortisol to investigate whether stress at the end of life was related to discomfort, psychological reasons, or 'organic stress'. According to the authors, 'organic stress' is a biological phenomenon related to deterioration of the body and destruction of homeostasis. The authors hypothesized that if elevated postmortem cortisol was due to psychological stress, the increase in cortisol would be inversely proportional to the severity of Alzheimer's disease due to decreased cognitive level and awareness. Furthermore, the administration of high dose morphine would be able to suppress a rise in cortisol if it was due to psychological reasons because morphine leads to analgesia, sedation, and sleep, which would limit psychological discomfort. After examining 85 Alzheimer's patients and 52 controls, they found that Alzheimer's patients displayed higher cortisol than controls and that those with severe disease displayed higher values than those with less severe disease. While studies of living people with Alzheimer's disease display an elevation in cortisol relative to controls (1.5-2.5-fold), the postmortem increase is much higher (20-fold) and is not correlated with postmortem interval (Erkut et al. 2004). Furthermore, although opiates, such as morphine, are known to suppress the HPA axis (Ambrogio et al. 2008); the individuals receiving high-dose morphine (n=19 controls, n=54 Alzheimer's patients) displayed no reduction in cortisol levels. Ultimately the authors argued that individuals on high-dose morphine and those with severe Alzheimer's disease would not experience psychosocial stress, thus, the elevated CSF cortisol is related only to biological factors. These conclusions are challenged by the known difficulties of distinguishing between

different aspects of stress experience and the assumption that because of their health or cognitive status, the individuals in the study are asocial beings.

While previous studies have examined the effects of the dying process on cortisol in blood and cerebrospinal fluid and determined that cortisol is often elevated, none have examined the effects of dying on HCC. Furthermore, although Erkut and colleagues (2004) argue that the increase in cortisol is related solely to 'organic stress', they do not consider quality of life or suffering from a biocultural perspective and did not examine variation in stress between individuals or multiple causes of death.

2.4.2. Cortisol in disease

Disease can interact with the HPA axis in complex ways through stress of disease burden, as well as immune response and inflammation. While few studies have investigated HCC values in disease, substantial research has explored HPA axis activation in the disease categories represented in the current study (Section 4.1.4 for a complete list of causes of death and corresponding categories). In most cases it is difficult to disentangle the relative effects of psychological stress related to disease burden and metabolic disturbances from pathology. Furthermore, while HPA axis activity is elevated in most cases of disease, variability in elevation between diseases has received little attention.

Some studies have found no relationship between HCC and non-cardiovascular disease such as cancers and obstructive pulmonary disorders (e.g., Manenschijn et al. 2013; Wester et al. 2015). In more specific studies of disease, HCC has been applied to

investigate diseases of cortisol metabolism, such as endogenous Cushing's syndrome, which is characterized by excessive production of endogenous glucocorticoids or the prolonged exposure to cortisol containing medications (Thomson et al. 2010). HCC is elevated in individuals with Cushing's syndrome, closely mirrors the clinical course of cyclic or periodic Cushing's syndrome, and improves after surgery (Thomson et al. 2010; Manenschijn et al. 2012; Hodes et al. 2017; Wester et al. 2017). Wester and colleagues (2017) suggest an upper cutoff of 31.1 ng/g in the identification of Cushing's syndrome with a sensitivity of 93% and specificity of 91%. In contrast, Addison's disease, characterized by cortisol insufficiency, has been associated with lower HCC values (Ibrahim and Van Uum 2014).

Cortisol levels in hair have been analyzed to investigate the experience of stress associated with disease. In women with endometriosis, a condition characterized by chronic pelvic pain, HCC was higher than in controls but did not correlate with pain intensity, suggesting a relationship with stress (van Aken et al. 2018). In contrast, HCC was not found to be higher in individuals with Parkinson's disease than in controls (van den Heuvel et al. 2020). In one unique case, elevated HCC was found in patients with active central serous chorioretinopathy, a disease that results in detachment of the retina, and in which cortisol plays a role the development of disease although the exact mechanism is unknown (Lenk et al. 2019).

2.4.2.1. Certain infectious or parasitic diseases

Cortisol plays a significant role in response to infection (Sibbald et al. 1977; Parker et al. 1985; Beishuizen et al. 2001; Vermes and Beishuizen 2001; Marik 2009). Elevated plasma and free cortisol have been commonly identified in acute infection (Drucker and Shandling 1985; Beishuizen et al. 2001; Ho et al. 2006; Manary et al. 2006; Marik 2009). As infection progresses, cortisol binding protein can increase leading to elevated serum cortisol and normalization of free cortisol (Parker et al. 1985; Vermes and Beishuizen 2001). The effects of chronic infection on HPA axis activity are complex. Adrenal failure may occur in prolonged infection (Marik 2009), cortisol hypersecretion may persist, or the HPA axis may become dysregulated (Beishuizen et al. 2001). In some stages of HIV, especially when less advanced, elevated serum cortisol is present (Christeff et al. 1997; Langerak et al. 2015). No correlation between severity of infection and plasma cortisol exists (Drucker and Shandling 1985; Schein et al. 1990), but a correlation with free plasma cortisol has been reported (Ho et al. 2006). In some severe infections adrenal insufficiency can occur while cortisol levels remain elevated (Salluh et al. 2006). Elevated plasma cortisol has been identified in bacterial versus viral infections although the severity of these conditions also differed (Chalupa et al. 2011), and in infections resulting from gram positive versus gram negative bacteria (Schein et al. 1990). Researchers have suggested that the variability observed in cortisol elevations are likely complexly related to the type of infection, duration, and pre-existing medical conditions (Schein et al. 1990).

Although some authors argue that tuberculosis is a cause of adrenal insufficiency, others argue that the adrenal gland is not involved in most instances of tuberculosis and that arguments regarding adrenal insufficiency are often based on misrepresentation of ACTH stimulation test results (Barnes et al. 1989; Kelestimur et al. 2004). However, in a small percentage of cases, tuberculosis can directly attack the adrenal glands which can eventually result in atrophy and lead to Addison's disease (Kelestimur et al. 2004). In contrast, in pulmonary tuberculosis cortisol levels can increase, which some authors argue could be a result of the stress of disease burden related to tuberculosis (Kelestimur et al. 2004).

2.4.2.2. Diseases of the circulatory system

Multiple studies have found elevated HCC associated with incidence of cardiovascular disease as well as factors that increase the risk of cardiovascular disease, such as high blood pressure, diabetes, smoking, higher than recommended waist circumference, total cholesterol levels, high body mass index, and adiposity (Manenschijn et al. 2013; Stalder et al. 2013; Wester et al. 2015; Bautista et al. 2019). Given the relative novelty of the field, these studies are largely small -and cross-sectional in nature (Iob 2019; Mazgelytė et al. 2019). Elevated HCC has also been associated with risk of a myocardial infarction and heart failure severity (Pereg et al. 2011; 2013).

Stress and increased HPA activity more generally have been linked with poor cardiovascular outcomes in the short-term by increasing risk for cardiovascular incidents such as myocardial infarction, left-ventricular dysfunction, or dysrhythmia and in the

longer term by encouraging atherosclerotic processes (Brotman et al. 2007; Iob 2019). Acute physical stressors, everyday stressors, and major life events have all been associated with negative cardiovascular outcomes (Brotman et al. 2007). Cortisol is associated with oxidative stress and may affect the coronary vasoconstricting response, which, along with other possible mechanisms that are still unknown, may account for the development of, and risk for, cardiovascular conditions including coronary vasospasm and myocardial ischemia, hypertension, and heart failure (Packer 1992; Whitworth et al. 2000; Hizume et al. 2006; Yamaji et al. 2009).

2.4.2.3. Diseases of the digestive system

No research is available exploring HPA axis in appendicitis or peritonitis; however, peritonitis is most often a result of infectious causes (see Section 2.4.2.1). Roughly a third of individuals with severe liver cirrhosis could be diagnosed with adrenal dysfunction (Thevenot et al. 2012; Fede et al. 2013).

2.4.2.4. Diseases of the nervous system

Morning levels of salivary cortisol were found to be higher in amyotrophic lateral sclerosis patients than in controls and higher in patients with spinal-onset disease as well as those with intermediate or rapidly progressing disease (Spataro et al. 2015). Researchers were able to exclude the effects of age, BMI, mood, depression, and degree of respiratory distress in elevating cortisol values and suggest that the elevated cortisol levels could be due to the significant psychological distress of disease burden (Pagnini 2013; Spataro et al. 2015). Cortisol was also elevated in myotonic dystrophy alongside markers of inflammation, which may suggest elevated cortisol was related to

inflammation (Johansson et al. 2000). Studies of HPA axis activity in cerebral palsy suggest that cortisol levels were higher in children with pain versus those without (Symons et al. 2015).

2.4.2.5. Diseases of the respiratory system

The relationship between stress, inflammation and pulmonary dysfunction is complicated and has not yet been disentangled in its entirety (Anda et al. 2008; Marinho et al. 2011). Cortisol levels were found to be low although inflammation was high in women with asthma (Anda et al. 2008). Cortisol levels and markers of inflammation were not found to be different between individuals with and without chronic obstructive pulmonary disease (COPD) despite depressive symptoms being higher in the COPD group (Marinho et al. 2011). Similarly, the HPA axis does not seem to be altered in cystic fibrosis although common treatments may affect cortisol (Rayas et al. 2019). Individuals with severe pneumonia can develop adrenal insufficiency while cortisol levels may be elevated and related to mortality risk (Salluh et al. 2006; Kolditz et al. 2010).

2.4.2.6. Mental, behavioral or neurodevelopmental disorders

Conditions associated with elevated cortisol levels, such as Cushing Syndrome, have been linked with hippocampal degeneration (Frimodt-Møller et al. 2019) and higher plasma cortisol levels have also been associated with more quickly progressing and more severe Alzheimer's dementia (Csernansky et al. 2006; de la Rubia Ortí et al. 2019). Urinary free cortisol was higher in patients with dementia (Alzheimer type and multi-infarct dementia) than in controls (Maeda et al. 1991). HCC has also been found to be elevated in depressed patients over time (Dettenborn et al. 2012b). These findings are

consistent with the majority of studies that found elevated cortisol in 24-hour urine and plasma suggesting an overactive HPA axis in patients with depression (Carroll et al. 1976; Gold et al. 1986; Brotman et al. 2007).

2.4.2.7. Neoplasms

Little research has explored cortisol levels in those with cancer or evaluated the relative effects of disease and psychosocial stress. The only study examining HCC, found that HCC was higher in patients with sarcoidosis than in controls (van Manen et al. 2019). The authors argue that the elevation is a result of chronic psychological stress related to disease burden rather than inflammation because HCC values correlated with anxiety, depression, and other scores of mental health but not to active disease symptoms (van Manen et al. 2019). Most studies of HPA axis activity in cancer have found changes associated with depression (Friedrich et al. 2006; 2010). Other studies have found that cancer treatment improves functional abilities, reduces inflammation, and normalizes cortisol levels (Schrepf et al. 2013). Elevated cortisol and altered diurnal cortisol rhythms were identified in individuals with metastatic breast cancer, which authors argue is indicative of allostatic load (Abercrombie et al. 2003).

2.4.3. Medication and cortisol

In general, studies of medication and HCC have not found a relationship (Kirschbaum et al. 2009; Karlén et al. 2011; Dettenborn et al. 2012b, Rippe et al. 2016). Stalder and colleagues (2012a) found no difference in the overall pattern of HCC when individuals taking medications such as antidepressants, antihypertensives, analgesics, non-steroidal antiallergic drugs, or thyroxine replacement medication were included in

the analysis or not. Fischer and colleagues (2017) argued that there seems to be little support for a relationship between HCC and medication use more broadly including topical steroids, antacids, cold medication, allergy medication, antibiotics, inhalers, diabetes medication, cardiovascular medication, and thyroid medication. The authors did not distinguish between Type I and Type II diabetes. In those studies that do not evaluate the effects of medications individually, the conflicting effects of different medications could mask any association (Fischer et al. 2017).

Many studies exploring the interaction of medications and hair cortisol have focused on steroid medications. Studies of HCC in patients with adrenal insufficiency receiving hydrocortisone treatment established that HCC correlated with hydrocortisone dose (Gow et al. 2011; Noppe et al. 2014b). Systemic, and even topical, glucocorticoid medications have been associated with elevated HCC (Abell et al. 2016; Gusman et al. 2020), although Guzman and colleagues (2020) found that systemic glucocorticoids were actually associated with lower HCC. In the case of non-steroidal medications, Stalder and colleagues (2017) found in their review that HCC was negatively associated with oral contraceptive use while other studies found a positive association (Staufenbiel et al. 2015b; Fischer et al. 2017). Painkillers and antidepressants have been associated with increased HCC (Fischer et al. 2017) although studies have found inconsistent effects of antidepressants on HPA axis activity, which might be a result of the class of antidepressant and clinical response to treatment (Dettenborn et al. 2012a). Abell and colleagues (2016) found higher HCC in individuals taking cardiovascular medications,

but the difference was not significant and could be a result of cardiovascular medications and elevated HCC both being associated with cardiovascular risk.

Clinical research has shown that a number of medications that have not been investigated in HCC can affect HPA axis activity (Table 2.4). Dexamethasone is a common steroid medication prescribed to dying people that has complex effects on the HPA axis. In those without adrenal insufficiency, corticosteroids such as dexamethasone inhibit the production of ACTH which in turn limits endogenous glucocorticoid production and may deplete cortisol levels (Ambrogio et al. 2008; van Esch et al. 2016).

Table 2.4. Summary of medications and known effects on cortisol levels.

Elevate Cortisol	**Decrease cortisol**	**No change**
• Synthetic glucocorticoids • Glucocorticoid antagonists • Serotonin receptor agonists* • Dopaminergic agonists and antagonists • Adrenergic antagonists • Opioid antagonists • Cholinesterase inhibitor • Tricyclic antidepressants • Cytokines • Synthetic progestational agents • Estrogen receptor modulator	• Cessation of glucocorticoids • Steroid inhibitors • Adrenergic agonists • Complete and partial opiate agonists • Mixed opiate agonist-antagonists • GABAergic agonist (long-term) • Serotonin, dopamine and histamine antagonsists • Growth hormone • Thyroid hormones • Estrogens • Dexamethasone • Cancer medication (cisplatin or carboplatin)	• GABAergic agonist (short-term)

Abbreviation: GABA = gamma-Aminobutyric acid
Notes: Information from Morrow et al. 2003; Ambrogio et al. 2008.
*Some examples, such as fluoxetine have shown no effect on hair cortisol concentrations.

2.4.4. Mortality risk

The relationship between cortisol and mortality risk has received substantial attention. Prolonged elevated cortisol is known to have negative effects on the body and brain including immunosuppression and metabolic dysregulation (Schoorlemmer et al. 2009; McEwen 2004). Higher HCC was found to predispose individuals to a myocardial infarction (Manenschijn et al. 2013) and poorer survival outcomes in pneumonia (Christ-Crain et al. 2007). Higher salivary cortisol was associated with increased mortality in an older sample over the course of 6 to 7.5 years (Schoorlemmer et al. 2009) and increased evening salivary cortisol was found to correlate with all-cause mortality in individuals with heart failure over 18 months following collection (Hammer et al. 2016). High serum cortisol is an independent predictor of mortality risk in those with systolic heart failure (Güder et al. 2007), chronic heart failure (Yamak et al. 2020), and severe pneumonia (Kolditz et al. 2010). Twenty-four-hour urinary cortisol was also correlated with cardiovascular mortality risk but not non-cardiovascular mortality over the course of six years in those with and without cardiovascular disease at time of sampling (Vogelzangs et al. 2010). In those with sepsis, high serum total cortisol in the first 24 hours of hospital admission predicted 28-day mortality risk (De Castro et al. 2019).

Stress has also been associated with mortality risk more broadly. Higher stress levels were associated with poorer survival among individuals with cancer (Giese-Davis et al. 2011) and with greater mortality risk in individuals with peripheral artery disease (Malik et al 2019). Recent episodes of stress were also found to increase all-cause mortality risk in a study of older males (Johnson et al. 2020). A relationship between

stress, cortisol, and mortality exists, but no studies have examined cortisol in the months directly preceding death and the mechanism by which cortisol may impact mortality is not clear.

2.5. Theoretical framework

This study adopts a biocultural framework built upon the concepts of embodiment and the ‘good death’. The concept of embodiment will lay the foundation for interpreting biochemical measures of stress. The framework of a 'good death' prioritizes understanding the dying process on its own terms and the importance of multifaceted factors in shaping experience at the end of life.

2.5.1. Biocultural framework

The human body is a product of biological and cultural forces (Freund and McGuire 2003; Ingold 2013; Palsson 2013b). Culture shapes the perception, understanding, and, ultimately, the experience of biology and biological events (Scheper-Hughes and Lock 1987; Van Wolputte 2004). In biomedicine, the Cartesian divide between the body and mind, nature and culture, and the individual and society persists (Scheper-Hughes and Lock 1987; Freund and McGuire 2003). However, such artificial dichotomies are poorly suited to understanding complex aspects of human experience, such as stress. Stressors can arise from the mind, body, nature, culture, an individual, or society (McElroy and Townsend 2009). The experience of stress can occur in any of these realms and the response to stressors depends upon perception, social support, and biological components (Thomas 1998). Because stress acts across the artificial divisions

between body and mind, nature and culture, and the individual and society, it is best studied through a biocultural framework.

The most commonly employed model of stress in bioarchaeology is a biocultural one (Goodman et al. 1984; Goodman and Armelagos 1989). In this model, stress is defined as a physiological disruption, stressors can be environmental and cultural, and mediating variables can be biological and social factors (Goodman and Armelagos 1989). Further developments emphasize the interconnectedness of environmental and cultural factors in shaping exposure and response to stressors and the biological and behavioral effects of stress (Klaus 2012). These models are predicated upon the assumption that the "degree of stress experienced by individuals long dead cannot be measured by direct physiological methods" (Goodman et al. 1988:177). Because the current study intends to break this core assumption, the model of stress employed in the study is modified from earlier versions and includes biomedical features (Figure 2.3). In this model, the environment and culture interact to produce stressors and buffering forces. When a stressor is perceived, biologically or psychologically, an individual adjusts and responds both biologically and behaviorally. If response is inadequate and/or adjustment persists, allostatic load and physiological disruption occurs. Disruption has consequences for individual morbidity and mortality and can impact the population, while shaping exposure and response to future stressors. HCC values can reflect stress at any stage of this model.

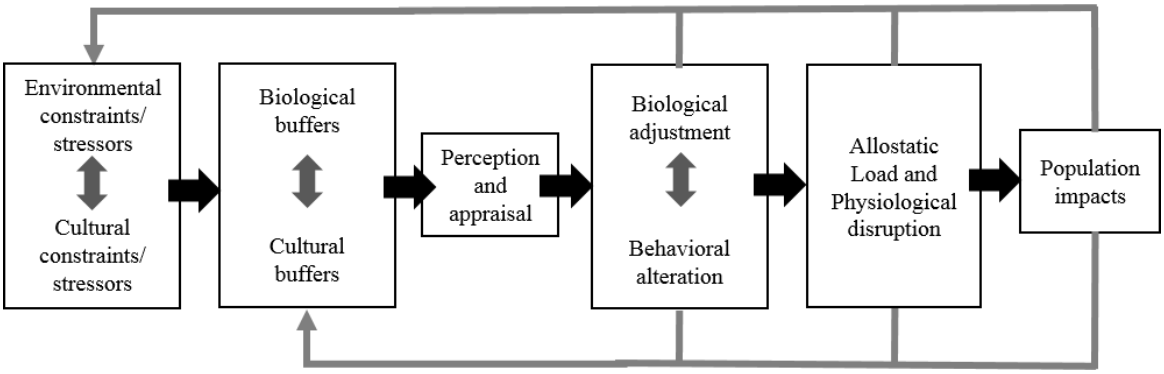

Figure 2.3. Biocultural model of stress. Adapted from Goodman and Armelagos 1989; McEwen 2007, and Klaus 2012.

2.5.2. Embodiment

The concept of embodiment is an extension of biocultural principles, predicated upon the body as a material entity (Sofaer 2006). It is the materiality of bodies that is created and shaped by experience, interacts with the world, and is the locus of perception and action (Csordas 1990; Weiss and Haber 1999; Van Wolputte 2004; Mascia-Lees 2011). Embodiment has been described as: how the "[m]ind speaks through the body, and the ways in which society is inscribed on the expectant canvas of human flesh" (Scheper-Hughes and Lock 1987: 10). It has also been defined as the expression of experience and relationships in material form or the manifestation of the environment in physiological form (Hoy 1999; Strathern and Stewart 2011; Hoke and McDade 2014). According to Krieger (2005b), embodiment is a construct, process, and reality that reveals individual experiences. Embodiment is the process by which bodies are created, recreated, and shaped across the life-course (Fox 1994; Hoy 1999; Ingold 2013; Palsson 2013a). Experiences and relationships as well as individual exposure, risk, and ability to resist all shape bodies and are a product of biology, society, and history (Csordas 1990; Krieger

2005b; Palsson 2013b). As a result, "[d]isease, disability, death, and health are embodied expressions of conditions under which organisms live" (Krieger 2005a: 4).

Materiality is a key component of the analysis and theorizing of archaeological human remains as it is the material remains that are available for study (Sofaer 2006). Krieger (2005a) argues that embodied bodies tell stories that individuals cannot, which is particularly applicable to the study of skeletal remains. In bioarchaeology, embodiment is understood as the way in which social and biological worlds constitute individuals and populations (Martin et al. 2013). The connection between lived experience and material expression is the foundation of bioarchaeology, especially in so far as it provides a lens for interpreting health and well-being, experience, and identity from skeletal remains in bioarchaeology (Sofaer 2006; Martin et al. 2013). While the concept of embodiment has implicitly provided the foundation for understanding relationships between social structures and inequities in health, patterns of behavior, or cultural practices, few of these studies explicitly speak to its utility (Zuckerman et al. 2014).

The model of embodiment is well suited to the study of stress as the biochemical aspects of stress cross the fluid and permeable boundaries between the biological and cultural, the mind and body, and the individual and social in ways that are consistent with conceptions of embodiment. Stress, inasmuch as it involves the biological consequences of perception and experience, can be understood as a literal reflection of the ways in which "the mind speaks through the body" (Scheper-Hughes and Lock 1987: 10). The biochemical features of stress, such as HCC values, have been understood to literally and

biologically embody experience and social structures (Pike and Williams 2006). Thus, stress is the mechanism by which experience is embodied in HCC values (Figure 2.4).

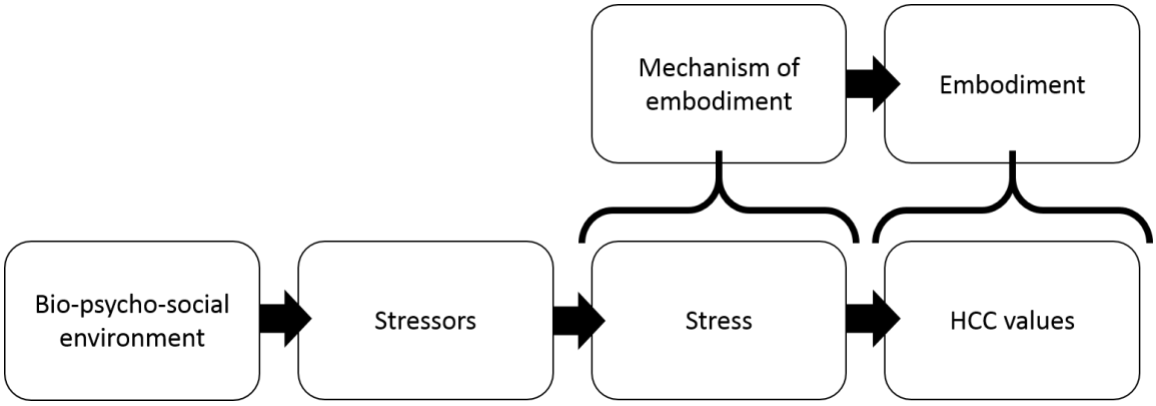

Figure 2.4. Flowchart depicting the relationship between the processes of embodiment and stress and the effects on HCC values.

2.5.3. Good death

Ariès (1974, 1981) introduced the rhetoric of a "tame death", in which death is a complex event that is shaped by cultural norms and requires warning, preparation, grief, and closure. This work set the foundation for theorizing about death, culture, and differences in experience of dying. Kübler-Ross (1969) established the stages of acceptance for dying people to achieve a positive death experience. From these foundations, and in tandem with the development of the modern hospice movement, the 'good death' concept developed (Palgi and Abramovitch 1984; Mak and Clinton 1999; Timmermans 2005; Hattori et al. 2006; Green 2008). Various definitions of a 'good death' exist. The World Health Organization (WHO 1990) suggested a 'good death' depends on place; pain management, maintenance of control, awareness, and autonomy; and acceptance and preparation for death. Hattori and colleagues (2006) argue that a

'good death' is a "multidimensional, ceaseless individual experience based on personal and sociocultural domains of life that incorporate a person's past, present, and future" (167).

While these definitions set a broad foundation for understanding and working towards a 'good death', researchers have attempted to identify more specific components of a 'good death'. Steinhauser and colleagues (2000) identified six components of a 'good death' through a series of interviews with medical and hospice staff, social workers, chaplains, patients, and family: 1) controlled pain and symptoms, 2) clarity in decision-making, 3) preparation, 4) culturally determined closure, 5) contributions to others, 6) whole person care. Many studies emphasize the importance of whole person care which considers spiritual and social factors in addition to biomedical care (Mount 1993; Lo et al. 1999; Steinhauser et al. 2000; Kayser-Jones 2002). Others emphasize the alleviation of suffering associated with pain and other symptoms (Nimocks et al. 1987; Steinhauser et al. 2000; Leichtentritt and Rettig 2001; Vig et al. 2002). Awareness, acceptance, closure, and preparation are important in some definitions (e.g., McNamara et al. 1995; Payne et al. 1996; Emanuel and Emanuel 1998; Johnson et al. 2000; Kayser-Jones 2002). Some focus on peacefulness and personal integrity (Hart et al. 1998; Johnson et al. 2000; Long 2001; Timmermans 2005). Research that centers the voices of the dying tend to find preferences of dying with dignity, a lack of pain and other symptoms, dying quickly, maintaining control, not being a burden on family, having time to prepare, and cleanliness in a 'good death' (Brody et al. 1997; Stephenson 1983; Payne et al. 1996; Emanuel and Emanuel 1998; Singer et al. 1999; Beckstrand et al. 2006).

Researchers have also explored the various factors that may shape how one defines and experiences a 'good death'. Cultural expectations can shape expectations of a 'good death' and may define the appropriate place to die, how much control a dying person should have, how long the process of dying should take, how involved family should be, and many other variables (Leichtentritt and Rettig 2001; Long 2001; Stephenson 2001; Timmermans 2005; Walter 2003; Goodwin-Hawkins and Dawson 2018). Culture can even shape acceptance and experience of pain depending on cultural meanings attributed to suffering (Cassel 1998; 1999; Seale and Van der Geest 2004). While some groups fear death and wish for a quick death free from pain, others view death with hope and prefer a long, slow, and potentially painful death that is a highly social process (Stephenson 1983; 2001).

Definitions and standards provide some key principles to be considered in the search for a 'good death'. However, the value of the concept of a 'good death' is that it provides a framework with which to approach and make sense of the dying experience. The 'good death' framework directly challenges the ideas of death as a failure or the opposite of life (Franklin and Lock 2001). It emphasizes the importance of examining the dying process on its own terms without relation to life and living (Hart et al. 1998; Landecker 2003; Timmermans 2005; Green 2008). The framework centers the dying person and establishes the importance of treating dying as a unique experience that is worthy of attention, and that can be improved independently of length of survival. Ultimately the 'good death' framework provides a means for examining and improving the dying experience that recognizes such an experience is biosocial, dynamic,

multifaceted, and a product of individual experience across multiple time scales (Hattori et al. 2006; Kayser-Jones 2007; Krikorian et al. 2020).

2.5.4. Combined framework

In bioarchaeology, researchers use the framework of embodiment to interpret lived experience from skeletal remains while the 'good death' framework provides a foundation for centering the dying experience and understanding the varied physical, social, emotional, and spiritual factors the shape the experience of dying in palliative care (Krieger 2005b; Hattori et al. 2006; Pike and Williams 2006; Green 2008). Key components of both embodiment and the 'good death' frameworks are that they are biosocial, dynamic, and multifaceted (Table 2.5; Krieger 2001; Hattori et al. 2006; Kayser-Jones 2007; Krikorian et al. 2020). The stress process also contains complex biological and cultural components and is dynamic as the body attempts to maintain allostasis (McEwen and Stellar 1993; Thomas 1998; Goodman and Martin 2002). Together, embodiment and the 'good death' provide a framework to study the dying process on its own terms that illustrates the connections between complex biosocial forces and the experience of dying, and which highlights the material consequences of such experiences. Thus HCC, as a biological embodiment of experience through the mechanism of stress provides a potential avenue for understanding dying as a multifaceted, biosocial experience in the past and present.

Table 2.5. Summary of key concepts and frameworks to interpret HCC at the end of life.

Stress	**Embodiment**	**Good death**	**Combined framework**
Biosocial Dynamic Material effects	Biosocial Dynamic Multifaceted Material effects	Biosocial Dynamic Multifaceted Individual Centers dying	Biosocial Dynamic Multifaceted Material effects Individual Centers dying

CHAPTER 3 - MATERIALS

3.0. Introduction

In order to address questions regarding stress experience associated with dying in the past and present, three samples from different places and times were investigated in this study: a modern sample, a historic sample, and an archaeological sample. These samples differed in many features that may influence the experience of dying, including cause of death, approaches to dying and death, and availability of medical treatments. In this investigation, a sample will refer to the subset of a collection or population that will be the largest unit of measurement in this study. Individuals comprise a sample and are the source of study material. A hair sample will refer to a length of hair collected from an individual while a segment will refer to a one-centimeter portion of a hair sample.

3.1. Sample characteristics

The number of hair segments per individual ranged from two to seventeen, resulting in a total of 1,007 hair segments (Table 3.1, Appendix A.0). Most individuals possessed six centimeters of hair or less (Figure 3.1).

Table 3.1. Contextual data and sample sizes for the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples.

Sample	Geographic Origin	Time Period	Number of individuals	Number of hair segments
Kellis 2 Cemetery	Dakhleh Oasis, Egypt	50-450 CE	130	531
Terry Collection	St. Louis, Missouri	1923-1960 CE	40	269
UCF Cadaver	Orlando, Florida	2016-2017 CE	40	207

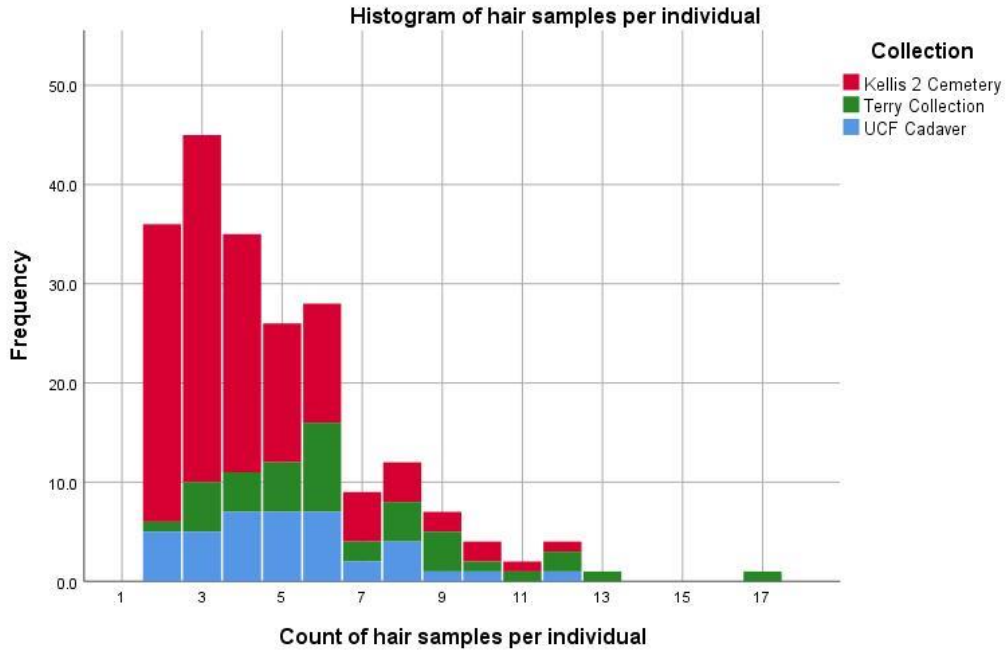

Figure 3.1. Histogram of hair segments per individual in the three study samples.

The Kellis 2 Cemetery sample contains individuals ranging from 1.5 to 70 years of age at death while the Terry Collection and the UCF Cadaver samples contained only adults (18+ years; Table 3.2; Appendix A.0).

Table 3.2. Age ranges for the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples.

Sample	Age range (years)	Adults	Juveniles	Total
Kellis 2 Cemetery	1.5-77	90	40	130
Terry Collection	18-91	40	0	40
UCF Cadaver	24-101	40	0	40

3.1.1. UCF Cadaver Sample

The University of Central Florida (UCF) sample was collected in the College of Medicine from bodies donated to the Anatomical Board of the State of Florida through

UCF's Willed-Body Program (Figure 3.2). This program accepts remains of individuals over the age of 18 years who died in Florida and provides them to universities throughout the state for the purposes of education and research. The samples for this study were collected between 2014 and 2016. The sample consists of 40 individuals. Limited information was provided for each individual to protect sensitive personal information and was made available in a spreadsheet produced by the UCF's College of Medicine Anatomical Facilities. The available data was collected from medical records and includes cause of death, contributing health problems, age, and sex. Information about medications prescribed or duration of disease was not available. Ages range from 24 to 101, with 21 males and 19 females represented; information about ancestry is not available (Figure 3.3). Individuals were selected by Dr. Williams to ensure a balanced sex ratio, wide range of ages, and a range of causes of death. Cause of death data was not made available to the author until after preliminary analyses.

Figure 3.2. Map of the United States of America © *OpenStreetMap contributors*. Red circle indicates the location of Orlando, Florida, the geographic origin of the UCF Cadaver sample. Red square indicates St. Louis, Missouri, the geographic origin of the Terry Collection sample. Scale is 1:28726935.

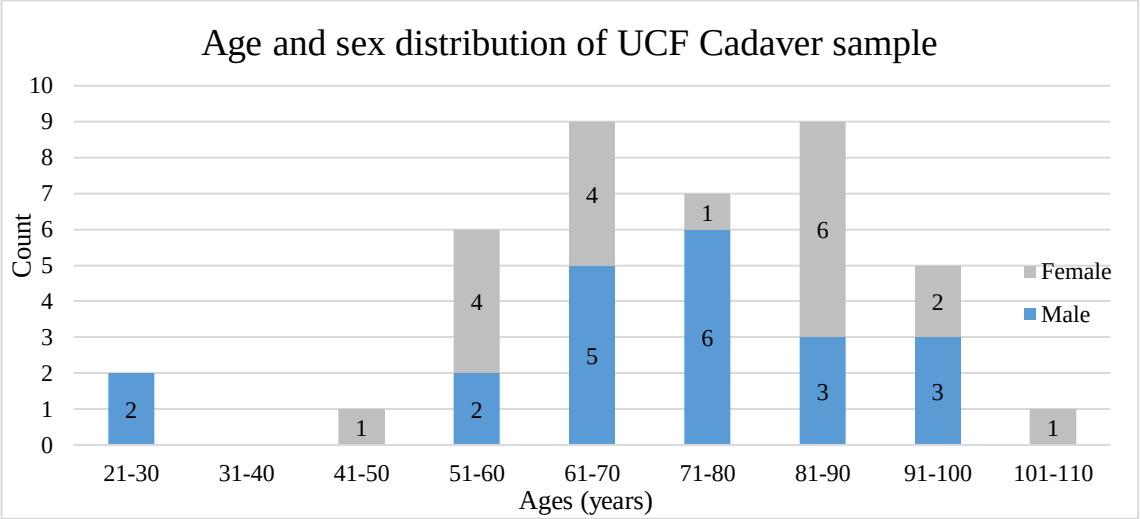

Figure 3.3. Bar graph depicting the age and sex distribution of the individuals in theUCF Cadaver sample.

3.1.2. The Terry Collection Sample

The second set of samples was collected from the Terry Collection that has been housed at the Smithsonian Institute in Washington, D.C., since 1967 but was collected in St. Louis Missouri and surrounding areas by Robert J. Terry and Mildred Trotter at Washington University (Figure 3.2; Hunt and Albanese 2005). Collection of unclaimed bodies from St. Louis area hospitals, almshouses, public health care institutions, and institutional morgues, which could be acquired by medical schools according to state law, began in 1905 (Hunt and Albanese 2005; de la Cova 2010). The individuals comprising the collection lived and died between 1828 and 1965 and were 14−102 years old at death (Hunt and Albanese 2005). The collection consists of the remains of 1728 individuals, 1089 of which have hair samples (Hunt and Albanese 2005). Because of the collection procedures, the Terry Collection does not reflect the demographic makeup of the population of St. Louis at any time (Hunt and Albanese 2005; de la Cova 2010).

The bodies comprising the Terry Collection underwent autopsy following death for research purposes and were then processed to obtain the skeleton, which are now stored at the Smithsonian Institute (Hunt and Albanese 2005). Hair samples are stored separately in envelopes labeled with individual numbers. Death certificates and morgue or autopsy records are associated with most of the individuals in the collection. These records provide some or all of the following information: age, 'race', occupation, state and country of birth, date of birth, date of death, name of hospital, amount of time in the hospital or duration of disease, cause of death, and contributing health conditions. Information regarding medical treatments was not available.

Ages from morgue records were confirmed from date of birth and death from death certificates. The procedure for identifying sex was not provided. At the time of sample collection, the definition of 'race' was largely social, and on autopsy records was ultimately recorded by the intake staff without clear guidelines, therefore it is unclear how the label related to how individuals identified themselves, skin color, or geographic area of ancestry (Hunt and Albanese 2005). While these problems are significant, other studies have found these labels to provide important distinctions in experience among individuals in the collection (Section 3.2.2.1).

The Terry Collection was included in this investigation because it is one of the few historical collections of hair samples in the world that contains information about cause of death. Individuals were selected for sampling to ensure a balanced sex ratio, a similar number of individuals described as 'Black' and 'White', and a representation of various causes of death. The individuals in this study ranged in age from 18−91 years and

included 20 males and 20 females (Figure 3.4). Twenty individuals are categorized as 'Black', 19 individuals characterized as 'White', and one individual described as 'Asian'. After initial analysis, further investigation revealed that one female individual was associated with conflicting records and she was removed from analyses of age, 'race', or cause of death. Available records indicate that the individuals analyzed in the current project were born between 1849−1920 CE and died between 1923−1960 CE. The hospitals and institutions in which they died were all located in St. Louis, Missouri, and included City Hospital, City Hospital #1 and #2, City Infirmary, City Sanitarium, Koch Hospital, and the Masonic Home. The sanitarium and Koch Hospital specifically treated tuberculosis while the City Infirmary was a psychiatric institution. A number of individuals were recovered from the City Morgue and no record regarding medical care is available.

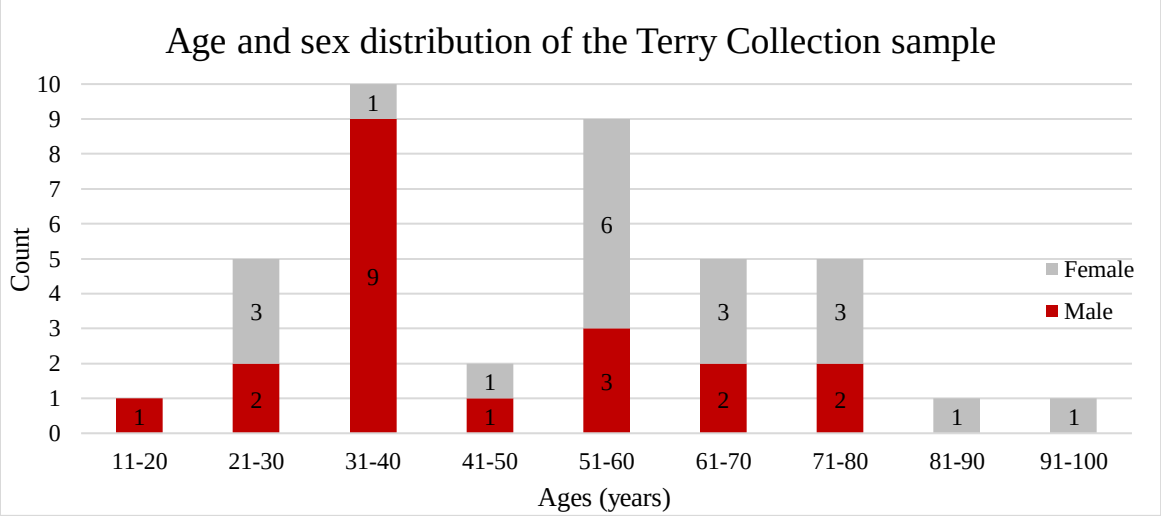

Figure 3.4. Bar graph depicting the age and sex distribution of the individuals in the Terry Collection sample.

3.1.3. Kellis 2 Cemetery Sample

The Kellis 2 Cemetery is located to the northeast of the settlement of Kellis in the Dakhleh Oasis, Egypt (Figure 3.5). The settlement was occupied from approximately 50 CE to 450 CE and the Kellis 2 Cemetery was in use between 100 CE and 450 CE during the Romano-Christian period (Dupras et al. 2016). Excavation began in 1992 and as of 2016 has recovered 770 individuals; the cemetery may contain the remains of 3,000−4,000 individuals (Molto 2002; Dupras et al. 2016; Appendix A.1).

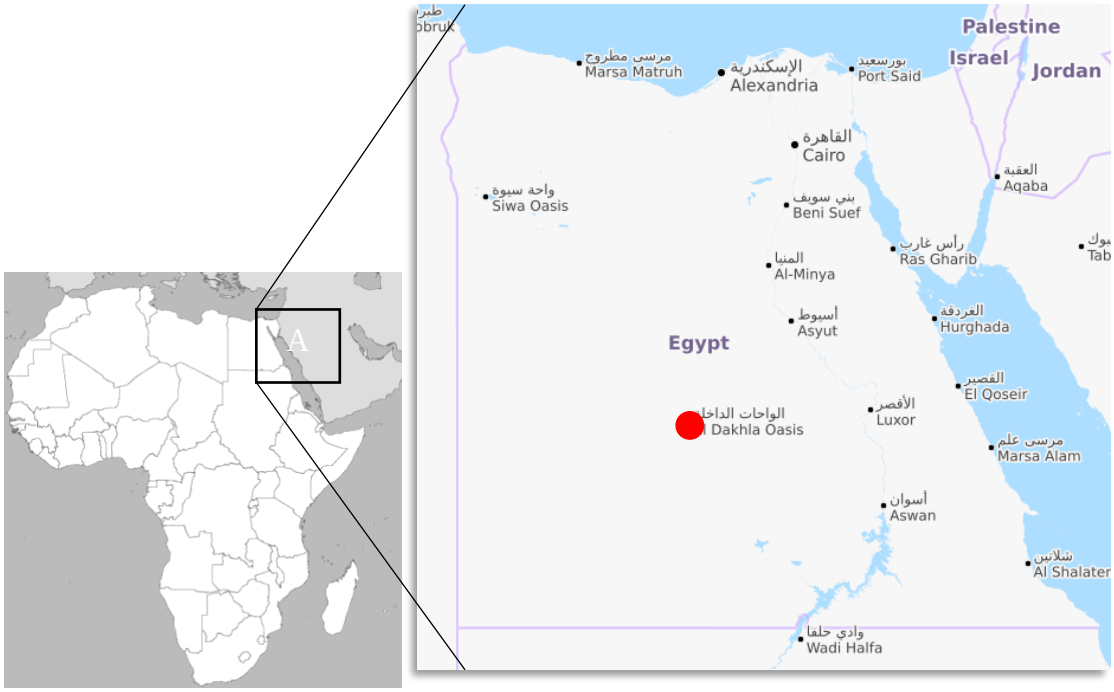

Figure 3.5. Map of Africa (left) © *www.mapsopensource.com*. Right image is magnified area within square © *OpenStreetMap contributors*. Red circle indicates the location of the Kellis settlement in the Dakhleh Oasis, modern day El Dakhla Oasis. North is up; scale is 1:7937074.

About one-third of the burials in the Kellis 2 Cemetery consist of mudbrick structures such as tombs, mastabas, or crypts which are surrounded by individual burial pits oriented East to West and likely organized according to kinship (See Appendix C for

cemetery map; Birrell 1999; Molto 2002; Bowen 2003; Wheeler 2009; Dupras et al. 2016). Each pit contains an individual, including preterm fetuses, wrapped in linen and placed in a supine position; features that are consistent with Christian burials during the period in Egypt (Bowen 2003; Wheeler 2009; 2012).

Of the 724 individuals analyzed from the cemetery thus far, ages range from fetuses to 72 years, 64% are juveniles and 36% are adults; 59% of the adults are female and 40% are male (Dupras et al. 2016). The high proportion of juveniles in the cemetery indicates a high fertility rate for the population and may account for the generally low average age at death for the Kellis 2 Cemetery population (16−20 years) as compared to upper-class Romans in other parts of the empire (26−29 years; Shaaban 1998; Molto 2001; Wheeler 2012).

In the Kellis 2 Cemetery sample, age was estimated by experienced members of the Dakhleh Oasis Bone Team and was provided to the author in a spreadsheet comprised of point estimates. Ages of adults were estimated according to the standards of Krogman and İşcan (1986) and Buikstra and Ubelaker (1994; Lana Williams personal communication: 7 March 2019). Ages of juveniles were estimated by Wheeler (2012) from dental eruption according to Moorrees and colleagues (1963) and Smith (1991), and epiphyseal fusion and development according to Krogman and İşcan (1986) and Baker and colleagues (2005). Juveniles were defined as individuals under the age of 15 because this was a biological and culturally significant age in Roman Egypt and the standard used in studies of the Kellis 2 Cemetery (Wheeler 2009). Sex was only estimated in individuals over the age of 15 in the Kellis 2 Cemetery sample using morphological features of the

pelvis and cranium according to the methods of Phenice (1969) and Buikstra and Ubelaker (1994) (Lana Williams personal communication: 7 March 2019). Sex was estimated by experienced members of the Dakhleh Oasis Bone team and provided to the author in a spreadsheet. Although the presence of migrants, based on historical records and stable isotope ratios, and diverse mitochondrial DNA profiles indicate a diverse population, individuals could not be classified according to ancestry (Parr 2002; Groff 2015).

The Kellis 2 Cemetery was chosen for analysis because of the excellent preservation and wide range of ages and sexes present. Furthermore, previous analyses of hair in the cemetery revealed favorable preservation of hair shafts and endogenous hormones such as cortisol and testosterone (Cole 2017; Tisdale et al. 2019). One hundred and thirty individuals from the Kellis 2 Cemetery were selected for this study. Individuals were chosen by Dr. Williams to reflect the general age and sex distribution of the cemetery (Figure 3.6). The sampling was limited to those individuals with hair which, in this case, only included individuals 18 months and older. The Kellis 2 Cemetery sample includes 40 juveniles, 32 males, and 58 females (Figure 3.7).

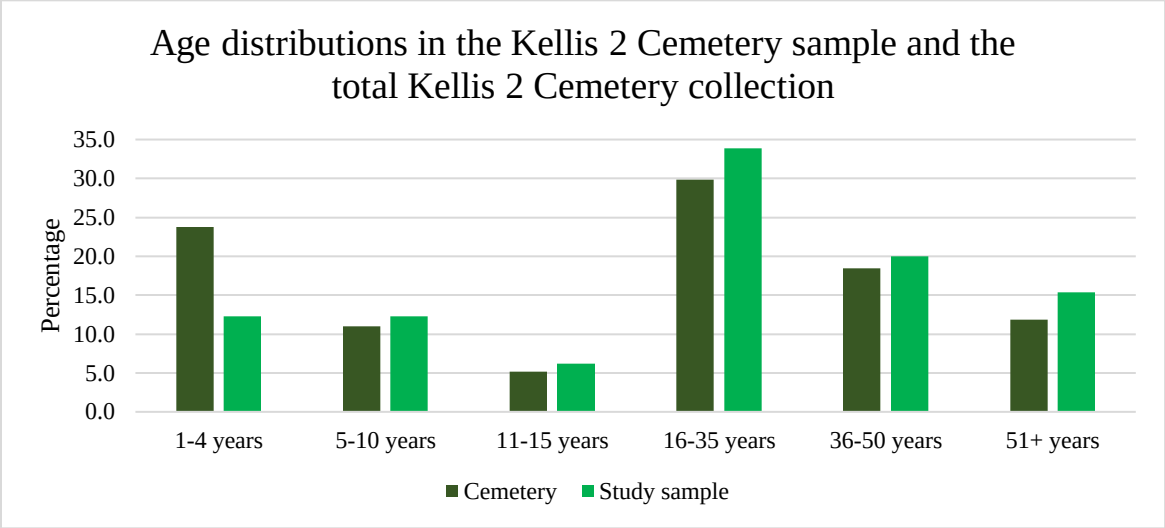

Figure 3.6. Bar graph depicting the age distribution of the Kellis 2 Cemetery sample in comparison to the age distribution of all individuals excavated from the Kellis 2 Cemetery.

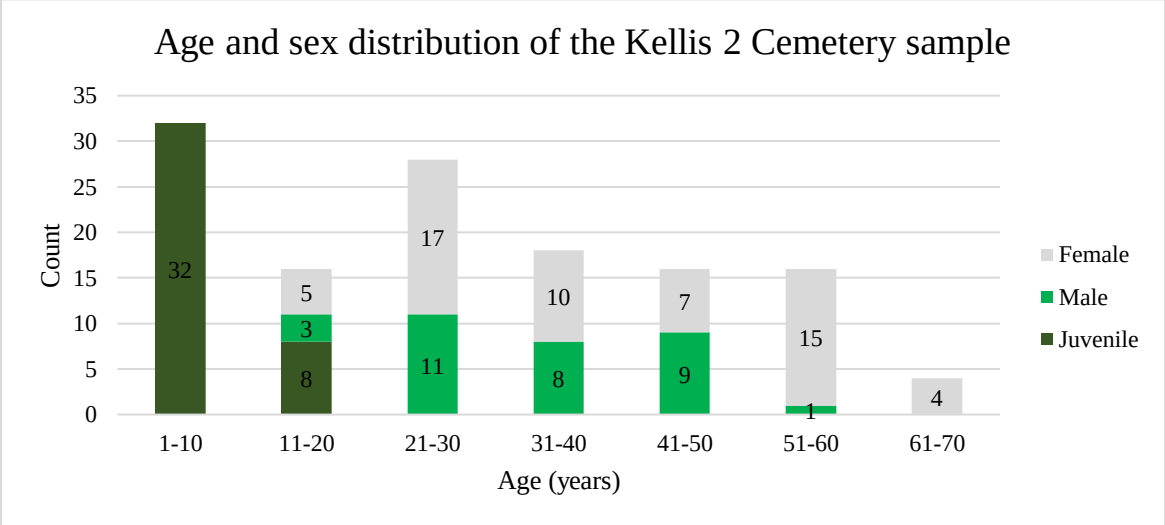

Figure 3.7. Bar graph depicting the age and sex distribution of individuals in the Kellis 2 Cemetery sample. Sex was not estimated for those under the age of 15 years.

3.1.4. Ethics and approvals

Given the use of materials from deceased persons, the destructive nature of this research, and the recommendations put forth by the American Association of Physical Anthropologists (AAPA Committee on Ethics 2003), the ethical implications of this work

have been thoroughly considered and appropriate approvals acquired. Sampling of hair from the UCF Cadaver materials was carried out with approval of UCF's Willed-Body Program and UCF ethics board as part of an ongoing project at UCF. The bodies were donated by individuals who consented prior to death for the purposes of education and research. The analysis of 40 hair samples for the current study was approved by the Hamilton Integrated Ethics Board (HiREB Project # 3819). Sampling of the materials from the Terry Collection was approved by the Smithsonian Institute sampling review board. The project was accepted on a preliminary basis, contingent upon the successful extraction of cortisol from an initial 10 hair samples. Because the hair samples were museum-curated for the purpose of research, HiREB did not require ethics approval. However, the ethical implications of the initial collection of these museum hair specimens cannot be ignored. Bodies were collected unethically, without consent from the deceased and were generally collected if the body was unclaimed (de la Cova 2010). Although the current study does not condone earlier collection methods, the Terry Collection represents a unique source of information which warranted the use of the material in the current study. Sampling and analysis of archaeological human remains do not require ethics approval; however, the Kellis 2 Cemetery samples were excavated by the Dakhleh Oasis project between 1993 and 2005 and collected with a permit issued for bone and hair samples to be used in chemical analysis under the supervision and approval of the Egyptian Supreme Council of Antiquities.

As Zuckerman and colleagues (2014) argued, if the analysis can be done holistically and with a recognition of the humanity of the dead, provides information that

is otherwise inaccessible or a voice for the marginalized, and can benefit modern society, the study of unethically collected human remains could be warranted. This study does not claim to speak for those who were marginalized in life and could not give consent to be studied in death or claim to benefit the dead or their descendent communities, but attempts were made to limit further harm and benefit living populations. As little hair as possible was used for the analysis and minced hair was returned to the Smithsonian Institute or UCF to be stored with the rest of the individual. No names or identifiable characteristics have been included in the current study and related documents. No denigrating or prejudiced language from the records were reproduced during the course of the research. Lastly, each individual was treated with respect and as a whole and complex human being.

3.2. Biocultural context of samples

3.2.1. UCF Cadaver

The UCF Cadaver sample is comprised of individuals born around the mid-1900s in the United States of America which means they lived through the Civil Rights Movement, Cold War and Vietnam War. They died in the late 2010s, during a time of ongoing social tensions, climate change, economic inequality, and foreign wars (Foner et al. 2016). These individuals lived and died during periods of relative economic prosperity (Foner et al. 2016). No medical histories or detailed information about socioeconomic circumstances are available for these individuals.

3.2.1.1. Causes of death

According to the Center for Disease Control the ten leading causes of death in the United States in 2016 were heart disease, cancer, accidents, chronic lower respiratory conditions, cerebrovascular diseases, Alzheimer's disease, diabetes mellitus, influenza and pneumonia, kidney disease, and suicide (Xu et al. 2018). Present causes of death in the UCF Cadaver sample are similar and are summarized along with definitions and symptoms in Table 3.3. Common symptoms reported for most dying people, regardless of underlying cause are pain, breathlessness, and fatigue (Emanuel and Emanuel 1998; Lo et al. 1999; Wenger and Rosenfeld 2001; Cherny 2011; Gysels and Higginson 2011). Individuals in the UCF Cadaver sample also died from accidents such as blunt head trauma and drowning that result in death in hours or days.

Table 3.3. Summary of causes of death in the UCF Cadaver sample and description and symptoms as reported in the literature. Symptoms experienced by each individual are not known.

Condition	**Description**	**Symptoms**
Amyotrophic lateral sclerosis	Degeneration of nerve cells controlling muscles	Muscle weakness, muscle spasm
Alzheimer's disease	Degenerative brain disease	Loss of memory and intellectual function, disorientation, confusion, anxiety
Cancer	Malignant growth or tumor resulting from abnormal cell growth	Loss of appetite, pain
Cerebral palsy	Damage to brain area controlling motor function	Seizures, pain, disordered sleep
Cerebrovascular diseases	Lack of oxygen supply to the brain	Pain, numbness, confusion, trouble seeing, lack of balance
Chronic obstructive pulmonary	Group of disorders that damage the lungs and make breathing increasingly difficult over time	Breathlessness, cough
Cystic fibrosis	Abnormal mucus production in lungs and digestive tract resulting in lung infections	Shortness of breath, discomfort, chronic cough
Diabetes mellitus	Insufficient production of or response to insulin resulting in damage to eyes, kidneys, blood circulation in legs, and the nervous system	Excessive thirst or urination, weight loss, fatigue, blurred vision
Heart and vascular conditions	Include arrhythmias, coronary heart disease, high blood pressure, congenital heart defects, heart attack,	Chest pain, shortness of breath, fatigue
Liver cirrhosis	Progressive liver damage	Fatigue, weakness, weight loss, confusion, and bleeding
Myotonic dystrophy	Gradual deterioration and weakening of muscles	Muscle weakness, cramps, and stiffness
Perforated appendix	Inflammation results in tear in appendix wall	Severe pain in the abdomen
Pneumonia	Infection of the lungs	Chest pain, shortness of breath, rapid breathing

Information from: Youngson 2001; Bashore et al. 2010; Giuliano and Hurvitz 2010; Carson-DeWitt et al. 2020; Division for Heart Disease and Stroke Prevention. n.d.; Rapoza 2017.

3.2.1.2. Approaches to dying and death

Research on attitudes and approaches to dying in contemporary America is limited by a lack of large and reliable studies (Field and Cassel 1997). Research from the 1970s and 1980s tended to focus on the avoidable suffering of death anxiety and death denial in America (Kübler-Ross 1969; Field and Cassel 1997). However, such work suffered from numerous methodological problems and more recent research has produced little evidence suggesting anxiety about death is a substantial component of day-to-day life for most Americans (Gallup and Newport 1991; Neimeyer and van Brunt 1995; Field and Cassel 1997). While studies have found an increase in anxiety in terminally ill people, researchers suggest it is associated with diagnosis, disease progression, treatment options, and fears about how one will die especially when considering pain, rather than death itself (Tilden 1999; Kolva et al. 2011).

It may not be possible to generalize about American approaches to dying as cultural differences account for varying approaches to advance directives, family roles in dying, patient autonomy, information disclosure, pain, and illness (Trill and Holland 1993; Field and Cassel 1997). However, key features shaping the contemporary American experience of dying include an interventionist medical profession, a cultural of individualism and exceptionalism, and the greater attention given to preparing for death and less to an afterlife (Field and Cassel 1997; Walter 2003; Green 2008). American patients tend to undergo higher rates of surgeries, tests and procedures than patients in other countries and know that they are dying, as 90% physicians report that they share terminal diagnoses with their patients (Field and Cassel 1997; Samuel 2013). The

individualist culture tends to manifest in individualized decisions about end-of-life care but also acts to constrain choices due to the nature of health care access resulting in fear of being a burden to one's family (Field and Cassel 1997). Parsons (1963) argues that the American approach to death is best characterized as "bringing to bear every possible resource to prolong active and healthy life" (61). As a result, tensions between cure, alleviating pain, prolonging life, and accepting death are common (Stephenson 2001; Kaufman and Morgan 2005; Timmermans 2005; Beckstrand et al. 2006; Green 2008). Death, it seems, can be accepted, but only when inevitable, at which point control of suffering becomes of paramount concern (Parsons 1963). Recent polls suggest that most Americans would prefer to receive care at home at the end of life and would seek hospice care (Seidlitz et al. 1995; Field and Cassel 1997).

3.2.1.3. Available medical treatments

In contemporary North America, pharmaceutical interventions are a significant component of the medical management of dying. While the medications prescribed differ between diseases (summarized in Table 3.4), steroid medications for the control of symptoms related to inflammation and opiates for the control of pain are commonly prescribed to dying people regardless of underlying condition (van Esch et al. 2016; Donegan and Bancos 2018).

Table 3.4. Summary of causes of death in the UCF Cadaver sample and associated treatments.

Condition	**Treatments**
ALS	Pain management, muscle relaxants, mechanical respiration
Alzheimer's disease	Mood stabilizers
Cancer	Surgery, chemotherapy, antibody therapy, hormone modulating therapy, radiation therapy
Cerebral palsy	Multiple surgeries
Cerebrovascular diseases	Thrombolytics to break up clots, surgical procedures to stop bleeding / repair damage
Cystic fibrosis	Physical therapy, antibiotics and anti-inflammatory drugs
Diabetes mellitus	Insulin to maintain appropriate levels of sugar in the blood, other medications to maintain/manage insulin and glucose metabolism, medications to prevent/ manage cardiovascular conditions
Heart and vascular conditions	Surgeries (implants; cardiac transplant; coronary revascularization); medications (beta blockers, nitrates, vasodilators, anticoagulants)
Liver cirrhosis	Pain management, surgeries to stop bleeding, relieve pressure, liver transplant (steroid treatments often required for immune suppression)
Perforated appendix	Surgery
Pneumonia	Antibiotics, bronchodilators, cough suppressants, pain medication, fever reducers

Abbreviation: ALS = Amyotrophic lateral sclerosis
Information from: Youngson 2001; Bashore et al. 2010; Giuliano and Hurvitz 2010; Carson-DeWitt et al. 2020; Division for Heart Disease and Stroke Prevention. n.d.; Rapoza 2017; Sgourakis and Dedemadi 2014.

3.2.2. Terry Collection

The individuals comprising the Terry Collection sample lived and died between 1849 and 1960 in St. Louis, Missouri, and were generally poor, unskilled laborers from urban settings who lived during multiple periods of turbulence in American history including slavery, the Civil War, Reconstruction, and the Great Depression (Hunt and Albanese 2005; de la Cova 2010). The Reconstruction period (1863−1877), in particular, was associated with racial prejudice, a struggling economy, overcrowded cities, job and

housing shortages, recovery from the Civil War and enslavement, financial panics, the 1918 flu pandemic, and industrialization (de la Cova 2011).

3.2.2.1. Previous bioarchaeological research of stress

Bioarchaeological investigations in the Terry Collection have identified high rates of periosteal new bone formation, growth disruptions, porotic hyperostosis, enamel hypoplastic defects, carious lesions, osteoarthritis, trauma, and infectious diseases (de la Cova 2011). De la Cova (2011) argued that the common presence of various types of indicators of stress is likely related to the overall low socioeconomic status of the individuals and the turbulent times in which they lived. Individuals described as 'Black' or African American display higher rates of infectious disease and arthritis at earlier ages than people identified as 'White' or European American. Those born during the Reconstruction era, in particular, display high rates of tuberculosis and treponematosis (Watkins 2003; de la Cova 2011). These findings are consistent with census records which indicate that African American mortality increased following the Civil War, while fertility decreased and remained low throughout the 20th century (de la Cova 2011). Records also indicate that rates of tuberculosis, malaria, measles, pneumonia, scrofula, and venereal diseases were higher among African American individuals during these times (de la Cova 2011).

Previous bioarchaeological research identified high rates of trauma in the Terry Collection. In a study of 374 males from the Terry Collection, 97% exhibited a fracture, which could be attributed to social tensions of the time (de la Cova 2010). The highest

rates were present among European American (i.e., 'White') males who also exhibited multiple healed fractures and antemortem tooth loss likely to be affiliated with interpersonal violence, and potentially, boxing (de la Cova 2010). A similar study of 256 females born between 1800 and 1877 from the Terry Collection identified higher rates of hip and radius fractures among European American (i.e., 'White') females consistent with accidents and osteoporosis and more cranial, nasal, and phalanx fractures among African American (i.e., 'Black') females, consistent with interpersonal violence (de la Cova 2012). Differences in peak bone mass could also be at play (Kelly et al. 1990).

While previous bioarchaeological studies have found differences in patterns and rates of trauma and skeletal indicators of stress between groups classified as 'Black' and 'White', much of this work is limited by sampling and mortality bias, bone remodeling, and the untenable relationship between skeletal indicators of stress and stress experience; limitations noted by Mays (2018) and Klaus (2014). Therefore, while present evidence indicates that the individuals in the Terry Collection experienced stress, little more can be said about stress in the Terry Collection especially regarding factors that may have influenced the last months of life.

3.2.2.2. Causes of death

National records indicate that the leading causes of death in 1900 were pneumonia, tuberculosis, intestinal conditions, heart diseases, intracranial lesions of vascular origin, nephritis, accidents, cancer and other malignant tumors, senility, and diphtheria (National Vital Statistics System n.d.). Information about cause of death is available for all but one of the individuals examined in this study from the Terry

Collection. The causes of death are consistent with those reported nationally and include accidents; suicides; and homicides; infections such as tuberculosis, malaria, meningitis, and pneumonia; cardiovascular conditions; and cancers. Other listed causes of death include heat stroke, gangrene, appendicitis, and gastrointestinal hemorrhage. Some causes of death such as, cancer, appendicitis, and pneumonia are also present in the UCF Cadaver sample (see Table 3.4). Causes of death that are unique to the Terry Collection sample are described in Table 3.5.

Table 3.5. Summary of causes of death in the Terry Collection sample with description and symptoms as reported in the literature. Symptoms experienced by each individual are not known.

Condition	**Description**	**Symptoms**
Arteriosclerosis (atherosclerosis)	Hardening and degeneration of the arteries leading to decreased blood flow to heart, brain, and other organs and may lead to heart attack or stroke	Chest pain, shortness of breath, dizziness, lightheadedness, headache, confusion
Cardiac Congestion	Heart cannot produce adequate blood supply	Fluid accumulation, breathlessness
Cerebral Hemorrhage	Deprivation of blood and oxygen to brain leading to swelling and damage	Headache, functional losses, loss of consciousness
Gangrene	Death of tissue, usually due to inadequate blood supply	Affected area turns dry and black, putrefaction of tissue,
Gastrointestinal hemorrhage	Escape of blood from blood vessel in gastrointestinal system	Bloody stool, weakness, fatigue, chest pain, shortness of breath, pain, discomfort
Heat stroke	Body temperature rises to dangerous levels	Headache, nausea, fatigue, dizziness, seizures
Hypertensive heart disease	Enlarged heart muscles impairs heart functioning	Chest pain
Malaria	Parasitic infection resulting in red blood cell destruction and kidney damage	Fever, shaking, headache, pain
Myocarditis	Inflammation of the muscular walls of the heart	Fever, irregular heartbeat, fatigue, shortness of breath, chest
Nephritis	Inflammation of the kidney	Swelling, vomiting, reduced urine flow, pain
Peritonitis	Inflammation of membrane lining the abdominal cavity, usually caused by infection	Fever, severe abdominal pain, rigidity of the abdominal muscles
Streptoci-meningitis	Infectious meningitis resulting in inflammation of the meninges surrounding the brain	Sore throat, fever, headache, stiffness of the neck, vomiting, confusion, drowsiness, coma
Tuberculosis	Bacterial infection, usually leading to destruction of lung tissue; may also impact the meninges, intestines, bones, lymph system, skin, spine, kidneys, and genitals	Fever, fatigue, loss of appetite and weight, night sweats and cough, fluid accumulation in pleural cavity

Information from: Youngson 2001; Colby and Davidson 2017; Lagasse and Columbia University 2018, Gale 2017.

3.2.2.3. Approaches to death through time

Farrell (1980) argues that the American approach to death can be broken into three stages: the living death (1600−1830 CE), the dying of death (1830−1945), and the resurrection of death (1945−Present). The majority of individuals in the Terry Collection Sample died during the second phase (only one individual died after 1945). Significant events in the 20th century also brought about changes in American approaches to death, such as the 1918 flu pandemic and World War I (Samuel 2013). Additionally, as more people moved to cities, mortality continued to increase, especially as a result of infectious disease and high infant mortality (Lightfoot 2019). As such, death became commonplace, and conceptions about life became more linear, with death representing an end (Samuel 2013; Lightfoot 2019). Hospitals became more common during this time, as did mortuary professionals (Samuel 2013). As a result, death became viewed as unnatural and associated with substantial fear, dread, and grief (Samuel 2013). It was not until the 1980s that leading medical associations began to encourage doctors to share terminal diagnoses; prior to that about 10% of doctors would (Samuel 2013). Therefore, while they likely suspected, individuals may not have been told they were dying, leading to greater uncertainty around death. Samuel (2013) claims that death denial grew during the 20th century, hitting its peak at the end of World War II (Samuel 2013). Spiritualism arose in response to the new uneasiness surrounding death (Samuel 2013; Lightfoot 2019). According to this belief system, the soul continued on after death and can influence the world of the living and death was likened to sleep (Samuel 2013; Lightfoot 2019).

Ultimately, understandings of death in this period were characterized by uneasiness as well as fear, grief, and dread.

3.2.2.4. Available medical treatments

The treatments available to the Terry Collection sample, who died between 1923 and 1960 CE, would have differed substantially from those available today. Although the specific treatments or medications prescribed to each individual in the sample is not known, possible treatments can be inferred from historical records. Records indicate that the primary treatment for infection was rest, for cancer was surgery and pain management, and for cardiovascular conditions was pain control. Although steroid medications can be prescribed for symptom management at the end of life today, these were not available to those comprising the Terry Collection.

Prior to 1944 (when antibiotics for the treatment of tuberculosis were discovered), tuberculosis treatment included a stay at a sanatorium that prescribed rest, good food, fresh air, and occasionally, surgical pulmonary collapse procedures (Hurt 2004; Daniel 2006). Rest was defined stringently according to a patient from 1944 as: "Absolute and utter rest of mind and body—no bath, no movement except to toilet once a day, no sitting up except propped by pillows and semi-reclining, no deep breath" (Hurt 2004: 350). The artificial pneumothorax or partial lung collapse was developed in 1894 to rest infected portions of the lungs and allow them to heal and by the mid-1900s thoracoplasty or removal of portion of the ribs to collapse the lung was developed (Daniel 2006), although it is not clear how often these treatments were performed.

Nineteenth and 20th century treatments of cancer included surgery or X-ray (Aronowitz 2001). In the early 20th century, complete and early mastectomy was the preferred treatment for breast cancer, which generally extended life by about two years (Lane-Claypon 1924). However, individuals often did not seek treatment early enough for a variety of reasons including fear of doctors and surgery, the use of alternative remedies, stigma, modesty, a lack of symptoms, or financial problems (Aronowitz 2001). If cancer was advanced or not in a location to be operated upon, few remedies were available and pain management became the priority (Aronowitz 2001).

For some conditions, pain management was the only treatment available. For mild to moderate pain, aspirin was the standard throughout the 20th century (Meldrum 2003). To minimize pain associated with surgical procedures, anesthesia and ether could be used starting in 1846 and chloroform was available from 1848 (Meldrum 2003). The most common course of action for heart conditions, beginning in the 1920s, was to treat the pain associated with cardiac conditions with regional nerve blocks (Meldrum 2003). For acute, severe pain, such as cancer, the prescription of opiates such as morphine was standard and carefully weighed against fears of addiction. Although it was not widely discussed at the time, many physicians advocated aggressive pain management to the seriously ill and dying, although how often this was practiced is not known (Meldrum 2003).

For those with neurological, psychiatric, or behavioral conditions, options were generally limited to institutionalization. The conditions of institutions for the treatment of psychological disorders were often inadequate in the 19th and 20th century (Lael et al.

2007; de la Cova 2012). Individuals were commonly institutionalized with little direct treatment for their conditions (Lael et al. 2007).

Steroid medications were not available to the individuals comprising the Terry Collection sample. Edward Kendall first isolated cortisone in 1935 and synthesized it in the 1940s (Hillier 2007). Corticosteroids were not used in medical treatment in America until 1948 when cortisone was used in the treatment of Addison's disease and rheumatoid arthritis, neither of which were identified in the Terry Collection sample (Saenger 2010; Benedek 2011). Dexamethasone was introduced in 1958 to treat neurologic conditions (Benedek 2011). Only one individual in the Terry Collection sample died after 1958 and was not diagnosed with a neurologic condition. One individual in the Terry Collection sample was diagnosed with a "Cushing-like syndrome" before death. At the time of their death, few treatments were available for the condition because replacement hormones did not exist (Lindholm 2000).

3.2.3. Kellis 2 Cemetery

The settlement of Kellis is located in the Dakhleh Oasis, approximately 300 km West of the Nile Valley in Egypt. Kellis was settled permanently around 50 CE and abandoned in the Romano-Christian period around 450 CE (Knudstad and Frey 1999; Hope 2001; Thanheiser et al. 2002). The area was hyper arid, but access to underground aquifers provided a year-round water supply, allowing for multiple crops throughout the year including exotic plants such as dates, olive oil, and wine which were in high demand across the empire (Bagnall 1993; Bowen 2001; Dupras and Schwarcz 2001; Thanheiser et al. 2002). The area was also rich in mineral resources and was located along a trade route

from the Nile Valley (Bagnall 1993; Hope 1998). Kellis was an important center of trade with a wealthy population that reached several thousand people by its height (Knudstad and Frey 1999; Hope 2001; Molto 2002). As such, Kellis was highly stratified, which is evident in differences in household architecture, goods, and mortuary treatments. Enslaved people were the most marginalized members of the population while male religious and administrative officials were the most powerful (Aufderheide et al. 1999; Bowen 2001; 2005). As a result of the close trade ties with the Nile Valley, Mediterranean, Red Sea, and Sudan, a significant portion of Kellis' population likely consisted of migrant farmers and merchants (Churcher 2002; Parr 2002; Groff 2015).

3.2.3.1. Previous bioarchaeological research

Previous bioarchaeological work provides some evidence for health conditions and cause of death in the Kellis 2 Cemetery. The investigation of skeletal indicators of stress faces many of the challenges outlined by Mays (2018) and Klaus (2017), as well as mortality bias (Wood et al. 1992), thus the conclusions that can be drawn about the amount and types of stress experienced by those in the Kellis 2 Cemetery is limited. While it is clear that stress was experienced, current bioarchaeological research provides little information to identify sources of stressors and how these may relate to stress associated with dying and death.

Systemic investigations of juvenile skeletons in the Kellis 2 Cemetery analyzed a suite of skeletal indicators of stress, patterns of growth, and dental health in individuals under the age of 15 years (Wheeler 2009; 2012). The pattern of skeletal indicators of stress are consistent with moderate levels of stress in juveniles, and the lower rate of

skeletal indicators of stress in the Kellis 2 Cemetery as compared to pre-Roman times could suggest an improvement in health (Wheeler 2009; 2012). Similar studies have not been carried out for adults.

Studies provide some insight into factors that may influence cause of death. Wheeler (2009; 2012) determined that the lack of skeletal indicators of stress or signs of disease in many infants was consistent with acute causes of death among juveniles. Paleodemographic studies suggest that the probability of dying was lowest for individuals between the ages of 10 and 15 (Sharman 2007). The high number of females of childbearing age, late term fetuses, and young infants in the cemetery indicates that pregnancy and infancy were dangerous periods, possibly due to infection, obstruction, and poor maternal health (Sharman 2007; Wheeler 2012). Williams (2008) calculated seasonal mortality based on grave alignment and stable isotope analysis of hair. She determined that peak mortality was reached in March/April during times associated with seasonal sandstorms, extreme temperatures, food shortages, and cycles of infection.

In a few rare cases, skeletal evidence from Kellis 2 Cemetery more directly suggests cause of death. Neural tube defects and osteogenesis imperfecta were identified in a number of juveniles (Mathews 2008; Cope and Dupras 2011). Among adults, eight individuals, mostly young males, display skeletal changes consistent with leprosy while three individuals exhibit evidence of tuberculosis (Molto 2002; Donoghue et al. 2005). A few individuals in the Kellis 2 Cemetery exhibit lesions likely associated with cancer. An approximately 40-year-old female displayed metastatic lesions (Dupras et al. 2013) while a three-to-five-year-old child presented lytic lesions consistent with lymphocytic

leukemia (Molto 2002). An 11-year-old child sustained multiple fractures around the time of death (Dupras et al. 2016). In another case, a pattern of fractures consistent with modern cases of child abuse were identified in a two-to-three-year-old (Wheeler et al. 2013). None of the individuals comprising the current study sample display skeletal evidence suggesting a cause of death.

3.2.3.2. Causes of death

Although bioarchaeological research in the Kellis 2 Cemetery sample identified a small number of potential causes of death; determining cause of death is not usually possible for archaeological human remains. Potential causes can be inferred from examples of preserved soft tissue, historical and geographic context, and mortality patterns.

Analyses of mummified individuals from the earlier Kellis 1 Cemetery associated with the settlement of Kellis have identified conditions that can contribute to death such as sand pneumoconiosis (Cook 1994; Cook and Sheldrick 2001), emphysema, anthracnosis, *Enterobius vermicularis* (pinworm), *Schistosoma hematobium* (schistosomiasis), and *Ascaris lumbricoides* (roundworm; Horne 2002; Aufderheide 2003; 2009; Zimmerman and Aufderheide 2010; Dupras et al. 2016). Atherosclerosis and cirrhosis of the liver has also been identified (Zimmerman and Aufderheide 2010; Branson 2013).

Studies of the same historical period from elsewhere in Egypt have identified plague and *Escherichia coli* (*E.coli*), which can cause death (Dzierzykray Rogalski 1980,

Zink et al. 2000). Common childhood diseases in Egypt today that could have resulted in death of children in the past include diphtheria, whooping cough, polio, tetanus, bacterial meningitis, measles, parasites, malaria, and tuberculosis (Jordan 2000, Yassin 2000a, 2000b; Youssef et al. 2004). The leading causes of death for infants in Egypt today are diarrheal disease and gastroenteritis, and malnutrition also plays a significant role (Yassin 2000a, 2000b; Winham et al. 2004). Cardiovascular disease, diabetes, cancer, and chronic respiratory disease are leading causes of death in Egypt today (WHO 2004), and in neighboring regions, such as Saudi Arabia, brucellosis from unpasteurized cow and goat milk remains a concern (Al Shaalan et al. 2002).

3.1.3.3. Approaches to dying and death

Kellis was a site of intermingling religious traditions as a result of its locations on the outskirts of the Roman Empire (Kaper 2002). During the 3rd and 4th centuries CE, Christianity spread quickly (Bowen 2002; 2003; Gardner 2008). However, at the same time, one of the latest built temples in Egypt dating to the late 2nd or early 3rd century CE resides on site (Hope 2001; Kaper 2002). Traditional Egyptian beliefs, Classical traditions, and Christianity interacted in complex ways and was encouraged by flexible religious doctrine in the area (Bowen 2003; Gardner 2008). The unique religious patterns in Kellis were reflected in mortuary practices at the site. The earlier Kellis 1 cemetery included rock cut tombs, anthropogenic mummification, grave goods, and flexibility in alignment (Molto 2002; Bowen 2003; Bowen et al. 2005). These Egyptian-style burial practices in the Kellis 1 Cemetery overlapped with the beginning of Christian style burials in the Kellis 2 Cemetery (Bowen 2003). Throughout their history, Egyptians

viewed life and death as cyclical, but death was still a source of grief and fear (Meskell 2001). Attempts were made to prolong life, although many conditions were considered incurable (van Middendorp 2010; Hajdu 2011). In contrast, conceptions of dying in early Christian history associated it with peaceful rest and hope while awaiting resurrection (Mutie 2015; 2017). While burial practices suggest a Christian view of the afterlife; other aspects of religious architecture in Kellis indicate that Pharaonic elements of religion persisted. Therefore, beliefs about dying and death during the use of the Kellis 2 Cemetery may have included both fear and hope.

3.1.3.4. Available medical treatments

The ancient Egyptian approach to medicine was advanced for its time. The Edwin Smith Papyri indicates that the systematic examination of a patient, diagnosis based on facts, and rational reason for treatment was well established in Egypt by the 17th century BCE (David 2004). The ancient Egyptians also had a detailed knowledge of anatomy and would make diagnoses based on suites of symptoms rather than single elements, which was unique for the time (David 2004). There is some evidence that palliative care was practiced, which included maintaining treatment of the condition, rest, and adequate nutrition (Campbell 2089). Opium and lotus flower were also available to treat pain although how often it was in use is not known (Nuun 2002; Norn et al. 2005).

Medical procedures were varied in ancient Egypt. Herbal remedies such as honey, milk, and cream could be used for conditions such as throat and chest infections, while spells could also be used to cast out spirits or transfer them to another creature (David 2004). Minerals such as alum were used as remedies for many ailments including open

wounds and diseases of the eye and genitals, as well as leprosy (Groff 2015). Broken bones could be expertly set and heal well in some cases while other instances show lack of alignment or healing (David 2004; Dupras et al. 2016). Simple surgery and amputation were available and usually associated with injuries (David 2004; Dupras et al. 2010). The Edwin Papyrus, dating to 3000 BCE, indicates that cancer could be identified and was known to be fatal; attempts at treatment included cauterization, salts, and arsenic paste, which were rarely effective (Hajdu 2011).

CHAPTER 4 - METHODS

4.0. Introduction

In order to evaluate the utility of hair cortisol concentration (HCC) analysis in the dead and investigate variation of HCC between individuals and groups, the current study compared average HCC and patterns of HCC in the last months of life between and within samples, and on the basis of age, sex, cause of death (when known), and duration of disease. Non-parametric statistical analysis was used to determine how HCC varied between and within groups.

4.1. Definitions and classifications

4.1.1. Stress

HCC values are not well suited to bioarchaeological definitions of stress because, unlike traditional indicators of stress in bioarchaeology, HCC values can reflect various aspects of the stress experience. Thus, the definition of stress in this study builds primarily on the biomedical definition of Goldstein (1995; Section 2.1). In the current study, I define stress as **a complex process that, at minimum, consists of exposure to a stressor, a state of disharmony, and a response; the experience of which can be inferred from indicators of stress.** Stress, as a process, is not directly observable, but can be inferred through various signs of physiological disruption and response, including skeletal lesions or HCC values, which will be referred to as indicators of stress.

4.1.2. Death

HCC analysis cannot provide insight about the moments around death itself because HCC reflects monthly averages and may not capture the last two weeks of life (Kapoor et al. 2018). **Therefore, death will be broadly defined as when biological functioning of the human body ceases.**

4.1.3. Dying

Given the focus on monthly averages of HCC, it is not possible to distinguish between specific stages of dying. Therefore, dying will be defined according to Kellehear (2009) as **a process leading to death in a relatively short period of time,** which includes both those dying of disease as well as those expecting to die, such as those who die by suicide. Data is not available for the individuals in the current study to identify the dying period, however, all of the individuals in this study have died. Thus, a majority of hair samples taken closer to the scalp are more likely to reflect experiences of dying. When the experience of dying cannot be confirmed, **stress in the months near death will be referred to only as experiences in the months leading up to death.** This definition accounts for complexities of identifying dying and ensures no assumptions are made that the experiences in the last months are directly related to dying.

4.1.4. Cause of death

A distinction is made in the current study between mechanism of death, terminal cause of death, and underlying cause of death (UCOD). A mechanism of death is defined as "a physiologic or biochemical disturbance produced by a cause of death" (Zumwalt

and Ritter 1987: 18). A terminal cause of death is the cause directly leading to death (WHO 2019b 2.18.1). The underlying cause of death is the condition that, if it had not been present, the individual would be alive, it is **"the disease or injury that initiated the train of morbid events resulting in death"** (Zumwalt and Ritter 1987: 18). For example, for individual 383 in the UCF Cadaver sample, the mechanism of death was listed as cardiopulmonary arrest, this was triggered by the terminal cause of death: multisystem organ failure, which in turn was a result of the UCOD: End Stage Alzheimer's Disease.

Mechanism of death is not useful for this study because examples such as, "respiratory arrest" provide no information about the condition leading to death (Kircher and Anderson 1987: Lauer et al. 1999). Similarly, terminal cause of death provides limited information about pathophysiology or experience of disease and dying over time. Additionally, a UCOD may result in various health conditions, and a single, terminal cause of death often cannot be determined (WHO 2019b: 2.17.1). For these reasons, the UCOD is most often tabulated for public health studies and is recommended by the World Health Organization (WHO 2019b: 2.17.1). The current investigation will primarily explore the UCOD.

Causes of death in the Terry Collection and UCF Cadaver samples were identified and classified according to the standards of the International Classification of Diseases and Related Health Problems (ICD) 11th Edition (WHO 2019a). The ICD is a comprehensive classification system developed and endorsed by the WHO that is designed to facilitate comparison and analysis of mortality data (WHO 2019b: 1.1; 1.2). According to the ICD, identification of causes of death are useful for "medical research,

monitoring of public health, evaluating health interventions, and planning and follow-up of health care" (WHO 2019b: 1.3). While the ICD guide is designed to be used with modern death certificates it can be applied to other health records (WHO 2019b: 1.1; 1.2).

A flowchart outlining the ICD-11 guidelines for identifying UCOD can be found in Appendix B.0. The first step is to work backwards from medical conditions listed in the records to determine if each one could be caused by other listed conditions (WHO 2019b: 2.19.5; 2.19.6). From here, the causal relationship between medical conditions listed in the records and the sequence of those conditions can be determined. 'Causal relationship' is defined as one condition being caused by another and 'sequence' indicates a series of events with causal relationships (WHO 2019b: 2.18.2). Notably, according to the ICD guide, a causal relationship may exist regardless of the order in which items are listed in the medical records and if unlisted steps could account for the relationship. The ICD guide (WHO 2019b: 2.21) provides specific instructions for determining the relationship between certain conditions (WHO 2019b: 2.19.4; Appendix B.0). In those situations in which the ICD guide does not provide specific information about causality, the A.D.A.M. Medical Encyclopedia was referenced (MedlinePlus 2020). With this approach, a disease that started the chain of events can be identified (WHO 2019b: 2.19.2).

The ICD-11 guidelines provide instructions for specific circumstances. If the UCOD cannot be distinguished between more than one medical condition but these conditions can both be accounted for under a broader subgroup, then the broader classification should be used. Second, if these conditions are not linked by a broader

subcategory but impact the same anatomical system, they should be coded to that anatomical system. Lastly, if two conditions are present that cannot be accounted for by the same subcategory or anatomical condition they should be categorized as "Other specified general symptoms, signs or clinical findings" (WHO 2019b: 2.22.1).

In the UCF Cadaver collection, medical conditions at death were provided in a spreadsheet and causal relationships between the listed health conditions were investigated to determine the UCOD. Cause of death was determined from morgue records rather than death certificates for the Terry Collection sample. According to Mitchell (2017), historical records must be evaluated on the basis of who wrote it, when it was written, and why it was written. Regarding death certificates, the qualifications of the writers are unknown, during the time period appropriate diagnostic tools did not always exist, and the physicians filling out the form may have been biased. Morgue records on the other hand, were recorded by researchers at the University of St. Louis, the diagnoses were made after autopsy when more information was available, and the researchers had no vested interest in diagnosis. Discrepancies between morgue records and the death certificate were present for only two individuals. Both individuals were in a psychiatric facility prior to death and cardiovascular conditions listed on the death certificate while autopsy records list pneumonia. The death certificate could reflect a lack of skill of the physician, lack of accurate diagnostic techniques, or a bias of the physician. Death certificates were used to identify cause of death in only two individuals who had no cause of death listed on their morgue records. In the Kellis 2 Cemetery sample, no individuals presented skeletal changes suggestive of cause of death and no attempt was made to

determine cause of death from skeletal remains. UCOD for each individual is presented in Appendix C.

A number of individuals in the UCF Cadaver sample were diagnosed with various forms of dementia, and in the Terry Collection sample one individual was diagnosed with dementia while a number of individuals were diagnosed with senility. Dementia is characterized by deterioration of the brain associated with problems with memory and judgement (Gale 2017). Senility is associated with mental decline in old age (CDC 1990). The CDC points out the diagnoses of senility and dementia often overlap, and that diagnosis of Alzheimer's disease and dementia are increasing while the diagnosis of senility is decreasing in light of better diagnosis and greater awareness (CDC 1990). In the Terry Collection, these conditions could not be distinguished at autopsy, thus the diagnosis may be interpreted broadly. Given the challenges of diagnosing dementia in the past and the similar disease experience for both dementia and senility those with either diagnosis will be cautiously considered together.

4.1.5. Classifying causes of death

The ICD proposes classifying each UCOD into a broader group category called a stem-code to facilitate statistical analysis and account for small sample sizes (WHO 2019b: 2.27.3). According to the ICD guide "A statistical classification of diseases must be confined to a limited number of mutually exclusive categories able to encompass the complete range of morbid conditions" (WHO 2019b: 1.2.1). There are 26 stem-code groups defined according to etiology, organ system, external causes, and factors influencing health status (WHO 2019b: 2.4). The ICD-11 system was adopted for this

study because it was developed according the most up to date scientific research with input from leading researchers in various fields, includes clear definitions, and is also widely used and standardized. The online coding tool was used to determine stem-codes (WHO 2019a: 2.13). The stem-code categories represented in the Terry Collection sample and the UCF Cadaver sample are summarized in Table 4.1 and Appendix C. 'External causes of morbidity or mortality / injury, poisoning or certain other consequences of external causes' will be referred to as 'External causes'; and 'Symptoms, signs or clinical findings, not elsewhere classified' will be referred to as 'Not elsewhere classified'.

Table 4.1. All causes of death in the UCF Cadaver and Terry Collection samples and corresponding category according to the ICD. Every underlying cause of death (UCOD) is listed only once.

ICD Category	**Underlying Cause of Death**	
Certain infectious or parasitic diseases	• Malaria • Meningitis	• Tuberculosis • Tuberculosis of the digestive system
Diseases of the circulatory system	• Acute Congestive heart failure • Arteriosclerosis • Atherosclerotic cardiovascular disease • Cardiomyopathy/Hypertension • Congestive heart failure • Coronary artery disease	• Coronary atherosclerosis • Heart disease • Heart failure • Myocardial infarction • Myocarditis • Peripheral vascular disease
Diseases of the digestive system	• Appendicitis • Cirrhosis	• Peritonitis • Peritonitis/ Perforated
Diseases of the nervous system	• Amyotrophic lateral sclerosis • Cerebral Hemorrhage • Cerebral Palsy	• End Stage Alzheimer's Disease • Myotonic Dystrophy
Diseases of the respiratory system	• Chronic obstructive pulmonary disease	• Cystic Fibrosis • Pneumonia
External causes of morbidity or	• Gunshot	• Suicide
Injury, poisoning or certain other consequences of external causes	• Blunt Head Trauma • Cranial Trauma • Drowning • Heat Stroke	• Hip Fracture • Stab Wound • Subdural hemorrhage
Mental, behavioral or neurodevelopmental disorders	• Dementia • Alzheimer Dementia	
Neoplasms	• Cancer Unknown Origin • Carcinoma Bladder • Cervical Cancer • Colon Cancer/Bladder Cancer • Colon Cancer • Esophageal Cancer • Liver Cancer	• Lung Cancer • Malignant Carcinoma of Transverse Colon • Mantle Cell Lymphoma • Metastatic Pancreatic Cancer
Symptoms, signs or clinical findings, not elsewhere classified	• Diseases Related to Advanced Age • Gastrointestinal hemorrhage	• Gangrene

4.1.6. Classifying duration of condition

To investigate the effects of duration of disease in HCC, the samples were split into a short-term and long-term disease group. Abrupt conditions were defined as those resulting in death in less than one day and are not considered in the comparisons. Given the definition of dying applied (Section 4.1.3), suicide is not considered an abrupt condition, and individuals with this cause of death are not included in analyses of duration of disease. In the Terry Collection sample, information for duration of disease or hospital stay is only available for 26 individuals from the sample investigated. The length of time individuals spent in hospital ranged from less than a day to two and a half years, while the duration of disease lasted less than a day to nearly three years. Death certificates listed either how long an individual had a disease or how long they had been under medical care. Whichever was available was used as a proxy for duration of condition. Although discrepancies likely existed between when an individual developed a condition and when they received medical care, the use of the broad categories of short-term and long-term, accounts for any error introduced by these inconsistencies. No consensus exists in the literature regarding the duration of diseases considered acute or chronic. Therefore, a number of comparisons were made in the current study employing different cut offs to distinguish acute from chronic conditions. Three-month intervals were used to capture the greatest amount of variation between groups and to account for any variability in monthly growth rates. In a series of comparisons, acute and chronic diseases will be defined as less than and greater than 1 month, 3 months, 6 months, 9 months, 1 year, 15 months and 18 months. Only 2-month average HCC was used for comparisons to ensure a

focus on the effects of duration of disease at the end of life, and not throughout the disease.

The second approach will compare acute to chronic conditions according to the definitions set forth by the WHO (2018). The definitions of acute and chronic differ considerably in the medical field, in stress research, and in HCC analysis. Medical definitions of chronic vary from seven weeks to years (Nachemson and Bigos 1984; Spitzer 1987; Frymoyer 1988; Patterson 2001; Fernández-Bañares et al. 2016), stress research defines chronic stress as ongoing stress or serious single events (McGonagle and Kessler 1990; Hammen et al. 2009; Rohleder 2016), and HCC studies define chronic stress as lasting weeks to months or any stress experience visible in HCC (Russell et al. 2012; Staufenbiel et al. 2013). According to the WHO, differences between acute and chronic conditions also relate to etiology and experience. The WHO (2018) defines chronic disease as non-communicable disease that are a result of genetic, biological, environmental, and cultural factors, which consist of cardiovascular disease, cancers (neoplasms), chronic respiratory diseases, and diabetes. This approach to classifying disease experience, does not take into account individual disease trajectories and is most often used in large scale studies. It is employed in this study as duration of disease is not known for all individuals, and a standard definition of acute and chronic across diseases is not available.

Notably, differences in experience between acute and chronic infections is not easily captured through either of these classification techniques. For example, in the Terry Collection, individuals who died from Tuberculosis infections, experienced disease

durations ranging from several days to more than two years. An ideal technique for exploring variation in disease experience would take into account both etiology and duration. However, no current definition exists for distinguishing acute from chronic infections. As such differences in these experiences could not be explicitly explored in the current study.

4.2. Hair Cortisol Analysis

A hair fiber contains keratin, water, lipids, pigments, and trace elements and is composed of a cortex surrounding a central medulla covered by a cuticle made up of flattened cells and with an overlying lipid layer (Wilson and Tobin 2010; Wilson 2017). The outer layer of the hair shaft is made up of overlapping keratin scales (Wilson and Tobin 2010). The keratin proteins that compose the hair shaft are synthesized in the hair follicle or bulb before erupting from the scalp surface during the anagen, or growth phase (Wilson and Tobin 2010). Hair also passes through a catagen or transition phase and a telogen or resting phase, in which hair is shed (Wilson 2017). Cortisol diffuses into the growing hair shaft during the anagen phase from surrounding blood vessels and can be deposited in all layers of the hair shaft (Russell et al. 2012; Greff et al. 2019). Kapoor and colleagues (2018) found that that radiolabeled cortisol could be identified in the hair of rhesus macaques 14 days after injection. Although the authors warn that, as in humans, the growth rate of hair in rhesus macaques varied considerably, mean growth rate was found to be 1.8 cm in 30 days (1 cm in 30 days is the widely reported average for humans; Kapoor et al. 2018). While rhesus macaques have been shown to be valid models

of cortisol metabolism in humans, further research is needed to verify the postulated two-week lag between systemic cortisol changes and alteration of HCC in humans.

Standard procedures of hair preparation dictate that hair be collected from the posterior vertex of the scalp (located halfway between bregma and lambda along the sagittal plane) to minimize variability between samples (Sauvé et al. 2007). Samples should be cut close to the scalp in sections about 5mm in diameter and should weigh between 10−30mg per cm. The root should be avoided as growth rate cannot be accounted for and adherent soft tissue or blood may contaminate cortisol values. After collection, samples should be kept away from UV radiation at room temperature in paper bags to avoid contamination and degradation (Wester et al. 2016; Wilson 2017). If being analyzed sequentially, hair samples should be taped to a piece of paper with the scalp end clearly noted. Samples can be stored in this way for extended periods of time (Russell et al. 2012). Hair is then cut using scissors with one centimeter corresponding to approximately one month of growth (Sauvé et al. 2007). After samples are washed in isopropyl alcohol to remove surface contaminants, each segment is minced using surgical scissors or a ball miller (Russell et al. 2015).

4.2.1. Hair collection

The procedure for hair collection varied between samples in the current study. The material from the UCF Cadaver sample was collected as part of a larger research program and was cut from the posterior vertex of the scalp, close to the scalp by Dr. Lana Williams (Anthropology, UCF) or trained technicians in the College of Medicine Anatomical Facility. After collection, the hair was wrapped with a small strip of parafilm

at the scalp end to maintain the direction of each hair relative to the scalp. The hair samples were then labeled by individual, placed in plastic bags, and shipped to McMaster University in two batches.

The hair samples in the Terry Collection were cut from the head at the time of autopsy between 1923 and 1960. Documentation indicates that all hair samples were taken from the scalp but does not indicate which region, how far from the scalp they were cut, or who collected them. However, some hair roots are present, some remnants of scalp tissue or cranial fragments are present, damaged ends consistent with natural hair ends are present, and the length of hair differed between individuals. These factors indicate that hair was not collected in standard lengths and that it was likely cut close to the scalp. The hair was then placed in small envelopes and labeled with the number corresponding with an individual body. It is clear that the hair samples were not washed at this time as many retain oils and particulate matter. Hair sample selection took place at the Smithsonian Institute's Museum Support Center in Suitland, Maryland. The goal weight for each hair sample was established by multiplying the total length of the hair sample by 10mg.

The hair samples from the Kellis 2 Cemetery were collected by members of the Dahkleh Oasis Project (Lana Williams Personal Communication: 7 March 2019). Sampling was limited by what part of the scalp was preserved. Additionally, due to shrinkage and desiccation of the skin as well as skin slippage in the postmortem period, the scalp pieces may not be in their original location and hair samples were likely collected from various scalp regions. Samples were transported and stored in Ampac 500 SealPAK specimen bags for long-term storage in humidity- and temperature-controlled

environments. Samples were chosen by Dr. Williams and mailed to McMaster University. Information about the individuals from which the samples came were not made available to the author until after preliminary analyses.

4.2.2. Hair sample preparation

Samples were prepared in the MJ Rieder hair cortisol laboratory in the Schulich School of Dentistry at the University of Western Ontario in London, Ontario. Detailed protocols for hair sample preparation are provided in Appendix B.1. Hair samples were sectioned into one-centimeter segments to reveal roughly one-month averages of HCC. Alterations and challenges specific to each sample are presented in this section.

The UCF Cadaver sample materials was shipped from the University of Central Florida to the MJ Rieder hair cortisol laboratory. Removal from plastic bags posed challenges from static causing hairs to stick to the inside of the bag. The use of parafilm also posed a significant challenge and resulted in the loss of material as hairs broke when removed from the parafilm and had to be discarded.

In the Terry Collection sample material, many of the samples were collected in braids or in curls with a clearly defined cut end that indicated the scalp end. However, some samples included only loose hairs or some loose strands that could not be articulated with the lock or braid. In this case, each hair or group of hairs were taped to a piece of white paper and any hairs that were much shorter than the others were removed because their relationship to complete hair strands could not be determined. A Keyence VHX 2000 microscope was employed to examine each hair or group of hairs under

magnification ranging from 400X to 1600X (Figure 4.1). The keratin scales comprising the outer layer of the hair shaft are deposited sequentially as the hair grows (Wilson and Tobin 2010). Therefore, the scales closer to the scalp overlap scales further from the scalp and can be used to determine which direction a hair grew.

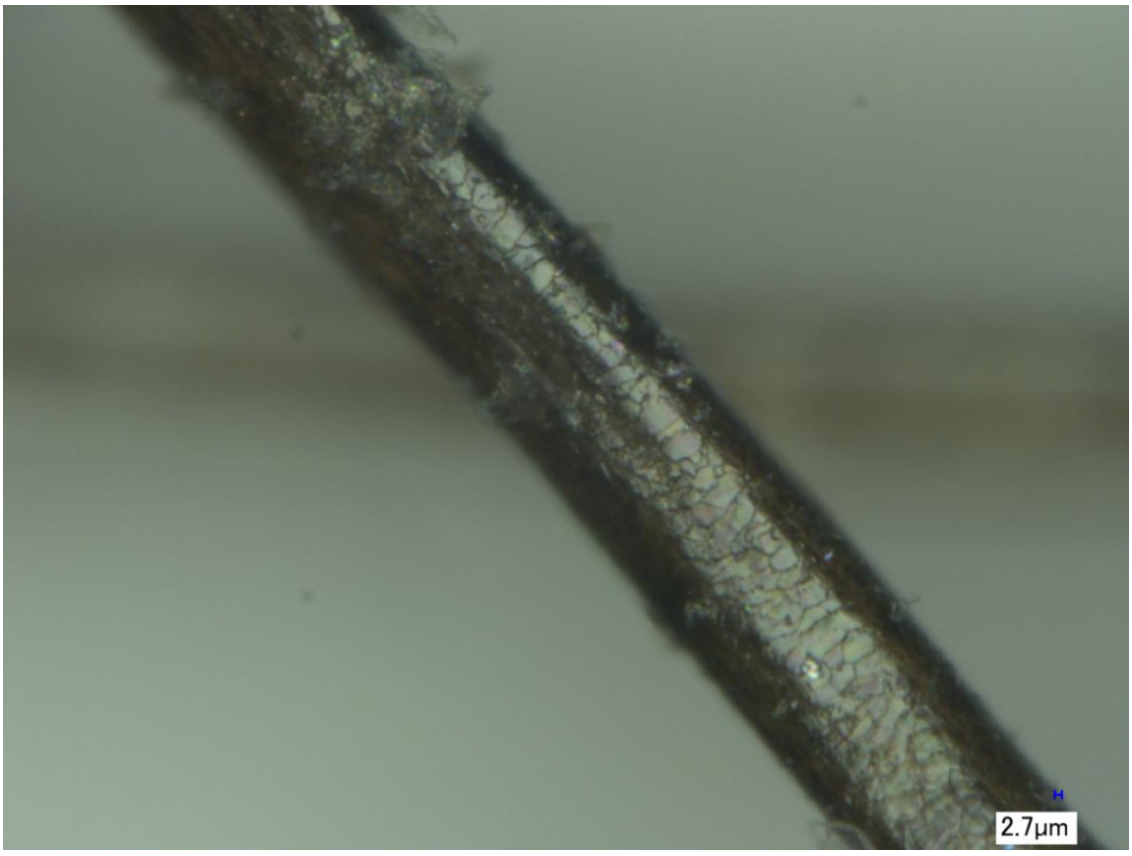

Figure 4.1. Image of hair shaft from Terry Collection sample Ind 740R taken with a Keyence VHX 2000 microscope showing closeup of keratin scales. Note the overlapping nature of the scales. Scalp end is to the left.

The Kellis 2 Cemetery Sample hair samples were shipped from UCF to the MJ Rieder hair cortisol laboratory in plastic bags. Static caused the hair to stick to the inside of the bag. Because the individuals had been buried, they retained adhered soil and soft tissue. These substances were removed by soaking the hair in petri dishes of distilled water. The gentle nature of washing ensured that desiccated hair follicles remained

adhered and that the hairs retained their consistent orientation. When the scalp end was not labeled with parafilm, it was identified on the basis of preserved roots, scalp tissue, and keratin scale orientation. While parafilm facilitated identification of the scalp end, this complicated removal of the hair samples and resulted in some breakage of hairs and loss of material.

4.2.3. Cortisol extraction and quantification

After the samples were prepared, the cortisol extraction followed the standards in the literature and described in detail in Appendix B.2 (e.g., Van Uum et al. 2008). Although research has indicated that a single methanol extraction method may only yield 40–60% of HCC (Slominski et al. 2015), this methodology was applied here to ensure consistency with previous studies and across the samples investigated in the current study.

A competitive solid-phase enzyme-linked immunosorbent assay (ELISA) was used to detect and quantify cortisol (see Appendix B.3 for detailed protocol). Other similar methods that have been employed for this purpose include luminescence immunoassay, radio immunoassay, or liquid chromatography-mass spectroscopy (LC-MS/MS; Greff et al. 2019). While some studies have found that ELISA kits show a strong correlation with LC-MS/MS other studies have found a low correlation, with measurements from ELISA tending to be two to three times higher than the same sample measured via LC-MS/MS (Russell et al. 2015; Slominski et al. 2015; Stalder et al. 2017). Although LC-MS is likely more sensitive and specific, it may underreport true cortisol

values (Russell et al. 2015; Slominski et al. 2015). ELISA was chosen for this study because of its low cost, reliability, and comparability to significant previous research.

The commercial ELISA kit ALPCO Diagnostics Salivary Cortisol ELISA kit (11-CORHU-E01-SLV) was employed in this study, as one for hair does not exist (Appendix B.4). The sensitivity of this kit was 1.0 ng/mL which is within the reported analytical sensitivities of most kits (0.09 and 1.0 ng/ml; Greff et al. 2019). This assay can have cross-reactivity with other steroid hormones such as cortisone and progesterone which may artificially increase HCC results, but studies have found this effect to be minimal at less than one percent (Slominski et al. 2015). Cross-reactivities of steroids in this kit included: cortisol 100%, prednisolone 13.6%, corticosterone 7.6%, deoxycorticosterone 7.2%, progesterone 7.2%, cortisone 6.2%, deoxycortisol 5.2%, prednisone 5.6%, and dexamethasone 1.6%. The lower limit of detection of cortisol for the kit is reported by the manufacturer as 1.0 ng/ml but this is heavily dependent on the quantity of hair. Inter-assay precision ranges from a coefficient of variation of 6.5% to 10.3%.

4.3. Quantitative Analysis

All statistical tests for this study were carried out in IBM SPSS Statistics 25. A lack of normality in HCC distribution and age distributions (Kolmogorov Smirnov test of normality) necessitated the use of non-parametric statistical tests (Huizingh 2007). As per standard conventions and because of the small sample sizes, statistical significance was set at $p \leq 0.05$ (Huizingh 2007). Outliers are defined as: 1.5 (interquartile range) + quartile three. Quartiles divide the data into four components of equal size. Outliers are removed for statistical comparisons between samples except where noted.

Mean HCC was determined across the complete hair shaft of an individual including the most proximal two centimeters (total average) and only the two centimeters closest to the scalp (2-month average) corresponding to the last two months of life in an individual. Employing 2-month average HCC ensures consistency across individuals with different hair lengths while also accounting for error introduced in hair growth rates by averaging more than one month of hair growth. The first month and all other months of HCC were compared, as were total average and 2-month average using a related-samples Wilcoxon signed-rank test. If total average HCC and 2-month average HCC were significantly different, comparisons were carried out with both variables.

Independent-samples Kruskal-Wallis and Independent-samples Mann-Whitney U tests were used to determine if average HCC differed across categories of sex, age, and 'race' within a sample depending on number of categories compared. Terry Collection sample 160 was removed from any analyses regarding demographic characteristics, cause of death, or duration of disease due to conflicting antemortem records. Terry Collection sample 1611 was removed from analyses regarding cause of death due to diagnosis of Cushing's disease prior to death, which is known to affect HCC (Section 2.4.2).

The standard deviation of monthly HCC values across the hair shaft was calculated for each individual from the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples; this value reflects how much stress experience varies over the course of the last months of life (Appendix A; Figure 4.2). The averages of the standard deviations for the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples were then determined, which represents the average amount of variation in the last month of

individual lives within each sample. The standard deviation of total average HCC in each sample was also calculated, which represents the variation in average HCC between individuals in each sample.

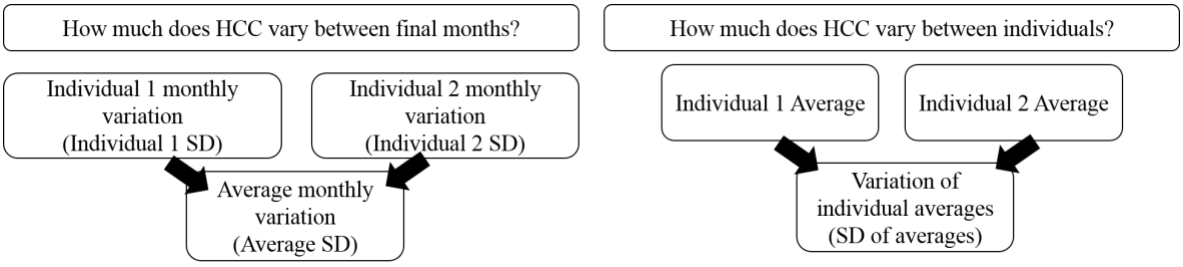

Figure 4.2. Flowchart depicting calculation of average standard deviation and standard deviation of average HCC. HCC = hair cortisol concentration; SD = standard deviation.

The mean HCC for each sample was compared to each other using an Independent-samples Kruskal-Wallis test (Huizingh 2007; Drennan 2009). To compare average HCC between individuals who died of different causes or of diseases of differing durations, an Independent-samples Mann-Whitney U test was used for two groups and an Independent-samples Kruskal-Wallis test was employed when three or more groups were of interest (Huizingh 2007 Drennan 2009). A Bonferonni correction was applied to the results of multiple comparisons. Given recent criticisms of applying a Bonferroni correction or any correction procedure on multiple comparisons, especially when the number of categories of comparison are high (Nakagawa 2004), results from before and after correction are presented. ‘External causes of morbidity or mortality’ and ‘Injury, poisoning or certain other consequences of external causes’ were combined to increase sample size and because they reflect the same general etiology. ‘Diseases of the genitourinary system’ was combined into ‘Symptoms, signs or clinical findings, not

elsewhere classified' as only one example was present. Given the similarity in causes of death represented and mean values and in order to increase sample sizes, the UCF Cadaver and Terry Collection sample were combined for analyses of cause of death categories.

If the percentage of individuals dying of particular category of cause of death in the Terry Collection and UCF Cadaver samples are plotted against average HCC a linear relationship can be calculated and the percentage of individuals dying of that cause could be predicted for the Kellis 2 Cemetery sample by extending that line. Insufficient information is available to test the validity of these predictions, therefore they only provide a means by which to determine if the values present in the Kellis 2 Cemetery sample could arise naturally, without taphonomic alteration. Only adults from the Kellis 2 Cemetery samples were investigated for these predictions to ensure consistency with the Terry Collection and UCF Cadaver samples. The relationship between various factors and HCC was also compared by calculating quartiles of average HCC in each sample and comparing age distribution and distribution of causes of death within and between these quartiles. A Kolmogorov-Smirnov test was used to compare age distributions, and an Independent-samples Kruskal-Wallis test was employed to explore differences in cause of death across quartiles.

In addition to average HCC, this analysis also examined the pattern of change in HCC in the last months by examining the curve of HCC values for each individual (where time in months=X; HCC= Y). Curves were compared between individuals and to historical records. An average curve for each cause of death was also calculated by

averaging Y values for each month. In this way the average curve, or the pattern of HCC change in the last months of life could be compared between causes of death using a Kolmogorov-Smirnov test (Huizingh 2007; DeWitte 2014). Month of death will always be displayed on the left-hand side of the graph, in accordance with standards for presenting stable isotope values from hair in bioarchaeology (e.g., D'Ortenzio et al. 2015).

ANOVA analysis was used to compare samples from the current study to previously published archaeological studies as only summary statistics were available for the Schaefer (2017) and López-Barrales and colleagues (2015) samples. Non-parametric Independent-samples Kruskal-Wallis tests were used to compare to previous archaeological studies for which raw data was available. Correlation between sample characteristics (e.g., sample size) and HCC values were investigated by calculating the R squared of the line of best fit.

4.4. Addressing limitations and obstacles

The materials and methods employed in this investigation are limited in a number of ways. Many of the limitations were unavoidable but carefully considered in the design of the study. Others arose during the course of the investigation and had to be accounted for with modifications to the analytical process or interpretation of results.

4.4.1. Well known HCC challenges

A number of factors impacting HCC have been identified in the literature. These include waning, UV radiation, contamination, hair treatment, and interlaboratory

variability (Section 2.3). Each of these has been carefully considered in the development of the research, sample preparation, and the interpretation of HCC results undertaken here.

In this study multiple hair segments were compared to the hair segment closest to the scalp in each individual to determine if a waning effect had occurred and many of the analyses took place on the proximal two centimeters of hair to limit the possibility of waning and to ensure consistency. The impact of UV radiation on degradation of HCC has been a subject of significant debate. The samples investigated in this study were all stored away from direct sunlight, in opaque and/or closed envelopes or containers. Furthermore, in many cases the hair most proximal to the scalp was the focus of analysis to limit the impact of UV degradation during life. To account for contamination of cortisol on the exterior hair shaft from sweat, sebum, or decomposition fluids, all of the hair samples were washed in isopropyl alcohol prior to extraction (after Greff et al. 2019)

Harsh chemical treatments were not available for the historical and archaeological samples in this study. Additionally, when the color of methanol was inspected, as suggested by Hoffman and colleagues (2014), no color changes were identified in any hair samples indicating that harsh chemical hair treatments were not applied to any of the hair samples. To account for variability between laboratories and methods of quantification (Russell et al. 2015; Greff et al. 2019), the primary analyses in this study relied on data generated in the same laboratory and with the same methods. However, in those cases where comparisons to previously published work were made, they were general and broad and only investigated studies that also used ELISA.

4.4.2. Sample size

Because HCC can vary considerably between individuals, studies of small numbers of individuals can limit the generalizability of findings. The current study was able to examine larger samples than any previous studies which ensures that sampling techniques could be sequential and consistent (1 cm segments) unlike previous studies and draw more conclusions about variation within and between samples. The sample sizes in this study were limited by the availability, cost, time, and labor involved in the preparation and extraction processes, and the destructive nature of analysis. Because an aim of this study was to explore variation in experience in the months leading up to death, sequential analysis of the hair shaft was required, further limiting the total number of individuals that could be investigated. In each case only those individuals with hair could be sampled and in the Terry Collection only those with cause of death data could be selected. Given these constraints, the largest number of individuals possible were chosen. Although the samples are much larger than any previous studies of archaeological individuals, they are much smaller than those employed in studies of modern populations which can involve several hundred subjects. The samples are large enough to permit basic statistical analysis across a number of categories. However, when classifying individuals within each sample into smaller groups for analysis, the number of categories and the number of individuals in each category had to be carefully considered and broad categories were chosen for analysis whenever possible.

4.4.3. Data collection

Given the expansive nature of the study I was not able to travel for sample collection to each location and the skeletons from the Kellis 2 Cemetery sample are located in Egypt, which the government of Canada has warned against travel to since 2017 (Government of Canada 2017). I was also not able to collect hair samples from the scalp of the deceased or collect data on age, sex, skeletal pathology, or cause of death first-hand as it was more efficient to analyze previously collected samples and I do not have the expertise to determine cause of death from observed health conditions or medical records. It was necessary to rely on the collection of materials and data by other researchers, some of which are no longer alive. However, I have carefully considered the procedures of collecting this data, qualifications of those who generated the data, and the context of the data. Therefore, the data I have compiled is reliable and I have accounted for any inconsistencies.

4.4.4. Scalp end identification

A significant obstacle that arose during analysis was the need to identify the scalp end of hair samples. In the Terry Collection material, it was not possible to determine how close to the scalp the hair was cut. For the reasons described above (Section 4.2.2) it is likely that the hair was cut at the scalp, however, this data must be cautiously interpreted. Furthermore, some of the Kellis 2 cemetery and Terry Collection samples did not have a clear scalp end, and scalp end was identified according to the methods described earlier (Section 4.2.2). This additional step introduced the possibility that some hairs may have been improperly identified. However, in these instances, as long as the

majority of hairs were identified correctly, the influence of the misidentified hairs on the final monthly averages would be minimal. Given the nature of the analysis and the general and broad nature of comparisons between months, individuals, and groups, the impact would have been small.

4.4.5. Hair growth rate

The purpose of sectioning each hair sample into one-centimeter segments was to reveal roughly one-month averages of HCC. In this study it was not possible to control what region of the scalp hair samples came from for the Terry Collection sample and the Kellis 2 Cemetery sample. Furthermore, growth rates of hair are known to differ considerably between individuals and groups, especially those differing in geographic region of ancestry (Loussouarn 2001; Kapoor et al. 2018). However, Carlitz and colleagues (2015) argue that hair from different regions can be mixed and is still useful for analysis, but noise will be introduced into the data. Despite the potential variation in growth rates, no significant difference exists in average HCC values between the UCF Cadaver sample that were collected from one region and the Terry Collection sample which were collected from various scalp regions (Independent-samples Mann-Whitney U test; $p > .05$) and the range and standard deviations in these samples are similar. Therefore, variation due to growth rate may not be significant for a study that is interested in broad comparisons that does not focus on single month HCC.

Furthermore, while Kapoor and colleagues (2018) identified a two-week lag in cortisol deposition in hair of rhesus macaques, how applicable this finding is in humans is still unknown. To date, no studies of HCC have examined the effects of dying or serious

disease on hair growth rate. Some researchers suggest that stress and cortisol may influence hair growth cycles but did not examine hair growth rate (Thom 2016).

Although not discussed in the clinical HCC literature, bioarchaeological research has identified the existence of a growth cycle error in analysis of stable isotope ratios from hair (Williams et al. 2011). A growth cycle error is defined as a lag in hair composition relative to blood because 10−15% of hair is not actively growing but may be retained in the scalp for up to four months. Thus, some hair may reflect experience of four months or more prior to analysis alongside hair reflecting current experience (Williams et al. 2011). D'Ortenzio and colleagues (2015) and Williams and colleagues (2011) both found that the proportion of hairs in each phase may differ with physiological stress as well. Williams and colleagues (2011) found no statistical differences in stable isotope ratio values between anagen only and mixed phase growth samples but, similar to D'Ortenzio and colleagues (2015) identified a growth cycle error of one to three months between them. In this study, given the large number of hair samples processed and the lack of need precise temporal control, hairs were not separated by growth phase.

Growth cycle error and variability of growth rates across the scalp will introduce noise into monthly averages and make it difficult to associate specific hair segments with particular months and events. However, exact temporal distinctions were not necessary for this study. In the case of comparing when someone became ill or entered the hospital with monthly HCC, this variability was easily accounted for by considering the HCC of the one-centimeter section believed to correspond with the month of the event, as well as the centimeters around it. Additionally, comparative analyses were not made using single

one cm segments of hair, but instead explored average HCC over two or more centimeters.

4.4.6. Contextual information

Information regarding cortisol-affecting medications prescribed to each individual was not available. This obstacle was partially accounted for by removing outliers which would be the most likely subjects of artificial alteration. Furthermore, the effects of medication can be inferred by comparing the Kellis 2 Cemetery and Terry Collection samples to the UCF Cadaver sample as they would not have had access to the same medications. Information about the contexts in which individuals died and their symptoms was also not available for any of the individuals in this study, which studies of modern people have shown can impact an individual's experience of death (e.g., Kübler-Ross 1969; Palgi and Abramovitch 1984; Steinhouser et al. 2000). However, this study was focused on exploring the effects of a limited number of variables on HCC, in the hope that future studies may apply the findings to delve into other specific features that may influence the stress associated with death.

CHAPTER 5 - RESULTS

5.0. Introduction

In this chapter I present the results of HCC analysis within and between the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples. The main units of measurement are monthly HCC, average HCC across the complete hair shaft of an individual (total average), and average HCC in the last two months of life in an individual (2-month average). Specifically, I demonstrate how average HCC and patterns of HCC differ between individuals dying of differing causes and dying in differing time periods. I also demonstrate changes in HCC values in the last months of individual lives in response to disease contraction and hospital entry and describe patterns of HCC values in individuals with different preexisting health conditions. Outliers are removed for comparisons between samples, except where noted and are defined as: 1.5 (interquartile range) + quartile three (Section 4.3).

5.1. General characteristics

Summary statistics for monthly HCC values in each sample are presented in Table 5.1. Raw data per individual is presented in Appendix D.0. The range of values for the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples is large and overlaps substantially in the Terry Collection and Kellis 2 Cemetery samples when outliers are not removed (Figure 5.1). Total average HCC values in the Kellis 2 Cemetery and the UCF Cadaver samples are not normally distributed ($p < 0.05$; Kolmogorov-Smirnov test of normality) but are normally distributed in the Terry Collection sample ($p = 0.57$; Kolmogorov-Smirnov test of normality). Figures 5.2 through 5.4 display the distribution

of total average HCC in each sample with outliers removed (Section 4.3). Two-month average HCC is not normally distributed in any samples ($p < 0.05$; Kolmogorov-Smirnov test of normality). Summary statistics for total average and 2-month average HCC are presented in Tables 5.2 and 5.3. Comparisons between samples will be presented in Section 5.2.

Table 5.1. Summary statistics for monthly hair cortisol concentration (HCC) values in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples.

Sample	N	Mean (ng/g)	Median (ng/g)	SD (ng/g)	Minimum (ng/g)	Maximum (ng/g)
Kellis 2 Cemetery	531	116.80	49.95	188.73	1.13	2942.29
Terry Collection	269	488.51	325.30	508.55	10.35	3011.41
UCF Cadaver	207	1058.02	569.73	1369.8	2.66	10785.10

Abbreviations: N = number of 1 cm hair samples; SD = Standard deviation; outliers not removed.

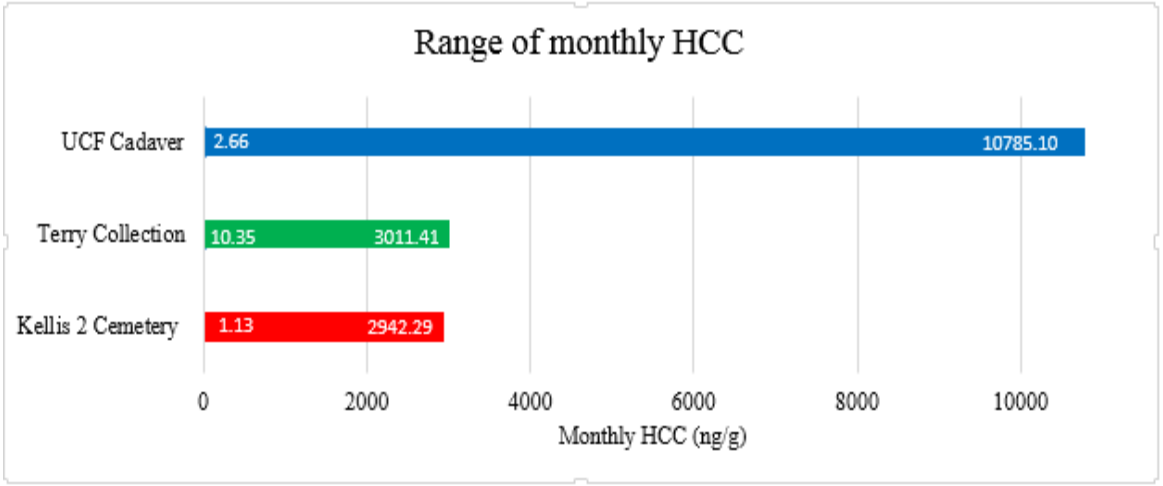

Figure 5.1. Bar graph comparing range of monthly hair cortisol concentration (HCC) values between three study samples. Labels for minimum and maximum values are presented in white.

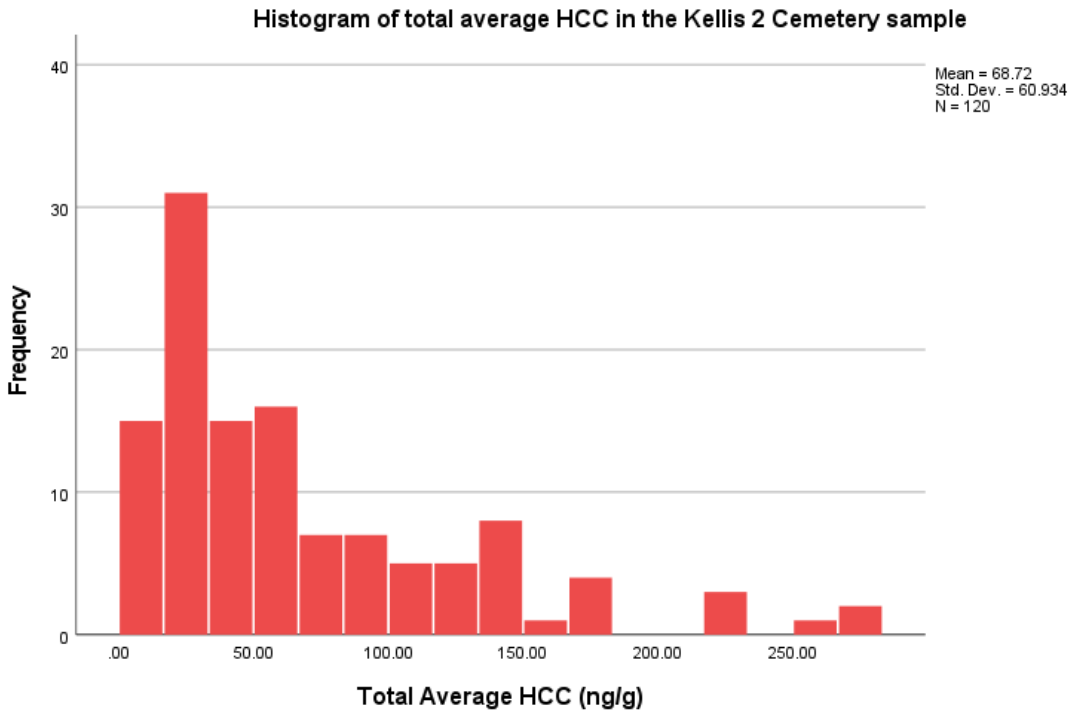

Figure 5.2. Distribution of total average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery sample (N=120); outliers removed (outliers > 268.63; N=10). X-axis differs from following graphs to show detail.

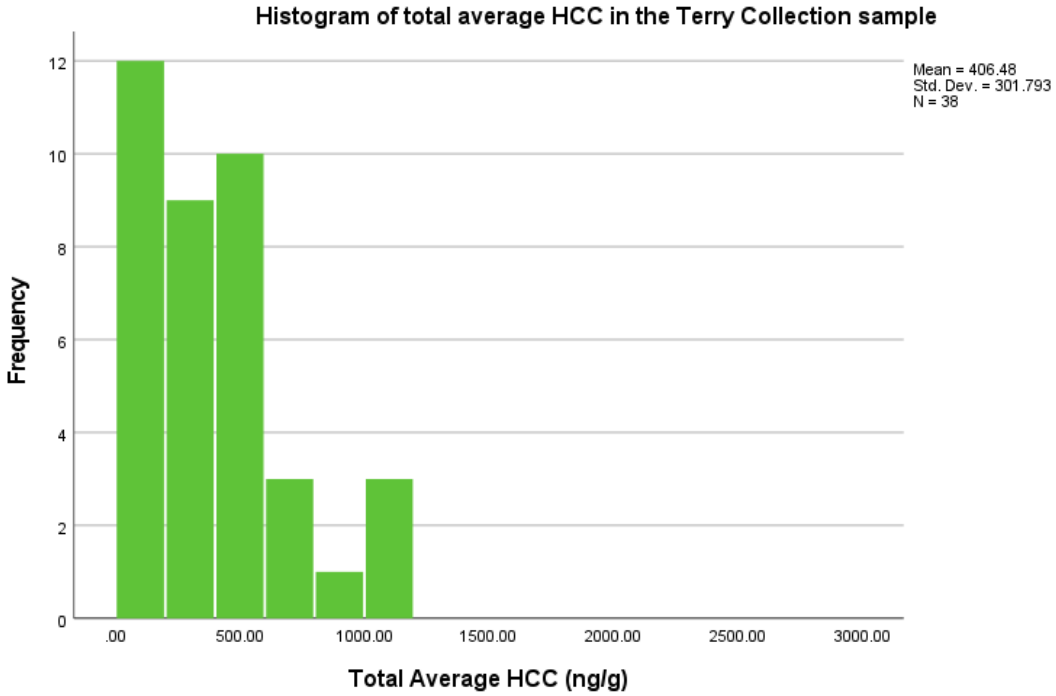

Figure 5.3. Distribution of total average hair cortisol concentration (HCC) values in the Terry Collection sample (N=38); outliers removed (outliers > 1197.99; N = 2).

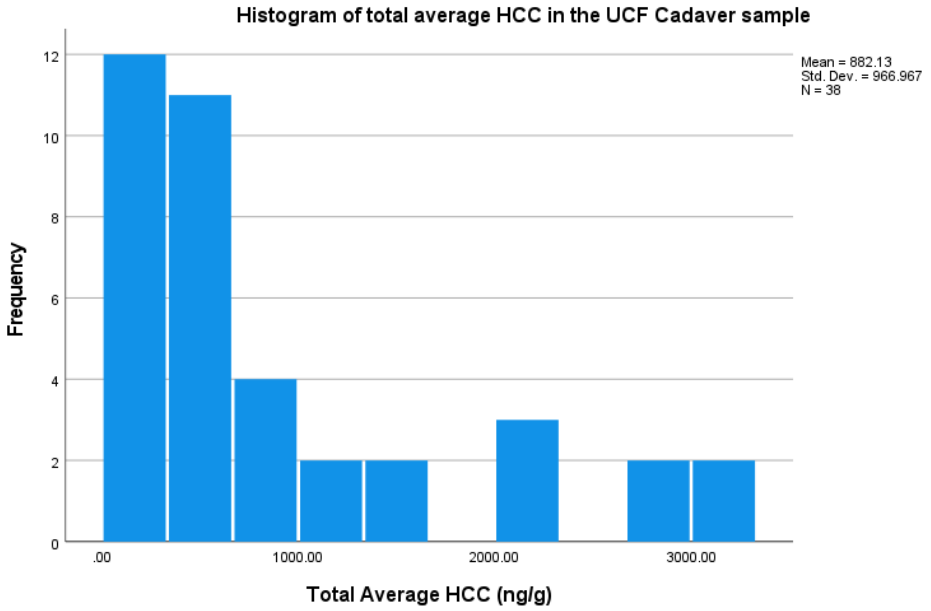

Figure 5.4. Distribution of total average hair cortisol concentration (HCC) values in the UCF Cadaver sample (N=38). Outliers removed (outliers > 3553.04; N = 2).

Table 5.2. Summary statistics for total average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples.

Sample	N	Mean (ng/g)	Median (ng/g)	SD (ng/g)	Minimum (ng/g)	Maximum (ng/g)
Kellis 2 Cemetery	120	68.72	45.90	60.93	1.16	269.51
Terry Collection	38	406.47	325.21	301.79	34.66	1190.49
UCF Cadaver	38	882.13	477.30	966.97	4.78	3310.49

Abbreviations: N = number of individuals; SD = Standard deviation
Notes: Outliers removed. Outliers defined as 1.5IQR +Q3: Kellis 2 Cemetery outliers > 268.63; N=10; Terry Collection outliers > 1197.99; N = 2; UCF Cadaver outliers > 3553.04; N = 2

Table 5.3. Summary statistics for 2-month average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples.

Sample	N	Mean (ng/g)	Median (ng/g)	SD (ng/g)	Minimum (ng/g)	Maximum (ng/g)
Kellis 2 Cemetery	122	69.59	41.45	64.42	1.13	270.24
Terry Collection	39	425.44	334.41	336.09	35.63	1327.45
UCF Cadaver	38	703.27	400.53	747.45	4.32	2676.27

Abbreviations: N = number of individuals; SD = Standard deviation
Notes: Outliers removed. Outliers defined as 1.5IQR +Q3: Kellis 2 Cemetery outliers > 280.52; N=8; Terry Collection outliers > 1328.60; N = 1; UCF Cadaver outliers > 3637.06; N = 2

The average standard deviation in each sample is less than the standard deviation of total average HCC for all individuals in each sample (Table 5.4), indicating that HCC varies more between individuals than between months for a single individual in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver sample.

Table 5.4. Standard deviation of hair cortisol concentration (HCC) values across individual hair shafts and standard deviation of average HCC values for all individuals in each sample.

Sample	Average SD (ng/g)	SD of individual average HCC
Kellis 2 Cemetery	33.79	79.91
Terry Collection	133.88	354.77
UCF Cadaver	276.98	966.97

Abbreviation: SD = standard deviation.

5.1.1. Age

Age is not normally distributed in the UCF Cadaver and Kellis 2 Cemetery samples, but age in the Terry Collection sample is normally distributed (Figures 3.3, 3.5, 3.8; Shapiro Wilk; $p < 0.05$). No correlation exists between total average HCC and age in the Kellis 2 Cemetery, Terry Collection, or UCF Cadaver sample (Table 5.5). Total average HCC between 10-year age categories did not differ significantly in the Terry Collection or UCF Cadaver sample but did in the Kellis 2 Cemetery (Table 5.5; Figure 5.5). Pairwise comparisons revealed no significant differences between age groups in the Kellis 2 Cemetery sample, but the highest total average HCC is in the 0-9 and 60-69 year groups while the lowest is in the 10-19 and 30-39 year groups. Juveniles (1.5-15 years) displayed significantly higher total average and two-month average HCC than adults (19-77 years) in the Kellis 2 Cemetery sample (Tables 5.6-5.7; Figures 5.6-5.7).

Table 5.5. Test results of correlation between age and hair cortisol concentration (HCC) values and comparison of average HCC year age categories.

Sample	Correlation Coefficient	Test	10-year	Test
Kellis 2 Cemetery	-0.096	Spearman's Rho	0.016*	Independent-samples Kruskal-Wallis
Terry Collection	0.174	Pearson Correlati	0.423	ANOVA
UCF Cadaver	-0.031	Spearman's Rho	0.447	Independent-samples Kruskal-Wallis

*indicates significance
Notes: Outliers removed

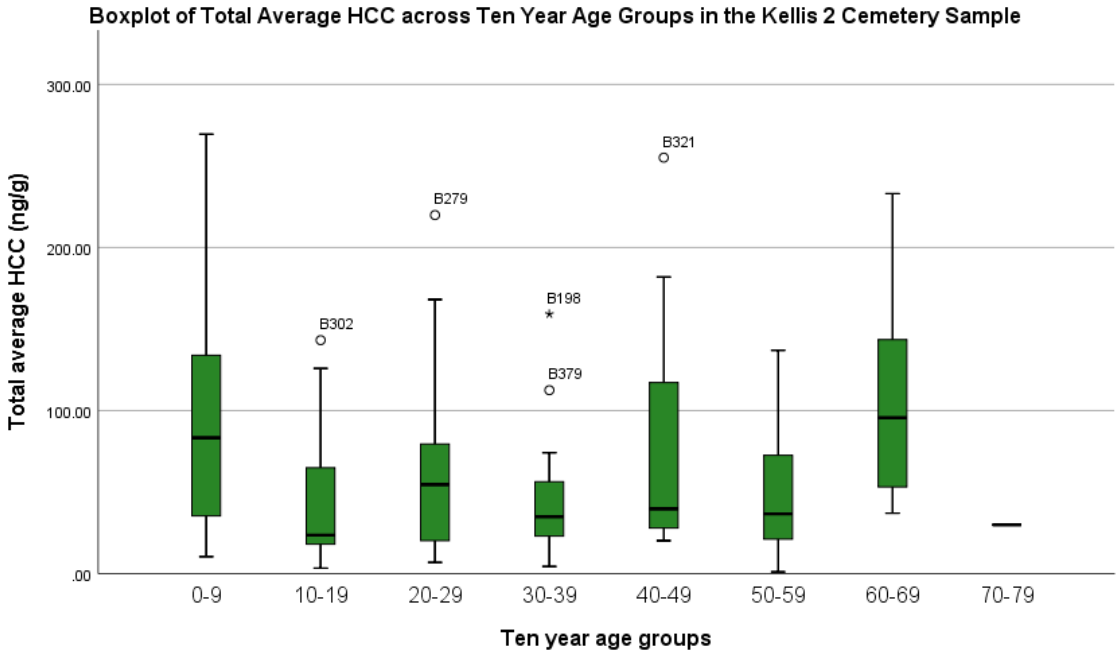

Figure 5.5. Boxplots of average hair cortisol concentration (HCC) in 10-year age groups in the Kellis 2 Cemetery sample. Circles indicate outlier values above 1.5IQR +Q3; asterisk indicates extreme outlier values outside above 3QR +Q3.

Table 5.6. Total and two-month average hair cortisol concentration (HCC) in adults and juveniles in the Kellis 2 Cemetery Sample and results of comparison using Independent-samples Mann-Whitney U tests.

	Juveniles			Adults			
	Mean (ng/g)	N	Age range	Mean (ng/g)	N	Age range	Adults vs juveniles (p-value)
Total average	85.74	38	1.5-15	58.43	81	19-77	0.037*
Two-month average	92.10	38		56.18	82		0.39

Notes: Outliers removed, * indicates significance at p <0.05; Outliers defined as 1.5IQR +Q3
Total average outliers: Juvenile outliers > 292.18, N=2; adult outliers > 249.85, N=9
Two-month average outliers: Juvenile outliers > 381.22, N=2; adult outliers > 224.94, N=8
Abbreviations: N = number of individuals

Table 5.7. Summary statistics for total average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery juvenile and adult samples.

Sample	N	Mean (ng/g)	SD (ng/g)	Minimum (ng/g)	Maximum (ng/g)
Juvenile	38	85.74	69.29	3.40	269.51
Adult	81	58.43	51.22	1.16	233..06

Notes: Outliers removed
Abbreviations: N = number of individuals; SD = Standard deviation

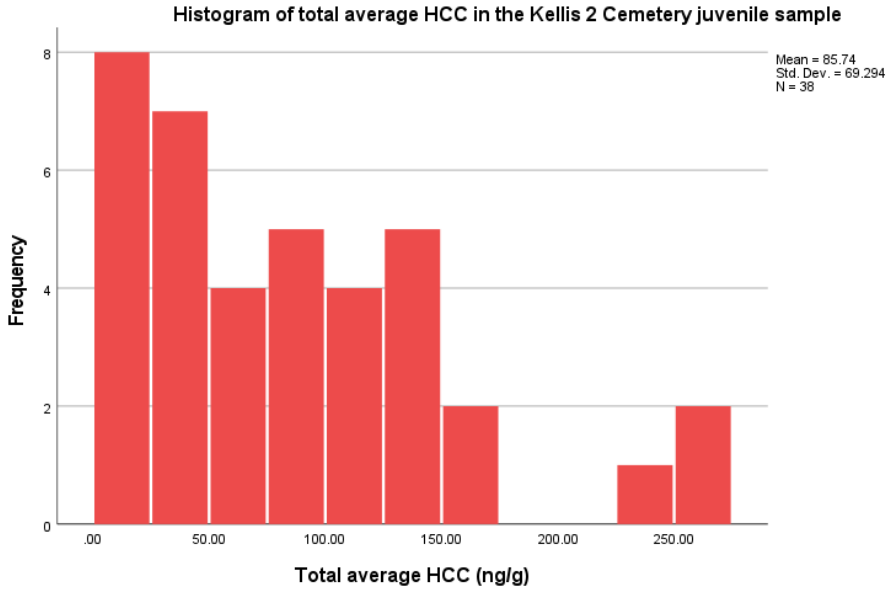

Figure 5.6. Distribution of total average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery juvenile sample. Outliers removed (outliers > 292.18; N=2).

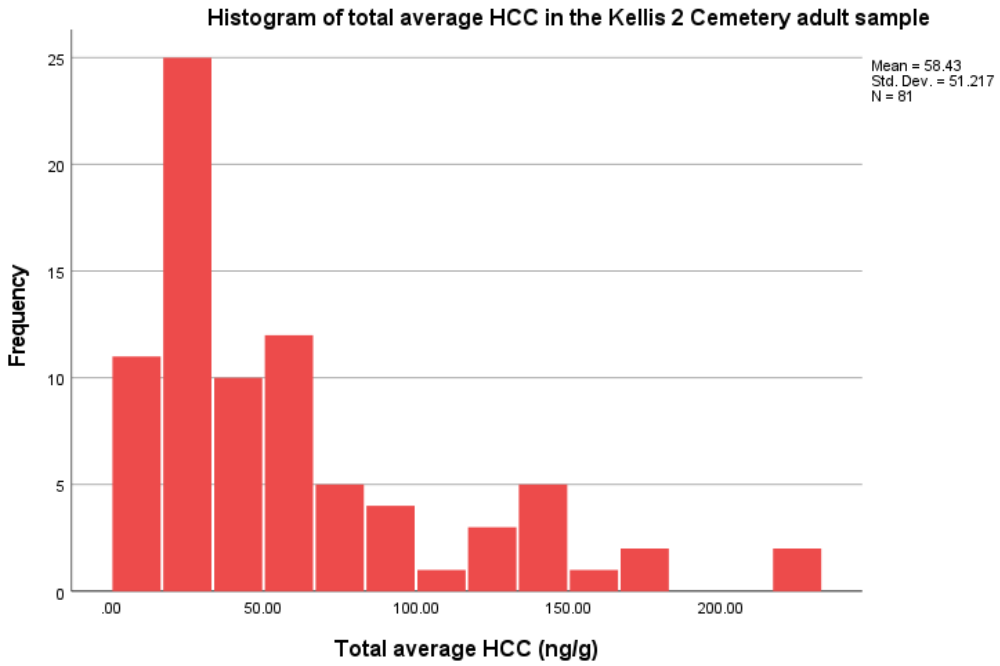

Figure 5.7. Distribution of total average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery adult sample. Outliers removed (outliers > 249.85, N=9).

In the combined Terry Collection and UCF Cadaver samples there was no difference in the age distribution between quartiles of total average HCC (Independent samples Kruskal-Wallis test; $p>.05$). The distribution of 10-year age cohorts did not differ significantly between all quartiles of total average HCC in the Kellis 2 Cemetery sample (Pearson chi square, $p = 0.109$); but did differ significantly between the 1st and 4th quartile (lowest and highest quarter of total average HCC) with more young individuals in the 4th quartile (Figure 5.8; Pearson chi square, $p = 0.038$). The distribution of 10-year age groups also differed significantly between the upper and lower 50th percentile of total average HCC (Pearson chi square, $p = 0.036$).

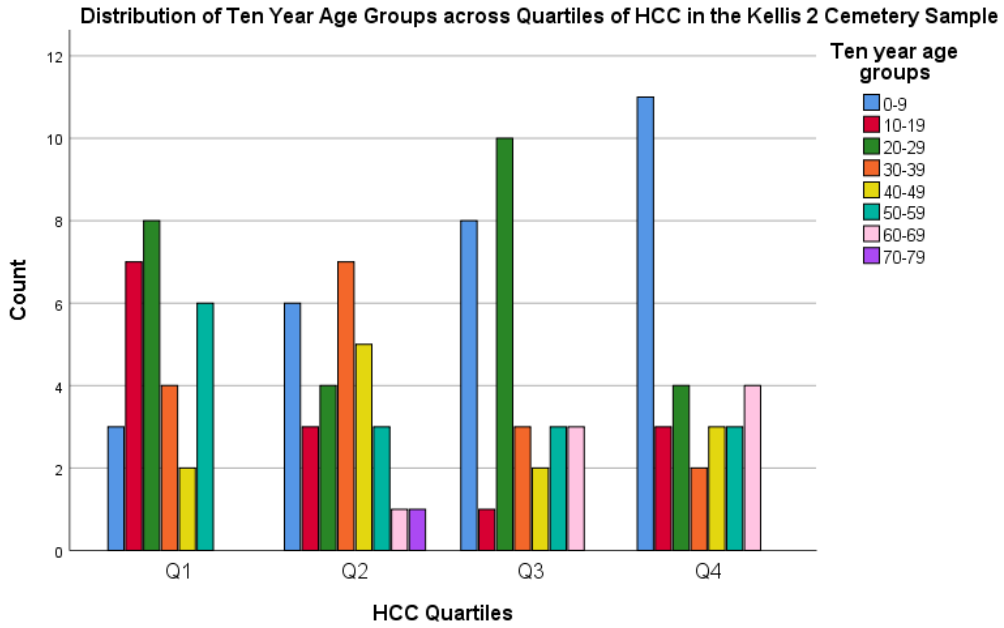

Figure 5.8. Bar graph displaying the number of individuals in each ten-year age cohort in the Kellis 2 Cemetery sample with total average hair cortisol concentration (HCC) that fall in each quartile. Q = Quartile. Quartile cutoffs = 25.20 ng/g, 45.90 ng/g, 95.23 ng/g.

5.1.2. Other demographic characteristics

No significant differences in average HCC between males and females were found in any of the samples in the current study (Table 5.8; Independent-samples Mann-Whitney U; p >0.05). When total average HCC is compared between ancestry groups in the Terry Collection (Section 3.1.2), no difference existed between 'Black' (n=18), 'White' (n-19), and 'Asian' (n=1) groups (Independent-samples Kruskal-Wallis; p >0.05) or 'White' and 'Black' groups (Independent-samples Mann-Whitney U; p >0.05).

Table 5.8. Distribution of males and females and results of comparison of total average hair cortisol concentration (HCC) using Independent-samples Mann-Whitney U tests.

Sample	Male (N)	Male mean HCC (ng/g)	Female (N)	Female mean HCC (ng/g)	Males versus females (p-value)
Kellis 2	29	66.64	53	57.65	0.35
Terry Collection	20	476.19	18	329.0	0.19
UCF Cadaver	21	692.43	17	1116.46	0.49

Notes: No signficant p-values present; outliers removed

5.1.3. Hair characteristics

In this study it was not possible to control the region of the scalp from which hair samples originated for the Terry Collection sample and the Kellis 2 Cemetery sample (Section 4.4.5). No significant difference exists between the UCF Cadaver sample values that were collected from one region of the scalp and the Terry Collection sample that were collected from various regions (Independent-samples Mann-Whitney U test; $p > 0.05$). Furthermore, the range and standard deviations in these samples are similar (Table 5.2, 5.3). This suggests that the variation introduced by sampling from various regions of the scalp may not be significant when relying on averages from several months.

When all samples are combined, average HCC of the most proximal segment of hair was significantly lower than segment 2, 3, 4, 5, and 9 but was not significantly different from any other segment (Appendix E.0; related-samples Wilcoxon signed-rank test; $p < 0.05$). In the Kellis 2 Cemetery sample segment 1 was significantly lower than segment 2, in the Terry Collection sample segment 1 was significantly greater than segment 9, and in the UCF Cadaver sample segment 1 was significantly lower than segments 3 through 5 (Appendix E.0). No systematic patterns of decline or increase were noted. There were also no significant differences between total average HCC and 2-month average HCC in the Kellis 2 Cemetery or Terry Collection sample, and a significant difference in the UCF Cadaver sample (Table 5.9).

Table 5.9. Comparison of total average hair cortisol concentration (HCC) to 2-month average HCC in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples using related-samples Wilcoxon signed-rank test.

Collection	Two-month average HCC vs overall average (p value)
Kellis 2 Cemetery	.298
Terry Collection	.898
UCF Cadaver	.003*

* indicates significance ($p < 0.05$)

5.2. Comparisons between the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples

No significant difference is present between the Terry Collection and UCF Cadaver sample when 2-month averages or total averages are considered (Independent-samples Mann-Whitney U test; $p = 0.528, 0.114$). Additionally, in those cause of death categories with greater than three samples for each sample (nervous system disorders, respiratory disorders, and neoplasms) no significant difference in total average HCC exist between the Terry Collection sample and UCF Cadaver sample in those categories (Independent-samples Mann-Whitney U test; $p > 0.05$).

Significant variation in total average HCC exists between the Kellis 2 Cemetery juvenile, Kellis 2 Cemetery adult, Terry Collection, and UCF Cadaver samples (Figure 5.9; Independent-samples Kruskal-Wallis test, $p = 0$). Pairwise comparisons revealed significant differences between the Kellis 2 Cemetery juvenile and adult samples and both the Terry Collection and UCF Cadaver samples (Bonferroni correction; $p = 0$).

The variation in 2-month averages between Kellis 2 Cemetery sample, the Terry Collection sample, and the UCF Cadaver is significant (Figure 5.10; Independent-samples Kruskal-Wallis; $p = 0$). The 2-month average HCC in the Terry Collection and UCF

Cadaver samples is significantly higher than in the Kellis 2 Cemetery sample. Pairwise comparisons reveal significant differences (Bonferroni correction; p = 0) between the Kellis 2 Cemetery sample and the others, and no difference between the Terry Collection and UCF Cadaver sample are present (Bonferroni correction; p >0.05).

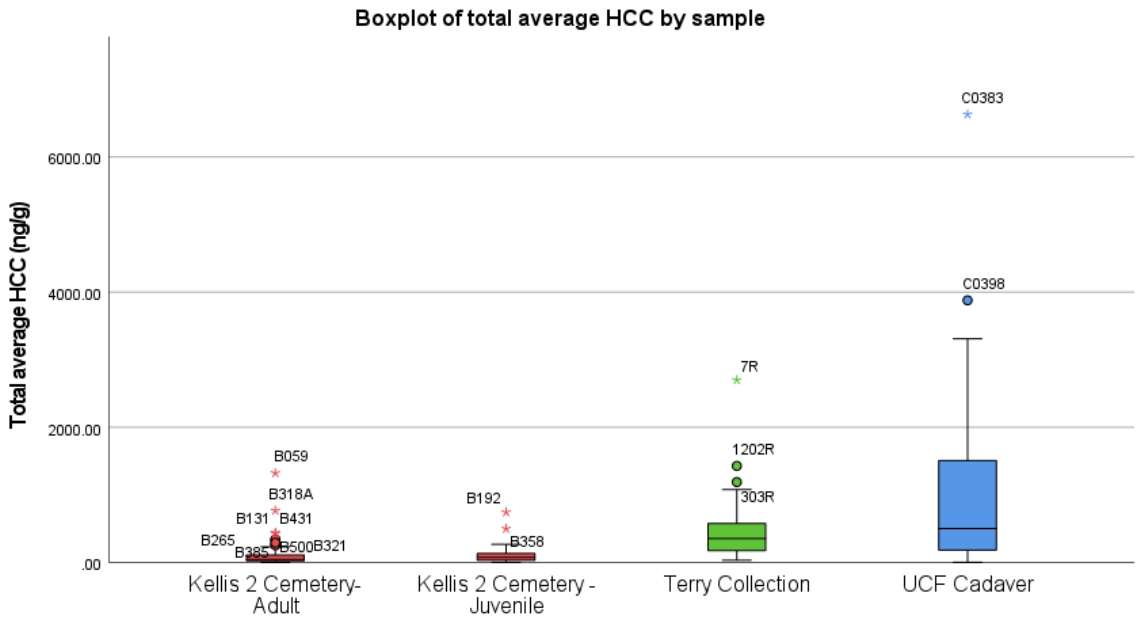

Figure 5.9. Box plot displaying distribution of average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery adult and juvenile, Terry Collection, and Cadaver samples. Circles indicate outlier values above 1.5IQR +Q3; asterisks indicate extreme outlier values outside above 3QR +Q3.

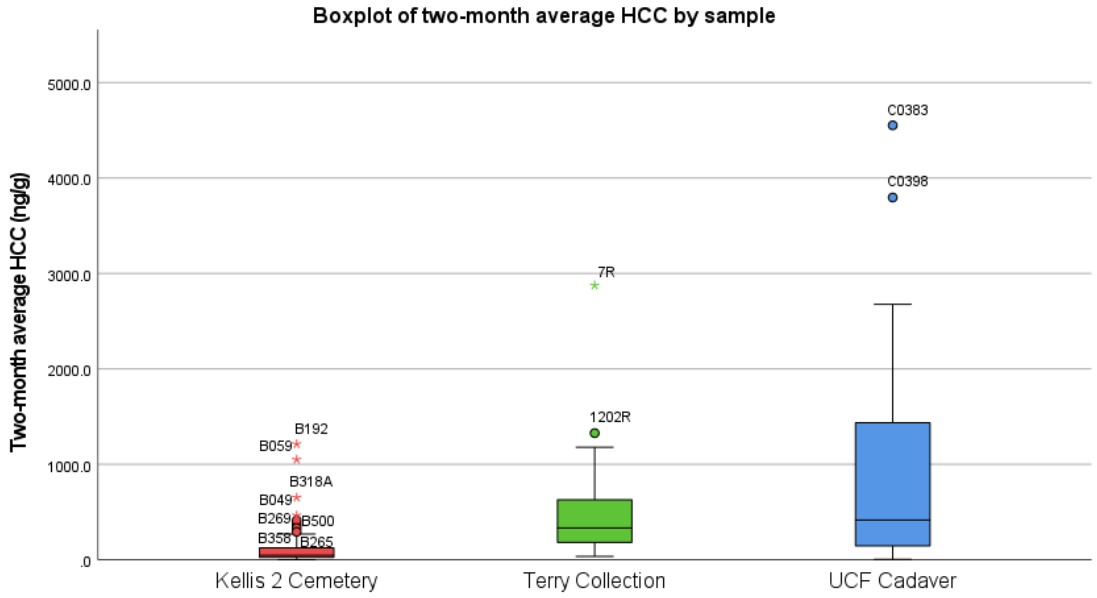

Figure 5.10. Box plot displaying distribution of 2-month average hair cortisol concentration (HCC) values in the Kellis 2 Cemetery, Terry Collection, and Cadaver samples. Circles indicate outlier values above 1.5IQR +Q3; asterisks indicate extreme outlier values outside above 3QR +Q3.

The distribution of total average HCC in the Kellis 2 Cemetery adult and juvenile samples are significantly different from each other and the Terry Collection and UCF Cadaver samples (Table 5.10; see Figures 5.6-5.7; 5.3-5.4). The distribution of HCC values in the Terry Collection and UCF Cadaver samples are not significantly different.

Table 5.10. Comparisons of distribution of total average HCC between samples using Kolmogorov-Smirnov tests.

	Kellis 2 Cemetery-Juvenile	Kellis 2 Cemetery-Adult	Terry Collection	UCF Cadaver
Kellis 2 Cemetery-Juvenile				
Kellis 2 Cemetery-Adult	.038*			
Terry Collection	<.001*	0.0*		
UCF Cadaver	<.001*	0.0*	.237	

* indicates significance p<0.05

5.3. Abrupt deaths

In those samples for which cause of death data was available (Terry Collection and UCF Cadaver samples), total average and 2-month average HCC was compared between those individuals who died of abrupt causes (e.g., accidents, homicides, and conditions lasting a day or less) and those who died of more long-standing conditions. Given the definition of dying established previously (Section 4.1.3), individuals dying by suicide were included in the not-abrupt category.

No significant differences in total average HCC exist between individuals who died abruptly and those who died of other conditions in the UCF Cadaver sample (Independent-samples Mann-Whitney U; $p > 0.05$) but do exist in the Terry Collection sample and combined sample (Table 5.11; Figure 5.11). No significant differences in 2-month average HCC exist between these groups in the UCF Cadaver or Terry Collection sample (Independent-samples Mann-Whitney U; $p > 0.05$) but do exist in the combined sample (Independent-samples Mann-Whitney U; $p = 0.019$). Differences in the distribution of age between abrupt and other conditions in the combined sample was not significant (Independent-samples Mann-Whitney U; $p > 0.05$).

Table 5.11. Comparison of total average hair cortisol concentration (HCC) between individuals dying abruptly and those dying of other conditions in the Terry Collection, UCF Cadaver, and combined samples.

Sample	Abrupt		All Other		p-value
	N	Mean (ng/g)	N	Mean (ng/g)	
Terry Collection	6	191.79	30	452.52	.046*
UCF Cadaver	2	346.64	36	911.88	.481
Combined	8	230.51	66	703.08	.049*

* indicates significance ($p < 0.05$).
Notes: All tests are Independent-samples Mann-Whitney U; outliers removed.

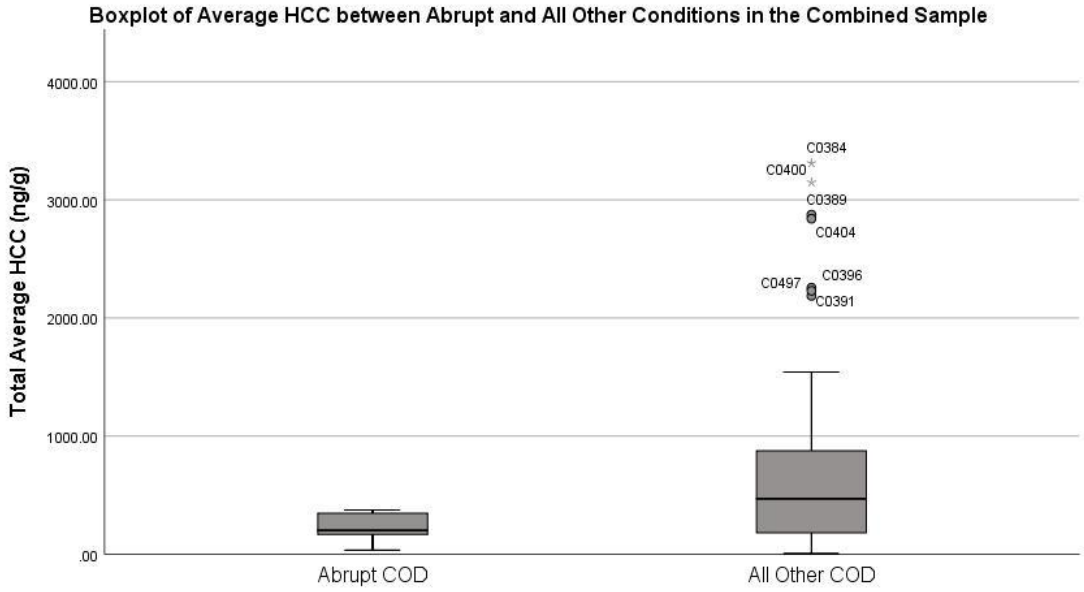

Figure 5.11. Boxplot displaying mean and varience of total average hair cortisol concentration (HCC) in individuals dying of abrupt and all other conditions in the combined Terry Collection and UCF Cadaver sample. Circles indicate outlier values above 1.5IQR +Q3; asterisks indicate extreme outlier values outside above 3QR +Q3.

The number of individuals dying of abrupt conditions was higher in the Terry Collection sample than the UCF Cadaver sample, and HCC values in the Terry Collection were lower. If the percentage of individuals dying abruptly in each sample is plotted against total average HCC in each sample, a regression equation can be written for the line connecting the two points. The line connecting the Terry Collection sample and UCF Cadaver sample could be expanded to include the Kellis 2 Cemetery sample adults (Figure 5.12; y = -36.514x + 1100.9). The resulting point estimate suggests that, assuming all other factors are equal between samples, the lower HCC values of the Kellis 2 Cemetery sample could be explained if 28% of the population died of abrupt causes. This estimation is limited by the effects of sample size and a lack of standard reference values for living healthy people. Therefore, the estimation should only be understood as a

general indicator that the Kellis 2 Cemetery values could be explained by a substantial increase in abrupt deaths from the other samples in the current study.

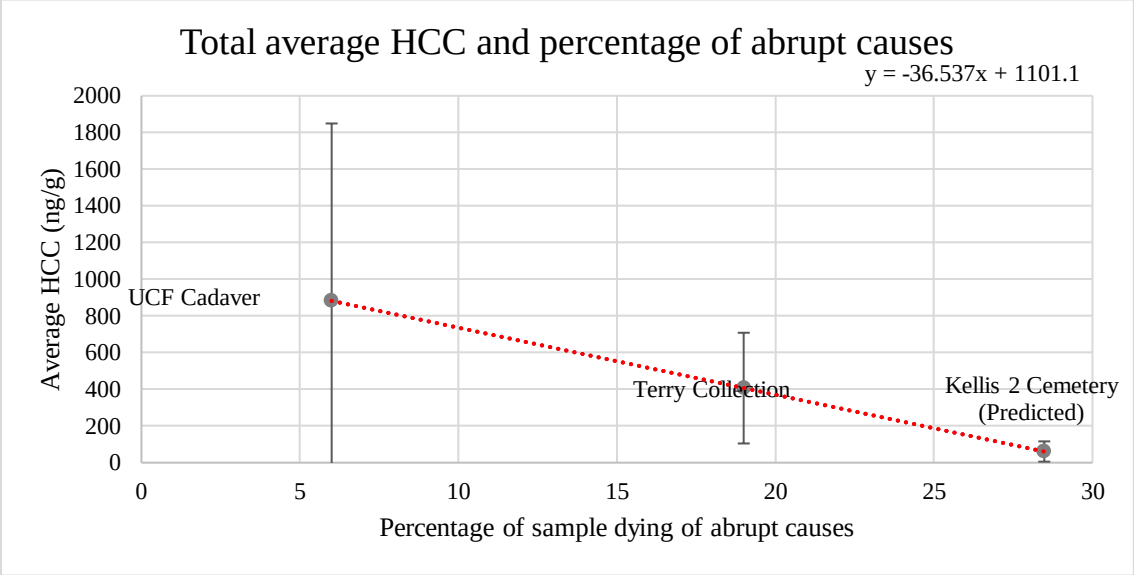

Figure 5.12. Scatterplot depicting relationship between percentage of individuals in a sample dying of abrupt causes and total average hair cortisol concentration (HCC) for that sample and the predicted percentage of individuals dying of abrupt causes in the Kellis 2 Cemetery sample.

In the Terry Collection sample, the number of people dying abruptly in each quartile of total average HCC was not significantly different (Pearson Chi Square; $p > 0.05$) and the number of people dying of abrupt conditions in the upper and lower 50th percentile was also not significant (Pearson Chi Square; $p > 0.05$). Due to the very small sample size of individuals dying abruptly (n=2) this pattern was not investigated in the UCF Cadaver sample. However, in the combined Terry Collection and UCF Cadaver sample, the difference in the number of people dying abruptly in each quartile was significant (Figure 5.13; Quartile cutoffs: 166.23ng/g, 407.79ng/g, 781.49 ng/g; Pearson Chi Square; $p = 0.01$).

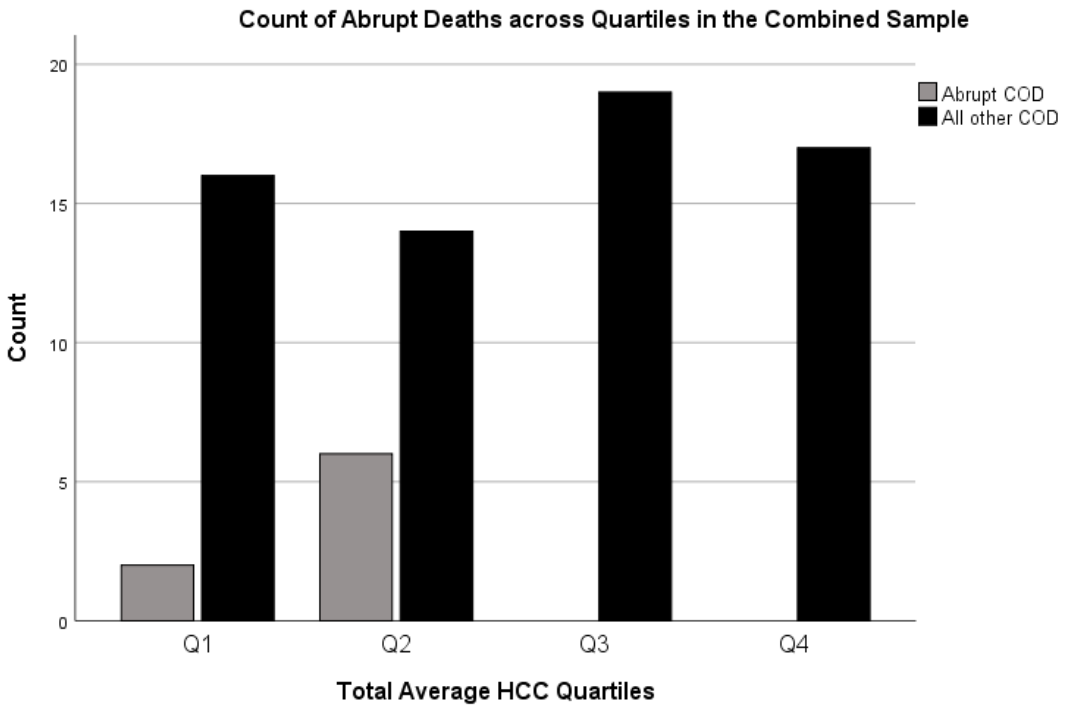

Figure 5.13. Bar graph depicting the number of individuals dying abruptly and of all other causes in each quartile of average HCC in the combined sample. Quartile cutoffs: 166.23ng/g, 407.79 ng/g, 781.49 ng/g. Terry Collection sample 1611 and 160, and outliers removed.

5.4. Cause of Death

Figure 5.14 shows the number of individuals in each ultimate causes of death (UCOD) category in the UCF Cadaver and Terry Collection sample (Section 4.1.5). Total average HCC and 2-month average HCC were compared in the Terry Collection and UCF Cadaver samples across UCOD categories. Tests were run with and without outliers to account for small sample sizes and ensure that extreme values were not driving the results. These comparisons revealed no significant differences between UCOD categories in the UCF Cadaver sample (Table 5.12). Significant differences in 2- month and total average HCC with and without outliers between UCOD categories were present in the Terry Collection and combined sample (Independent-samples Kruskal-Wallis; $p < 0.05$).

Total average HCC and 2-month average HCC for each cause of death category and results of statistical comparisons are presented in Appendix E.1.

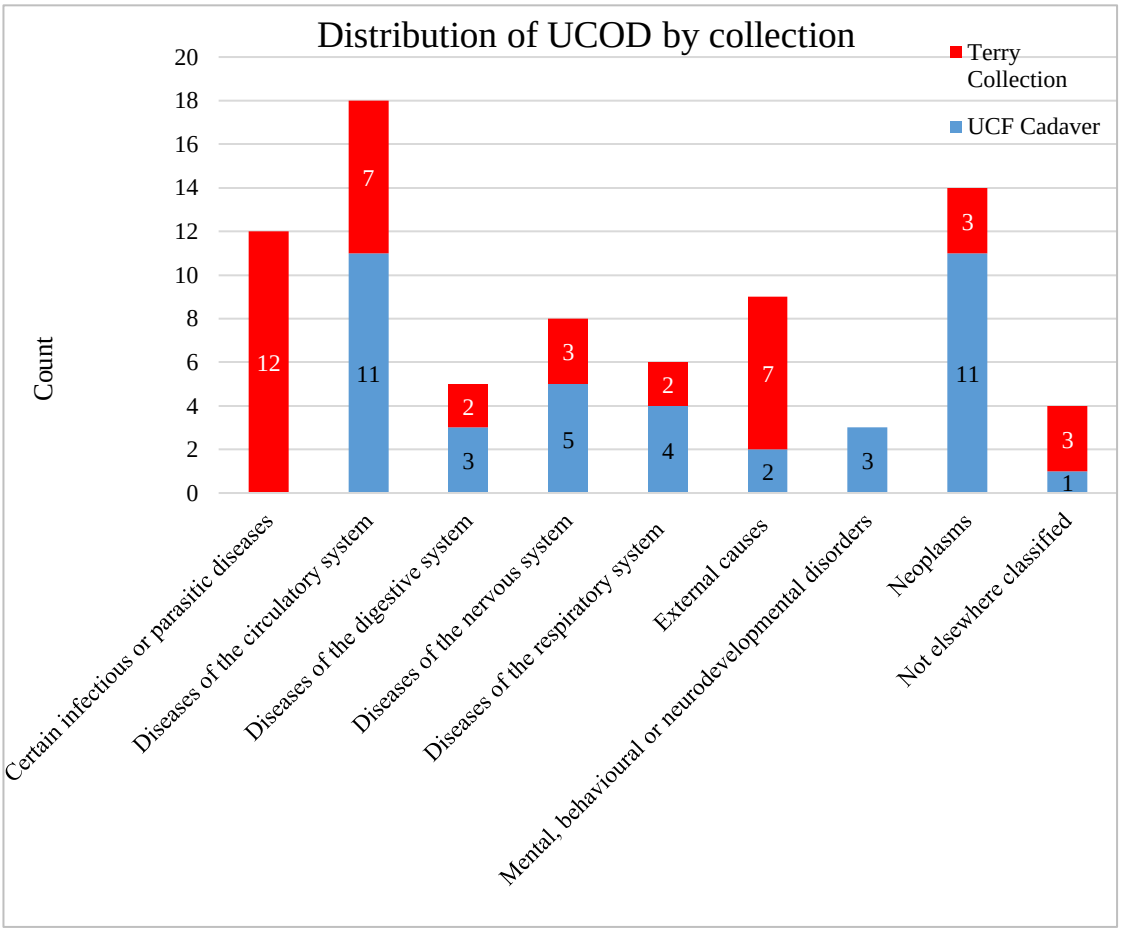

Figure 5.14. Bar graph depicting the number of individuals dying of each ultimate cause of death (UCOD) category in the Terry Collection and UCF Cadaver sample.

Table 5.12. Results of comparisons (p-values) between cause of death categories in the Terry Collection, UCF Cadaver, and combined samples. All tests are Independent-samples Kruskal-Wallis tests.

Collection	Total Average HCC	2-month average HCC
Terry Collection	0.037*	0.014*
UCF Cadaver	0.071	0.126
Combined	0.019*	0.013*

Notes: Outliers removed; * indicates signifiance ($p < 0.05$)
Abbreviation: HCC = Hair cortisol concentration.

Pairwise comparisons revealed significant differences in total average HCC and 2-month average HCC between pairs of UCOD categories in the combined Terry Collection and UCF Cadaver sample when outliers are removed (See Appendix E.1 for total results of pairwise comparisons). Total average HCC in 'Neoplasms' was significantly higher than 'Certain infectious or parasitic diseases', 'Diseases of the nervous system', 'Diseases of the respiratory system', 'External causes' and 'Not elsewhere classified'. Total average HCC in 'Diseases of the circulatory system' was significantly higher than 'Diseases of the nervous system', 'Diseases of the respiratory system', and 'External causes'. When a Bonferroni correction was applied only the difference between 'Neoplasms' and 'Diseases of the Respiratory system' remained significant.

Two-month average HCC in 'Neoplasm' is significantly higher than 'Certain infectious or parasitic diseases', 'Diseases of the nervous system', 'Diseases of the respiratory system', 'External causes', and 'Not elsewhere classified'. Two-month average HCC in 'Diseases of the circulatory system' was significantly higher than 'Diseases of the nervous system'. Two-month average HCC in 'Diseases of the digestive system' was significantly lower than 'Diseases of the nervous system'. When a

Bonferroni correction was applied no relationships remained significant. See Appendix D for complete comparisons. In general, 'Neoplasms' and 'Diseases of the circulatory system' display the highest total average HCC and 'External causes' and 'Diseases of the respiratory system' display the lowest HCC values (Figure 5.15; See Appendix E.1 for boxplot of 2-month average HCC across UCOD categories).

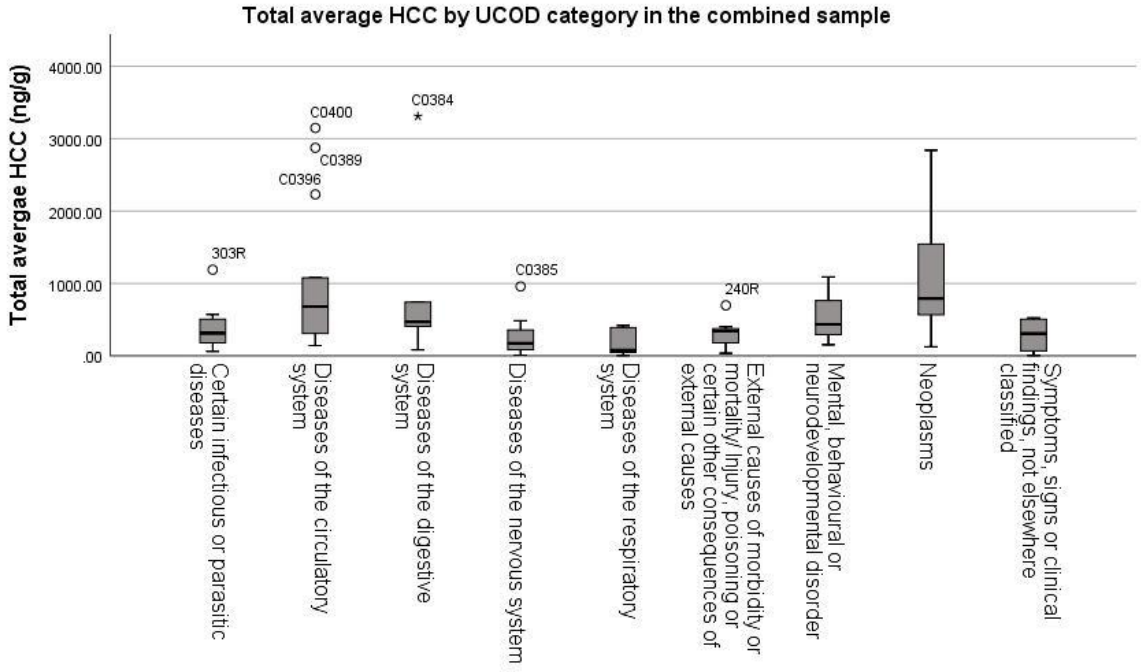

Figure 5.15. Boxplot displaying mean and varience of total average hair cortisol concentration (HCC) across UCOD categories in the combined Terry Collection and UCF Cadaver sample; outliers are removed. Circles indicate outlier values above 1.5IQR +Q3; asterisks indicate extreme outlier values outside above 3QR +Q3.

Total average HCC for the combined Terry Collection and UCF Cadaver samples were broken into quartiles (cut offs at 162.67 ng/g; 390.43 ng/g; 603.09 ng/g; sample 160 and outliers were removed). The number of individuals dying of each cause of death in each quartile was significantly different (Figure 5.16; Table 5.13; Pearson chi square, p =

.042). When the same was done for 2-month average HCC (cut offs at 162.88 ng/g; 354.70 ng/g; 656.47 ng/g; sample 160 and outliers were removed); the number of individuals dying of each cause of death in each quartile was not significantly different (Pearson chi square, p >0.05). See Appendix E.1 for distribution of UCOD across quartiles for total and 2-month average HCC with and without outliers.

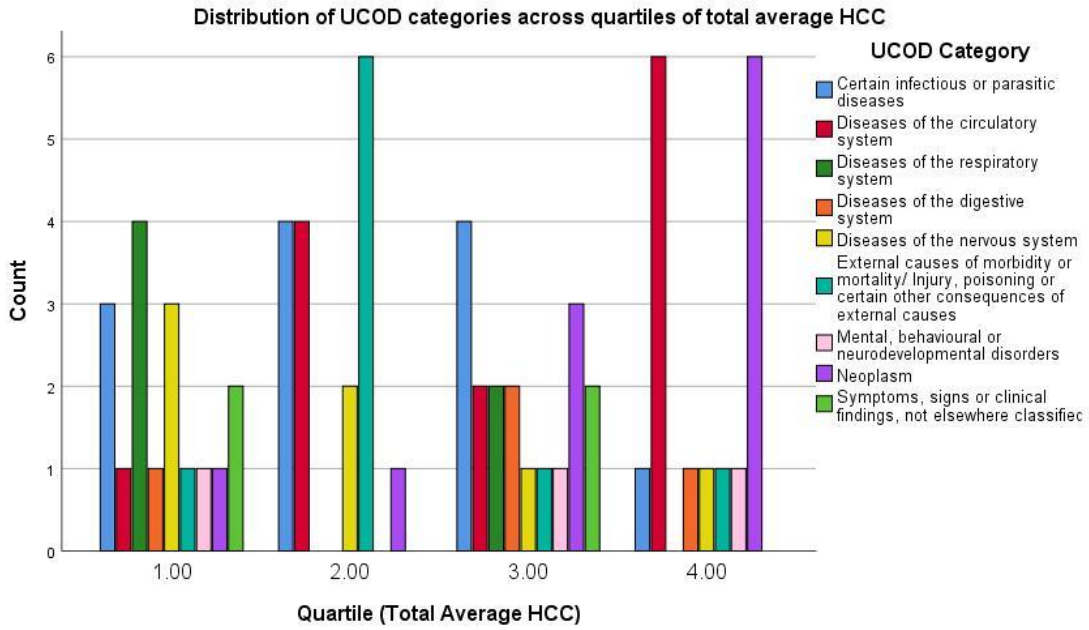

Figure 5.16. Distribution of cause of death categories across quartiles of total average hair cortisol concentration (HCC) in the combined Terry Collection and UCF Cadaver samples (outliers removed).

Table 5.13. Distribution of UCOD across quartiles of total average hair cortisol concentration in the combined Terry Collection and UCF Cadaver sample (outliers removed). Numbers in parentheses indicate percentage of total individuals dying in each category of UCOD and are rounded to nearest whole number.

Quartiles	Certain infectious or parasitic diseases	Diseases of the circulatory system	Diseases of the digestive system	Diseases of the nervous system	Diseases of the respiratory system	External causes	Mental, behavioural or neurodevelopmental disorders	Neoplasms	Not elsewhere classified
1	3 (25%)	1 (8%)	1 (25%)	3 (43%)	4 (67%)	1 (11%)	1(33%)	1 (9%)	2 (50%)
2	4 (33%)	4 (31%)	0	2 (29%)	0	6 (67%)	0	1 (9%)	0
3	4 (33%)	2 (15%)	2 (50%)	1 (14%)	2 (33%)	1 (11%)	1(33%)	3 (27%)	2 (50%)
4	1 (8%)	6 (46%)	1 (25%)	1 (14%)	0	1 (11%)	1(33%)	6 (55%)	0

The number of individuals dying of infections was higher in the Terry Collection sample (n = 12; 31%) than in the UCF Cadaver sample (n= 0; 0%). As indicated above, average HCC of those dying of 'Certain infectious or parasitic diseases' were generally lower than those dying of 'Neoplasms'. If the percentage of individuals dying of infections in the Terry Collection and UCF Cadaver samples are plotted against average HCC a linear relationship can be calculated ($y = -14.678x + 882.24$). The resulting point estimate suggests that the lower HCC values of the Kellis 2 Cemetery sample could be explained if 55.4% of the population died of infectious causes (Figure 5.17).

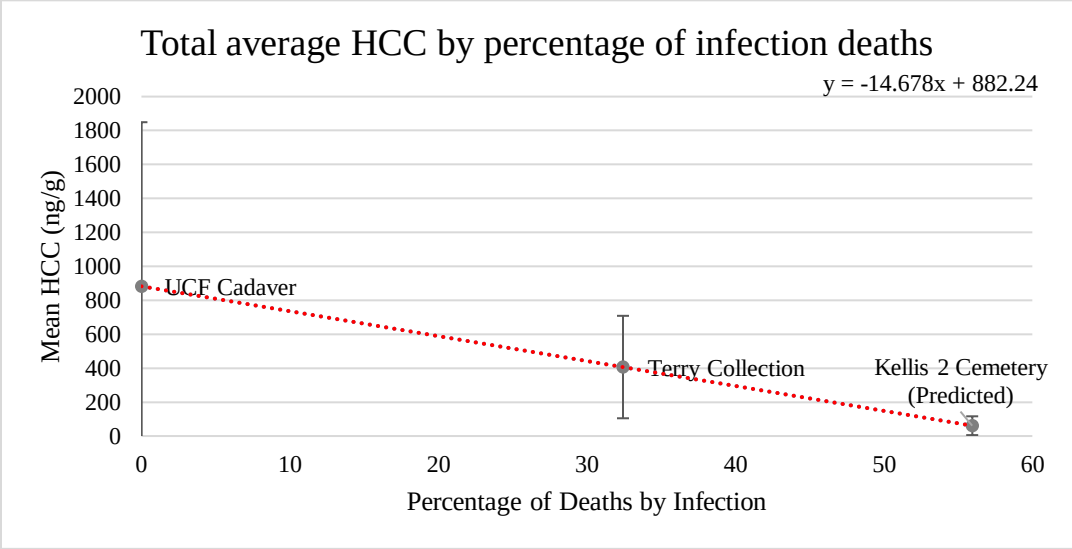

Figure 5.17. Scatterplot depicting relationship between percentage of individuals in a sample dying of infectious disease and total average hair cortisol concentration (HCC) for that sample and a predicted percentage of individuals dying of infectious disease in the Kellis 2 Cemetery.

5.4.1. Patterns of change

If monthly HCC values for each individual are plotted against time, a curve is displayed. If the mean of the HCC values is calculated at each month, a curve for each UCOD is generated. Figure 5.18 illustrates the pattern of HCC values in each UCOD category leading up to death (Table 5.14). The curve for each individual can then be compared to the curve for their cause of death (See Appendix figures E.2-E.11).

The average HCC curve for 'Injury, poisoning or certain other consequences of external causes', 'Diseases of the respiratory system', and 'Certain infectious or parasitic diseases' displays little change in the last months of life and generally low HCC values. The curve for 'Diseases of the circulatory system' displays a general increase in HCC values with substantial monthly variation. 'Diseases of the nervous system' displays a

spike in HCC values in the months before death followed by decrease until death. ‘Neoplasms’ displays a general decline towards death with a number of spikes. ‘Diseases of the digestive system’ displays a significant spike in the months before death and a general decline in HCC values. ‘Mental, behavioural or neurodevelopmental disorders’ displays a gradual decline followed by a small increase and stabilization.

However, individuals dying of each cause display unique curves that do not always match the average curve, as evidence by the low percentages of individuals with similar patterns to the average in certain UCOD groups (see Table 5.14). Therefore, it may be more accurate to examine which patterns of HCC change are most common in each UCOD category; these are summarized in Table 5.15 (See Appendix figures E.2-E.11). By this approach ‘Diseases of the circulatory system’ are most commonly associated with high values and substantial monthly variation, ‘Diseases of the nervous system’ with low values and little variation, ‘Neoplasms’ with high values and a general decline towards death, ‘Diseases of the digestive system’ with high values and little change, and a ‘Mental, behavioural or neurodevelopmental disorders’ with gradual decline or little change.

Table 5.14. Summary of patterns of HCC in each UCOD average curve and the percentage of individuals displaying that pattern.

Cause of death category	**Average curve of HCC**	**% displaying pattern**
'Injury, poisoning or certain other consequences of external causes'	Little change in the last months of life and relatively low HCC values overall	44% (n=4/9)
'Diseases of the respiratory system'		50% (n=3/6)
'Certain infectious or parasitic diseases'		50% (n=6/12)
'Diseases of the circulatory system'	General increase in HCC values with substantial monthly variation	16% (n=3/18)
'Diseases of the nervous system'	Spike in HCC values in the months before death followed by decrease until	0% (n=0/8)
'Neoplasms'	A general decline towards death with a number of spikes	14% (n=2/14)
'Diseases of the digestive system'	A significant spike in the months before death and a general decline in HCC	0% (n=0/5)
'Mental, behavioural or neurodevelopmental disorders'	A gradual decline followed by a small increase and stabilization	0% (n=0/3)

Table 5.15. Summary of patterns of HCC in each UCOD average curve and the percentage of individuals displaying that pattern.

Cause of death category	**Most common pattern of HCC**	**% displaying pattern**
'Injury, poisoning or certain other consequences of external causes'	Little change in the last months of life and relatively low HCC values overall	44% (n=4/9)
'Diseases of the respiratory system'		50% (n=3/6)
'Certain infectious or parasitic diseases'		50% (n=6/12)
'Diseases of the circulatory system'	High values and substantial monthly variation	61% (n=11/18)
'Diseases of the nervous system'	Mostly low values with little variation	63% (n=5/8)
'Neoplasms'	High values and a general decline towards death	71% (n=10/14)
'Diseases of the digestive system'	High values and little change	60% (n=3/5)
'Mental, behavioural or neurodevelopmental disorders'	Gradual decline or little change	100% (n=3/3)

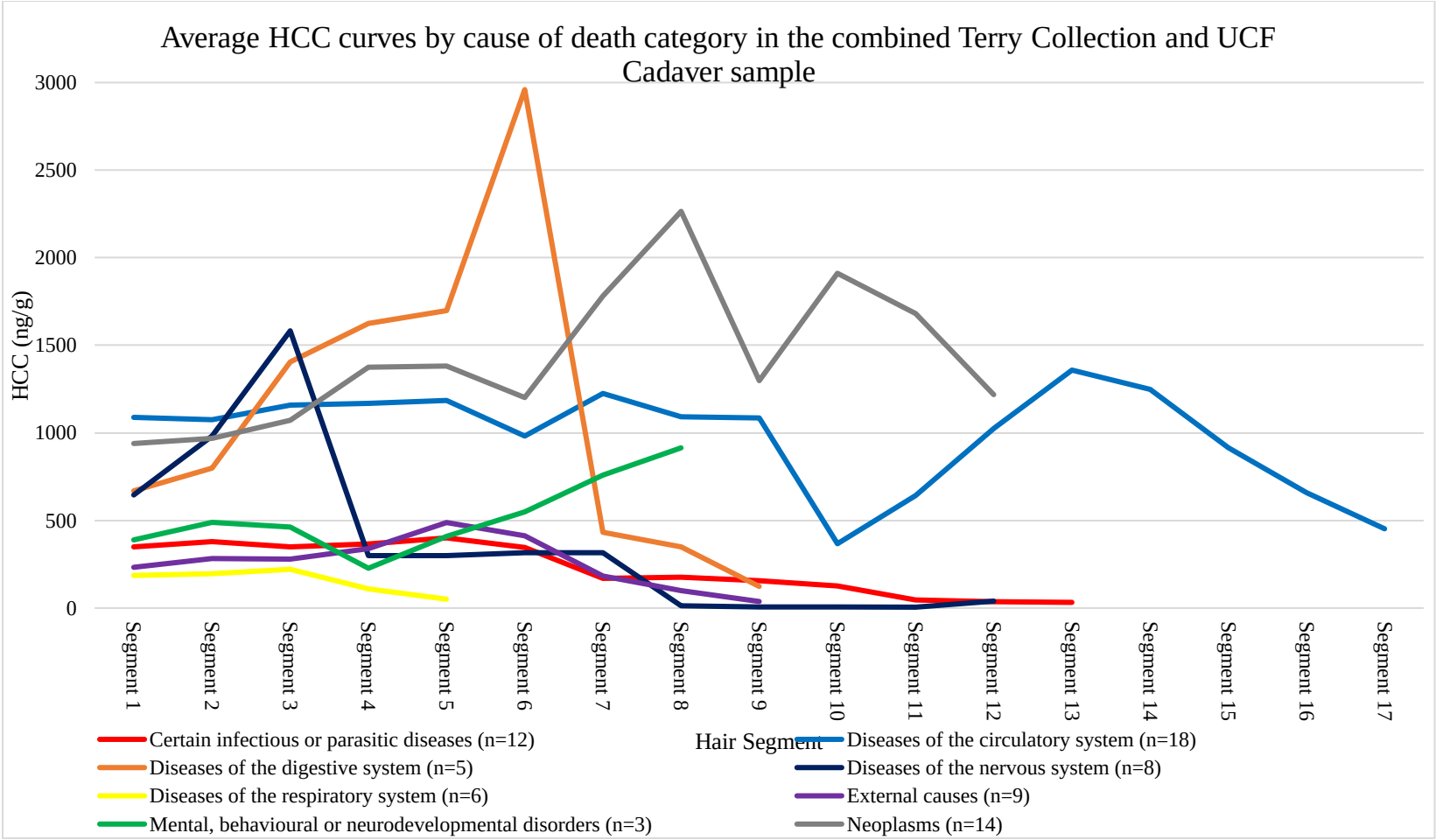

Figure 5.18. Line graph of average hair cortisol concentration (HCC) in each month across UCOD categories in the combined Terry Collection and UCF Cadaver sample. Death is to the left. 'External causes' = External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes. Symptoms, signs, or clinical findings not elsewhere classified not included due to variability in etiology.

5.5. Duration of disease

Two approaches were taken to the analysis of short-term and long-term medical conditions (Section 4.1.6). Abrupt conditions were excluded, Terry Collection sample 37R was removed due to the complexities surrounding dying by suicide, and Terry Collection sample 160 was removed because UCOD could not be determined. In the Terry Collection, sample no significant differences exist in 2-month average HCC between short- and long-term causes of death for any tested cutoff (1 month, 3 months, 6 months, 9 months, 1 year, 15 months and 18 months; Independent-samples Mann-Whitney U; $p > 0.05$; Appendix E.2). When conditions are categorized as chronic or acute according to the WHO definition (Section 4.1.6), the difference in 2-month average HCC is significant in the Terry Collection sample (Independent-samples Mann-Whitney U; Table 5.16), as was the difference in total average HCC ($p = 0.041$). In the UCF Cadaver sample and combined sample, the difference between acute and chronic conditions was not significant for total average (Independent-samples Mann-Whitney U; $p = 0.790$, 0.103) or 2-month average HCC (Independent-samples Mann-Whitney U; Table 5.16).

Table 5.16. Comparison of 2-month average hair cortisol concentration between acute and chronic conditions as defined by the WHO.

Sample	Acute		Chronic		p-value
	N	Mean (ng/g)	N	Mean (ng/g)	
Terry Collection	21	382.21	9	696.60	0.017*
UCF Cadaver	4	764.15	32	732.35	0.610
Combined	25	443.32	41	724.43	0.171

* indicates signifiance ($p < 0.05$)

5.6. Response to change

Information about hospital entry and disease diagnosis was only available for the Terry Collection and only a small number of individuals possessed sufficient hair to reflect hospital entry or disease diagnosis. The challenges of distinguishing disease diagnosis from beginning of disease has been discussed previously (Section 4.1.6.).

5.6.1. Dying and entry into hospital

For the Terry Collection sample, date of hospital entry and duration of illness or medical condition is available. While the date of hospital entry is most likely correct, the date of diagnosis and contracting of a disease are likely conflated with hospital entry (Section 4.1.6). Of the 39 people for whom cause of death data was available, 25 have hair long enough to potentially reflect when they entered the hospital. Twelve of these individuals entered hospital long enough before death to be visible in HCC values (two weeks before death; Section 4.2; Table 5.17). Given the possibility of growth rate errors and inaccuracies in sampling procedures, the month of hospital entry and the months before and after hospital entry were investigated for these changes.

Table 5.17. Number of individuals with the potential to show change in monthly hair cortisol concentration (HCC) values with entry to hospital in the Terry Collection sample.

	Number of individuals	Show change in HCC in the segments corresponding to hospital entry
Total Terry Collection samples with records	39	
Enough hair to reflect hospital entry	31	
Hospital entry more than 2 weeks before death	12	10

One individual (299R) displayed decreasing monthly HCC values following hospital entry, but insufficient hair is present to determine if this is a change from previous months (Appendix E.3). Another individual (769) displayed a trend of decreasing HCC values, but this decline started prior to hospital entry (Appendix E.3). Therefore 10 of the 12 individuals who had hair samples long enough to reflect the time of hospital entry while also having entered the hospital more than two weeks before death show a change in HCC values around hospital entry. Of the 10 individuals who exhibit a response to hospital entry, nine individuals experienced a decrease in HCC values around hospital entry (Table 5.18). These changes varied in degree and were determined by calculating the difference between the month of admission and the following month. Figure 5.19 depicts an example of a response to hospital admission six months before death and corresponding decline in HCC values between month six and month five that continues until the individual's death. One individual experienced an increase in HCC values with hospital admission (Table 5.18). However, this individual's hospital admission corresponded with an acute intestinal obstruction three weeks before death (Figure 5.20). Graphs for the other individuals are presented in Appendix E.3.

Table 5.18. Summary of characteristics of individuals exhibiting hair cortisol concentration (HCC) response to hospital entry in the Terry Collection sample.

Sample number	Months in hospital	Direction of change	Amount of HCC change	Segment of change	Cause of death	Duration of disease
1415R	1	Increase	100.03	2-1	Acute intestinal obstruction	.75 months
568	7	Decrease	17.54	8-7	Tuberculosis	10
834R	3	Decrease	254.79	3-2	Degenerative heart disease	N/A
78R	4	Decrease	144.47	4-3	Bronchopneumonia	.25
764	6	Decrease	20.03	6-5	Tuberculosis	8 months
125	1	Decrease	80.57	2-1	Tuberculosis	1 month
1034	3	Decrease	52.86	3-2	Tuberculosis	3 months
464	3	Decrease	34.70	4-3	Myocarditis	14
1544	6	Decrease	412.93	6-5	Tuberculosis	24
1227	1	Decrease	26.05	2-1	Pneumonia	N/A

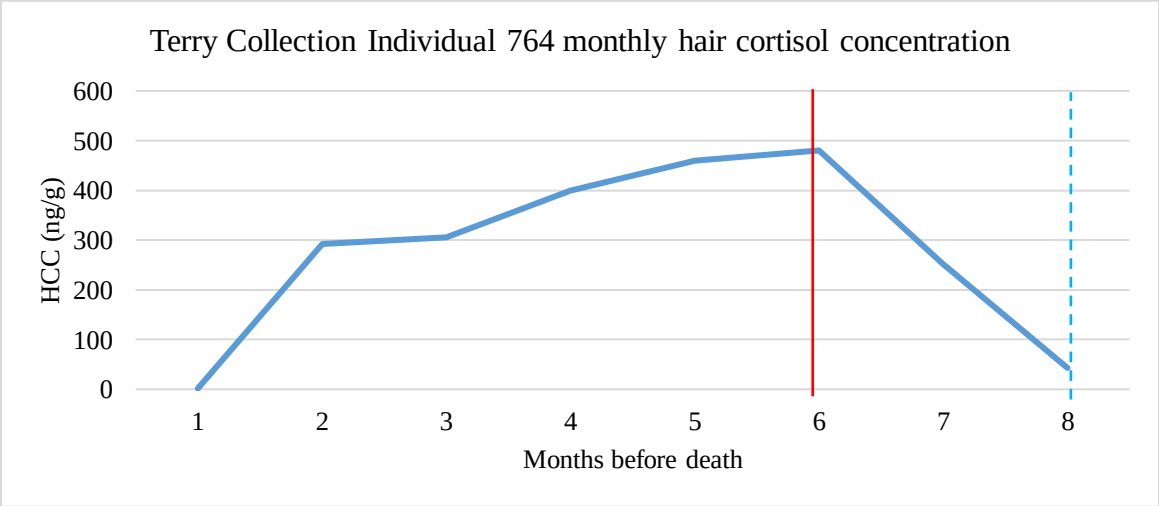

Figure 5.19. Monthly Hair cortisol concentration (HCC) values for individual 764 from the Terry Collection sample exhibiting a decrease in monthly HCC around month of hospital entry. The red solid line indicates the month of hospital entry; the blue dotted line indicates the month of diagnosis.

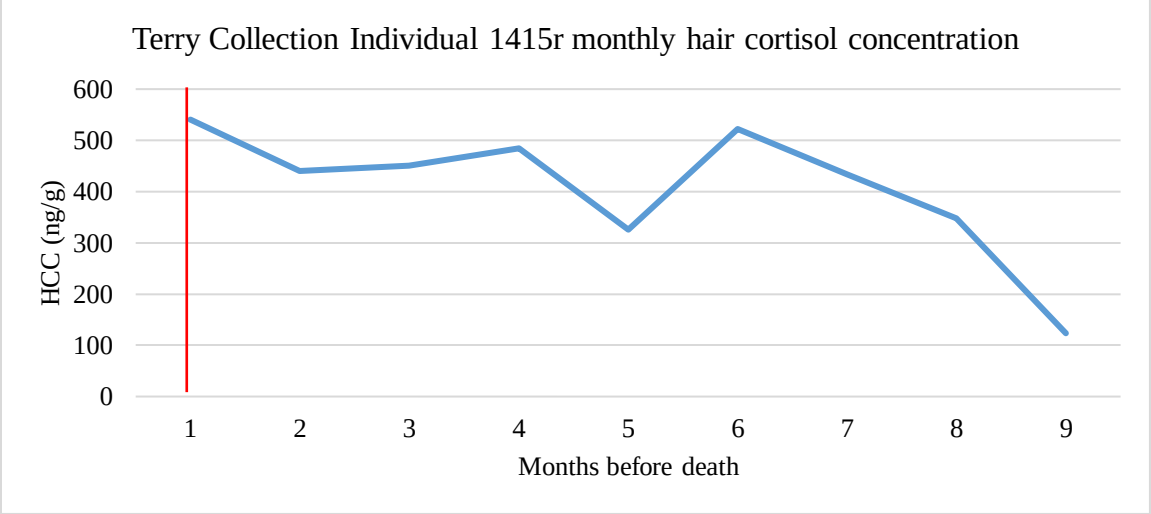

Figure 5.20. Monthly Hair cortisol concentration (HCC) values for individual 1415R from the Terry Collection sample exhibiting an increase in monthly HCC around the month of hospital entry. The red solid line indicates the month of hospital entry.

5.6.2. Contraction of disease

Three individuals have hair samples that overlap with the beginning or diagnosis of the medical conditions that led to their death and can be definitively distinguished from the date of hospital entry. All three of these individuals displayed significant increases in HCC values in the month immediately following the beginning/ diagnosis of a disease (Table 5.19). Figure 5.21 depicts one such example. Individual 568 was diagnosed with tuberculosis 10 months before death. The difference in the monthly values of month 10 and nine show a small increase, although the values decrease again with hospital entry at month eight. Notably after a small decrease around hospital entry, the HCC values

continue to increase almost until death. Graphs for other individuals are presented in Appendix E.3.

Table 5.19. Summary of characteristics of individuals exhibiting response to diagnosis of disease in the Terry Collection sample.

Sample number	Duration of illness	Direction of change	Amount of change	Months of change	Cause of death
764	8 months	Increase	209.22	8-7	Tuberculosis
568	10 months	Increase	19.71	10-9	Tuberculosis
480	11 months	Increase	35.69	11-10	Myocarditis

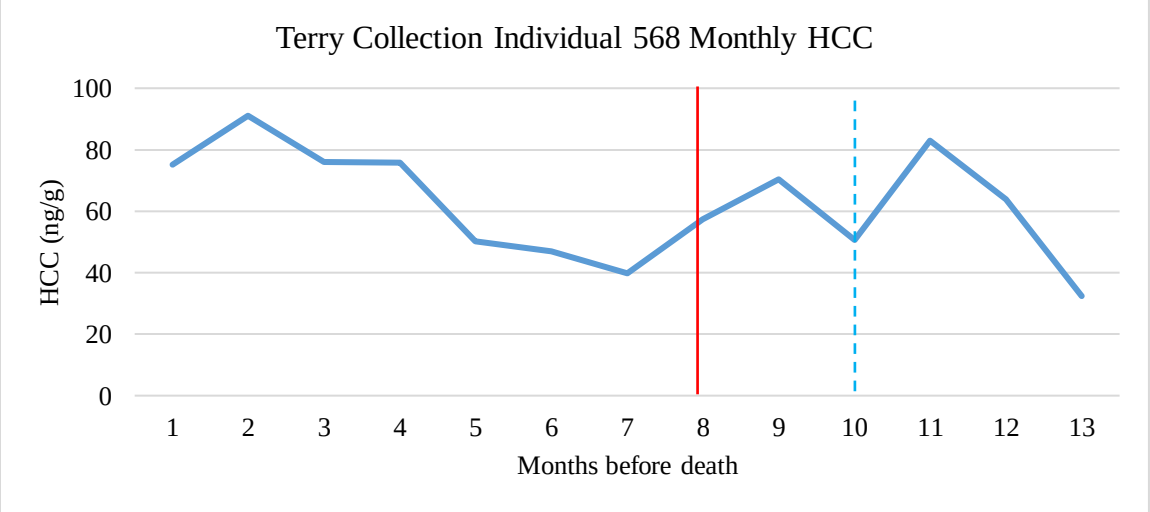

Figure 5.21. Monthly hair cortisol concentration (HCC) values for individual 568 from the Terry Collection sample exhibiting an increase in monthly HCC around the month of diagnosis. The red solid line indicates the month of hospital entry; the blue dotted line indicates the month of diagnosis.

5.6.3. Institutionalization

The records for two individuals from the Terry Collection indicate that their address prior to hospitalization and/or death was a psychiatric facility. The duration of time spent in this facility and the condition that precipitated the stay are not listed in the available records. The pattern of HCC in each individual is substantially different.

Individual 834R exhibits HCC values far above that for living healthy people and displays extreme variation in HCC values in the 17 months before death while individual 1066R displays HCC values within the range of living healthy people with little change in the last five months of life (Table 5.20; Figure 5.22).

Table 5.20. Hair cortisol concentration (HCC) values and summary of characteristics of individuals experiencing spending time in a psychiatric facility before death.

Sample ID	Sample	Age	Sex	Average Total HCC	Cause of Death
834 R	Terry Collection	87	Female	1079.164	Pneumonia
1066R	Terry Collection	62	Male	56.314	Pneumonia

Abbreviation: HCC = Hair cortisol concentration

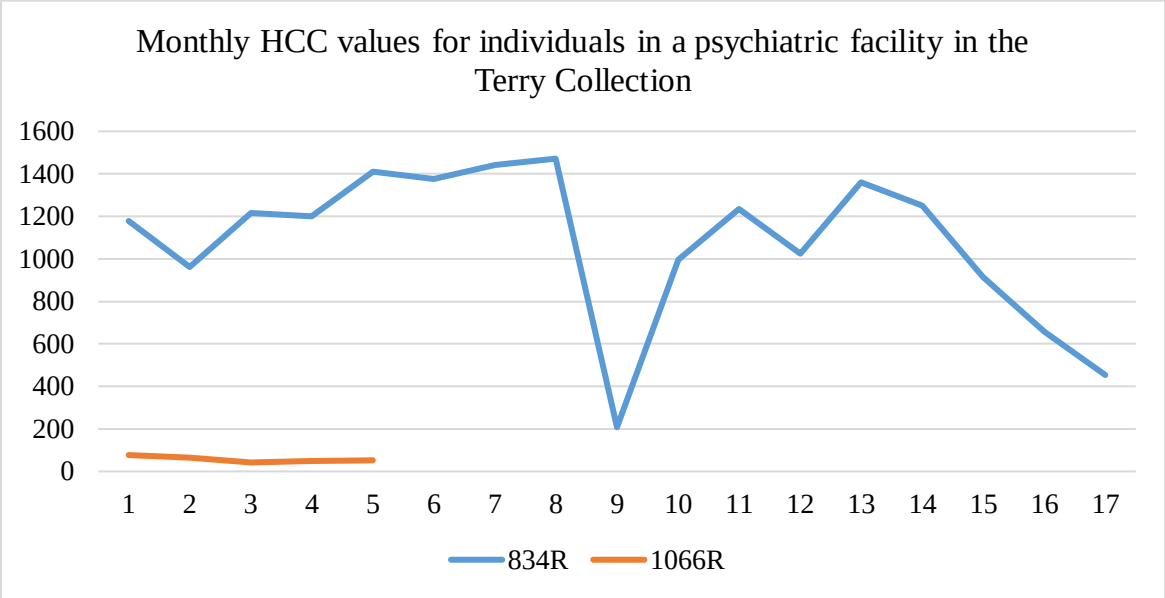

Figure 5.22. Monthly hair cortisol concentration (HCC) values for two individuals from the Terry Collection who spent time in a psychiatric facility before death.

5.7. Preexisting medical conditions

5.7.1. Depression

In the UCF Cadaver sample, two individuals were reported to have depression. In the Terry Collection sample, one individual died by suicide. In all of these cases, HCC values were higher than in healthy living individuals and increased in the months before death (31.3-75.9 ng/g according to Greff et al. 2019; Table 5.21). Two individuals (37R and CO403) display values similar to the average value of living healthy people in their earliest hair segments but experienced significant increases in HCC until death (Figure 5.23). However, no significant difference was identified in HCC values between those experiencing depression and those not in the samples from the current study, although the sample size was small (Independent-samples Mann-Whitney U test; $p > 0.05$). Therefore, all three individuals exhibiting symptoms of depression displayed HCC values higher than healthy living people, but their HCC values were consistent with the other samples of the dead from the current study. In each case, HCC increased from the earliest available hair segment. In the one individual who died by suicide, HCC increased from healthy living people values to more than 20 times that value at the time of death. Because no disease was diagnosed during autopsy and dissection, this elevated HCC and increase towards death is unlikely to be a result of disease. Another individual displays early values consistent with healthy living people but dies with HCC values 10 times those. This individual died of cardiomyopathy.

*Table 5.21. H*air cortisol concentration (HCC) values and summary of characteristics of individuals experiencing depression at the time of death.

Sample ID	Sample	Age	Sex	2-month average HCC	Cause of Death
37R	Terry	55	Female	606.58	Suicide
CO537	UCF Cadaver	65	Female	1178.48	Coronary heart
CO403	UCF Cadaver	97	Female	296.56	Cardiomyopathy

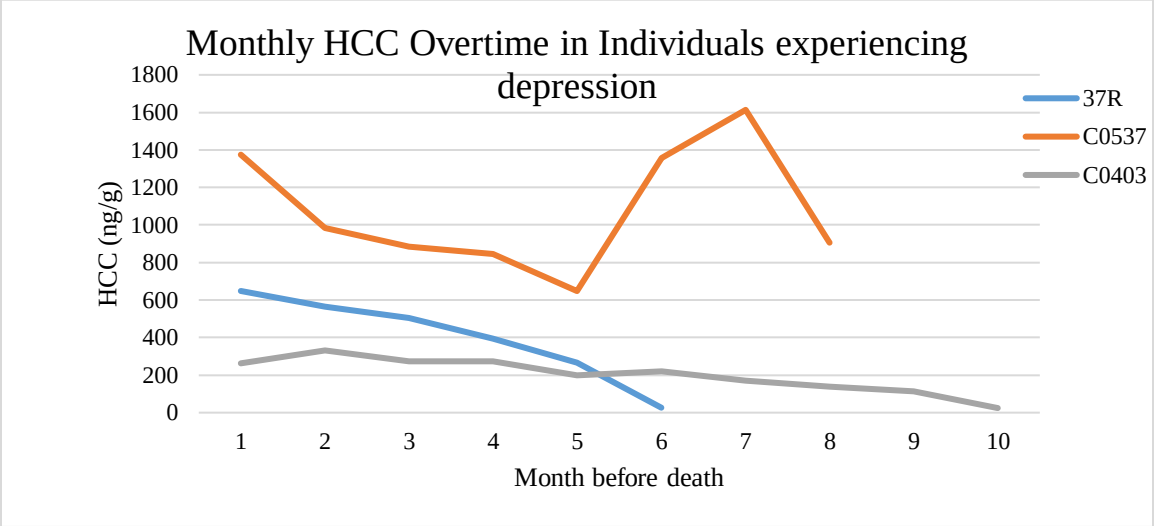

Figure 5.23. Monthly hair cortisol concentration (HCC) values in individuals experiencing depression in the months before death.

5.7.2. Senility and dementia

Four individuals in the UCF Cadaver sample were diagnosed with dementia or Alzheimer's disease. In the Terry Collection sample, one individual was diagnosed with dementia and two with senility (Section 4.1.4). Two-month average HCC varied considerably in this group. While most of these individuals exhibited HCC values higher than healthy living people (31.3-75.9 ng/g according to Greff et al. 2019), not all of them did. The average values of this group were not significantly different from the other samples of the dead (Table 5.22; Independent-samples Mann-Whitney U; $p > 0.05$). The small sample size of this group challenges the validity of any statistical analysis. Notably,

individual CO383 is an outlier in the UCF Cadaver sample and 'Diseases of the nervous system' category. None of the other examples are outliers for their sample or in their UCOD category.

Table 5.22. Hair cortisol concentration (HCC) values and summary of characteristics of individuals experiencing dementia or senility at the time of death.

Sample ID	Sample	Age (years)	Sex	2-month average HCC (ng/g)	Diagnosis	Cause of Death
9R	TC	71	M	978.935	Dementia	Arteriosclerosis
1227	TC	75	F	673.09	Senility	Gangrene
78R	TC	91	F	40.83	Senility	Pneumonia
CO393	UCF	80	M	1079.95	Dementia	Pneumonia
CO422	UCF	64	F	76.48	Alzheimer's Disease/ Dementia	Alzheimer's Disease
CO395	UCF	84	F	159.89	Alzheimer Dementia	Alzheimer's Disease
CO383	UCF	87	F	4552.53	End Stage Alzheimer's Disease	Alzheimer's Disease
CO403	UCF	97	F	296.56	Dementia	Cardiomyopathy

Abbreviations: TC = Terry Collection, UCF = UCF Cadaver, M = male, F = female

5.7.3. Cushing's disease

One individual from the Terry Collection sample has a death certificate listing 'Cushing-like syndrome' for 20 years before death and experienced a gastrointestinal hemorrhage one day before death. This individual did not exhibit HCC values similar to living healthy people (Figure 5. 5.24; 31.3-75.9 ng/g according to Greff et al. 2019) but they are similar to dying samples.

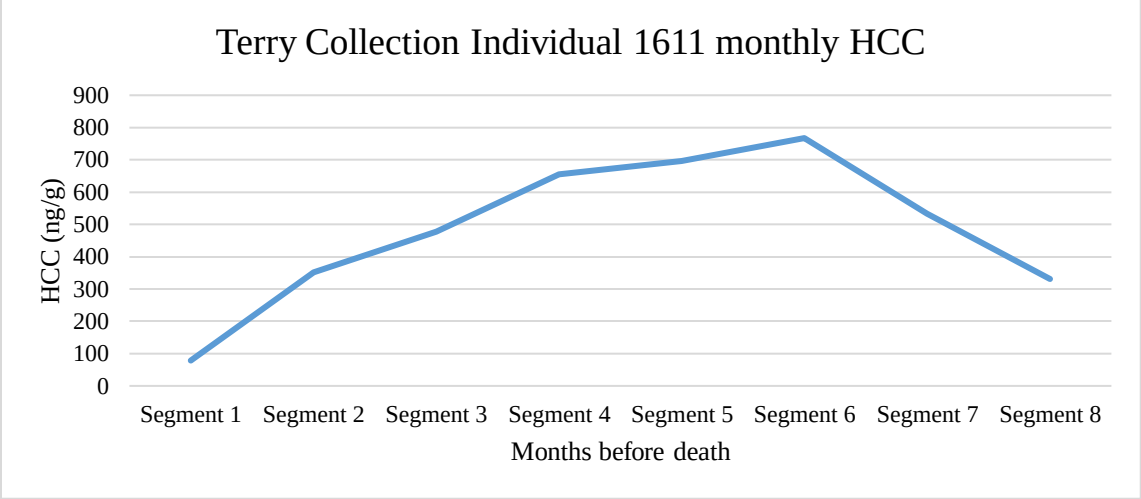

Figure 5.24. Monthly hair cortisol concentration (HCC) for individual 1611, diagnosed with a 'Cushing-Like Syndrome'.

CHAPTER 6 - COMPARATIVE RESULTS WITH EXISTING LITTERATURE

6.0. Introduction

In this chapter I will compare total average hair cortisol concentration (HCC) between the samples from the current study (the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples) and previous archaeological studies as well as studies of HCC in living people.

6.1. Comparison to samples of the dead

The archaeological studies used for comparison are presented in Table 6.1 and 6.2 and represent the only currently published HCC studies of the dead. The studies of Webb and colleagues (2010; 2014; and 2015) and López-Barrales and colleagues (2015) do not report an isopropyl wash, which is used to remove surface contamination of cortisol that could elevate HCC (Greff et al. 2019). The data from these studies are interpreted with caution. Total average HCC was used for samples from the current study for consistency with studies that employed bulk analysis.

Table 6.1. Contextual data for current and previously published archaeological studies.

Collection	Geographic origin	Burial environment	Age (Years before present)
Kellis 2 Cemetery*	Kellis, Dakhleh Oasis, Egypt	Arid	2000
Terry Collection	St. Louis, Missouri, USA	None	60
UCF Cadaver	Orlando, Florida, USA	None	1-3
Webb et al. 2010	Cajamarquilla, Leymebamba, Puruchuco, Tucume and Nasca, Peru	Arid	500-1500
Webb et al. 2014	Nasca Region, Peru	Arid	1000-2000
López-Barrales et al. 2015	San Pedro de Atacama, Chile	Arid	500-1000
Webb et al. 2015	Nasca Region, Peru	Arid	1000-2000
Schaefer 2017	Lambayeque Valley, Peru	Arid	500-1000
Tisdale et al. 2019*	Kellis, Dakhleh Oasis, Egypt	Arid	2000

*The Kellis 2 Cemetery sample and the Tisdale et al. 2019 sample are derived from the same cemetery sample.

Table 6.2. Summary of hair cortisol concentration (HCC) results of previously published archaeological studies of HCC in addition to the total average HCC from this study.

Collection	Mean (ng/g)	SD (ng/g)	Min (ng/g)	Max (ng/g)	N
Kellis 2 Cemetery- Juveniles	85.74	69.29	3.40	269.51	38
Kellis 2 Cemetery- Adults	60.86	55.34	1.16	255.11	82
Terry Collection	406.47	301.79	34.66	1190.49	38
UCF Cadaver	882.13	966.97	4.70	3310.49	38
Webb et al. 2010	281	35	91	707	10
Webb et al. 2014	522	368	87	2392	14
López-Barrales et al. 2015	76.0	35.3	33.7	152.0	19
Webb et al. 2015	1444	402	757	2507	5
Schaefer 2017	75.00	71.40	18.02	247.95	10
Tisdale et al. 2019	343.70	70.15	272.50	467.00	10

Abbreviations used: SD=standard deviation, Min= miniumum, Max=maximum, N = sample size

Notes: Means for each sample represent the mean of individual average HCC; outliers from current study removed; samples from current study represented above double line.

The average values for each of the samples in the current study fall within those of previously reported averages of archaeological samples. However, pairwise comparisons following ANOVA analysis, reveal that the Kellis 2 Cemetery juvenile and adult samples are significantly different from the Terry Collection sample, the UCF Cadaver sample, the Webb et al. (2014) sample, and the Webb et al. (2015) sample (Table 6.3; Tukey HSD Post-hoc test).

Table 6.3. Results (p-values) of multiple pairwise comparisons (Tukey HSD Post-hoc test) between samples from the current study sample and those reported in the literature.

	Kellis 2 Cemetery-Juveniles	Kellis 2 Cemetery-Adults	Terry Collection	UCF Cadaver	Webb et al. 2010	Webb et al. 2014	López-Barrales et al. 2015	Webb et al. 2015	Schaefer 2017	Tisdale et al. 2019
Kellis 2 Cemetery-Juveniles										
Kellis 2 Cemetery-Adults	1.00									
Terry Collection	0.02	0.00								
UCF Cadaver	0.00	0.00	0.00							
Webb et al. 2010	0.93	0.82	0.99	0.00						
Webb et al. 2014	0.02	0.00	0.99	0.12	0.91					
López-Barrales 2015	1.00	1.00	0.10	0.00	0.95	0.06				
Webb et al. 2015	0.00	0.00	0.00	0.10	0.00	0.00	0.00			
Schaefer 2017	1.00	1.0	0.37	0.00	0.98	0.18	1.00	0.00		
Tisdale et al. 2019	0.72	0.52	1.00	0.01	1.00	0.99	0.79	0.00	0.89	

Notes: Shading indicates significant p values <0.05.

In those samples for which raw data was available (Kellis 2 Cemetery, Terry Collection, UCF Cadaver, Webb et al. 2010; 2014; 2015, Tisdale et al. 2019), non-parametric tests were possible. The average values of the samples in the current study were not normally distributed, therefore non-parametric tests are more appropriate and

reliable than ANOVA. Significant variation was identified (Independent-samples Kruskal Wallis; p=.000), and pairwise comparison revealed significant differences between the Kellis 2 Cemetery sample and all other samples with raw data (Bonferroni post hoc test; p=.000) except for the Webb and colleagues (2010) sample from Peru (see Table 6.1; Bonferroni post hoc test; p=1.0). Unlike the earlier comparison using parametric tests, the non-parametric analysis revealed significant differences between the Kellis 2 Cemetery and the Tisdale et al. (2019) samples which are derived from the same cemetery sample.

When plotting HCC values against time since death for each sample, no relationship exists (Figure 6.1; $R^2 = 0.0035$). When those studies that did not report an isopropyl wash are removed, time since death has a very weak effect on HCC values (Figure 6.2; $R^2 = 0.37$).

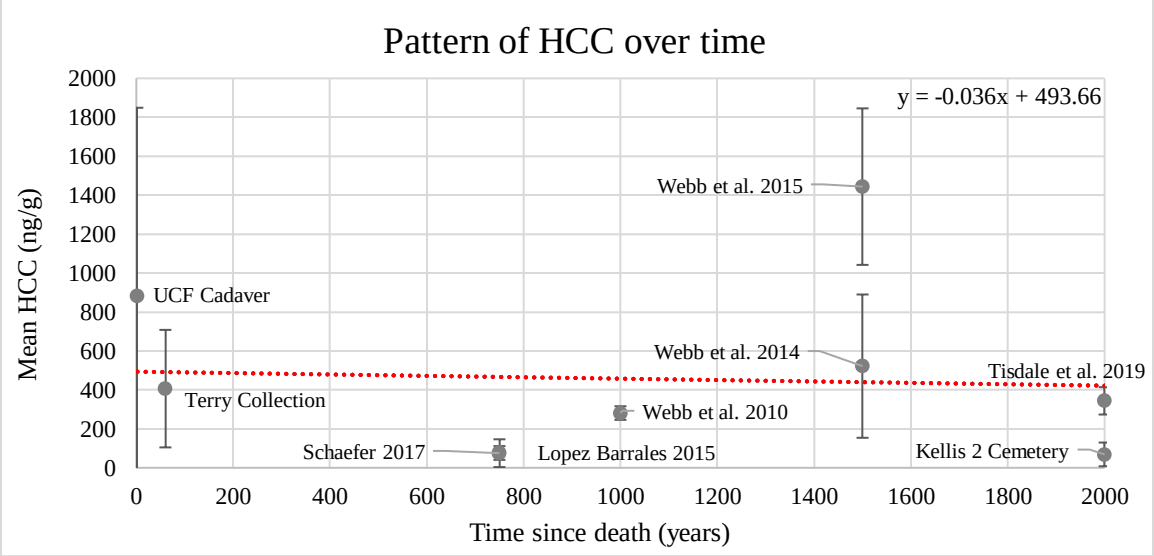

Figure 6.1. Scatterplot plotting mean hair cortisol concentration (HCC) values against time since death (in years). Error bars reflect 1 standard deviation.

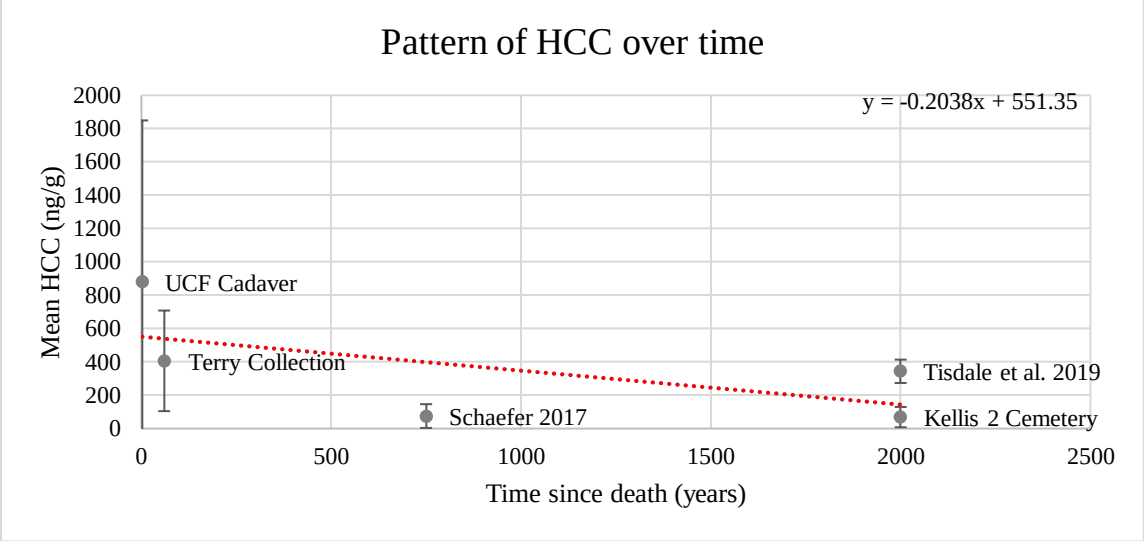

Figure 6.2. Scatterplot plotting mean hair cortisol concentration (HCC) values against time since death in years after exclusion of those studies that did not carry out an isopropyl wash. Error bars reflect 1 standard deviation.

When comparing HCC values against sample size of the current and previous published studies, sample size has no effect on HCC values (Figure 6.3; $R^2 = 0.09$). If only including those samples that carried out an isopropyl wash, the effect is even smaller (Figure 6.4; $R^2 = 0.07$).

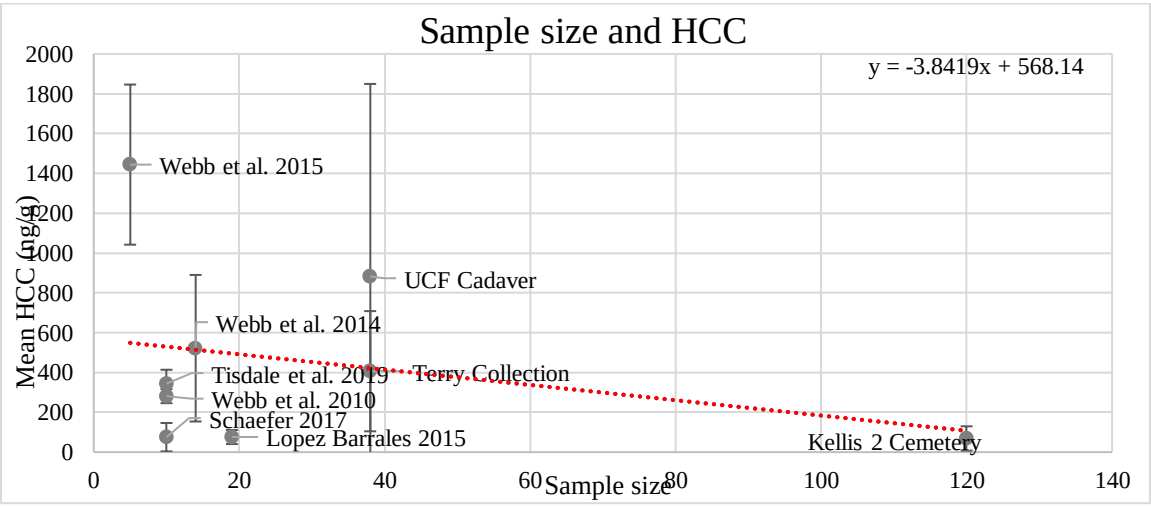

Figure 6.3. Scatterplot plotting mean hair cortisol concentration (HCC) values against sample size. Error bars reflect 1 standard deviation.

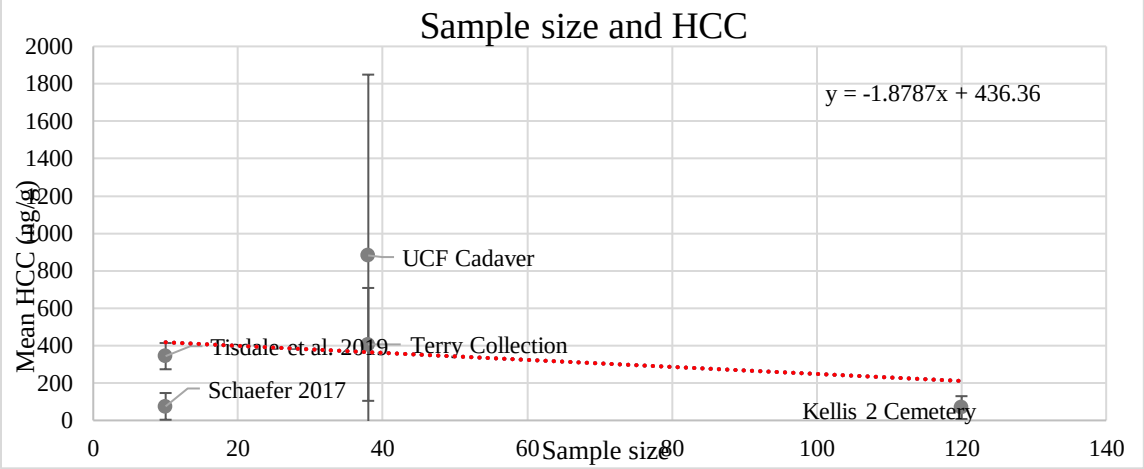

Figure 6.4. Scatterplot plotting mean hair cortisol concentration (HCC) values against sample size with those studies that did not carry out an isopropyl wash excluded. Error bars reflect 1 standard deviation.

6.2. Comparisons to living reference values

The ranges of values from the currently investigated samples and the Thomson and colleagues (2010) standards for healthy (defined as not experiencing symptoms of Cushing's Syndrome) people are presented in Figure 6.5. The standard presented by Thomson and colleagues (2010) has been used in one other archaeological study (Schaefer 2017). ANOVA comparison revealed that HCC values from Thomson and colleagues (2010) were significantly different from that of the Terry Collection ($p = 0.016$) and the UCF Cadaver sample ($p = 0.0$) but not the Kellis 2 Cemetery sample ($p > 0.05$). A number of individuals with different known causes of death from the Terry Collection and UCF Cadaver samples displayed HCC values within the range of healthy living people reported by Thompson and colleagues (2010; Figure 6.6.)

However, if employing the standards for living healthy people established by Greff and colleagues (2019; 31.3−75.9 ng/g), only five individuals fall within that range. Two of these individuals died of 'External causes' and three died of 'Diseases of the

respiratory system'; three are from the Terry Collection sample while two are from the UCF Cadaver sample.

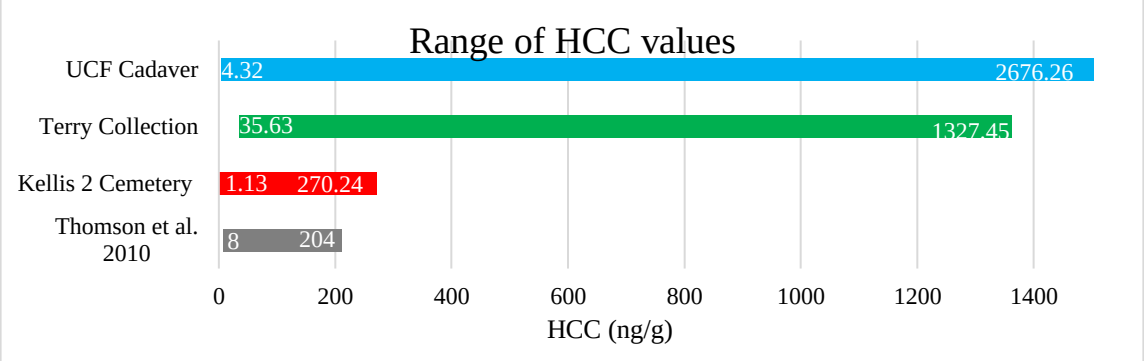

Figure 6.5. Comparison of hair cortisol concentration (HCC) ranges between samples of the dead and living healthy people (Thomson et al. 2010). Two-month average HCC, outliers removed. Minimum and maximum values are labeled in white.

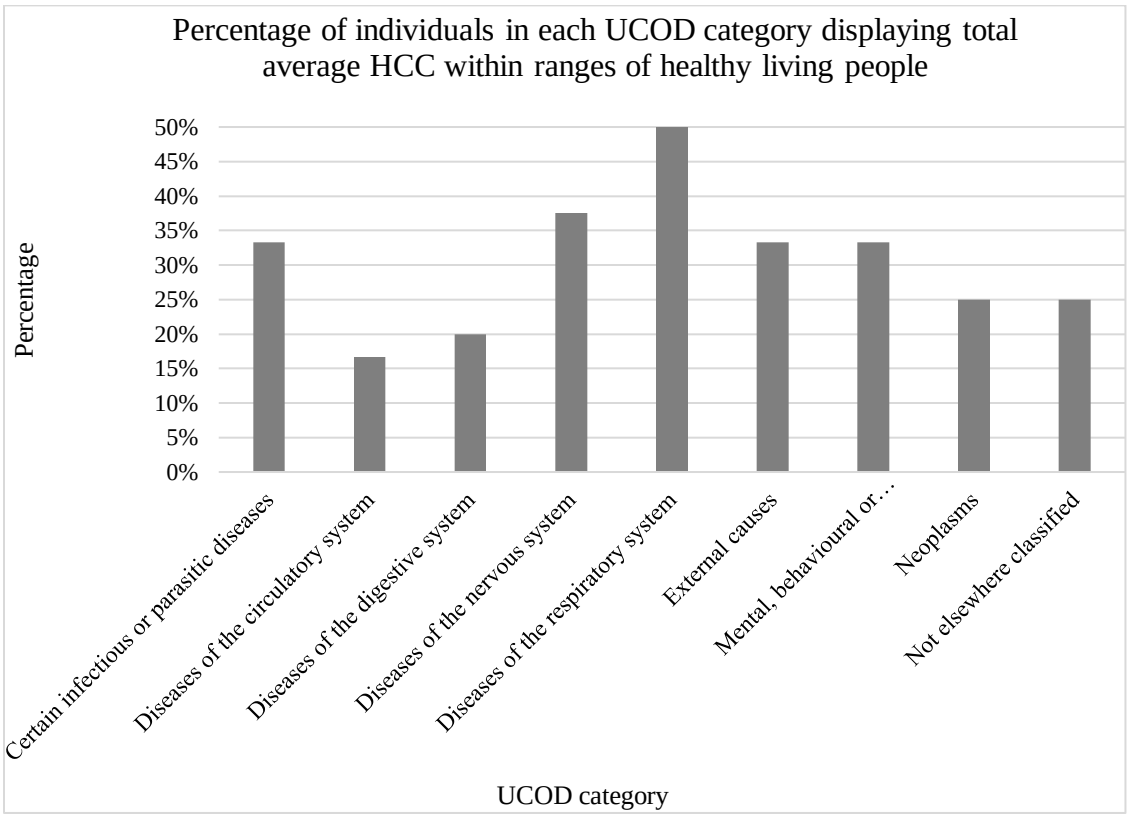

Figure 6.6. Percentage of individuals in each ultimate cause of death (UCOD) category reflecting hair cortisol concentration (HCC) values with that reported for modern healthy people by Thompson et al. (2010).

6.3. Comparison to living samples

Comparisons were made between 2-month average HCC in the Kellis 2 Cemetery adults, the Terry Collection, and the UCF Cadaver samples and the control groups of healthy living individuals from previously published studies of HCC. The studies all employed an immunoassay, had clearly defined summary statistics for a study group and/or control group, and reported data that was not log transformed (Appendix D.1). For the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples 2-month average HCC was chosen to ensure comparability with the reference papers that often focus on average values over one-to-three months. The intention is to make general comparisons to discern broad trends that may be present. In general, the samples of the dead have higher HCC than the samples of healthy controls from previous publications (Figure 6.7). While the Terry Collection samples (green) and the UCF Cadaver samples (blue) occupy the upper extreme of the distribution, the Kellis 2 Cemetery sample (red) falls much lower on the graph.

Two-month average HCC in samples of the dead are higher than most living people with various health conditions or from social conditions which may impact stress levels (Figure 6.8; Appendix D.1). In general, individuals with syndromes leading directly to elevated cortisol (hypercortisolemia and Cushing's syndrome) or those in very high stress circumstances (First Nation's communities in Canada and individuals from a slum settlement in Kenya) have values similar to the dead. Individuals with chronic pain and experiencing myocardial infarction also display similar ranges to samples of the dead.

Summary statistics for archaeological dead, modern dead, healthy living, and stressed living groups are presented in Table 6.4 (2-month average used for samples from this study). The variability between these four groups is significant (Independent-Samples Kruskal-Wallis; p=.001). If the dead groups are combined, a comparison of average values from studies of living people compared to studies of dead people revealed significant differences between dead groups and both the healthy living groups and the group with disease and high stress (Table 6.5; Figure 6.9). If the living groups are also combined, the difference in HCC between living and dead groups is also significant (Independent-samples Mann-Whitney U; p = .005).

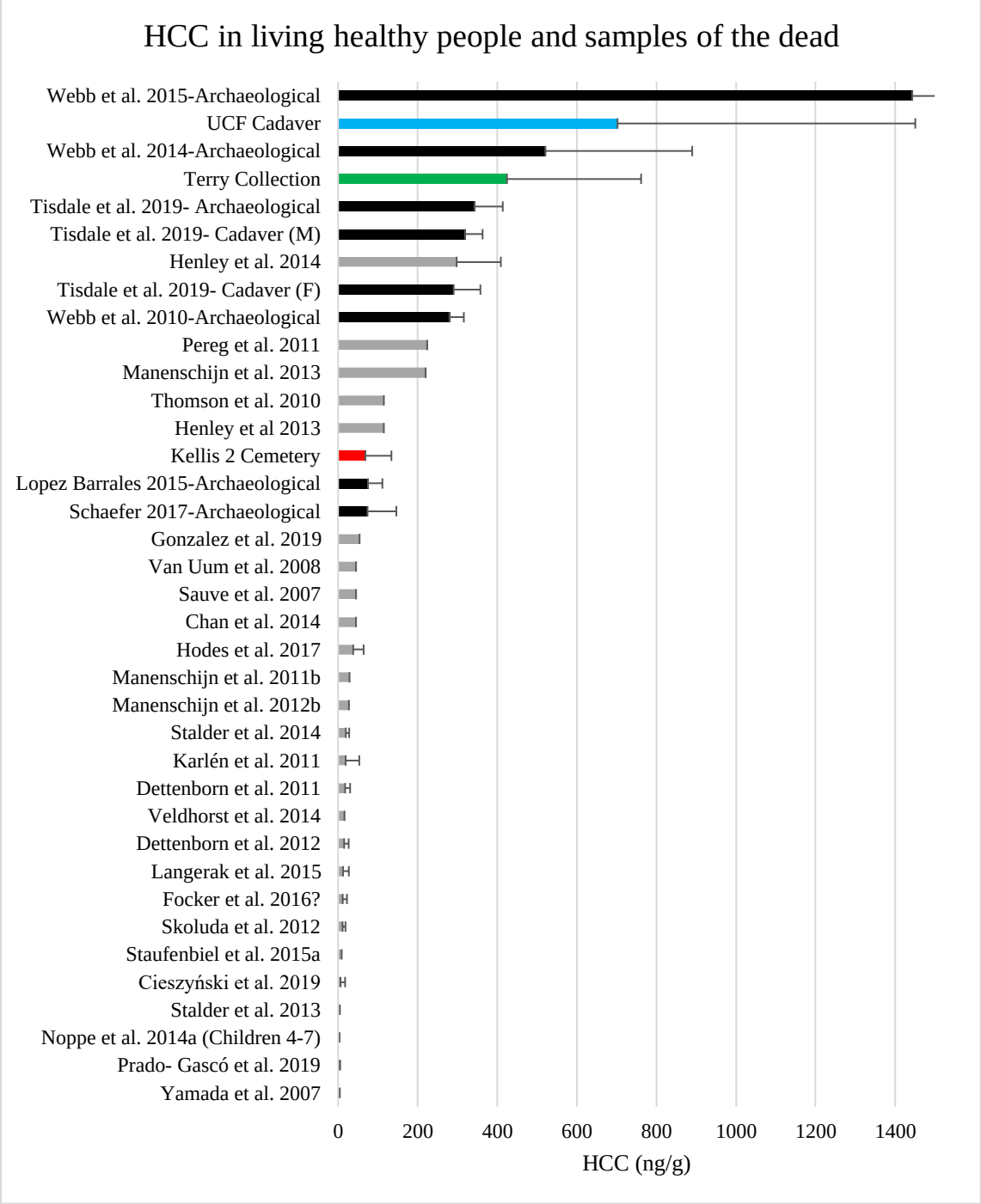

Figure 6.7. Average hair cortisol concentration (HCC) for the control groups of healthy living people (grey) from previously published studies compared to the previous studies of the dead (black), the Kellis 2 Cemetery (red), Terry Collection (green), and UCF Cadaver (blue) samples. Two-month average HCC without outliers used for samples from this study. Error bars reflect 1 SD only when SD was reported in original literature.

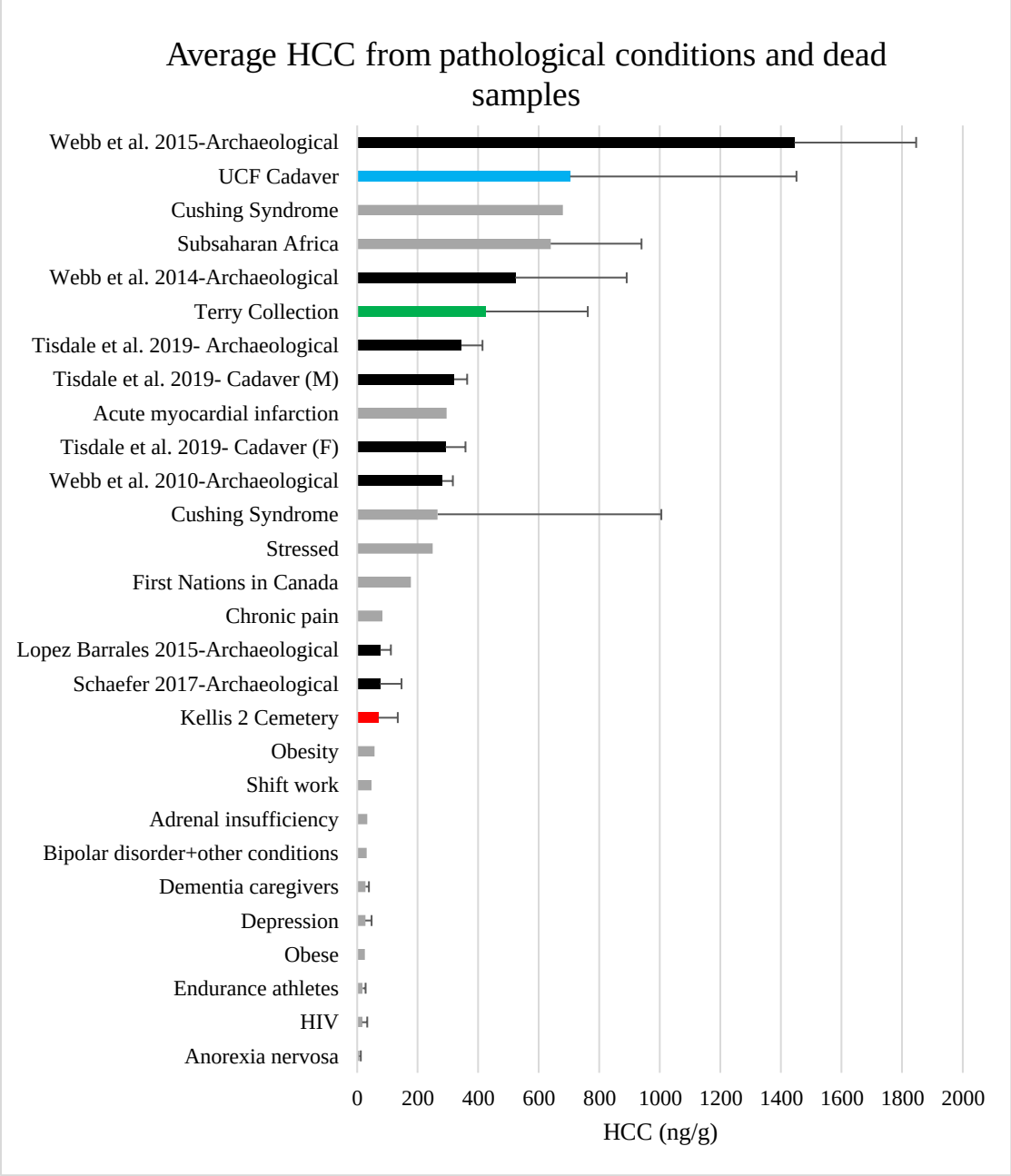

Figure 6.8. Average hair cortisol concentration (HCC) from individuals with various health conditions and sources of stress as compared to previous studies of the dead (black), the Kellis 2 Cemetery (red), Terry Collection (green), and UCF Cadaver (blue) samples. Two-month average HCC used for samples from this study. Error bars reflect 1 SD only if SD reported in original publication (see appendix for references for studies of pathological conditions).

Table 6.4. Summary statistics of hair cortisol concentration (HCC) from various studies of living and dead groups (See Appendix C for breakdown of studies).

	Mean	SD	Number of Studies
Cadaver	438.27	229.93	3
Dead (combined archaeological and cadaver)	413.78	393.78	11
Archaeological	404.59	453.94	8
Living (stressed)	157.78	211.45	18
Living (healthy)	53.26	77.01	27
Living (combined healthy and stressed)	93.64	151.24	44

Abbreviation: SD = Standard deviation

Table 6.5. Results of Bonferroni corrected post hoc pairwise comparisons of mean hair cortisol concentration (HCC) between living healthy group, living pathological groups, and the dead. * indicates significance at $p<0.05$.

	Dead	Living (healthy)	Living (pathological)
Dead			
Living (healthy)	.00*		
Living (pathological)	.047*	.089	

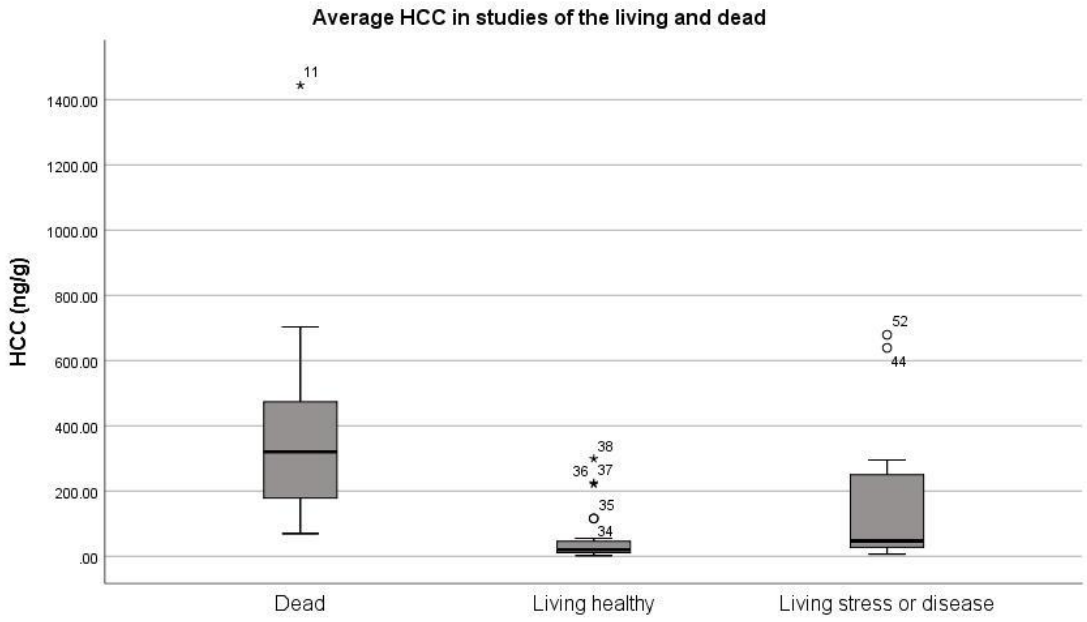

Figure 6.9. Boxplot of average hair cortisol concentration (HCC) in dead, living healthy, and living stressed or diseased samples. Circles indicate outlier values above 1.5IQR +Q3; asterisks indicate extreme outlier values outside above 3QR +Q3.

CHAPTER 7 - DISCUSSION

7.0. Introduction

The aims of the current study were two-fold: first, to evaluate the utility of HCC analysis in the study of stress in the dead and dying and second, to evaluate the relationships between HCC, disease, dying, and medical treatment to determine how stress experience varies in the last months of life. Chapter 7 includes a discussion of major findings as related to the literature on HCC analysis and the study of dying and death in bioarchaeology and palliative care. This discussion is directed at determining if HCC is a valid measurement of stress; if dying is stressful; if individual experiences of stress at the end of life vary as a result of causes of death, medical interventions, or preexisting disease; and if stress in the last months of life fluctuates within an individual. This is followed by a discussion of connections between the study undertaken and current theory and practice in bioarchaeology and palliative care. The chapter concludes with a discussion of the limitations of the current study and recommendations for future research.

7.1. HCC analysis validity in the dead

HCC analysis is a valid and useful measurement of stress experience in the last months of life because elevated HCC in the dead from the current study cannot be explained by non-stress related factors and because HCC shows responsiveness to stressors at the end of life (Sections 5.6, 6.3, 6.4). Research on the living has determined that HCC reliably measures long-term or chronic stress (defined as weeks or months) in living people (Davenport et al. 2006; Stalder and Kirschbaum 2012; Vives et al. 2015).

However, unlike in the living, studies of HCC in the dead and dying cannot always exclude or account for individuals receiving medication or experiencing alteration of the HPA axis due to disease, especially in past populations. HCC in the living is also not affected by decomposition. Given the lack of previous research, it is necessary to account for the possible influences of these unique factors. Despite the potentially unique circumstances surrounding the dead and dying, HCC from dead samples is reflective of stress experience.

Medication for the management of serious disease and the control of symptoms related to dying are not often investigated in HCC studies of the living. A notable exception to this rule is the study of hydrocortisone treatments (Gow et al. 2011). The results of the current study were able to exclude medications as a significant factor influencing HCC in samples of the dead. While specific medications administered to the individuals in the samples from the current study were not known, broad effects of medication could be inferred through comparison between samples with and without access to a number of advanced medical treatments (including synthetic steroids; Section 3.2.2.4). HCC values between the Terry Collection and UCF Cadaver samples do not differ significantly, even when accounting for cause of death, despite differences in the availability of medical treatments (Section 3.2.1.3; 3.2.2.4; 5.2). In contemporary North American groups, pharmaceutical interventions directed at curing disease and managing symptoms are often significant in the last months of life (van Esch et al. 2016; Donegan and Bancos 2018). In a study of nursing home residents in the United States the average number of prescriptions per individual at the end of life was 7.90 ± 3.27 (Chun et al.

2010) while in a study of individuals receiving hospice care, the average number of medications was 15.7 (range 1-100) (Sera et al. 2014). Kaufman and colleagues (2002) found that 91% of community dwelling males over the age of 65 and 94% of females were prescribed at least one medication (65% of the UCF Cadaver population was over the age of 65). Prescriptions of multiple medications is also common among individuals experiencing chronic conditions, and in the UCF Cadaver sample 80% of individuals died of a chronic condition according to WHO definitions (Elseviers et al. 2016; Section 5.4). At the same time, for those not receiving palliative care or in nursing home facilities, medication non-adherence for chronic conditions can be as high as 40-50% (Kleinsinger 2018). Information regarding the prescription of specific medications or location of death in the UCF Cadaver sample was not available. Therefore, while most of the individuals in the UCF Cadaver sample were likely taking at least one medication in the months before death it is possible that a minority were not.

According to the clinical literature, a number of contemporary medications, most notably steroids, prescribed to dying people or those with serious disease could have an impact on HPA axis activity (Section 2.4.4; Saenger 2010; Benedek 2011), and were not available to those in the Terry Collection sample. Therefore, medication is unlikely to be the primary factor accounting for generally elevated HCC values in the dead due to the lack of significant difference in HCC values between the Terry Collection and UCF Cadaver samples (Section 5.2).

Alteration of cortisol metabolism related to serious disease or dying is another factor that does not affect most living people that have been studied via HCC but must be

accounted for in studies of the dead. Given the high HCC across multiple cause of death groups in the current study and significant variation between individuals (Sections 5.1; 5.4), abnormal cortisol metabolism is unlikely to account for all elevation of HCC in individuals, or for variation between individuals. According to the clinical literature, HPA axis alteration is common in disease, however the degree of alteration and whether elevation is due to inflammation, immune response, or psychosocial stress of disease burden is rarely explored (Section 2.4). In those cases in which researchers have attempted to parse out the relative effects of the factors, psychological stress of disease burden has been identified as the primary factor influencing elevated HCC (Section 2.4; e.g., van Manen et al. 2019). Therefore, without further research regarding relative HPA axis activity in various diseases, it is not possible to exclude disease as a substantial factor influencing HCC at the end of life. Given the definition of stress employed in this study (a process involving recognition of a stressor, homeostatic imbalance, and response) changes to the HPA axis resulting from disease is still an experience of stress. However, if the HPA axis is dysregulated to the point that it no longer responds to other aspects of stress, then HCC would not be useful as a biocultural measure of stress at the end of life.

Fortunately, the dynamic nature of stress at the end of life convincingly indicates that HCC does reflect biocultural experience at the end of life. Fluctuations in HCC in response to diagnosis or contraction of disease, entry to hospital, and institutionalization identified in the Terry Collection sample indicate that HCC responds to changes in stress experience even in the last months of life (Section 5.6). That HCC responds to stressors and changes in the environment throughout the last months of life just as it does at other

points in life (e.g., Gao et al. 2014) is consistent with HCC as a measure of stress. Furthermore, fluctuations in HCC are present in individuals in the current study for which no information is available about the biocultural context of care. Notably, the fluctuations do not correspond with expected trajectories of decline in the particular disease present. For example, the end of life for those with heart failure is often characterized by fluctuations within a general long-term decline in health while a steadier decline is expected for those with cancer (Lunney et al. 2003). The most common patterns for 'Disease of the circulatory system' (high HCC and significant variation) and 'Neoplasms' (high values with general decline) (Section 5.4.1) are inconsistent with the expected pattern of functional decline reported for heart failure and cancer.

While decomposition is another factor unique to HCC analysis in samples of the dead, and therefore requires consideration, it is unlikely to account for the elevated HCC in the samples from the current study. Decomposition could affect HCC through decay of the hair matrix, breakdown of cortisol itself, and contamination by decomposition fluids. Decomposition of cortisol would lead to a decrease in HCC over time, which is not or only weakly present (Section 6.1) while decomposition of the hair matrix would be expected to increase HCC with time, which is also not present (Section 6.1). Furthermore, sweat and sebum are well recognized sources of contamination in the literature (e.g., Greff et al. 2019), and decomposition fluids would be likely to act in a similar fashion. However, those samples that were collected before decomposition occurred displayed elevated HCC (Section 5.2) and an isopropyl wash was employed for all samples, as

recommended by Greff and colleagues (2019) to account for possible external contamination.

The validity and reliability of HCC analysis in the dead is further supported by previous studies of cortisol in the dead, the finding that the values displayed by the samples in the current study are not outside of what is biologically possible, and because elevated HCC in the dead is visible in previously published archaeological studies. Limited previous research has found elevated cortisol in plasma and cerebrospinal fluid just before and after death, even in patients with severe Alzheimer's disease and patients on high-dose morphine (Sandberg et al. 1956; Swaab et al. 1994; Erkut et al. 2002; 2004). Furthermore, while HCC values of dying people are generally higher than living people, they are similar to individuals in very high stress circumstances (e.g., First Nation's communities in Canada and individuals from a slum settlement in Kenya), those with chronic pain, and those at risk for myocardial infarction (Section 6.3). Thus, the HCC values displayed by the dead are biologically plausible and consistent with high stress scenarios. The consistently high HCC in hair samples from the dead across multiple studies verifies that HCC analysis is reliable across time periods, archaeological contexts, and laboratories (Section 6.3).

The validity of HCC analysis as an accurate measure of stress in the archaeological dead is challenged by low HCC values in the Kellis 2 Cemetery sample. The limitations posed by the potential of leaching in the Kellis 2 Cemetery sample are discussed in depth below (Section 7.5). However, the ability to identify potential leaching

in the Kellis 2 Cemetery sample verifies that HCC analysis is valid and reliable in the dead in general, especially in non-archaeological samples.

7.2. Stress and dying

According to the definition of dying proposed by Kellehear (2009), dying individuals are those expecting to die in a short period of time and includes those experiencing severe disease symptoms, diagnosed with terminal disease, and expecting to end their own lives. HCC was higher in individuals dying of disease, as well as those expecting to die in the Terry Collection and UCF Cadaver samples (Section 5.3), suggesting that dying is a stressful experience across various times and places. High HCC among individuals dying of disease in the Terry Collection and UCF Cadaver samples could be accounted for by mortality risk associated with elevated HCC and the accumulation of allostatic load. However, the best explanation for high HCC at the end of life overall, among those dying of various conditions, including suicide, is that the months before death are a period of substantial stress associated with the dying process.

Dead individuals in all the samples investigated in the current study exhibit higher HCC than living people in modern communities (Section 6.3). Since HCC in samples of the dead is unlikely to be a result of only non-stress related factors (Section 7.1), dead individuals from the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples and previous archaeological studies display higher stress levels than most living people (Section 6.3; 6.4). Furthermore, in the Terry Collection and UCF Cadaver samples, those dying of disease display significantly higher HCC than those who died of abrupt events (Section 5.4). People who die suddenly of abrupt conditions or events may not experience

an extended dying period and display low levels of HCC in the Terry Collection and UCF Cadaver samples (Section 5.3).

One individual in the Terry Collection sample is reported to have died by suicide and displays elevated HCC (Section 5.7.1). The definition of dying employed in this study is from Kellehear (2009), and explicitly argues that individuals who die by suicide experience a dying period. Therefore, while the elevated HCC observed in this individual is less likely to be related to dying as distance from death increases, the effects of dying on HCC in this individual cannot be excluded. Thus, the elevated HCC in an individual dying by suicide, is not inconsistent with the finding that elevated HCC at the end of life is a result of the stress of dying.

Furthermore, according to the definition of Kellehear (2009; Section 4.1.3), not all people who die of disease experience dying if they do not expect to die. While such individuals cannot be parsed out with the information available, all the conditions causing death in the Terry Collection and UCF Cadaver samples were known to cause death at the time both groups died. Thus, most individuals likely knew or suspected they were dying as their conditions worsened. As doctors in contemporary North America are very likely to share terminal diagnosis with patients (Samuel 2013), the UCF Cadaver sample almost certainly knew if their conditions were terminal, and this may explain the finding of the highest HCC (Section 5.2) in this group.

Previous studies have established that elevated cortisol and stress can be associated with increased mortality risk in the days or years before death (Section 2.4.2). However, no studies have examined the pattern of cortisol in the months directly

preceding death. The mechanism by which cortisol may impact all-cause mortality is not clear and it is often difficult to disentangle the causation from correlation in previous studies. For example, researchers have claimed that high HCC in those experiencing myocardial infarction or poorer survival outcomes in instances of pneumonia, indicates that higher HCC predisposes individuals to a potential lethal event (Christ-Crain et al. 2007; Manenschijn et al. 2013). These studies did not report the presence of any underlying health conditions that could have affected both cortisol and mortality risk. However, the studies did not consider the possibility of high stress levels among dying people or confounding factors that could contribute to both high stress and mortality risk. In the Terry Collection and UCF Cadaver samples, individuals often did not die in the month with the highest HCC (Section 5.4.1). Therefore, the elevated HCC in samples of the dead is unlikely to be a result of people being most likely to die during periods of greatest stress. While stress may contribute to mortality risk in complex ways, the general elevation in HCC present in samples of the dead is likely because dying results in significant stress.

Allostatic load can also result in elevated HCC but is unlikely to account for high HCC in the dead in the current sample. Studies have identified allostatic load from high HCC in older living people (Section 2.3.1) but there is no relationship between HCC and age in the Terry Collection and UCF Cadaver samples (Section 5.1.1). While dying individuals could experience wear and tear regardless of age, the substantial fluctuations in HCC in the last months of life and the relatively low HCC in individuals dying of certain diseases of long duration suggest that allostatic load does not account for elevated

HCC. The variation in HCC indicates that stress in the last months of life does not accumulate across the duration of a disease (Section 5.5). Furthermore, certain causes of death such as 'Diseases of the nervous systems' or 'Diseases of the respiratory system' display relatively low HCC in comparison to other causes of death despite a long duration (Section 5.4). Similarly, life-long diseases, such as cystic fibrosis, do not reflect especially elevated HCC, which would be expected if elevated HCC was simply a result of allostatic load. Instead, the substantial and variable stress experience in the last months is accurately reflected by elevated and dynamic monthly HCC values in samples of the dead.

The high HCC values in dead individuals and the relationship between HCC and cause of death (Sections 5.4; 6.3) suggest that the effects of dying on HCC values are significant. Because dying and death have such substantial effects on HCC at the end of life, HCC cannot reflect stress in an entire population or throughout an individual's life; HCC values are subject to mortality bias (Section 2.2.2). Therefore, HCC values in the dead cannot be analyzed to understand stress levels in living healthy people in the society or in the dead individual at other times in their life; this does not diminish the utility of the method in exploring experience relating to dying and death.

Although high HCC values at the end of life are likely to be associated with the stress of dying, the beginning of the dying period could not be identified from patterns of HCC in samples from the current investigation. Little research exists on how to identify the dying period or how long it may last, however if HCC values are related to experiences of dying, one would expect that stress levels would be lower before dying

began. HCC values did not differ between the most proximal segment of HCC and more distal segments of hair in any of the samples currently investigated (Section 5.1.3). The majority of individuals in the current study had less than six months of hair present for analysis (Section 5.1.3). Research has found that symptoms relating to dying of some diseases, such as heart disease, can be present for more than six months before death (McCarthy et al. 1996). Furthermore, individuals may be admitted to hospice when they have approximately 6 months left to live but may live longer as health decline is not linear or easy to predict (National Institute on Aging n.d.). One study found that 14% of individuals who died at 65 years or older were fully functional one year before death but that a similar number (10%) were severely restricted (Lentzner et al. 1992). Therefore, it is not possible to know how long individuals may experience a dying period in the current study. Additionally, the inclusive definition of dying, in which individuals expecting to die are included, may account for the extended periods of elevated HCC in the current study.

While suffering, distress, and poor quality of life are common experiences of dying people according to the palliative care literature, the current study is one of few studies to explicitly investigate the experience of stress associated with dying (Kellehear 2009; Cherny 2011). The relationship between stress, suffering, distress, and quality of life is complex and has been discussed in detail elsewhere (Section 2.2.1.1). The finding that dying is a period of substantial stress across places and times is consistent with previous research of dying people reporting that suffering is often substantial and common, even with the development of palliative care (Rummans et al. 2000; Armstrong-

Coster 2004; Kellehear 2009; Cherny 2011). These studies emphasized that suffering is tied to physical conditions as well as social, cultural, emotional, and spiritual components (Cherny 2011). As suggested earlier (Section 2.2.1.1), stress is an aspect of experience that is holistic, broad, and may encompass distress and suffering; therefore, the study of stress provides a more holistic perspective on human experience than the study of distress or suffering alone. Additionally, although stress is still only one aspect of quality of life, the measurement of stress is closely related to quality of life; therefore, the analysis of stress can provide a closer approximation of quality of life than the measurement of suffering or distress. The high HCC at the end of life, across space and time, suggests that stress, as a component of quality of life, is an important area for future research of dying people.

7.2.1. Stress and dying cross-culturally

Stress associated with dying does not appear to be limited to a particular time or place. While the relationship between fear and dying likely has a long history (Kaufman 2005), the relationship between stress and dying has received little explicit attention. HCC values were high in both the Terry Collection and the UCF Cadaver sample relative to living populations and did not differ significantly between the samples (Section 5.2; 6.3, 6.4). In the approximately 60 years between the deaths of individuals in each collection, medical treatment has improved considerably (Section 7.1); social conditions have changed substantially; approaches to death have changed; and longevity, especially with chronic disease, has increased. Because HCC values at the end of life do not differ substantially between these samples, it is unlikely that stress at the end of life is tied to

medication, social conditions, approaches to death, or age at death. Instead, stress may be a common and deep-seated feature of the dying process.

The socio-economic conditions of the individuals comprising the UCF Cadaver sample are unknown, however, the social conditions likely differed considerably from those experienced by the Terry Collection sample individuals. The Terry Collection sample was comprised of individuals with overall low socioeconomic status who lived during periods of great social unrest (Section 3.2.2). Those in the UCF Cadaver sample lived and died during periods of relative economic prosperity alongside social tensions and climate concerns (Section 3.2.1). Given the substantially different socioeconomic contexts of the samples, the similarity in HCC values between them further suggests that HCC values at the end of life are closely tied to stress during the dying period but not necessarily socioeconomic status.

Comparing beliefs around death is challenging because they are distinctly personal and difficult to generalize (Sections 3.2.1.2, 3.2.2.3; Field and Cassel 1997). However, according to Farrell (1980) the individuals comprising the Terry Collection and UCF Cadaver samples died during two distinct periods regarding approaches to death in the United States: the dying of death (1830−1945) and the resurrection of death (1945−Present). During the "dying of death period" uneasiness with death grew, leading to fear and the development of belief systems to cope with the uncertainty of death (Samuel 2013; Lightfoot 2019). The unwillingness of doctors to share terminal diagnoses with patients contributed to feelings of uncertainty around dying (Samuel 2013). In contemporary United States, attitudes towards death are difficult to generalize and

research is more limited, but fear of death may be less significant than the fear of the experience of dying especially regarding pain and other symptoms, lack of control, and impacts on family members (Field and Cassel 1997; Emanuel and Emanuel 1998; Tilden 1999; Beckstrand et al. 2006). An interventionist approach is common and a willingness to accept death is present only when all possibilities for cure have been exhausted (Parsons 1963). Ultimately, while the specific details of the approaches to death differed between the samples, stress could be an aspect of the dying experience in each case.

Age at death differed significantly between the Terry Collection and UCF Cadaver sample (Section 5.1.1). This pattern is reflective of broader trends of individuals living longer and dying more often of chronic disease in the modern day (Walter 2003; Green 2008). People dying at older ages tend to experience greater disability for longer periods before death (Field and Cassel 1997). Research has argued that people dying of chronic illness, especially in old age, experience dying differently and require different systems of care and support than those who die of more acute conditions or die at younger ages (Field and Cassel 1997). The similarity in HCC values between the Terry Collection and UCF Cadaver samples, therefore, indicates that age at death and duration of disease or disability does not affect HCC at the end of life.

Individuals within the Terry Collection and UCF Cadaver samples differed in access to medical treatments, social conditions, approaches to death, and age at death, and yet experienced similar degrees of stress at the end of life. When considering previously published archaeological studies, the variation in factors influencing the dying process is even greater and still, HCC was elevated in the last months of life in all studies (Section

6.1). Kellehear (2007) argues that, despite changes in what people die of and how they die, death has been a source of fear throughout history. The findings of the current study indicate that stress, similar to fear, may be deeply rooted in the process of dying.

7.3. Individualized experience of stress at the end of life

Although stress was found to be consistently higher in samples of the dead than in the living, substantial variation in stress experience was discovered between individuals in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples at the end of life. In fact, variation is higher in the samples of the dead than in studies of living people as indicated by the much higher ranges and standard deviations among samples of the dead (Section 6.3). Furthermore, the variation in HCC values between individuals is higher than the amount of variation across most individual's hair shafts (Section 5.1), indicating that stress is shaped more by factors that vary between individuals, than factors that change across an individual's last months of life.

Studies of the 'good death' and care for dying people emphasize that while there are many similarities in dying experiences, significant variability between individuals precludes the development of a single, universally applicable model of care for dying people (Field and Cassel 1997). In fact, Hattori and colleagues define dying as a: "multidimensional, ceaseless individual experience based on personal and sociocultural domains of life that incorporate a person's past, present, and future" (2006:167). Substantial individual variability is also consistent with stress being an embodiment of complex, biocultural forces at multiple temporal scales (Dressler 1991). The lack of effect of duration of disease on stress experience in the Terry Collection and UCF Cadaver

samples underscores that stress experience is a complex, holistic and often difficult to predict experience from the measurement of component parts (Section 5.5). Significant variation is therefore present as expected, verifying the relationship between HCC and biocultural stress experience. The reasons for this variability are complex and difficult to disentangle.

Given the lack of effect of modern medical treatments on stress reduction (Section 7.1), medical treatment is unlikely to account for high levels of individual variation. It is possible that medical care, beyond treatment, could account for differences between individuals given the finding of a reduction in stress upon hospital entry, even when effective medical treatment was not available in the Terry Collection sample (Section 5.6). Insufficient information about quality and type of care was available to explore the effects of care on HCC variation between individuals. However, information regarding changes in care for certain individuals was available and is discussed in detail below (Section 7.4).

7.3.1. Cause of death

Cause of death can account for some of the variability in stress experience at the end of life. In the combined Terry Collection and UCF Cadaver sample, HCC values differed significantly between cause of death categories and between chronic and acute causes of death, although no difference exists with duration of disease (Sections 5.4; 5.5). Patterns of stress in the last months of life also differed between cause of death categories (Table 7.1; See section 5.4.1 for details) and often do not correlate with expected disease trajectories (Section 7.1). It should be noted that, for the individuals who died of

tuberculosis, adrenal atrophy is not reported, but cannot be excluded as a cause of declining HCC at the end of life.

Table 7.1. Common patterns of change in hair cortisol concentration (HCC) in the last months of life in cause of death categories.

Cause of death category	**Pattern of HCC**
'Injury, poisoning or certain other consequences of external causes'	Little change in the last months of life and relatively low HCC values overall
'Diseases of the respiratory system'	
'Certain infectious or parasitic diseases'	
'Diseases of the circulatory system'	High values and substantial monthly variation
'Diseases of the nervous system'	Mostly low values with little variation
'Neoplasms'	High values and a general decline towards death
'Diseases of the digestive system'	High values and little change
'Mental, behavioural or neurodevelopmental disorders'	Gradual decline or little change

Although significant variation in HCC values exists between cause of death categories, pairwise comparison revealed no significant difference when a Bonferroni correction was applied to account for multiple comparisons. There is some debate in the literature about statistical power and increase in false negative results with the use of the Bonferroni correction or any correction procedure on multiple comparisons, especially when the number of categories of comparison are high (Nakagawa 2004). Without the correction, pairwise comparisons reveal that 'Neoplasms' and 'Diseases of the circulatory system' display the highest HCC and most significant differences in the combined sample (Table 7.2; Section 5.4).

Table 7.2. Summary of prominent relationships from pairwise comparisons of total average hair cortisol concentration (HCC) between cause of death categories.

Cause of death category	**Relation-ship**	**Cause of death categories**
'Neoplasms'	>	'Certain infectious or parasitic diseases', 'Diseases of the nervous system', 'Diseases of the respiratory system', 'External causes' and 'Not elsewhere classified'
'Diseases of the circulatory system'	>	'Diseases of the nervous system', 'Diseases of the respiratory system', and 'External causes'

Notes: > = indicates "greater than"

The significant overlap in HCC values indicates that no cause of death category can be distinguished from others on the basis of HCC alone. However, some general patterns are present. 'Certain infectious or parasitic diseases', 'Diseases of the respiratory system', 'Diseases of the nervous system', and 'External causes' tend to display lower HCC values while 'Diseases of the circulatory system' and 'Neoplasms' tend to be higher (Section 5.4). Furthermore, 'Neoplasms', 'Diseases of the circulatory system', 'Diseases of the nervous system', and 'Diseases of the digestive system' display the most substantial variability in HCC values in the last months of life (Section 5.4.1). The literature on cortisol in disease provides little data about relative elevations in cortisol or patterns of HPA axis activity between diseases with which to compare these findings (Section 2.4.2).

The finding of significant variation in HCC values between cause of death categories (Section 7.2.4) is consistent with previous work exploring HCC in the dead and dying and those with serious disease (Section 2.4). The higher HCC values of individuals dying of chronic disease in the combined Terry Collection and UCF Cadaver

samples is consistent with previous findings that chronic disease is associated with elevated plasma cortisol with and without infectious origin (Lechin et al. 1995' Section 2.4.2). Furthermore, in the palliative care literature, multiple researchers have argued that cause of death affects the experience of dying (Vig et al. 2002; Long 2003). The high levels of stress and the variation between causes of death is also consistent with research finding that the experience of dying is generally similar across different diseases although important differences do exist (Gysels and Higginson 2011). The reasons for differences in experiences of dying between those dying of different causes is generally unknown in palliative care. Some studies have pointed to the importance of quality of medical care and symptoms in shaping the experience of dying, both of which are known to vary with cause of death (Emanuel and Emanuel 1998; Horne and Payne 2004). It is also possible that diseases differ in their direct or indirect effects on cortisol metabolism which could account for HCC variation between causes of death.

Medical treatments associated with causes of death are unlikely to account for variation in HCC values between cause of death categories for reasons discussed previously (Section 7.1). However, some research has indicated systemic differences in the quality of care and treatment available to people dying of different causes, specifically heart failure and those dying of cancer (Horne and Payne 2004). The sudden decline in HCC values following hospital entry in the Terry Collection sample in individuals for whom limited effective treatments were available indicates that medical care can alter an individual's stress experience independently of effective treatment (Section 7.4). While the quality of end-of-life care is unknown for each individual in the Terry Collection and

UCF Cadaver samples and cannot be directly compared, approaches to the care of dying people has changed between the deaths of those comprising each sample. More aggressive curative and interventionist approaches are available for those dying in the UCF Cadaver sample, and palliative care has developed (Section 3.2.1, 3.2.2). However, HCC does not vary substantially between the Terry Collection and UCF Cadaver samples for the same cause of death, therefore, systematic disparities in care are unlikely to account for variability in stress experience between cause of death categories.

Symptoms associated with cause of death are likely to shape an individual's end of life, and therefore their stress experience. Proponents of a 'good death' have argued that symptoms, symptom control, and fear of symptoms heavily influence the experience of dying (e.g., Emanuel and Emanuel 1998; Beckstrand et al. 2006). Specific symptoms experienced by each individual in the Terry Collection and UCF Cadaver samples are not known. While the range of symptoms present is substantial, there are no obvious differences in the possible symptoms experienced by those with high and low HCC at the end of life in the combined Terry Collection and UCF Cadaver sample (Table 7.3). Furthermore, multiple researchers have found that the most prominent symptoms at the end of life are quite similar across diseases and most often include pain, breathlessness, and fatigue (Cherny 2011; Gysels and Higginson 2011). Studies have not explored variability in the severity of this suite of symptoms between cause of death categories. Therefore, while symptoms likely play a role in shaping stress experience at the end of life, without specific information about type and severity of symptoms experienced by those in the Terry Collection and UCF Cadaver samples, it is not possible to determine

the relative effect of symptoms in producing variation in stress experience. Further research regarding types and severity of stressors in the months leading up to death could clarify how symptoms affect stress experience in the last months of life.

Table 7.3. Symptoms for conditions in cause of death categories that differ significantly in total average hair cortisol concentration (information summarized from Tables 3.3 and 3.5).

Category	Symptoms
'Neoplasms'	Loss of appetite, pain
'Diseases of the circulatory system'	Chest pain, shortness of breath, fatigue, dizziness, lightheadedness, headache, confusion, fever, irregular heartbeat
'Certain infectious or parasitic diseases'	Fever, fatigue, loss of appetite and weight, night sweats and cough, fluid accumulation in pleural cavity, sore throat, headache, stiffness of the neck, vomiting, confusion, drowsiness, coma, severe abdominal pain, rigidity of the abdominal muscles, irregular heartbeat, shortness of breath, chest pain, shaking, pain
'Diseases of the nervous system'	Headache, functional losses, loss of consciousness, muscle weakness, muscle spasm, loss of memory and intellectual function, disorientation, confusion, anxiety, frustration, seizures, pain, disordered sleep, muscle cramps, stiffness
'Diseases of the respiratory system'	Shortness of breath, discomfort, chronic cough, chest pain, rapid breathing, breathlessness
'Symptoms, signs or clinical findings, not elsewhere classified'	Bloody stool, weakness, fatigue, chest pain, shortness of breath, pain, discomfort, putrefaction of tissue, general illness

Notes: Those above thick black line display generally higher HCC than those below. 'External causes' are not associated with symptoms persisting for greater than a day.

The differences in HCC between cause of death categories could be explained by metabolic factors specific to each disease. Pathophysiology and its effect on HPA axis in disease was discussed in Section 2.4.2. Although significant literature exists exploring the

effects of various diseases on the HPA axis, insufficient information is available to contextualize the findings of the current study, in which most dying people display elevated HCC. Therefore, the effects of various diseases on HPA axis activity is a likely but difficult to explore explanation for variation in HCC between cause of death categories.

Duration of disease may influence the experience of dying (Long 2003). No correlation was found between HCC values and duration of disease in the current study, which indicates that duration of disease does not directly account for the difference in stress experience between causes of death categories (Section 5.5). However, disease duration may relate to symptomology, type and quality of care, or other factors of a 'good death' such as control, awareness, preparation, and autonomy. The relationship between these factors has not be explored in the literature and could not be explored in the current study.

7.3.2. Multiple conditions

In the Terry Collection and UCF Cadaver samples, individuals experiencing depression and various types of dementia did not display higher HCC values at the end of life than those without (Section 5.7). There is limited evidence in the literature to suggest that depression and dementia may lead to elevated cortisol levels in living people (Erkut et al. 2004; Dettenborn et al. 2012b). In the current investigation, individuals with these conditions displayed elevated HCC relative to healthy living people but similar values to other dead and dying people. If HCC is related to number and type of stressors, one would expect individuals experiencing cortisol affecting conditions in addition to dying,

to display even higher HCC values than other dead individuals. Instead, HCC values and stress experience do not have a straightforward or predictable relationship with the number of stressors, even when those stressors are medical conditions that are known to impact HCC. Because the literature on the effects of depression and dementia on HCC is mixed, the finding that multiple cortisol affecting conditions do not proportionally alter HCC, should be considered preliminary until further research explores the phenomenon.

The preliminary finding that dying individuals who experienced conditions which could have affected cortisol do not display HCC values higher than expected for their cause of death suggests that HCC analysis is a holistic measure of stress experience and is well suited to capturing the outcome of complex experiences. Dowlati and colleagues (2010) reported similar findings that HCC did not differ considerably between individuals with depression or coronary artery disease alone, or both diseases simultaneously. The results of the present study are also consistent with research into stress, which argues that responses to stressors are a product of multiple factors and not just the number and duration of stressors (McEwen 2012; Staufenbiel et al. 2013). The unexpectedly low HCC values in those with multiple cortisol-affecting conditions indicates that stress experience, as embodied in HCC, is a product of the complex interaction between multiple stressors and stress responses. As a result, it is clear that stress experience at the end of life cannot easily be predicted by measuring type and number of stressors. However, many quality-of-life questionnaires measure separate components of experience and rely on the expertise of the researcher to combine the scores into an overall quality of life score (Test and Simmons 1996, Stewart et al. 1999). Therefore, the complex interaction of multiple

stressors persists to the end of life, suggesting that the determination of overall quality of life by traditional means may be inadequate and that HCC offers a new and promising approach to capturing these experiences.

7.4. Dynamic stress experience in the last months of life

In the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples, stress levels rarely increased linearly until death. Although quality of life and functional decline is rarely linear at the end of life, that perception persists, even among those responsible for the care of dying people (Bretscher et al. 1999). However, most individuals in the samples investigated for the current study exhibited complex fluctuations in stress levels, and often displayed a decrease in stress levels in the last months of life (Sections 5.1, 5.4.1). For those in the Terry Collection sample with detailed background information and sufficient hair, a relationship was present between HCC and hospital entry, diagnosis of disease, and institutionalization.

Nearly all individuals in the Terry Collection sample with sufficient hair for observation experienced a sudden decrease in HCC with hospital entry despite limited medical treatments available (Section 5.6.1). The diseases represented consist of tuberculosis, degenerative heart disease, myocarditis, and pneumonia. The only treatment available for these conditions would have been opiates to control pain which are known to suppress HPA function (Section 2.4.4; Meldrum 2003). Opiates were often used sparingly for fear of dependence and were not reportedly administered in accounts of tuberculosis treatment (Hurt 2004; Daniel 2006). While many physicians advocated for aggressive pain management of the dying (Meldrum 2003), it is not known how often opiates were

administered at the time of hospital entry or how long opiates would have to be consumed before they may alter HCC values. Various scenarios might account for the improvement in stress levels with admittance to hospital. Receiving hospital care could alleviate stress through support, stability, rest, increased hope, or a reduction in emotional and physical labor. Care could also improve stress through opioid administration and/or access to a regular and well-balanced diet.

All three individuals who had hair long enough to overlap with the diagnosis or contraction of disease displayed significant increases in HCC in the month immediately following the beginning/ diagnosis of a disease (Section 5.6.2). The challenges of interpreting disease diagnosis or contraction from available records has been discussed previously (Section 4.1.4). Given these constraints, it is not possible to determine if the increase in HCC values was related to being diagnosed, contracting disease, or beginning of treatment. If related to diagnosis, the increased stress could relate to psychological stress and fear although there is disagreement about the effects on mental well-being of sharing poor prognoses with patients (Mack and Smith 2012). On the other hand, if the increased HCC values are a result of contraction of disease, that could be a result of the onset of symptoms or immune responses. Further research that can distinguish between disease diagnosis and contraction could clarify the cause of the increase in HCC values found in the Terry Collection sample and provide important data to distinguish the emotional impact of prognosis from the immune response to disease.

Two individuals in the Terry Collection died in a psychiatric facility. One of these individuals exhibits HCC within healthy living people ranges with little variability, while

the other individual exhibits sudden and extreme changes in HCC suggesting considerable variation in stress experience and high levels of stress. Research into stress levels of institutionalized individuals is currently lacking. However, previous bioarchaeological and historical studies of institutionalization in the Terry Collection and the 19th century found that accidents and interpersonal violence were common, suggesting poor conditions (de la Cova 2012). Historic reports also indicate violence was common and care was often inadequate within psychiatric facilities, including in St. Louis in the 19th and early 20th Century (Lael et al. 2007; de la Cova 2012). Today, violence in psychiatric facilities is a result of overcrowded wards, poor staff training, unsafe conditions, and staff shortages, which could have also contributed to poor conditions in the past (Birnbach 1981, Owen et al. 1998). Limited existing work exploring the effects of mental health on HCC suggests that the effect size of such conditions is smaller than stress (Staufenbiel et al. 2013), however, without knowing what condition the individuals in the Terry Collection sample experienced, it is not possible to compare their stress experience with published reports. The findings of this study suggest that the experience of institutionalization varies between individuals and can be highly stressful and potentially unstable. Further research examining stress experience of individuals living with mental health conditions or in institutionalized settings would be necessary to determine the generalizability of these results.

Although fluctuations in HCC are clearly visible in all individuals from the current study, the effects of growth cycle error should be recognized. It is possible that the retention of a large proportion of hairs in a resting phase could increase the lag

between HCC values in anagen phase and mixed phase hairs. This lag could obscure the pattern of HCC somewhat or affect the absolute values of HCC in the months leading up to death. While such a lag may dampen some of the extreme values visible in patterns of HCC, it would not significantly affect average HCC values and would not alter the general pattern observed in HCC. When investigating temporal changes, the lag may obscure responses to events such as hospital entry, but these errors were accounted for by examining more than one month of HCC for change.

Fluctuations in stress at the end of life, especially the stress reduction associated with hospital entry indicates that the HCC is a dynamic and valid measure of stress in this period and is influenced by more than disease and physical decline. Stress is a dynamic experience at the end of life and is shaped by interventions, changes in health, and, possibly, changes in perception of health. The palliative care literature has long argued that quality of life, physical decline, and suffering can all fluctuate in the period leading up to death (Bretscher et al. 1990; Krikorian et al. 2012). Research on stress also attests to its dynamic and responsive nature in living people (Goldstein and McEwen 2002). Thus, the dynamic nature of stress at the end of life is not surprising and provides further support that assessments of experience in this period must take into account change. Furthermore, given that the goal of palliative care is ultimately to relieve suffering and improve quality of life (Terry et al. 2006), the response of HCC to hospital entry in the Terry Collection sample provides promising insight into the effects of care in the final months of life. The improvement of stress with hospital entry is consistent with other research suggesting that medical treatment alone is insufficient to improve quality of life

at the end of life (Kayser-Jones 2002; Mount 2003). Although the specific aspect of hospital care that accounts for improved stress levels cannot be identified from the available data, it is clear that it is not related to advanced medical care or cure of disease. Thus, care has great potential to improve stress experience at the end of life, and HCC analysis is well suited to capturing the outcomes.

7.5. HCC in human remains

Although the HCC values of the Kellis 2 Cemetery sample are higher than many reported for samples of the living (Section 6.2 and 6.3), the values are remarkably low in comparison to the majority of other archaeological studies. Possible explanations for such low values include sample composition and unique stress experience or instrument error or leaching. HCC values in the archaeological Kellis 2 Cemetery sample were significantly lower than the Terry Collection or UCF Cadaver samples and most previously published archaeological studies (Sections 5.2; 6.2). Unlike the current study, some previous archaeological studies do not report carrying out an isopropyl wash to remove surface contamination (Section 6.2); which could make the Kellis 2 Cemetery sample values appear more abnormal than they are. At the same time, the Kellis 2 Cemetery sample was not significantly different from two previously published archaeological studies (López-Barrales 2015; Schaefer 2017), one of which did carry out an isopropyl wash (Schaefer 2017) or from values for living healthy people (Section 6.3, 6.4). Additionally, the highest HCC values in the Kellis 2 Cemetery sample are similar to the other samples in the current study and the minimum values are similar to the lowest values from the UCF Cadaver sample (Section 5.1). Furthermore, the range of values in

the Kellis 2 Cemetery sample is greater than the difference between the average values for the Kellis 2 Cemetery sample and all other samples (Section 5.1). The HCC values of the Kellis 2 Cemetery vary considerable and generally overlap with the other samples in the study, which could suggest that the low average HCC is a product of sample composition or differences in stress experience between the populations. However, the Kellis 2 Cemetery sample displays values significantly lower than the Tisdale and colleagues' (2019) study which is drawn from the same sample and did report carrying out an isopropyl wash (Section 6.2). Therefore, the very low values of the Kellis 2 Cemetery sample are concerning and suggest leaching is present.

The possibility of leaching raises questions about the validity of the results from the Kellis 2 Cemetery sample and other archaeological samples. Without being able to account for the effects of leaching on the data, drawing further conclusions about changing causes of death, approaches to death, and lifetime stress experience from the Kellis 2 Cemetery sample is limited at the present time. The low HCC values in the Kellis 2 Cemetery sample also highlight the problems of comparing HCC values in the dead to those of the living. Without comparing the Kellis 2 Cemetery sample with other samples of dead and dying individuals, low values would not have been questioned and the possibility of leaching would not have been identified (see Section 7.5.3).

7.5.1. Sample composition

HCC values display considerable individual variation in the current study (Section 5.1) and the Kellis 2 Cemetery sample comprises the largest sample of dead individuals to date. Therefore, sampling bias and factors related to sample composition, such as cause of

death, could have an effect on HCC values. When plotted against mean HCC in all samples from the current and previous studies, sample size has a weak negative effect (Section 6.2). This effect is even weaker when those samples that do not report an isopropyl wash are removed (Figure 6.4). Furthermore, the standard deviation in the Kellis 2 Cemetery sample and the Tisdale and colleagues (2019) sample are not statistically different. Therefore, sample size does not explain the smaller HCC values in the Kellis 2 Cemetery sample.

Differences in the distribution of causes of death could lead to low values in a sample. In the Terry Collection and UCF Cadaver samples individuals dying of abrupt conditions display significantly lower HCC than those dying of chronic conditions while individuals dying of 'Certain infectious or parasitic diseases' tend to display lower HCC values than most other causes of death (Section 5.4). Based on the HCC values and percentage of individuals dying abruptly in the Terry Collection and UCF Cadaver samples, a higher number of individuals in the Kellis 2 Cemetery sample would have had to die of abrupt conditions, to account for the low values of the sample without leaching (Section 5.3). Thirty-four percent of a 268 individual sub-sample of the Kellis 2 Cemetery sample displayed evidence of skeletal trauma (Graham 2016), which may suggest that accidents or violence were common. However, studies on modern populations find no correlation between lifetime fracture prevalence and cause of death (Scholes et al. 2014; Office for National Statistics 2020). Alternatively, extending the linear relationship between the Terry Collection and UCF Collection sample, indicates that a much higher percentage of individuals in the Kellis 2 Cemetery sample would have had to die of

infectious conditions to explain the low values (Section 5.3). Distribution of causes of death have changed throughout history. In the 21st century, more people die of chronic conditions and fewer die from infection than in the past (Olshansky et al. 1997; Omran 1998; Kellehear 2007; Green 2008).

It is very likely that more individuals in the Kellis 2 Cemetery died of abrupt causes and infectious causes than in the other samples in the current study, which could account for lower HCC values in the sample. Inferring the exact percentage of individuals dying from particular causes is not possible, and the predictions above should be considered only broadly. However, the predictions show that differences in the distribution of various causes of death could each, independently explain the low HCC values in the Kellis 2 Cemetery sample in ways that are consistent with historical records. Changes in causes of death cannot account for the significantly lower values relative to Tisdale and colleagues' (2019) study.

7.5.2. Stress and experiences

The stress associated with dying is a complex process subject to numerous biocultural variables, which can be shaped by differing approaches to and expectations of dying and death as well as previous stress experience across life (Steinhauser et al. 2000). Any one of these factors could result in low HCC values. During the early Christian period, Egyptians may have viewed death as both a source of fear and hope, and attempts were made to postpone as well as manage dying and death, although individual beliefs cannot be known from the materials available (Meskell 2001; Campbell 2008; van Middendorp 2010; Mutie 2015). Therefore, beliefs about death could have differed from

the Terry Collection or UCF Cadaver samples (Section 3.2) although the amount of variation cannot be known. Beliefs about dying in the Kellis 2 Cemetery are further complicated by the presence of infants and young children as their views on dying are not known from the records available. Given the possibility that individual beliefs could have still included aspects of anxiety, fear, pain, and ultimately stress, differences in beliefs around dying are a potential but unlikely explanation for the substantially lower HCC values in the Kellis 2 Cemetery sample.

In each sample from the current study, the number of possible stressors to which individuals may be exposed is innumerable. Sociocultural contexts and differences between the Terry Collection and UCF Cadaver collection have been discussed previously (Section 7.2.1). While the settlement of Kellis was of high socioeconomic status with abundant resources, the society also faced seasonal cycles of food shortages, extremes in weather, infection, social stratification, long distance travel, dehydration, and dangerous childbirth (Hope 2001; Churcher 2002; Parr 2002; Williams 2008; Dupras et al. 2016). Given the limited information available, it is not possible to predict how overall stress levels might have differed between populations prior to the dying period. Thus, sociocultural differences in stress, unrelated to dying, cannot be excluded as a factor in explaining the lower HCC values in the Kellis 2 Cemetery sample.

7.5.3. Leaching

The very low HCC values of the Kellis 2 Cemetery sample compared to other samples of dead is best explained by leaching. Leaching refers to the loss of HCC following death due to decomposition of the hair shaft and subsequent removal of cortisol

via water activity (Webb et al. 2010). Previous research has discussed the risk that leaching presents in archaeological samples, but no method for assessing the potential or degree of leaching has been developed. Webb and colleagues (2010) argued that leaching could be excluded as a cause of concern in their study because values were not uniformly low, variation across the hair shaft was consistent with biological processes, and the burial environment was not conducive to leaching (i.e., because it is dry and arid). In the Kellis 2 Cemetery sample, although the burial environment is not conducive to leaching and variation across the hair shaft is present, values are generally low.

The leaching in the Kellis 2 Cemetery sample is unlikely to be a systematic decline due to length of burial. No decline in HCC over time is present when samples from the current study are compared to previously published studies (Section 6.2). The significantly lower values in the Kellis 2 Cemetery sample as compared to the previous data obtained from the same cemetery by Tisdale and colleagues (2019) is the most striking evidence that leaching could have occurred in the Kellis 2 Cemetery sample and that not all samples from the cemetery were affected evenly. The similar maximum values in the Tisdale et al. (2019) and the Kellis 2 Cemetery sample but much lower minimum value in the Kellis 2 Cemetery value further suggest leaching may have occurred unevenly across the sample. Water washes during sample preparation could be a possible source of the leaching. These were deemed necessary in the current study to remove adherent sediment, resin, and mummified tissue, which could not have been removed otherwise and would have contaminated HCC results. While Tisdale and colleagues (2019) reported washing in water for five minutes, the duration of washing was more

extensive and differed between samples in the current study, depending on the amount and type of adhered substance. Webb and colleagues (2010) suggest that soaking hair in water can result in loss of cortisol, but others disagree (Hamel et al. 2011) and water washes are reported in some archaeological studies (Tisdale et al. 2019) while most do not report how they removed particulate matter or mummified tissue.

The possibility of leaching is supported by the current evidence. Previous studies, especially the Tisdale et. al (2019) study, did contain very small sample sizes and many did not report having carried out an isopropyl wash. Differences in cause of death as well as population health between the Kellis 2 Cemetery sample and previously published archaeological studies are also possible. Additionally, the Tisdale et al (2019) study only examined hair in the anagen phase which could introduce error when comparing the last months of life. Despite these factors, at this time, leaching is the best explanation for the low values in the Kellis 2 Cemetery sample and future validation studies are recommended.

7.6. Embodiment and the 'good death'

The results of the current study suggest that HCC values at the end of life in the past and the present are best interpreted through a framework that combines aspects of embodiment and the 'good death'. The substantial variation in HCC across individual hair shafts in the samples in the current study, indicate that monthly experiences can shape the biochemical composition of a body in a material fashion, even at the end of life. The dynamic natures of embodiment and the 'good death' is often overlooked. In bioarchaeology studies of skeletal indicators of stress focus on cumulative effects of

embodiment (Martin et al. 2013), while studies of quality of life in dying people often rely on measurements or questionnaires taken at a single moment in time (Krikorian et al. 2013). Furthermore, elderly individuals, especially those close to death, are often believed to be unresponsive to social context (Pickard 2013). However, many authors have discussed embodiment as a dynamic process in which bodies are created and recreated across the life course (Fox 1994; Hoy 1999; Ingold 2013; Palsson 2013a). Similarly, the 'good death' framework argues that the creation of a 'good death' is an ongoing, multidimensional process (Hattori et al. 2006). The response of HCC to change in the current study emphasizes the importance of a dynamic approach to embodiment and the 'good death'.

The complexity of correlating variation in HCC values with purely medical or biological factors support the assertion that material outcomes of embodiment, in this case HCC, is the result of the complex interaction of multiple biocultural factors that are holistically embodied. In the Terry Collection and UCF Cadaver samples HCC values cannot be easily predicted by duration of disease, types of preexisting conditions, or access to medical treatments. Although cause of death accounts for some variation in HCC values, much of the variation cannot be explained by the biological data available. The literature on embodiment emphasizes that experience is a product of interconnected biological and cultural factors acting at multiple scales to shape material bodies (Csordas 1990; Palsson 2013b). Similarly, the 'good death' framework highlights the importance of physical, social, and emotional factors in shaping the dying experience (Hattori et al. 2006). However, the limited existing research on cortisol at the end of life has focused on

physical factors. For example, Erkut and colleagues (2004) argued that elevated cortisol in postmortem cerebrospinal fluid was a result of "organic stress" because individuals with severe Alzheimer's disease and those receiving high dose morphine display elevated HCC but would be unlikely to experience psychological stress. This tendency to view those in in old age, especially nearing death, as biomedical, asocial beings is common in clinical research (Pickard 2013).

Embodiment and the 'good death' concepts are valuable, complementary approaches to the study of the dead. High stress at the end of life in the current samples confirms that dying is a unique experience that must be understood on its own terms. Substantial individual variation in the dead and the challenges of correlating HCC with strictly biological factors, indicates that dying is a uniquely personal experience shaped by myriad biosocial factors. Fluctuations in HCC across individual hair shafts, especially in response to change, highlight the dynamic nature of stress and embodiment at the end of life. Therefore, the findings of the current study demonstrate the dynamic nature of embodiment, establish the importance of considering embodiment at the end of life, and affirm that experiences are embodied holistically. Bioarchaeology could benefit from a focus on dying as a complex process while making an effort to emphasize the dynamic and holistic nature of embodiment. Because stress is a significant component of the experience of dying, and a mechanism of embodied experience, stress offers a valuable approach to understanding the experience of dying within a 'good death' framework.

7.7. Implications for practice in bioarchaeology

HCC analysis is best suited to the study of the dying period in bioarchaeology and can shed light on a unique and universal aspect of human experience while providing insight into the relationship between dying, death, and skeletal remains. In the current investigation, HCC is generally high in samples of the recent and historical dead, individual variation is high, and cause of death affects HCC values in samples of the dead. Although the HCC values of the Kellis 2 Cemetery sample are lower than other archaeological studies, they are still higher than HCC values reported for most living healthy people or living people with pathological conditions (Section 6.3). The high stress levels and significant variation in the final months of life suggest that there is much to be learned from investigating this complex and stressful period but the effect of cause of death on HCC indicates that patterns of HCC in samples of the dead are shaped by mortality bias (Section 2.2.2). However, the difficulty of identifying the variables responsible for differences in HCC values between samples, highlights the need for caution when comparing stress levels in groups from substantially different contexts. Comparisons between populations may only be useful when the variation in stressors between them is small or only broad conclusions are desired.

The high HCC in samples of the modern and historical dead as compared to the living and the effects of cause of death on HCC, suggest that dying impacts HCC at the end of life. As such, HCC values in samples of the dead are subject to mortality bias; they do not reflect stress in a population or throughout an individual's life and cannot be compared appropriately to samples of living healthy individuals. Furthermore, the

beginning of the dying period could not be identified from HCC, thus the effects of dying on HCC cannot be excluded at any point along the hair shaft. The effects of mortality bias do not reduce the utility of HCC analysis. Instead, HCC can be used to investigate experiences of dying and may even be useful in exploring the effects of factors creating assemblages of human remains, such as cause of death (Section 2.2.2). For example, it is likely that cause of death differs with age in the Kellis 2 Cemetery sample. In the Kellis 2 Cemetery sample, total average HCC differed significantly between 10-year age groups (Section 5.1) which could be due to the significant variability in HCC values between causes of death (Section 5.4). Contemporary data indicates that cause of death differs considerably with age (Centers for Disease Control and Prevention 2018). Therefore, the current study may provide evidence that cause of death can shape age distribution in assemblages of human remains. Cause of death is rarely identifiable from the skeleton; thus, this relationship cannot be explored directly in skeletal assemblages although it has implications for paleodemography and studies of skeletal indicators of stress.

Reframing studies of HCC around dying and death could also provide contextual information for case studies of disease. Although HCC cannot be used to determine cause of death, it can be used to shed light on changes in stress experience in the months leading up to death that could contextualize or support a diagnosis from other lines of evidence such as historical documents or mummified tissues. While this approach would have limited utility, paleopathological case studies still hold value in the field and may benefit future studies, diagnoses, and syntheses (Stojanowski and Buikstra 2005; Mays 2012).

In the current study HCC values from juveniles and adults in the Kellis 2 Cemetery sample are higher than values reported for most living populations, providing limited evidence of stress at the end of life (Section 6.2, 6.3). Additionally, HCC values among the 0-9 age group are higher than the other age groups in the Kellis 2 Cemetery sample, which is consistent with findings from Binz and colleagues (2017) and De Kruijff and colleagues in living groups (2020; Section 2.3.2). Given the challenges of validating the data from the Kellis 2 Cemetery sample using the current sample, these results should be interpreted cautiously but suggest that HCC values in adults and juveniles should be compared prior to combining the samples together.

Recent applications of HCC analysis have shed light on the possibility of HCC analysis in archaeological settings but have been unable to consider the effects of dying on HCC and therefore the utility of HCC in studying the dying period remained unverified (e.g., Webb et al. 2010). The relationship between mobility, trauma, or dietary changes and stress levels, have been explored in previous bioarchaeological research, but the findings of the current study suggest the effects of dying and death can never be excluded as possible explanations for patterns of HCC values in dead individuals, especially when values are high. As such, bioarchaeological studies should first consider the impact of disease and dying and factors related to dying to explain patterns of HCC values and should limit comparison to living people. Framing studies of HCC in bioarchaeology on the dying period has the potential to shed light on a unique experience, provide data about the effects of mortality bias, and add contextual information to case studies.

7.8. Implications for practice in palliative care

HCC presents an effective new analytical tool in the evaluation of experience of dying and care of dying people. Substantial individual variation in HCC in the current investigation confirms that HCC can accurately and sensitively identify differences in experience at the end of life. The change in HCC in response to hospital entry in the Terry Collection verifies the use of HCC analysis to track the effects of interventions.

The application of HCC analysis in the current study revealed that stress levels at the end of life have been high throughout history and remain high today, despite medical advancements (Section 7.2.1). In the 60 years between the deaths in the Terry Collection and UCF Cadaver samples, the ability to treat and cure a large number of conditions has led to individuals living longer lives and dying of more chronic conditions, yet the stress of dying has not improved. Stress is closely tied to distress, suffering, and quality of life and high levels of stress at the end of life are consistent with a high level of distress and suffering and lower quality of life (Section 2.2.1.1). However, individuals in the Terry Collection sample displayed a short-term alleviation of stress following hospital entry although curative treatments were not available, and they died a few months later (Section 7.4). The improvement in stress levels suggests that care, but not necessarily medical treatment, can have a profound effect on stress levels, and by extension, distress, suffering, and quality of life. Palliative care has long argued that medical care at the end of life is often inadequate to alleviate suffering (Shega et al. 2008). Some have even suggested that medical treatment has a limited effect on quality of life at the end of life (Stewart et al. 1999) and that prolonged curative treatment can increase suffering (Cassel

1998; Tilden 1999). The findings of the current study support the assertions that medical treatment alone is insufficient to improve suffering at the end of life and provides promising support for the potential of whole person care to improve stress and quality of life, even at the end of life (Mount 2003).

A substantial obstacle to the improvement of end-of-life care is the limitation of current methods for measuring the effectiveness of hospice care, impact of management strategies, quality of life at the end of life, and changes in experience over time in dying people (Sprangers and Aaronson 1992; Brody et al. 1997; Cohen et al. 1997; Mak and Clinton 1999; Hanson et al. 2002). Although distress, suffering, and quality of life have been important areas of research, few evaluation methods currently in use in palliative care explicitly consider stress (Section 2.2.1.1; Smith et al. 2005). Key features of a successful tool for the measurement of experience at the end of life have been discussed previously (Section 2.2.1.2, Table 2.1). HCC analysis addresses many of the needs of an effective tool for measuring end of life experience.

7.8.1. Methodological strengths of HCC analysis

HCC analysis is accurate and reliable, cost effective, non-invasive, comparable, objective, and can be used retrospectively or prospectively. Unlike questionnaires which are validated only by their consistency with each other (Cohen et al. 1995; 1997), the high HCC in individuals dying of disease, changes in response to hospital entry, and the extensive testing of HCC analysis to measure stress in living people, validate the accuracy of HCC analysis as an independent measure of stress at the end of life (Section 7.1). ELISA is low cost, especially if analyzing multiple samples at once. Minimal training is

needed to collect and prepare samples, thus existing staff can carry out much of the process. Furthermore, unlike more invasive testing and even prolonged questionnaires which may have negative effects on quality of life in dying individuals (Kellehear 2009), the collection of samples for HCC analysis is minimally invasive and can limit further distress. HCC analysis is also well suited to comparing care outcomes between health care settings, types of providers, or approaches to care which cannot be controlled for in practice (Stewart et al. 1999). The best comparisons would measure an individual's HCC before and after changes in care, but comparison between dying individuals is possible.

Unlike most tools to measure experience at the end of life, HCC analysis can be used in still living people to track the effects of interventions and inform care strategies as well as in dead individuals to investigate experience up until the very end of life. Identification of the dying period is often only possible after death, therefore most studies of dying tend to focus on those with terminal disease such as cancer, as the dying period can be more easily identified (McCormick and Conley 1995; Fowler et al. 1999; Leichtentritt and Rettig 2001; Kellehear 2009). Given the definition of dying proposed by Kellehear (2009; Section 4.1.3), high HCC in an individual dying of suicide in the Terry Collection sample may indicate that dying could be a stressful period even when it may not be identifiable prior to death. The ability of HCC to provide retrospective insight about dying for individuals for whom the dying period may be difficult to identify before death, is an important contribution of HCC analysis (Stewart et al. 1999). Such research could have important implications for improving care for future patients and broadening the understanding of dying across causes of death.

7.8.2. Conceptual advantages of HCC analysis

Dying is a complex process that is inherently difficult to study. HCC analysis provides a number of novel and conceptual benefits over previous methods in the studying of dying: it is patient centric, holistic, responsive to interventions, and relevant to the dying period.

Patients deserve to be heard and take an active role in their own care and proxies are often inaccurate or unreliable (Sprangers and Aaronson 1992; Terry et al. 2006). Krieger (2005a) has argued that embodied bodies tell stories that individuals cannot. Thus, HCC can speak about individuals' experience of stress and dying in ways that dying people may not be able. Analysis may provide insight into experience of those who are unconscious, experiencing dementia and are unable to communicate, or do not speak English (Kayser-Jones 2002). Researchers have specifically called for measurements of quality of life in these groups as they are often underserved in current palliative and hospice care settings (Rummans et al. 2000; Kayser-Jones 2002). Furthermore, because the dying period is often only recognized with hindsight, HCC may be the only way to assess experience from the patient's perspective after death.

Most assessment tools measure various components of quality of life separately and relies on the patient or researcher to combine these into an 'overall summary' of quality of life (Test and Simmons 1996; Stewart et al. 1999). The result is a vague and ambiguous measure of end-of-life experience which cannot account for the multiple and complex dimensions involved in experience and quality of life and the ways they interact (Test and Simmons 1996). Unlike other measures, HCC reflects stress the way it is

experienced by individuals instead of artificially broken down into component parts, which avoids assumptions regarding the relative importance of different components or how multiple stressors may interact. In the current study, the difficulty of predicting stress levels through the measurement of component parts, such as preexisting conditions, duration of disease, medical care, and context of care, confirms that HCC measures holistic stress experience. Such an approach is particularly important when considering psychosocial aspects of stress and wellbeing at the end of life, which health care providers are often unable to assess accurately (Rummans et al. 2000).

A clear and concise measure of response to intervention is necessary for health care workers to understand the effects of interventions and plan improvements in care (Test and Simmons 1996). HCC analysis is well suited to this goal as a single hair sample can reflect stress levels before and after an intervention. Such an approach is highly accurate and objective as it only compares values within an individual.

Many measures of quality of life at the end of life do not take into account unique experiences associated with dying, may focus on health outcomes, or compare the current state of a dying person to a previous healthy state (Emanuel and Emanuel 1998; Bretscher et al. 1999). Patterns of HCC in the current sample under investigation have shown that stress can fluctuate in the last months of life, independently of physical decline (Section 7.4). Therefore, HCC values can be used to evaluate experience in the last months of life without reference to living people, previous functional ability, or health status.

7.9. Limitations and future directions

As the first study to explore stress at the end of life, while explicitly considering the effects of dying, the current study was able to identify key patterns of HCC necessary to answer important research questions and provide a foundation for future work. However, the study was faced with small sample sizes, lack of contextual information, and possible leaching. As a result, and given the number of variables that can influence stress and the experience of dying, various details about the experience of dying were beyond the scope of the research.

The size of the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples were sufficient for addressing the main research questions. However, when broken down, group sizes were often small and the number of individuals with sufficient hair or contextual information was limited. Small sample size in each cause of death category limited the utility of pairwise comparisons and the identification of generalizable patterns. Furthermore, the number of individuals who had data regarding, and hair reflecting, diagnosis of disease and entry into hospital was limited. For those individuals with a duration of disease recorded, it is not clear if it reflects the beginning of the disease, the appearance of symptoms, or the diagnosis of disease. Therefore, the relationship between HCC and hospital entry or disease diagnosis could not be rigorously tested and the finding that duration of disease may not affect HCC at the end of life may have limited scope.

Perhaps the most significant limitation of the current study is that leaching cannot be excluded as a factor influencing the Kellis 2 Cemetery sample. As a result, attempts to

explore patterns of stress experience or cause of death in an archaeological sample were limited and the investigation could not draw rigorous conclusions about universal aspects of the dying process from ancient times until today. Furthermore, because the Kellis 2 Cemetery sample was the only group in the current study to contain juvenile human remains, the ability to evaluate the experience of dying across age categories was limited.

The current study highlights promising avenues of future research in bioarchaeology and palliative care. Reanalysis of a small number of hair samples would be useful in verifying the results from the Kellis 2 Cemetery sample or determining if leaching occurred and was exacerbated by the laboratory methods employed in the current study. Examining hair degradation in the context of HCC analysis more generally would also be beneficial, especially in regard to developing an understanding of the mechanisms of leaching and tools for identifying it. To verify the results of the current study regarding the impact of care on stress levels at the end of life, an investigation of different types and aspects of care on stress levels would be necessary. Such studies could explore hospital versus home or hospice care, medical interventions, and medication and would benefit care of dying people as well as the interpretation of HCC in bioarchaeology. The final step needed to establish the utility of HCC in palliative care is to compare HCC results against preexisting methods for evaluating quality of life and care at the end of life. Comparing findings from a study of HCC with results from questionnaires could support the validity of HCC as a useful component of measuring suffering and quality of life, especially in response to changes in care strategies. Future

studies examining the stress of not just the dying person, but their families and caregivers would also be a valuable contribution to studies of dying experience in modern people.

CHAPTER 8 - CONCLUSIONS

The aims of the current study were to validate the use of HCC analysis in the study of stress in the dying and dead, to assess the presence of individual and monthly variation in stress experience, and to evaluate the effects of biocultural factors on variation in stress experience in the last months of life. The experience of dying, disease, and care was explored through the comparison of HCC in the dead and the living and investigation of the relationships between HCC, cause of death, duration of disease, and medical care and treatment. The results clearly illustrate that HCC is a valid and useful measure of stress in samples of the dead. In investigating stress experience, patterns of HCC in the Kellis 2 Cemetery, Terry Collection, and UCF Cadaver samples revealed that stress levels are high in the last months of life, stress experience differs substantially between individuals, and stress experience can vary considerably over the last months of an individual's life. The combination of embodiment and the 'good death' perspectives allowed this study to interpret lived experience from the chemical alterations of human hair. At the same time, the investigation could prioritize understanding the dying experience and considering the complex interaction of biocultural factors in the production of these experiences. These results indicate that the stress of dying is considerable throughout time and that stress at the end of life is an individualized experience resulting from the interaction of multiple biocultural factors that continues to be dynamic and may be improved.

8.0. HCC applications to the dead

Despite the limitations posed by the potential of leaching in the Kellis 2 Cemetery sample, the suite of findings from the current study validates the relationship between HCC and stress at the end of life and verify the utility of HCC analysis to study stress in the dead and dying. The high HCC in the last months of life in the Terry Collection, and UCF Cadaver samples cannot be easily accounted for by non-stress related factors, are within possible biological ranges, and continue to be dynamic and responsive to change during the dying process. Although HCC has been applied in archaeological studies of the dead, such studies have not considered the potential unique factors and circumstances surrounding serious disease, dying, and death on HCC values. By addressing this gap in the literature, the current study confirms the utility of HCC analysis in studies of the dead and sets the foundation for further investigations.

8.1. Stress of dying

The findings of the current study verify that stress is high at the end of life and that it is related to the experience of dying. HCC is high among the dead from the current study as well as previous studies of dead individuals. The high HCC persisted across time despite dramatic changes in access and effectiveness of medical treatments, socioeconomic conditions, beliefs about death, and life span. Individuals that did not die of disease but may have expected to die because they died by suicide, also exhibit high HCC.

Although elevated HCC is consistent with higher mortality risk associated with stress and high cortisol levels, the pattern of HCC at the end of life indicates that HCC can fluctuate considerably at this time and that individuals are not more likely to die when their cortisol values are highest. Similarly, while high HCC may suggest the accumulation of allostatic load, the variation in monthly HCC is more consistent with the embodiment of significant and dynamic stress experience in the months leading to death. If dying individuals are defined broadly as those expecting to die in a short period of time, high HCC in samples of the dead suggests that dying is a stressful experience. That the last months of life have consistently been periods of significant stress across multiple times and places speaks to the shared nature of this experience and, in accordance with a 'good death' framework, highlights the value of focusing on the dying experience. Substantial previous research indicates that suffering and distress are high at the end of life and quality of life can be low (Kellehear 2009; Cherny 2011). As the first to directly measure stress experience in the months before death, the current study offers concrete validation of these previous studies in a biocultural, diachronic context.

8.2. Individual variability in dying experience

Significant individual variation in HCC in samples of the dead speaks to the complex biocultural, and ultimately individual nature of dying across times and space. Although HCC was particularly high in the historic and modern samples under investigation, significant variation was present between individuals. Variation was higher between individuals than across the last months of a single individual's life and was greater than in most studies of the living. The substantial variation in HCC values

between individuals emphasizes that stress at the end of life is a highly individual experience that can be captured in HCC. Some factors that were expected to influence HCC did not, such as duration of disease, presence of cortisol affecting conditions, and medical treatment. The lack of effect of these factors point to the complex and biocultural nature of stress and of dying. Cause of death accounts for some variation in stress which could relate to pathophysiology, symptomology, or experiences of disease. However, much of the variation between individuals could not be explained by the data available. These findings, building from a 'good death' framework, indicate that the stress experience in the last months of life is a complex product of the interaction of multiple factors and that it cannot, necessarily, be predicted by analyzing each factor independently.

8.3. Dying is a dynamic experience

HCC values fluctuate considerably in the last months of individual lives suggesting that stress at the end of life continues to be a complex and dynamic experience. Changes in stress experience were particularly noticeable in response to complex biocultural experiences of hospital entry, disease diagnosis, and institutionalization. The substantial variation in HCC in the dead emphasizes that stress is a dynamic process that persists, even at the very end of life and further validates HCC as an embodiment of stress in the last months of life. Stress was not tied solely to worsening health status as death approached, highlighting the holistic nature of stress experience in this period. An increase in stress levels in response to disease diagnosis emphasizes that stress response is dynamic but determining whether the increase was due to the

psychological effects of poor diagnosis or pathophysiological effects of disease onset was not possible. The improvement in stress levels in response to hospital entry indicates that the experience of disease or dying does not prevent stress levels from improving in response to care, even when effective treatments were not available. The erratic HCC levels in an institutionalized individual could be tied to unstable and often violent conditions of psychiatric facilities or the complex biosocial experience of mental health conditions; further information would be necessary to disentangle these factors. The variable and responsive nature of HCC in the final months of life further validates the relationship between HCC and stress experience in this period and highlights that stress at the end of life is an ongoing, dynamic and complex experience for each individual.

8.4. Contributions to bioarchaeology and palliative care

While the findings that stress is high, individual, and dynamic are not unexpected, the current study is the first to provide concrete and objective evidence of stress at the end of life across time. As such, the results provide an important and necessary foundation for the study of HCC and stress in dying and dead people and makes significant contributions to bioarchaeology and palliative care. In general, the findings of the current study emphasize that stress in the months before death is a complex and biocultural process, HCC is well suited to the study of dying experiences, and HCC analysis would make a promising addition to current research tools used to investigate the dying period.

This study has revealed that the last months are diverse and dynamic periods of individual lives that hold great value for study in bioarchaeology. The high HCC at the end of life suggests that studies of HCC in bioarchaeology must seriously consider the

effects of dying on HCC and employ comparisons to appropriate reference samples. At the same time, the high variability identified in dying individuals provides evidence that dying can be studied in the dead and that sufficient variability exists for fruitful investigation. Because HCC is closely tied to dying experience, HCC analysis holds great potential in the investigation of aspects of mortality bias in assemblages of human remains. The low HCC values in the Kellis 2 Cemetery sample highlight a need for caution in the analysis of HCC from archaeological samples, but suggest that with appropriate comparisons, the potential of leaching can be identified. By employing a framework of embodiment and the 'good death', HCC analysis in bioarchaeology can investigate HCC as an embodiment of lived experience and focus on the dying experience. Such analyses have the potential to shed light on the experience of disease and dying in the past and present and the forces shaping assemblages of human remains.

The results of the current study also indicate that HCC analysis holds great potential in the study of the dying experience in modern contexts. As an accurate reflection of stress experience and changes in stress in response to care, HCC can be effectively used to understand stress experience at the end of life and how it changes in response to interventions. Because stress experience is complexly related to various biosocial factors, a holistic measure of stress such as HCC analysis is better suited to capturing overall experience than previous methods that breakdown aspects of experience and then combine them into a single measure of quality of life. Dying is a stressful experience across time that has seen little improvement with advances in medicine but the response of HCC to hospital entry in the Terry Collection sample provides hope that

access to care can contribute to the alleviation of stress in the short-term. The results of the current study point to the complex nature of stress in the last months of life and the need for holistic, not just medical, approaches to the quality of life in this period. Ultimately, the current study highlights the potential utility of HCC in the field of palliative care and pursuit of a 'good death' as it is a holistic and dynamic reflection of stress experience that does not prioritize living over quality of life and can be used to track response to, and the effectiveness of, interventions.

8.5. Summary

HCC is a valid measure of stress in dying people. Stress is high at the end of life, varies between individuals, and fluctuates across the last months of an individual's life. Together these results, within a 'good death' and embodiment framework, reveal that the stress of dying is substantial and relatively consistent across cultures and time periods. Additionally, the experience of dying is highly individual and while it can be partially shaped by cause of death, it is a holistic experience that cannot be explained fully by biological data. Furthermore, the experience of dying is dynamic and dying people can still interact with their biosocial environment. HCC has great potential in bioarchaeology if the study can be reframed to focus on the dying period and be cautious of the possibility of leaching. Although this period has received little attention in bioarchaeology, it holds great potential to improve understanding of a universal biosocial human experience across time and space while increasing our understanding of mortality bias and providing context for case studies on disease and dying. The current study also offers new insight into the last months of life of modern people by demonstrating that the

HPA axis still responds to change despite declining health; that stress experience in the last months of life cannot be predicted by the type, number, and duration of stressors; and that stress experience in the last months of life has not improved with modern medical intervention. Thus, HCC analysis represents an innovative approach to the exploration, understanding, and improvement of stress experience leading up to death in the past and present.

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APPENDIX A - SAMPLE DATA

A.0. SAMPLE DEMOGRAPHIC AND HAIR DATA

Table A.1. Background data for the UCF Cadaver sample.

Sample ID	Sex	Age-at-death	# of hair samples
C0380	M	92	2
C0381	M	92	4
C0382	M	72	5
C0383	F	87	3
C0384	M	90	6
C0385	F	49	7
C0386	M	99	3
C0388	M	90	7
C0389	F	83	9
C0390	M	86	4
C0391	F	86	4
C0392	M	69	6
C0393	M	80	3
C0394	F	101	4
C0395	F	84	5
C0396	M	75	5
C0397	M	71	2
C0398	F	79	3
C0399	F	94	6
C0400	F	90	4
C0401	M	73	3
C0402	F	89	6
C0403	F	97	10
C0404	F	66	8
C0407	F	55	12
C0409	M	69	4
C0411	F	58	8
C0417	M	60	5
C0422	F	64	8
C0429	M	63	6
C0430	F	56	4
C0439	M	63	5
C0447	M	59	2
C0479	M	72	2

Sample ID	Sex	Age	# of hair samples
C0482	M	26	5
C0488	M	24	2
C0497	F	55	5
C0528	F	64	6
C0529	M	64	6
C0537	F	65	8

Abbreviations: M = Male; F= Female

Table A.2 Background data for the Terry Collection sample.

Sample ID	Sex	Age	Race	Duration of disease (days/365)	Hair samples
125	M	31	BL	0.083	3
160*	F	N/A	BL	N/A	3
181	M	38	WH	0.002	6
464	F	64	WH	1.166	9
480	F	77	WH	0.958	11
530	F	57	BL	0.008	5
568	F	27	BL	0.833	13
764	M	58	WH	0.667	8
769	F	70	BL	0.063	6
776	M	40	BL	0.500	3
989	M	30	WH	0	4
1034	F	48	BL	0.135	10
1057	M	34	AS	2.750	7
1227	F	75	BL	0.060	8
1367	M	32	BL	0.030	4
1532	M	76	WH	0.016	5
1544	F	23	BL	2.000	6
1546	M	52	WH	0.014	6
1611	F	59	WH	0.003	8
1066R	M	62	WH	0.888	5
1202R	F	56	WH	N/A	12
1249R	F	40	BL	0.500	4
131R	M	39	WH	0.014	5
1415R	M	34	BL	0.074	9
146R	F	30	BL	0.008	12
20R	F	78	WH	0	8
240R	F	63	WH	0.041	6
24R	F	52	BL	0.016	6
296R	M	69	WH	0.833	4
299R	M	49	BL	0.135	3
301R	M	36	WH	0	9
303R	M	30	WH	N/A	6
37R	F	55	WH	0.003	6
489R	M	32	WH	0	7

740R	M	18	BL	0.008	3
78R	F	91	BL	0.378	6
7R	M	55	BL	1.000	5
834R	F	87	WH	0.266	17
903R	F	51	BL	0	9
9R	M	71	BL	2.000	2

Abbreviation: M = Male; F= Female; BL = Black, WH = White, AS = Asian
* Morgue records and death certificate differ in age. No further information could be inferred.

Table A.3. Background data for the Kellis 2 Cemetery sample.

Sample ID	Sex	Age-at-death	# of hair samples
B019	F	45	2
B021	F	45	3
B022	F	22	8
B026	F	19	6
B044	F	63	2
B049	U	7	4
B052	F	30	2
B053	F	38	3
B059	M	45	3
B060	M	37	4
B064	U	6	3
B067	U	11	3
B069	M	29	3
B070	U	1.5	2
B071	U	2.5	3
B073	F	38	4
B081	M	40	3
B091	F	55	4
B105	F	58	2
B107	M	27	3
B111	M	37	2
B124	M	30	3
B131	F	23	10
B132	M	19	2
B137	U	6	3
B149	U	12	5
B159	M	20	2
B168	F	19	6
B169	F	23	3

Sample ID	Sex	Age-at death	# of hair samples
B177	F	55	3
B189	F	50	8
B190	F	19	6
B192	U	7	7
B198	F	38	4
B204	F	25	4
B210	F	54	3
B214	F	23	4
B218	M	29	3
B239	U	15	4
B240	M	46	2
B242	M	25	2
B243	U	15	4
B254	F	32	2
B259	M	44	4
B261	F	70	5
B263	U	13	2
B265	M	50	3
B268	M	23	2
B269	F	55	2
B270	F	27	3
B274	M	20	2
B275	F	55	5
B278	U	3	4
B279	F	23	4
B280	F	60	3
B281	M	60	2
B282	F	27	9
B284	F	33	3

Sample ID	Sex	Age-at-death	# of hair samples
B288	U	15	11
B289	F	34	4
B293	M	45	5
B294	F	45	5
B300	F	60	4
B302	U	12	4
B303	M	23	2
B305	M	29	2
B306	F	65	3
B307	F	23	6
B308	M	35	3
B309	M	35	4
B310	M	21	3
B314	F	53	3
B318A	F	27	6
B321	M	40	3
B322	F	60	6
B327	F	21	4
B330	U	4	6
B358	U	2.5	4
B360	U	5	3
B373	U	8	6
B374	U	7	8
B377	F	55	2
B379	M	35	5
B382	U	1.6	5
B385	M	27	3
B388	M	45	5
B393	M	45	6
B396	U	2	4
B399	U	10	3
B403	F	20	12
B410	F	25	5
B412	M	50	2
B416	U	9	2
B423	F	23	7
B424	F	22	4
B425	F	40	5
B426	F	27	6
B431	F	22	7
B434	F	33	5
B437	F	40	3
B443	F	60	3
B454	U	5	2
B458	F	50	4
B459	F	55	3
B464	U	3	5
B467	F	55	2
B468	U	13	8
B469	M	38	2
B470	F	50	5
B486	F	33	7
B487	U	2	5
B488	M	50	2
B500	F	45	4
B505	U	7	3
B515	U	8	2
B520		7	3
B523	F	20	3
B527	U	4	6
B528	F	55	3
B534	U	1.5	7
B541	F	65	4
B543	M	25	3
B550	U	4	2
B555	U	4	2
B556	U	8	9
B562	U	1.5	10
B582	U	10	2
B584	U	5	4
B592	U	1.5	2
B620	U	2	6

Abbreviation: M = Male; F= Female; U = Undetermined because juvenile

A.1. KELLIS 2 CEMETERY MAP

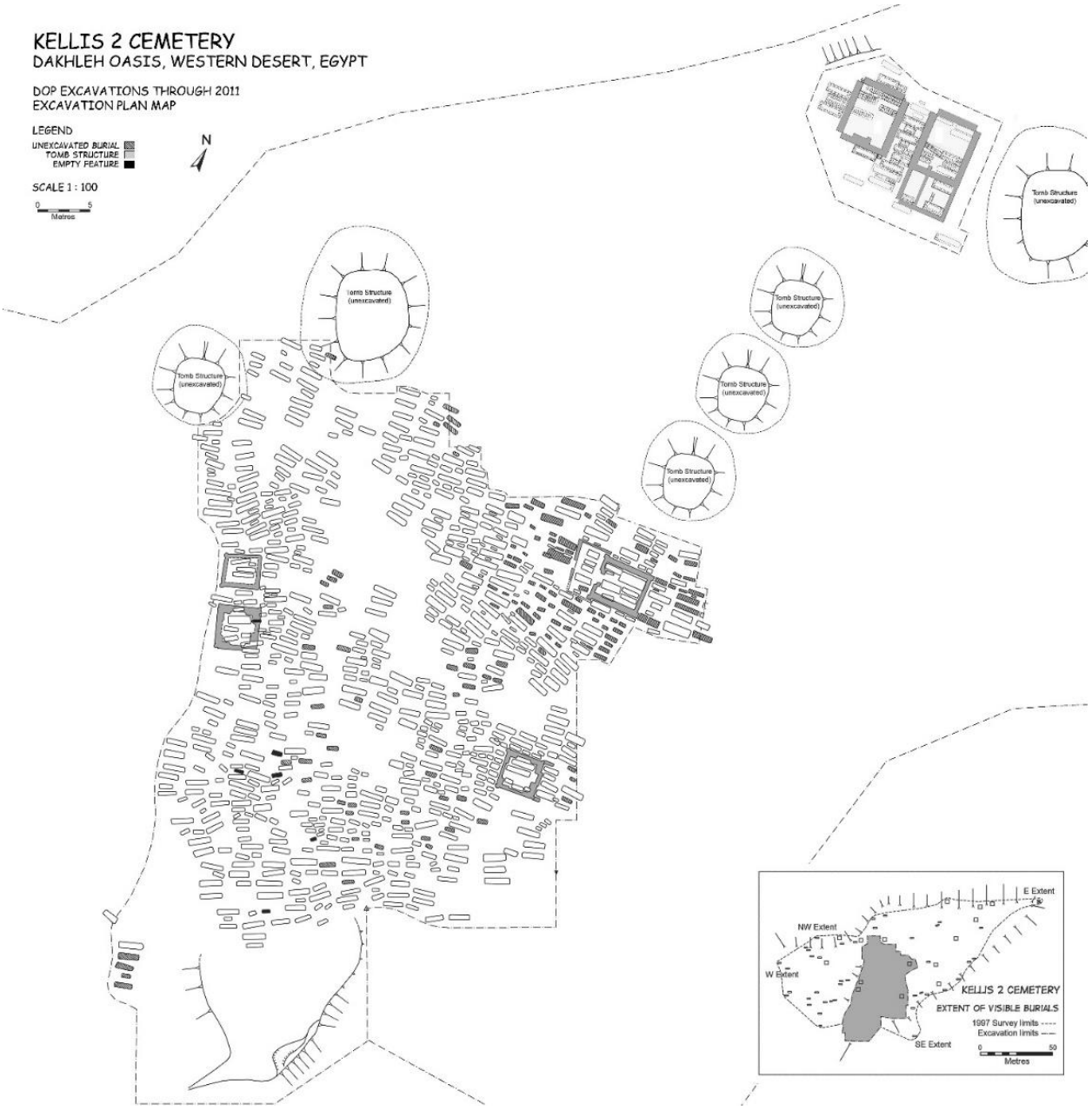

Figure A.1. Map of features excavated from the Kellis 2 cemetery as of 2011 (Courtesy of the Dakhleh Oasis Project Bioarchaeology Team).

APPENDIX B − METHODS AND PROCEDURES

B.0. PROCEDURE FOR IDENTIFYING UNDERLYING CAUSE OF DEATH (UCOD).

UCOD was identified via guidelines from the International Classification of Diseases for Mortality and Morbidity Statistics, eleventh revision (ICD-11). For details, exceptions, and examples see reference guide at https://icd.who.int/icd11refguide/en/index.html.

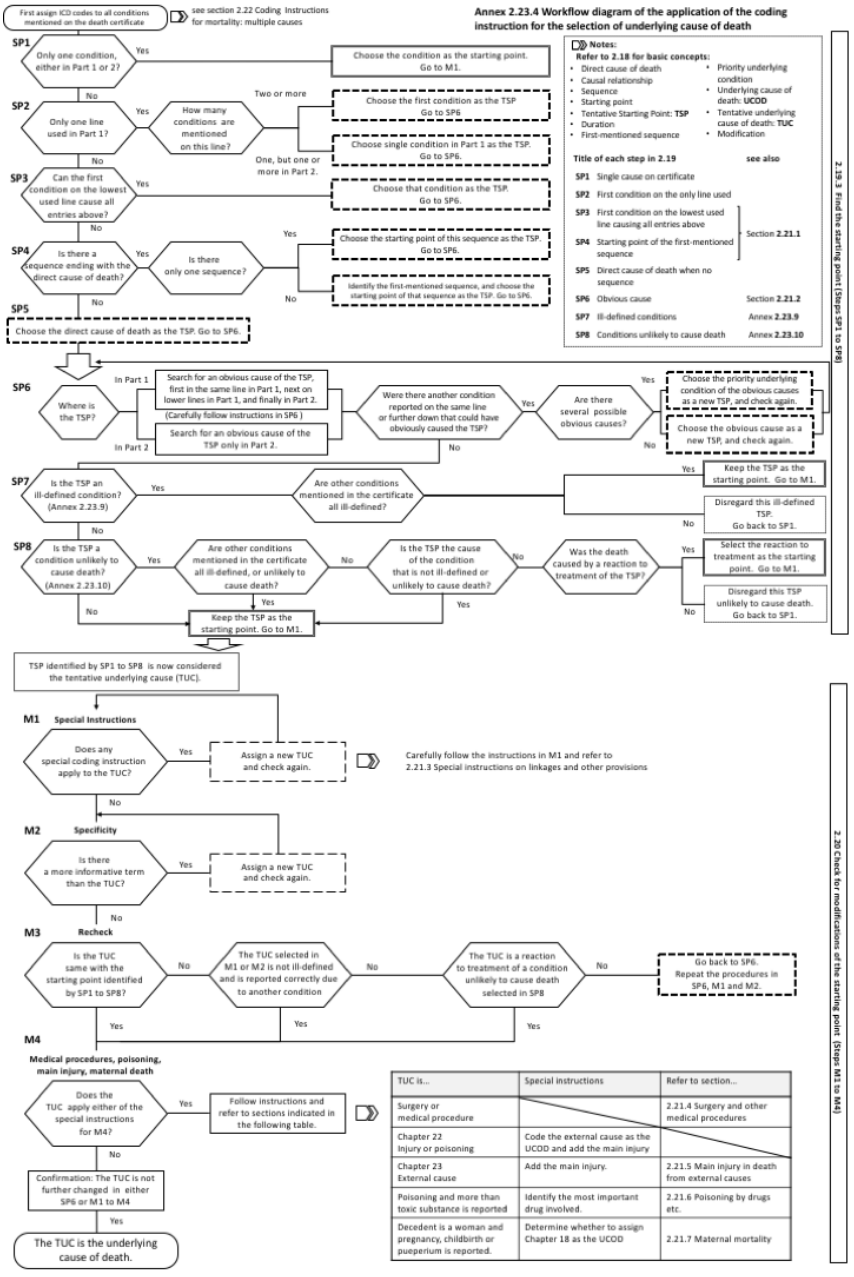

Figure B.1. Workflow to identify UCOD (from ICD-11 Reference guide Section 2.19.2).

B.1. HAIR PREPARATION (MODIFIED FROM VAN UUM ET AL. 2008)

1. Receive items in lab, label with an internal lab number, and transfer to clean paper envelopes.
2. If surface contaminants visible, gently soak hair in petri dishes of water until sediment and contaminants are dislodged. Remove water and add clean water as needed until sediment, tissue, resin and other visible contaminants are removed. Each round may last 10 to 20 minutes.
3. Dry in fume hood overnight.
4. Identify scalp end by parafilm marking, hair follicles, or microscopic identification of keratin scales.
5. Line up hair strands and place on white paper next to centimeter ruler.
6. Label glass vial and weigh; record weight.
7. Use scalpel to cut hair strands into 1 cm segments (remove hair follicles). Use forceps to transfer 1 cm segments to clean glass tube.
8. If surface contaminants still adhered, add water to glass vial and vortex. Pipette out water and contaminants and dry hair in fume hood.
9. Weigh filled glass tube, record weight.
10. Aim for between 10 and 30 mg per hair segment.
11. Add two milliliters of isopropyl ethanol to each vial for three minutes then pipette off with care to avoid removing any hairs. Repeat (two times total).
12. Let hair dry overnight in fume hood.

B.2. CORTISOL EXTRACTION (MODIFIED FROM VAN UUM ET AL. 2008)

1. Add one to two milliliters of methanol to each glass vial containing a 1 cm segment.
2. Finely mince hair with surgical scissors, cap vial.
3. Place vials on a rocker overnight at 52 °C.
4. Pipette liquid off; transfer supernatant to a glass culture tube.
5. Place glass tube in a fume hood to evaporate the liquid under a stream of nitrogen.
6. Seal with parafilm and freeze until ready for ELISA analysis.

B.3. ELISA PROCEDURE (REPRODUCED FROM INSTRUCTIONS FOR ELISA KIT ALPCO DIAGNOSTICS SALIVARY CORTISOL ELISA KIT ([-CORHU-E01-SLV])

1. Reconstitute samples in 150–250 µL of phosphate buffered saline (PBS, pH 8.0), centrifuge
2. All reagents must reach room temperature before use. Calibrators, controls and samples should be assayed in duplicate.
3. Prepare working solutions of the cortisol-HRP conjugate and wash buffer.
4. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
5. Pipette 50 µL of each calibrator, control and sample into correspondingly labeled wells in duplicate.
6. Pipette 100 µL of the conjugate working solution into each well. (It is recommended to use a multichannel pipette.)

7. Incubate on a plate shaker (approximately 200 rpm) for 45 minutes at room temperature.
8. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
9. Pipette 150 µL of TMB substrate into each well at timed intervals.
10. Incubate on a plate shaker for 15–20 minutes at room temperature (or until calibrator A attains dark blue color for desired OD).
11. Pipette 50 µL of stop solution into each well at the same timed intervals as in Step 9.
12. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stop solution.

B.4. CALCULATIONS (REPRODUCED FROM INSTRUCTIONS FOR ELISA KIT ALPCO DIAGNOSTICS SALIVARY CORTISOL ELISA KIT [-CORHU-E01-SLV])

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 100 ng/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

APPENDIX C – CAUSE OF DEATH DATA

Table C.2. Medical record information and underlying cause of death data in the UCF Cadaver sample.

Sample ID	Conditions Listed on Available Records	Underlying Cause of Death (UCOD)	ICD Category	WHO Category (Acute vs Chronic)**
C0380	Cardiorespiratory failure, Constrictive cardiomyopathy, Pleural effusion, Congestive heart failure / Chronic obstructive pulmonary disease, Hypertension	Cannot be identified*	Diseases of the circulatory system	Chronic
C0381	Ischemic cardiomyopathy, Coronary atherosclerosis	Coronary atherosclerosis	Diseases of the circulatory system	Chronic
C0382	Pancreatic adenocarcinoma, Cerebrovascular accident, Diabetes mellitus / Hypertension, Hyperlipidemia	Pancreatic adenocarcinoma	Neoplasms	Chronic
C0383	Cardiopulmonary arrest, Multisystem organ failure, Failure to thrive, End stage Alzheimer's	End Stage Alzheimer's Disease	Diseases of the nervous system	Chronic
C0384	Respiratory failure, Peritonitis, Perforated appendix/ Laparoscopic appendectomy	Peritonitis/ Perforated appendix	Diseases of the digestive system	Acute
C0385	Cerebral palsy	Cerebral palsy	Diseases of the nervous system	Chronic
C0386	Chronic obstructive pulmonary disease	Chronic obstructive pulmonary disease	Diseases of the respiratory system	Chronic
C0388	Lung cancer, Chronic obstructive pulmonary disease	Lung cancer	Neoplasms	Chronic
C0389	Atherosclerotic cerebrovascular disease; Chronic obstructive pulmonary disease	Atherosclerotic cardiovascular disease	Diseases of the circulatory system	Chronic
C0390	Chronic obstructive pulmonary disease	Chronic obstructive pulmonary disease	Diseases of the respiratory system	Chronic
C0391	Lung cancer	Lung cancer	Neoplasms	Chronic

C0392	Congestive heart failure, Cardiac arrhythmia	Congestive heart failure	Diseases of the circulatory system	Chronic
C0393	Aspiration pneumonia, Multi-organ failure, Dementia, Depression	Dementia	Mental, behavioural or neurodevelopmental	Chronic
C0394	Diseases related to advanced age	Diseases related to advanced age	Symptoms, signs or clinical findings, not elsewhere classified	Chronic
C0395	Alzheimer dementia	Alzheimer dementia	Mental, behavioural or neurodevelopmental	Chronic
C0396	Acute congestive heart failure, Acute renal failure, Metastatic prostate cancer, Respiratory failure	Acute Congestive heart failure	Diseases of the circulatory system	Chronic
C0397	Liver cirrhosis, Diabetes mellitus II, Coronary artery disease, Chronic obstructive pulmonary	Cannot be identified*	Diseases of the digestive system	Chronic
C0398	Respiratory failure, Aspiration pneumonia, Myocardial infarction	Myocardial infarction	Diseases of the circulatory system	Chronic
C0399	Blunt head trauma	Blunt head trauma	Injury, poisoning or certain other consequences of external causes	N/A
C0400	Thoracic aortic aneurysm, Peripheral vascular disease	Peripheral vascular disease	Diseases of the circulatory system	Chronic
C0401	Colon cancer	Colon cancer	Neoplasms	Chronic
C0402	Mantle cell lymphoma, Bone metastasis lumbar	Mantle cell lymphoma	Neoplasms	Chronic
C0403	Cardiomyopathy/hypertension, Congestive heart failure, Coronary artery disease, Diabetes mellitus II, Dementia, Depression	Cardiomyopathy/Hypertension	Diseases of the circulatory system	Chronic
C0404	Small bowel obstruction, Colon cancer/Bladder cancer, Pulmonary metastasis	Colon cancer/Bladder cancer	Neoplasms	Chronic
C0407	Myotonic dystrophy	Myotonic dystrophy	Diseases of the nervous system	Chronic

C0409	Atherosclerotic cardiovascular disease, Hypertension, Multiple diseases of the elderly, Non-insulin-dependent diabetes mellitus	Atherosclerotic cardiovascular disease	Diseases of the circulatory system	Chronic
C0411	Acute on chronic respiratory failure, Amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis	Diseases of the nervous system	Chronic
C0417	Right middle cerebral artery stroke, Chronic obstructive pulmonary disease, Malignant cerebral edema, PVD	Peripheral vascular disease	Diseases of the circulatory system	Chronic
C0422	Alzheimer's disease/ Dementia, Hypothyroidism	Alzheimer's disease/ Dementia	Mental, behavioural or neurodevelopmental	Chronic
C0429	Carcinoma bladder metastatic to pancreas, Retroperitoneal nodes and left cerebellum	Carcinoma bladder	Neoplasms	Chronic
C0430	Cervical cancer, Respiratory failure	Cervical cancer	Neoplasms	Chronic
C0439	Hypoxemic delirium, Retroperitoneal and psoas muscle metastasis with left renal mass, Cancer unknown origin with history of malignancies colon and pancreas	Cancer unknown origin	Neoplasms	Chronic
C0447	Cardiorespiratory arrest, cirrhosis	Cirrhosis	Diseases of the digestive system	Acute
C0479	Chronic obstructive pulmonary disease	Chronic obstructive pulmonary disease	Diseases of the respiratory system	Chronic
C0482	Anoxic encephalopathy, Drowning	Drowning	Injury, poisoning or certain other consequences of external causes	N/A
C0488	Respiratory failure, Cystic fibrosis	Cystic fibrosis	Diseases of the respiratory system	Chronic
C0497	Cardiorespiratory arrest, Esophageal cancer	Esophageal cancer	Neoplasms	Chronic
C0528	Amyotrophic lateral sclerosis	Amyotrophic lateral sclerosis	Diseases of the nervous system	Chronic

C0529	Metastatic pancreatic cancer	Metastatic pancreatic cancer	Neoplasms	Chronic
C0537	Acute cardiopulmonary arrest, Coronary artery disease, Diabetes mellitus	Coronary artery disease	Diseases of the circulatory system	Chronic
*A single UCOD could not be identified from the records available, the broadest classification that encompasses all medical conditions listed was chosen. **Abrupt conditions not categorized according to WHO standards for acute vs chronic disease and are listed as N/A. Abbreviations: ICD = International Classification of Disease; WHO = World Health Organization				

Table C.3. Morgue record information and underlying cause of death data in the Terry Collection sample.

Sample ID	Conditions Listed on Morgue Records	Underlying Cause of Death (UCOD)	ICD Category	WHO Category (Acute vs Chronic) †
125	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
160*	N/A	N/A	N/A	N/A
181	Morphine poisoning, cerebral hemorrhage	Cerebral hemorrhage	Diseases of the nervous system	N/A
464	Chronic myocarditis	Myocarditis	Diseases of the circulatory system	Chronic
480	Chronic myocarditis, arteriosclerosis	Myocarditis	Diseases of the circulatory system	Chronic
530	Meningitis	Meningitis	Certain infectious or parasitic diseases	Acute
568	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
764	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
769	Apoplexy	Cerebral hemorrhage	Diseases of the nervous system	Acute
776	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
989	Homicide	Gunshot	External causes of morbidity or mortality	N/A
1034	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
1057	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute

1227	Gangrene of the left foot, senility	Gangrene	Symptoms, signs or clinical findings, not elsewhere classified	Acute
1367	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
1532	Cancer of liver	Liver cancer	Neoplasms	Chronic
1544	Tuberculosis	Tuberculosis	Certain infectious or parasitic diseases	Acute
1546	Cardiac disease, cancer	Cancer	Neoplasms	Chronic
1611	Hemorrhage in the gastro-intestinal tract	Gastrointestinal hemorrhage	Symptoms, signs or clinical findings, not elsewhere classified	N/A
1066R	Pneumonia	Pneumonia	Diseases of the respiratory system	Acute
1202R	Malignant carcinoma of transverse colon	Malignant neoplasm of transverse colon	Neoplasms	Chronic
1249R	N/A**	Tuberculosis	Certain infectious or parasitic diseases	Acute
131R	Appendectomy	Appendicitis	Diseases of the digestive system	Acute
1415R	Generalized peritonitis	Peritonitis	Diseases of the digestive system	Acute
146R	Pulmonary TB	Tuberculosis	Certain infectious or parasitic diseases	Acute
20R	Heat stroke	Heat stroke	Injury, poisoning or certain other consequences of external causes	N/A
240R	Fracture of the hip	Hip fracture	Injury, poisoning or certain other consequences of external causes	Acute
24R	Cerebral hemorrhage	Cerebral hemorrhage	Diseases of the nervous system	Acute
296R	Chronic myocarditis	Myocarditis	Diseases of the circulatory system	Chronic
299R	Heart disease	Heart disease	Diseases of the circulatory system	Chronic
301R	Stab wound	Stab wound	Injury, poisoning or certain other consequences of external causes	N/A
303R	Malarial fever	Malaria	Certain infectious or parasitic diseases	Acute
37R	Phenol poison	Suicide (Intentional self-harm, person intended to die)	External causes of morbidity or mortality	N/A
489R	Fracture of the skull	Cranial trauma	Injury, poisoning or certain other consequences of external causes	N/A

740R	TB peritonitis	Tuberculosis of the digestive system	Certain infectious or parasitic diseases	Acute
78R	Probable coronary disease	Coronary atherosclerosis	Diseases of the circulatory system	Chronic
7R	Cardiac congestion	Congestive heart failure	Diseases of the circulatory system	Chronic
834R	Cardiac failure, pneumonia	Pneumonia	Diseases of the respiratory system	Acute
903R	N/A**	Subdural hemorrhage (Traumatic subdural hemorrhage)	Injury, poisoning or certain other consequences of external causes	N/A
9R	Central nervous system (C.N.S.) lues	Arteriosclerosis	Diseases of the circulatory system	Chronic

* Morgue records and death certificate differ in age. Cause of death could not reliably be inferred.
** No cause of death indicated on morgue record, death certificate used to establish UCOD.
†Abrupt conditions not categorized according to WHO standards for acute vs chronic disease and are listed as N/A.
Abbreviations: ICD = International Classification of Disease; WHO = World Health Organization, TB = Tuberculosis

APPENDIX D - HCC DATA

D.0. RAW HCC DATA FROM UCF CADAVER, TERRY COLLECTION, AND KELLIS 2 CEMETERY

Table D.4. Monthly HCC data in the UCF Cadaver sample. HCC values in ng/g.

Sample ID	Seg. 1	Seg. 2	Seg. 3	Seg. 4	Seg. 5	Seg. 6	Seg. 7	Seg. 8	Seg. 9	Seg. 10	Seg. 11	Seg. 12
C0380	641.28	595.57										
C0381	83.67	135.71	174.66	170.57								
C0382	193.75	475.63	674.09	1054.10	1226.08							
C0383	3115.22	5989.83	10785.10									
C0384	1418.35	2350.53	2974.87	3709.94	4012.18	5397.08						
C0385	1280.22	1086.88	928.68	758.69	788.02	962.36	906.36					
C0386	72.09	65.56	4.13									
C0388	207.34	198.36	269.89	310.66	293.03	286.59	281.92					
C0389	1543.02	1850.35	2297.41	2313.41	2813.84	3042.47	3371.75	3692.67	4938.35			
C0390	230.60	544.71	617.76	168.64								
C0391	1241.11	2085.95	2443.14	3253.52								
C0392	204.62	713.29	871.52	737.42	1126.75	795.50						
C0393	984.95	1174.95	1111.29									
C0394	3.96	4.69	5.48	5.02								
C0395	112.10	207.68	133.70	121.19	188.89							
C0396	2428.72	2125.34	2123.80	2375.65	2096.27							
C0397	83.47	84.31										
C0398	4099.87	3493.05	4043.99									
C0399	56.69	55.79	52.05	25.81	825.04	1026.06						
C0400	2422.61	2930.57	2931.58	4306.72								
C0401	481.49	482.60	739.89									

C0402	474.86	252.10	293.48	512.95	582.91	569.73						
C0403	261.60	331.51	274.86	274.70	197.95	221.70	169.88	137.90	114.30	24.22		
C0404	2117.91	2069.13	2348.02	2863.08	3019.80	3437.73	3643.87	3220.99				
C0407	5.89	26.00	2.99	3.65	3.79	2.66	3.65	5.49	7.54	7.69	5.14	40.27
C0409	204.19	250.79	149.13	60.81								
C0411	25.26	20.51	17.44	17.89	18.31	19.88	35.61	19.02				
C0417	486.13	340.70	390.47	594.04	801.33							
C0422	68.02	84.94	143.81	333.51	630.73	549.54	759.52	914.48				
C0429	1814.38	1465.00	1081.58	1507.77	1429.36	1560.90						
C0430	1199.03	1268.69	1511.87	2191.20								
C0439	144.99	119.11	193.55	104.08	68.84							
C0447	517.61	422.10										
C0479	542.67	294.70										
C0482	46.09	312.44	355.74	463.92	587.03							
C0488	4.83	4.89										
C0497	1610.91	1776.68	1665.80	2014.38	3866.42							
C0528	260.41	184.98	349.27	524.46	681.05	908.31						
C0529	683.64	540.10	809.03	660.22	1080.97	983.66						
C0537	1374.40	982.55	883.12	845.59	647.87	1356.29	1613.37	905.67				

Table D.5. Monthly HCC data in the Terry Collection sample. HCC values in ng/g.

Sample ID	Seg. 1	Seg. 2	Seg. 3	Seg. 4	Seg. 5	Seg. 6	Seg. 7	Seg. 8	Seg. 9	Seg. 10	Seg. 11	Seg. 12	Seg. 13	Seg. 14	Seg. 15	Seg. 16	Seg. 17
125	677.42	757.99	278.33														
160	243.97	236.02	220.05														
181	120.29	214.95	182.01	304.04	175.94	35.37											
464	341.81	350.05	398.51	433.21	438.93	418.61	317.15	112.47	33.64								
480	438.05	361.55	327.58	451.52	390.43	456.31	434.09	223.90	134.32	81.60	45.90						
530	494.77	532.90	502.44	136.82	357.49												
568	75.16	91.05	75.90	75.80	50.22	47.04	39.82	57.37	70.40	50.69	83.05	63.89	32.39				
764	138.57	292.63	305.60	399.47	460.24	480.27	251.50	42.28									
769	135.03	192.71	255.83	285.04	295.19	211.36											
776	84.35	102.73	91.01														
989	26.97	44.28	38.90	28.50													
1034	203.75	164.90	217.76	152.38	130.15	158.20	137.79	497.24	335.19	304.12							
1057	291.21	341.53	334.84	334.84	337.30	376.84	324.10										
1227	660.07	686.12	523.63	620.90	599.08	558.46	325.13	228.22									
1367	180.65	465.12	607.82	708.31													
1532	531.68	470.28	617.49	662.86	656.48												
1544	139.82	137.66	60.71	73.83	75.01	487.94											
1546	1142.71	997.30	1013.69	1301.49	1610.10	75.76											
1611	78.53	350.99	476.95	656.19	696.55	767.41	533.91	330.67									
1066 R	76.84	63.81	42.08	48.18	50.66												
1202 R	1308.97	1345.92	1343.00	1449.64	1378.01	1490.54	1422.53	1308.56	1296.83	1910.36	1680.58	1217.73					
1249 R	193.70	196.96	264.01	131.73													
131R	793.04	698.77	791.22	678.07	756.34												
1415 R	540.58	440.55	451.14	485.06	325.30	522.18	433.52	348.24	123.55								

146R	203.13	251.84	200.58	235.16	227.17	142.38	88.88	103.75	65.32	26.43	10.91	10.35					
20R	97.02	161.58	197.90	158.21	264.33	178.52	156.51	88.73									
240R	351.82	455.96	651.09	854.82	967.32	904.11											
24R	222.20	136.70	140.30	200.88	121.96	74.10											
296R	639.48	662.36	838.38	985.75													
299R	442.06	485.96	593.70														
301R	228.01	276.10	247.58	319.74	324.77	253.47	243.33	119.29	22.46								
303R	1263.15	1096.49	1093.61	1396.84	1567.60	725.23											
37R	648.23	564.93	504.22	395.06	266.70	26.23											
489R	473.43	451.07	234.95	523.11	538.45	375.10	29.96										
740R	583.39	479.36	507.00														
78R	18.81	62.86	45.65	190.11	98.98	193.41											
7R	2983.26	2768.73	2160.51	2580.76	3011.41												
834R	1178.93	960.39	1215.17	1198.39	1409.54	1374.52	1440.43	1471.23	207.71	996.08	1235.92	1024.94	1358.54	1248.69	914.01	657.79	453.50
903R	165.93	210.28	229.84	289.87	131.84	136.11	307.73	95.38	52.47								
9R	904.66	1053.21															

Table D.6. Monthly HCC data in the Kellis 2 Cemetery sample. HCC values in ng/g.

Sample ID	Seg. 1	Seg. 2	Seg. 3	Seg 4	Seg. 5	Seg. 6	Seg. 7	Seg. 8	Seg. 9	Seg. 10	Seg. 11	Seg. 12
B019	18.13	22.28										
B021	74.81	273.00	71.74									
B022	21.10	33.65	69.20	55.62	13.97	15.45	10.44	18.94				
B026	13.82	45.07	64.36	58.57	47.15	13.14						
B044	18.64	79.94										
B049	317.04	613.51	77.86	63.12								
B052	7.98	20.92										
B053	61.92	43.27	22.00									
B059	845.68	1254.78	1869.61									
B060	1.94	5.33	4.20	6.31								
B064	42.94	39.78	18.53									
B067	80.43	40.15	74.34									
B069	4.27	5.92	33.02									
B070	142.87	132.28										
B071	60.85	69.19	55.76									
B073	6.69	8.84	10.95	8.08								
B081	85.01	83.82	85.68									
B091	16.74	13.63	19.72	24.80								
B105	27.95	24.02										
B107	12.99	44.26	25.30									
B111	14.55	58.93										
B124	60.41	30.14	8.51									
B131	79.34	61.70	6.40	1.76	1.96	2.42	1319.50	2942.29	5.44	2.96		
B132	18.25	32.50										
B137	41.04	24.79	47.68									

B149	0.25	2.01	2.51	8.10	4.11							
B159	4.38	11.06										
B168	6.89	6.70	4.48	4.16	3.38	6.27						
B169	40.36	30.95	167.25									
B177	1.33	0.94	1.22									
B189	22.10	21.73	104.91	184.53	137.61	30.78	78.54	73.89				
B190	15.89	28.47	18.58	9.24	16.93	19.59						
B192	970.32	1454.27	987.38	636.19	286.45	299.03	581.36					
B198	135.35	97.77	201.81	202.01								
B204	12.96	43.62	37.13	12.02								
B210	34.06	46.60	35.84									
B214	3.50	8.98	7.40	7.84								
B218	41.86	9.60	9.15									
B239	21.26	23.43	22.72	10.12								
B240	59.10	130.06										
B242	89.45	31.34										
B243	24.19	27.81	57.61	35.40								
B254	5.01	33.80										
B259	51.49	31.57	49.38	15.48								
B261	12.97	82.84	30.74	13.26	9.67							
B263	125.37	126.34										
B265	303.05	364.33	363.11									
B268	81.69	36.90										
B269	260.05	325.91										
B270	47.32	36.55	69.15									
B274	167.26	169.06										
B275	155.42	154.66	105.69	109.00	85.61							

B278	64.85	114.25	96.92	92.34								
B279	171.29	140.19	232.60	335.41								
B280	160.78	127.36	160.70									
B281	35.82	82.43										
B282	57.64	18.50	22.70	20.61	29.02	9.31	10.68	5.23	3.70			
B284	19.55	45.17	14.95									
B288	172.23	190.02	249.34	142.18	149.16	104.63	92.84	51.38	44.25	21.58	14.21	
B289	31.13	48.27	70.28	75.47								
B293	43.25	36.86	26.74	26.82	27.89							
B294	44.32	18.03	21.70	30.83	14.35							
B300	218.23	251.42	321.33	141.27								
B302	209.97	139.86	107.69	115.23								
B303	58.26	58.16										
B305	109.88	132.43										
B306	182.73	171.27	58.16									
B307	26.45	14.36	9.47	7.43	27.19	9.64						
B308	26.72	29.38	29.65									
B309	31.89	41.60	38.12	44.26								
B310	99.61	109.17	38.04									
B314	6.78	7.32	7.55									
B318A	503.84	802.92	746.11	756.13	1055.56	744.55						
B321	313.10	218.22	234.01									
B322	27.89	74.75	187.54	152.70	202.88	146.53						
B327	54.95	63.44	68.30	65.52								
B330	186.31	251.67	124.85	178.79	197.17	97.29						
B358	425.29	313.45	470.46	795.73								
B360	31.80	33.20	39.09									

B373	178.98	183.86	167.61	189.81	144.94	177.80						
B374	165.86	131.60	113.40	54.28	52.94	62.88	71.24	193.10				
B377	57.47	69.57										
B379	86.47	111.91	226.35	80.57	57.40							
B382	36.56	60.94	61.40	246.60	246.19							
B385	197.52	306.67	305.61									
B388	214.10	152.69	227.76	200.51	114.72							
B393	37.64	42.34	20.81	56.30	61.62	36.34						
B396	59.48	75.99	72.14	95.65								
B399	26.98	19.74	13.00									
B403	26.32	30.82	28.72	6.01	10.59	37.01	21.36	25.05	10.39	21.48	42.22	21.75
B410	25.12	54.76	103.09	113.73	84.62							
B412	131.53	142.38										
B416	26.06	37.23										
B423	220.25	103.99	128.32	113.86	43.43	15.43	30.45					
B424	186.91	95.78	59.07	40.03								
B425	15.12	41.17	26.10	40.82	27.20							
B426	48.34	71.54	94.16	89.34	38.67	44.05						
B431	96.10	119.23	186.84	603.44	607.37	533.90	166.80					
B434	10.02	19.17	38.32	42.35	42.02							
B437	29.03	23.71	20.14									
B443	79.97	63.85	27.27									
B454	20.49	20.03										
B458	71.69	50.52	65.13	14.73								
B459	34.72	37.19	38.20									
B464	8.62	38.73	83.82	140.84	40.05							
B467	13.11	33.92										

B468	31.00	20.32	25.43	10.57	27.29	2.90	28.18	30.22				
B469	95.86	52.34										
B470	195.82	98.74	117.98	166.43	105.31							
B486	11.86	24.52	88.38	94.52	49.95	78.35	47.44					
B487	98.80	168.56	128.89	129.72	48.34							
B488	4.95	6.29										
B500	382.10	441.03	438.78	448.50								
B505	91.53	157.48	69.02									
B515	11.32	17.67										
B520	104.61	75.67	83.83									
B523	16.25	20.70	14.34									
B527	97.47	15.55	13.63	17.28	18.40	10.45						
B528	30.82	26.89	17.73									
B534	312.94	178.97	138.38	43.64	46.73	84.29	158.47					
B541	59.98	17.22	54.65	16.39								
B543	24.92	36.54	27.02									
B550	7.47	13.41										
B555	35.61	36.65										
B556	117.72	236.38	179.78	224.08	435.86	189.10	243.33	544.46	254.88			
B562	34.89	63.18	140.43	70.94	45.37	34.56	91.51	30.54	83.83	27.09		
B582	6.90	15.39										
B584	252.64	287.85	194.94	167.61								
B592	67.42	89.66										
B620	69.88	33.31	71.49	105.20	125.81	129.22						

Table D.7. UCF Cadaver sample summary statistics per individual.

Sample ID	2-month Average HCC (ng/g)	Total Average HCC (ng/g)	Standard Deviation (ng/g)
C0380	618.43	427.21	32.32
C0381	109.69	172.94	42.12
C0382	334.69	582.52	420.24
C0383	4552.53	4072.03	3874.81
C0384	1884.44	2542.12	1387.09
C0385	1183.55	793.91	179.16
C0386	68.82	125.36	37.49
C0388	202.85	258.42	43.60
C0389	1696.68	2139.69	1041.49
C0390	387.66	339.62	223.77
C0391	1663.53	1583.45	834.47
C0392	458.95	613.76	302.42
C0393	1079.95	748.84	96.71
C0394	4.32	85.69	0.64
C0395	159.89	177.51	42.82
C0396	2277.03	1660.11	158.76
C0397	83.89	158.94	0.60
C0398	3796.46	2422.78	335.38
C0399	56.24	316.81	457.95
C0400	2676.59	2180.25	808.90
C0401	482.05	435.60	148.86
C0402	363.48	397.13	141.57
C0403	296.56	237.01	91.74
C0404	2093.52	2319.05	602.28
C0407	15.95	51.61	11.54
C0409	227.49	190.49	81.64
C0411	22.89	64.29	6.12
C0417	413.41	441.38	183.49
C0422	76.48	397.06	324.91
C0429	1639.69	1168.87	236.89
C0430	1233.86	1109.46	452.66
C0439	132.05	161.80	46.69
C0447	469.86	361.43	67.54
C0479	418.68	347.09	175.34
C0482	179.26	324.75	201.82
C0488	4.86	130.43	0.04
C0497	1693.79	1640.89	951.60

C0528	222.69	437.56	274.98
C0529	611.87	668.83	206.44
C0537	1178.48	921.09	331.17

Table D.8. Terry Collection sample summary statistics per individual.

Sample ID	2-month Average HCC (ng/g)	Total Average HCC (ng/g)	Standard Deviation (ng/g)
125	717.70	571.24	256.85
160	240.00	233.35	12.18
181	167.62	172.10	90.26
464	345.93	316.04	145.41
480	399.80	304.11	155.72
530	513.83	404.88	164.38
568	83.11	62.52	17.78
764	215.60	296.32	152.54
769	163.87	229.20	61.15
776	93.54	92.70	9.31
989	35.63	34.66	8.32
1034	184.33	230.15	116.76
1057	316.37	334.38	25.27
1227	673.10	525.20	163.90
1367	322.88	490.47	229.39
1532	500.98	587.76	83.95
1544	138.74	162.50	163.05
1546	1070.01	1023.51	516.64
1611	214.76	486.40	227.80
1066R	70.33	56.31	13.95
1202R	1327.45	1429.39	192.39
1249R	195.33	196.60	54.04
131R	745.91	743.49	52.86
1415R	490.57	407.79	128.18
146R	227.48	130.49	91.26
20R	129.30	162.85	55.63
240R	403.89	697.52	253.07
24R	179.45	149.36	54.07
296R	650.92	781.49	162.60
299R	464.01	507.24	78.03
301R	252.06	226.08	97.08
303R	1179.82	1190.49	291.46
37R	606.58	400.90	226.79
489R	462.25	375.15	183.84

740R	531.38	523.25	53.89
78R	40.83	101.64	74.51
7R	2875.99	2700.93	349.00
834R	1069.66	1079.16	356.57
903R	188.11	179.94	86.35
9R	978.93	978.93	105.04

Table D.9. Kellis 2 Cemetery sample summary statistics per individual.

Sample ID	2-month average HCC (ng/g)	Total average HCC (ng/g)	Standard Deviation (ng/g)
B019	20.21	20.21	2.93
B021	173.90	139.85	115.32
B022	27.38	29.80	21.58
B026	29.45	40.35	22.00
B044	49.29	49.29	43.34
B049	465.27	267.88	258.14
B052	14.45	14.45	9.15
B053	52.59	42.39	19.97
B059	1050.23	1323.36	515.40
B060	3.63	4.44	1.88
B064	41.36	33.75	13.27
B067	60.29	64.97	21.71
B069	5.10	14.40	16.14
B070	137.57	137.57	7.49
B071	65.02	61.93	6.78
B073	7.76	8.64	1.78
B081	84.41	84.84	0.94
B091	15.18	18.72	4.75
B105	25.99	25.99	2.78
B107	28.62	27.52	15.75
B111	36.74	36.74	31.38
B124	45.27	33.02	26.07
B131	70.52	442.38	969.04
B132	25.38	25.38	10.08
B137	32.91	37.83	11.78
B149	1.13	3.40	2.96
B159	7.72	7.72	4.73
B168	6.80	5.31	1.49
B169	35.65	79.52	76.12
B177	1.13	1.16	0.20

B189	21.92	81.76	58.62
B190	22.18	18.12	6.24
B192	1212.30	745.00	420.39
B198	116.56	159.24	51.61
B204	28.29	26.43	16.32
B210	40.33	38.83	6.79
B214	6.24	6.93	2.38
B218	25.73	20.20	18.75
B239	22.34	19.38	6.24
B240	94.58	94.58	50.18
B242	60.39	60.39	41.09
B243	26.00	36.25	14.99
B254	19.41	19.41	20.36
B259	41.53	36.98	16.89
B261	47.90	29.90	30.73
B263	125.86	125.86	0.69
B265	333.69	343.50	35.04
B268	59.30	59.30	31.67
B269	292.98	292.98	46.57
B270	41.93	51.01	16.61
B274	168.16	168.16	1.27
B275	155.04	122.07	31.40
B278	89.55	92.09	20.46
B279	155.74	219.87	86.06
B280	144.07	149.61	19.27
B281	59.13	59.13	32.96
B282	38.07	19.71	16.55
B284	32.36	26.55	16.28
B288	181.12	111.98	75.69
B289	39.70	56.29	20.50
B293	40.05	32.31	7.43
B294	31.18	25.85	12.00
B300	234.82	233.06	74.77
B302	174.91	143.19	46.59
B303	58.21	58.21	0.07
B305	121.15	121.15	15.94
B306	177.00	137.39	68.85
B307	20.40	15.76	8.87
B308	28.05	28.58	1.62
B309	36.75	38.97	5.35
B310	104.39	82.27	38.60

B314	7.05	7.22	0.39
B318A	653.38	768.18	175.99
B321	265.66	255.11	50.84
B322	51.32	132.05	67.62
B327	59.20	63.05	5.76
B330	218.99	172.68	54.84
B358	369.37	501.23	207.12
B360	32.50	34.70	3.87
B373	181.42	173.83	15.95
B374	148.73	105.66	54.03
B377	63.52	63.52	8.55
B379	99.19	112.54	66.51
B382	48.75	130.34	106.42
B385	252.10	269.93	62.72
B388	183.39	181.95	47.05
B393	39.99	42.51	14.75
B396	67.74	75.81	14.99
B399	23.36	19.91	6.99
B403	28.57	23.48	10.79
B410	39.94	76.27	36.30
B412	136.96	136.96	7.67
B416	31.64	31.64	7.90
B423	162.12	93.68	71.15
B424	141.34	95.45	65.22
B425	28.15	30.08	11.02
B426	59.94	64.35	24.05
B431	107.67	330.53	237.88
B434	14.59	30.38	14.85
B437	26.37	24.30	4.47
B443	71.91	57.03	27.01
B454	20.26	20.26	0.33
B458	61.10	50.52	25.44
B459	35.95	36.70	1.79
B464	23.67	62.41	51.39
B467	23.52	23.52	14.72
B468	25.66	21.99	10.18
B469	74.10	74.10	30.77
B470	147.28	136.86	42.29
B486	18.19	56.43	31.83
B487	133.68	114.87	44.68
B488	5.62	5.62	0.94

B500	411.57	427.60	30.62
B505	124.51	106.01	45.97
B515	14.49	14.49	4.49
B520	90.14	88.04	14.92
B523	18.47	17.10	3.26
B527	56.51	28.80	33.76
B528	28.85	25.14	6.72
B534	245.95	137.63	93.83
B541	38.60	37.06	23.49
B543	30.73	29.49	6.19
B550	10.44	10.44	4.20
B555	36.13	36.13	0.74
B556	177.05	269.51	134.58
B562	49.04	62.23	35.83
B582	11.14	11.14	6.00
B584	270.24	225.76	54.50
B592	78.54	78.54	15.73
B620	51.59	89.15	37.44

D.1. REFERENCE HCC DATA

Table D.7. HCC studies used for comparison between living and dead samples.

Study	Category	Condition	Mean (ng/g)	SD* (ng/g)
López-Barrales 2015	Archaeological	N/A	76	35.3
Schaefer 2017	Archaeological	N/A	75	71.4
Tisdale et al. 2019	Archaeological	N/A	343.7	70.15
Webb et al. 2010	Archaeological	N/A	281	35
Webb et al. 2014	Archaeological	N/A	522	368
Webb et al. 2015	Archaeological	N/A	1444	402
Chan et al. 2014	Living-healthy	N/A	46	
Cieszyński et al. 2019	Living-healthy	N/A	7.17	10.4
Dettenborn et al. 2012a	Living-healthy	N/A	18.7	11.5
Dettenborn et al. 2012b	Living-healthy	N/A	16.28	10.3
Föcker et al. 2016	Living-healthy	N/A	12.55	9.69
Gonzalez et al. 2019	Living-healthy	N/A	55	
Henley et al 2013	Living-healthy	N/A	116	
Henley et al. 2014	Living-healthy	N/A	299	110
Hodes et al. 2017	Living-healthy	N/A	38.9	25.3
Karlén et al. 2011	Living-healthy	N/A	19.93	33.35
Langerak et al. 2015	Living-healthy	N/A	13.5	13.5
Manenschijn et al. 2011	Living-healthy	N/A	29.72	
Manenschijn et al. 2012	Living-healthy	N/A	28.18	
Manenschijn et al. 2013	Living-healthy	N/A	221	
Noppe et al. 2014a (adults)	Living-healthy	N/A	7.5	
Noppe et al. 2014a (Children 4-7)	Living-healthy	N/A	4.6	
Pereg et al. 2011	Living-healthy	N/A	224.9	
Prado- Gascó et al. 2019	Living-healthy	N/A	3.26	1.88
Sauve et al. 2007	Living-healthy	N/A	46.1	
Skoluda et al. 2012	Living-healthy	N/A	12.43	6.2
Stalder et al. 2013	Living-healthy	N/A	5.5	
Stalder et al. 2014	Living-healthy	N/A	20.5	7.3
Staufenbiel et al. 2015a	Living-healthy	N/A	10.07	
Thomson et al. 2010	Living-healthy	N/A	116	
Van Uum et al. 2008	Living-healthy	N/A	46.1	
Veldhorst et al. 2014	Living-healthy	N/A	17	
Yamada et al 2007	Living-healthy	N/A	2.22	2.11

Chan et al. 2014	Living-pathological	Obesity	57	
Dettenborn et al. 2012a	Living-pathological	Depression	26.7	20.8
Föcker et al. 2016	Living-pathological	Anorexia nervosa	6.72	5.19
Henley et al 2013	Living-pathological	First Nations in Canada	177	
Henley et al. 2014	Living-pathological	Subsaharan Africa	639	300
Hodes et al. 2017	Living-pathological	Cushing Syndrome	266	738.4
Langerak et al. 2015	Living-pathological	HIV	16.4	16.8
Manenschijin et al. 2011	Living-pathological	Shift work	47.32	
Manenschijn et al. 2012	Living-pathological	Bipolar disorder+other conditions	31.84	
Pereg et al. 2011	Living-pathological	Acute myocardial infarction	295.3	
Skoluda et al. 2012	Living-pathological	Endurance athletes	18.18	9.6
Stalder et al. 2014	Living-pathological	Dementia caregivers	27.4	11.3
Thomson et al. 2010	Living-pathological	Cushing Syndrome	679	
Van Uum et al. 2008	Living-pathological	Chronic pain	83.1	
Gonzalez et al. 2019	Living-pathological	Stressed	250	
Staufenbiel et al. 2015a	Living-pathological	Adrenal insufficiency	33.89	
Veldhorst et al. 2014	Living-pathological	Obese	25	
Tisdale et al. 2019- Cadaver (F)	Modern	N/A	291.54	66.13
Tisdale et al. 2019- Cadaver (M)	Modern	N/A	320.02	43.18

Studies chosen that used immunoassays, similar units of measurements that could be easily translated into ng/g, and were not log transformed.
*Standard deviation reported only when provided in original publication.
Abbreviations: SD = Standard Deviation; M = Male, F = Female

APPENDIX E − TEST RESULTS

E.0. HAIR CHARACTERISTICS TEST RESULTS

Table E.1. Results of Related-Samples Wilcoxon Signed Rank test between the average hair cortisol concentration (HCC) of Month 1 (the most proximal segment of hair) and each other month in all sample combined.

Month of comparison	N	Mean month HCC (ng/)	P value	Segment 1 Mean HCC (ng/g)
Month 2	210	339.14	.028*	306.76
Month 3	174	425.61	.022*	344.84
Month 4	129	458.14	.006*	355.83
Month 5	94	526.43	.027*	390.04
Month 6	68	507.72	.313	379.63
Month 7	40	505.61	.502	391.16
Month 8	31	575.44	.769	380.09
Month 9	19	410.77	.049*	367.52
Month 10	12	289.52	.117	332.35
Month 11	8	389.74	.779	426.08
Month 12	6	396.49	.116	466.40
Month 13	2	695.46	.655	627.04
Month 14	N/A	N/A	N/A	N/A
Month 15	N/A	N/A	N/A	N/A
Month 16	N/A	N/A	N/A	N/A
Month 17	1	435.50	.317	1178.93

Notes: * indicates significance $p<0.05$, N = sample size

Table E.2. Results of Related-Samples Wilcoxon Signed Rank test between the average hair cortisol concentration (HCC) of Month 1 (the most proximal segment of hair) and each other month in the Kellis 2 Cemetery sample.

Month of comparison	N	Mean month HCC (ng/)	P value	Segment 1 Mean HCC (ng/g)
Month 2	130	109.28	.035*	95.00
Month 3	100	121.22	.252	106.27
Month 4	65	124.15	.554	112.48
Month 5	41	116.70	.771	109.75
Month 6	27	108.70	.280	131.60
Month 7	15	196.81	.609	155.98
Month 8	10	391.51	.799	72.82
Month 9	6	67.08	.600	81.36
Month 10	4	18.28	.068	78.20
Month 11	2	28.21	.650	99.28
Month 12	1	21.7	.317	26.32

Notes: * indicates significance $p<0.05$, N = sample size

Table E.3. Results of Related-Samples Wilcoxon Signed Rank test between the average hair cortisol concentration (HCC) of Month 1 (the most proximal segment of hair) and each other month in the Terry Collection sample.

Month of comparison	N	Mean month HCC (ng/)	P value	Segment 1 Mean HCC (ng/g)
Month 2	40	490.37	.536	483.04
Month 3	39	468.74	.675	472.23
Month 4	34	551.37	.039	481.93
Month 5	30	590.45	.079	511.49
Month 6	25	418.78	.657	418.61
Month 7	16	405.40	.836	401.45
Month 8	14	359.09	.300	404.18
Month 9	10	234.19	.017*	468.43
Month 10	6	561.55	.600	567.99
Month 11	5	611.27	.893	640.85
Month 12	4	579.23	.068	691.54
Month 13	2	695.47	.655	627.04
Month 14	1	1248.69	.317	1178.93
Month 15	1	914.01	.317	1178.93
Month 16	1	657.79	.317	1178.93
Month 17	1	453.50	.317	1178.93

Notes: * indicates significance $p<0.05$, N = sample size

Table E.4. Results of Related-Samples Wilcoxon Signed Rank test between the average hair cortisol concentration (HCC) of Month 1 (the most proximal segment of hair) and each other month in the UCF Cadaver sample.

Month of comparison	N	Mean month HCC (ng/)	P value	Segment 1 Mean HCC (ng/g)
Month 2	40	934.96	.452	818.70
Month 3	35	1247.23	.028*	884.52
Month 4	30	1076.12	.026*	740.15
Month 5	23	1173.32	.015*	731.27
Month 6	16	1320.03	.030*	737.29
Month 7	9	1198.43	.173	764.85
Month 8	7	1270.89	.499	770.87
Month 9	3	1686.73	.593	603.50
Month 10	2	15.95	.655	133.75
Month 11	1	5.14	.317	5.89
Month 12	1	40.27	.317	5.89

Notes: * indicates significance $p<0.05$, N = sample size

E.1. CAUSE OF DEATH TEST RESULTS

Table E.5. Mean HCC (ng/g) for UCOD in categories using total averages in the combined Terry Collection and UCF Cadaver sample, the UCF Cadaver sample, and the Terry Collection sample.

Stem Code	UCOD Category			With outliers	Without outliers
	COMBINED	**N**			
1	Certain infectious or parasitic diseases	12		379.63	379.63
11	Diseases of the circulatory system	18(13)		1236.96	571.83
13	Diseases of the digestive system	5 (4)		1003.10	426.26
8	Diseases of the nervous system	8(7)		813.56	289.35
12	Diseases of the respiratory system	6		169.86	169.86
22	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes	9		307.82	307.82
6	Mental, behavioural or neurodevelopmental disorders	3		559.56	559.56
2	Neoplasms	14 (11)		953.56	816.66
21	Symptoms, signs or clinical findings, not elsewhere classified	4		286.72	286.72
	UCF Cadaver				
1	Certain infectious or parasitic diseases	0			
11	Diseases of the circulatory system	11 (10)		1417.94	1171.84
13	Diseases of the digestive system	3		1288.08	1288.08
8	Diseases of the nervous system	5 (4)		1620.97	368.70
12	Diseases of the respiratory system	4		215.31	215.31
22	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes	2		346.64	346.64
6	Mental, behavioural or neurodevelopmental disorders	3		559.56	559.56
2	Neoplasms	11		1202.31	1202.31
21	Symptoms, signs or clinical findings, not elsewhere classified	1		4.79	4.79
	TERRY COLLECTION				
1	Certain infectious or parasitic diseases	12		379.63	379.63
11	Diseases of the circulatory system	7 (6)		952.56	661.16
13	Diseases of the digestive system	2		575.64	575.64
8	Diseases of the nervous system	3		183.55	183.55
12	Diseases of the respiratory system	2		78.98	78.98
22	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes	7		296.73	296.73
6	Mental, behavioural or neurodevelopmental disorders	0			
2	Neoplasms	3 (2)		1013.55	805.63
21	Symptoms, signs or clinical findings, not elsewhere classified	3		380.70	380.70

Notes: Number in parentheses indicates sample size with outliers removed. Terry Collection sample 160 removed due to conflicting records. Outliers defined as 1.5IQR +Q3 (combined sample > 2138.71; N = 10; Terry Collection outliers > 1197.99; N = 2; UCF Cadaver outliers > 3553.04; N = 2).

Table E.6. Mean HCC (ng/g) for UCOD using two-month averages in categories in the combined Terry Collection and UCF Cadaver sample, the UCF Cadaver sample, and the Terry Collection sample.

Stem Code	UCOD Category		With outliers	Without outliers
	COMBINED	**N**		
1	Certain infectious or parasitic diseases	12	374.39	374.39
11	Diseases of the circulatory system	18(14)	1140.83	636.35
13	Diseases of the digestive system	5	734.93	734.93
8	Diseases of the nervous system	8(7)	813.56	279.43
12	Diseases of the respiratory system	6	165.20	165.20
22	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes	9	257.03	257.03
6	Mental, behavioural or neurodevelopmental disorders	3	438.77	438.77
2	Neoplasms	14	953.56	953.56
21	Symptoms, signs or clinical findings, not elsewhere classified	4	279.91	279.91
	UCF Cadaver			
1	Certain infectious or parasitic diseases	0		
11	Diseases of the circulatory system	11 (10)	1249.98	995.33
13	Diseases of the digestive system	3	812.73	812.73
8	Diseases of the nervous system	5(4)	1199.52	361.27
12	Diseases of the respiratory system	4	220.00	220.00
22	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes	2	117.75	117.75
6	Mental, behavioural or neurodevelopmental disorders	3	438.77	438.77
2	Neoplasms	11	950.13	950.13
21	Symptoms, signs or clinical findings, not elsewhere classified	1	4.33	4.33
	TERRY COLLECTION			
1	Certain infectious or parasitic diseases	12	374.39	374.39
11	Diseases of the circulatory system	7(6)	969.32	651.54
13	Diseases of the digestive system	2	618.24	618.24
8	Diseases of the nervous system	3	170.31	170.31
12	Diseases of the respiratory system	2	55.58	55.58
22	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes	7	296.83	296.83
6	Mental, behavioural or neurodevelopmental disorders	0		
2	Neoplasms	3	966.15	966.15
21	Symptoms, signs or clinical findings, not elsewhere classified	3	371.78	371.78

Notes: Number in parentheses indicates sample size with outliers removed. Terry Collection sample 160 removed due to conflicting records. Outliers defined as 1.5IQR +Q3: combined sample > 2195.895; N=5; Terry Collection outliers > 1328.60; N = 1; UCF Cadaver outliers > 3637.06; N = 2]

Table E.7. Results of Independent-Samples Kruskal-Wallis Tests between UCOD categories. Asterisk indicates significance ($p < .05$).

Variable	**P value**
COMBINED Sample	
Total average	.002*
Total average w/o outliers	.019*
Two-month average	.003*
Two-month average w/o outliers	.013*
Minimum	.001*
Maximum	.008*
UCF CADAVER	
Total average	.119
Total average w/o outliers	.071
Two-month average	.188
Two-month average w/o outliers	.126
Minimum	.063
Maximum	.106
TERRY COLLECTION	
Total average	.012*
Total average w/o outliers	.037*
Two-month average	.010*
Two-month average w/o outliers	.014*
Minimum	.120
Maximum	.059

Table E.8. Results of pairwise comparisons between total average HCC in each UCOD category in the combined Terry Collection and UCF Cadaver sample, outliers not removed. Parentheses indicate p-value after Bonferroni correction. Asterisk indicates significance (p > .05) before correction, gray color indicates significance after correction.

UCOD category-stem code	Certain infectious or parasitic diseases-1	Diseases of the circulatory system-11	Diseases of the digestive system-13	Diseases of the nervous system-8	Diseases of the respiratory system-12	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	Mental, behavioural or neurodevelopmental disorders-6	Neoplasms-2	Symptoms, signs or clinical findings, not elsewhere classified-21
Certain infectious or parasitic diseases-1									
Diseases of the circulatory system-11	0.022 (0.793)*								
Diseases of the digestive system-13	0.319 (1.0)	0.524 (1.0)							
Diseases of the nervous system-8	0.902 (1.0)	0.032 (1.0)*	0.303 (1.0)						
Diseases of the respiratory system-12	01707 (1.0)	0.001 (0.039)*	0.044 (1.0)*	0.243 (1.0)					
External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	0.703 (1.0)	0.012 (0.444)*	0.210 (1.0)	0.818 (1.0)	0.326 (1.0)				
Mental, behavioural or neurodevelopmental disorders-6	0.593 (1.0)	0.415 (1.0)	0.799 (1.0)	0.553 (1.0)	0.145 (1.0)	0.5441 (1.0)			
Neoplasms-2	0.010 (0.372)*	0.662 (1.0)	0.359 (1.0)	0.016 (0.585)*	0.001 (0.018)*	0.06 (0.221)*	0.297 (1.0)		
Symptoms, signs or clinical findings, not elsewhere classified-21	0.669 (1.0)	0.047 (1.0)*	0.246 (1.0)	0.756 (1.0)	0.496 (1.0)	0.896 (1.0)	0.438 (1.0)	0.027 (.963) *	

Table E.9. Results of pairwise comparisons between total average HCC in each UCOD category in the combined Terry Collection and UCF Cadaver sample, outliers removed. Parentheses indicate p-value after Bonferroni correction. Asterisk indicates significance ($p > .05$) before correction, gray color indicates significance after correction.

UCOD category-stem code	Certain infectious or parasitic diseases-1	Diseases of the circulatory system-11	Diseases of the digestive system-13	Diseases of the nervous system-8	Diseases of the respiratory system-12	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	Mental, behavioural or neurodevelopmental disorders-6	Neoplasms-2	Symptoms, signs or clinical findings, not elsewhere classified-21
Certain infectious or parasitic diseases-1									
Diseases of the circulatory system-11	0.175 (1.0)								
Diseases of the digestive system-13	0.724 (1.0)	0.553 (1.0)							
Diseases of the nervous system-8	0.394 (1.0)	0.043 (1.0)*	0.331 (1.0)						
Diseases of the respiratory system-12	0.116 (1.0)	0.007 (0.257)*	0.095 (1.0)	0.495 (1.0)					
External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	0.663 (1.0)	0.090 (0.942)*	0.510 (1.0)	0.673 (1.0)	0.261 (1.0)				
Mental, behavioural or neurodevelopmental disorders-6	0.541 (1.0)	0.817 (1.0)	0.802 (1.0)	0.246 (1.0)	0.095 (1.0)	0.379 (1.0)			
Neoplasms-2	0.026 (0.925)*	0.334 (1.0)	0.213 (1.0)	0.006 (0.206)*	0.001 (0.026)*	0.012 (0.448)*	0.410 (1.0)		
Symptoms, signs or clinical findings, not elsewhere classified-21	0.625 (1.0)	0.149 (1.0)	0.492 (1.0)	0.845 (1.0)	0.436 (1.0)	0.881 (1.0)	0.375 (1.0)	0.038 (1.0)*	

Table E.10. Results of pairwise comparisons between 2-month average HCC in each UCOD category in the combined Terry Collection and UCF Cadaver samples, outliers not removed. Parentheses indicate p-value after Bonferroni correction. Asterisk indicates significance ($p > .05$) before correction, gray color indicates significance after correction.

UCOD category-stem code	Certain infectious or parasitic diseases-1	Diseases of the circulatory system-11	Diseases of the digestive system-13	Diseases of the nervous system-8	Diseases of the respiratory system-12	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	Mental, behavioural or neurodevelopmental disorders-6	Neoplasms-2	Symptoms, signs or clinical findings, not elsewhere classified-21
Certain infectious or parasitic diseases-1									
Diseases of the circulatory system-11	0.026 (0.932)*								
Diseases of the digestive system-13	0.0263 (1.0)	0.642 (1.0)							
Diseases of the nervous system-8	0.697 (1.0)	0.018 (0.635)*	0.175 (1.0)						
Diseases of the respiratory system-12	0.150 (1.0)	0.001 (0.037)*	0.030 (1.0)*	0.316 (1.0)					
External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	0.469 (1.0)	0.005 (0.175)*	0.101 (1.0)	0.771 (1.0)	0.449 (1.0)				
Mental, behavioural or neurodevelopmental disorders-6	0.787 (1.0)	0.107 (1.0)	0.292 (1.0)	0.996 (1.0)	0.441 (1.0)	0.828 (1.0)			
Neoplasms-2	0.034 (1.0)*	0.989 (1.0)	0.636 (1.0)	0.022 (0.801)*	0.001 (0.052)*	0.007 (0.248)*	0.113 (1.0)		
Symptoms, signs or clinical findings, not elsewhere classified-21	0.673 (1.0)	0.052 (1.0)	0.211 (1.0)	0.915 (1.0)	0.461 (1.0)	0.899 (1.0)	0.928 (1.0)	0.057 (1.0)	

Table E.11. Results of pairwise comparisons between 2-month average HCC in each UCOD category in the combined Terry Collection and UCF Cadaver samples, outliers removed. Parentheses indicate p-value after Bonferroni correction. Asterisk indicates significance ($p > .05$) before correction.

UCOD category-stem code	Certain infectious or parasitic diseases-1	Diseases of the circulatory system-11	Diseases of the digestive system-13	Diseases of the nervous system-8	Diseases of the respiratory system-12	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	Mental, behavioural or neurodevelopmental disorders-6	Neoplasms-2	Symptoms, signs or clinical findings, not elsewhere classified-21
Certain infectious or parasitic diseases-1									
Diseases of the circulatory system-11	0.222 (1.0)								
Diseases of the digestive system-13	0.233 (1.0)	0.780 (1.0)							
Diseases of the nervous system-8	0.280 (1.0)	0.032 (1.0)*	0.050 (1.0)*						
Diseases of the respiratory system-12	0.376 (1.0)	0.052 (1.0)	0.071 (1.0)	0.862 (1.0)					
External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes-22	0.439 (1.0)	0.056 (1.0)	0.080 (1.0)	0.732 (1.0)	0.874 (1.0)				
Mental, behavioural or neurodevelopmental disorders-6	0.773 (1.0)	0.292 (1.0)	0.261 (1.0)	0.635 (1.0)	0.734 (1.0)	0.816 (1.0)			
Neoplasms-2	0.022 (0.809)*	0.288 (1.0)	0.615 (1.0)	0.002 (0.083)*	0.004 (0.158)*	0.004 (0.134)*	0.088 (1.0)		
Symptoms, signs or clinical findings, not elsewhere classified-21	0.653 (1.0)	0.191 (1.0)	0.182 (1.0)	0.685 (1.0)	0.797 (1.0)	0.892 (1.0)	0.923 (1.0)	0.041 (1.0)*	

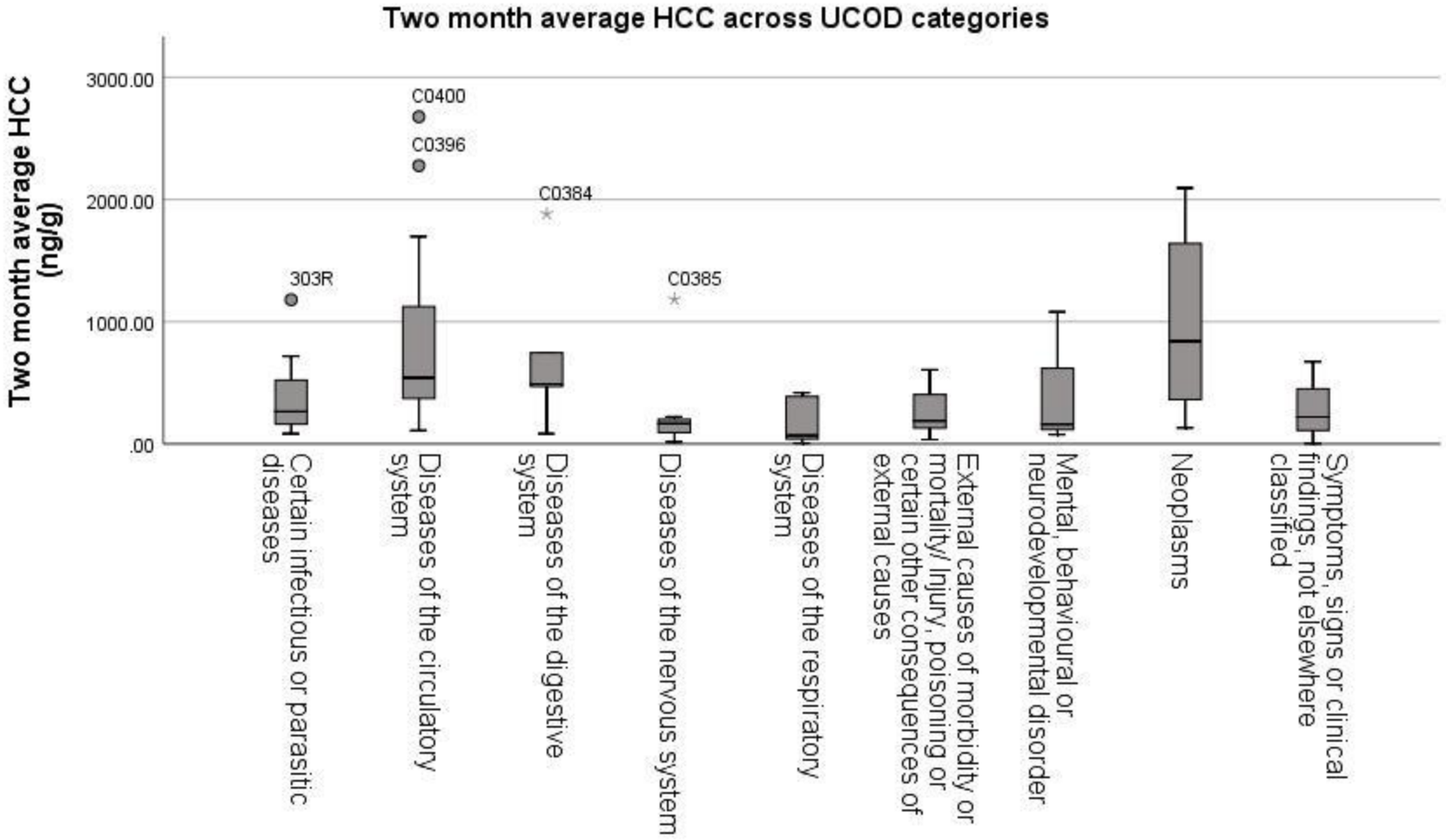

Figure E.1. Box plot displaying mean and variance of two-month average HCC across UCOD categories in the combined Terry Collection and UCF Cadaver samples.

Table E. 12. Distribution of UCOD across two-month average quartiles with outliers Quartile cutoffs (ng/g): 167.62; 399.80; 978.80; Pearson Chi square = .119. Likelihood ratio = .026.

	Certain infectious or parasitic diseases (1)	Diseases of the circulatory system (11)	Diseases of the digestive system (13)	Diseases of the nervous system (8)	Diseases of the respiratory system (12)	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes (22)	Mental, behavioural or neurodevelopmental disorders (6)	Neoplasms (2)	Symptoms, signs or clinical findings, not elsewhere classified (21)
1	3	1	1	4	4	3	2	1	1
2	5	4	0	2	1	3	0	3	2
3	3	6	3	0	1	3	0	3	1
4	1	5	1	2	1	0	2	7	0
Total	12	16	5	8	7	9	4	14	4

Table E.13. Distribution of UCOD across two-month average quartiles without outliers. Quartile cutoffs (ng/g): 162.88; 354.70; 656.47; Pearson Chi square = .110; Likelihood ratio = .023.

	Certain infectious or parasitic diseases (1)	Diseases of the circulatory system (11)	Diseases of the digestive system (13)	Diseases of the nervous system (8)	Diseases of the respiratory system (12)	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes (22)	Mental, behavioural or neurodevelopmental disorders (6)	Neoplasms (2)	Symptoms, signs or clinical findings, not elsewhere classified (21)
1	3	1	1	2	4	3	2	1	1
2	5	3	0	4	0	3	0	2	2
3	2	6	2	0	2	3	0	4	0
4	2	3	2	1	1	0	1	7	1
Total	12	13	5	7	7	9	3	14	4

Table E.14. Distribution of UCOD across total average quartiles with outliers. Quartile cutoffs (ng/g): 172.10; 435.57; 958.75; Pearson Chi Square .046; Likelihood ratio = 0.16.

	Certain infectious or parasitic diseases (1)	Diseases of the circulatory system (11)	Diseases of the digestive system (13)	Diseases of the nervous system (8)	Diseases of the respiratory system (12)	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes (22)	Mental, behavioural or neurodevelopmental disorders (6)	Neoplasms (2)	Symptoms, signs or clinical findings, not elsewhere classified (21)
1	3	2	1	4	4	2	1	1	2
2	5	3	1	1	2	6	1	1	0
3	3	5	2	2	0	1	0	5	2
4	1	6	1	1	1	0	2	7	0
Total	12	16	5	8	7	9	4	14	4

Table E.15. Distribution of UCOD across total average quartiles without outliers. Quartile cutoffs (ng/g): 162.67; 390.43; 603.09; Pearson Chi square .042; Likelihood ratio = .019.

	Certain infectious or parasitic diseases (1)	Diseases of the circulatory system (11)	Diseases of the digestive system (13)	Diseases of the nervous system (8)	Diseases of the respiratory system (12)	External causes of morbidity or mortality/ Injury, poisoning or certain other consequences of external causes (22)	Mental, behavioural or neurodevelopmental disorders (6)	Neoplasms (2)	Symptoms, signs or clinical findings, not elsewhere classified (21)
1	3	1	1	3	4	1	1	1	2
2	4	4	0	2	0	6	0	1	0
3	4	2	2	1	2	1	1	3	2
4	1	6	1	1	0	1	1	6	0
Total	12	13	4	7	6	9	3	11	4

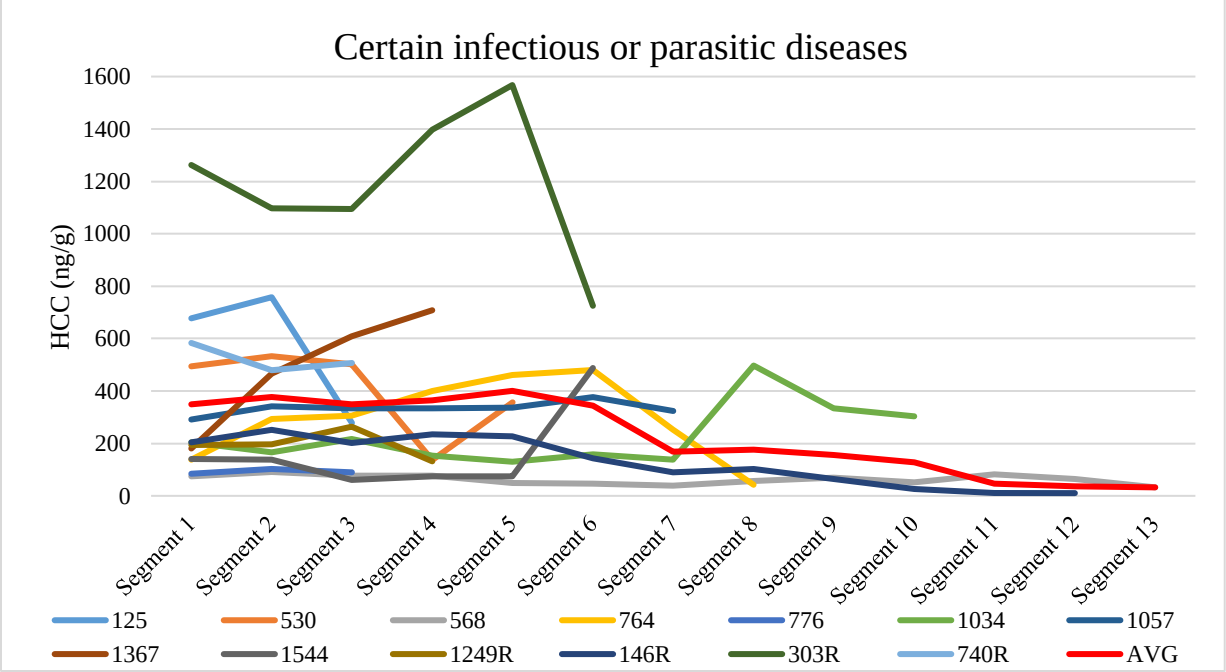

Figure E.2. Line graph of monthly hair cortisol concentration (HCC) for individuals dying of 'Certain infectious of parasitic diseases' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

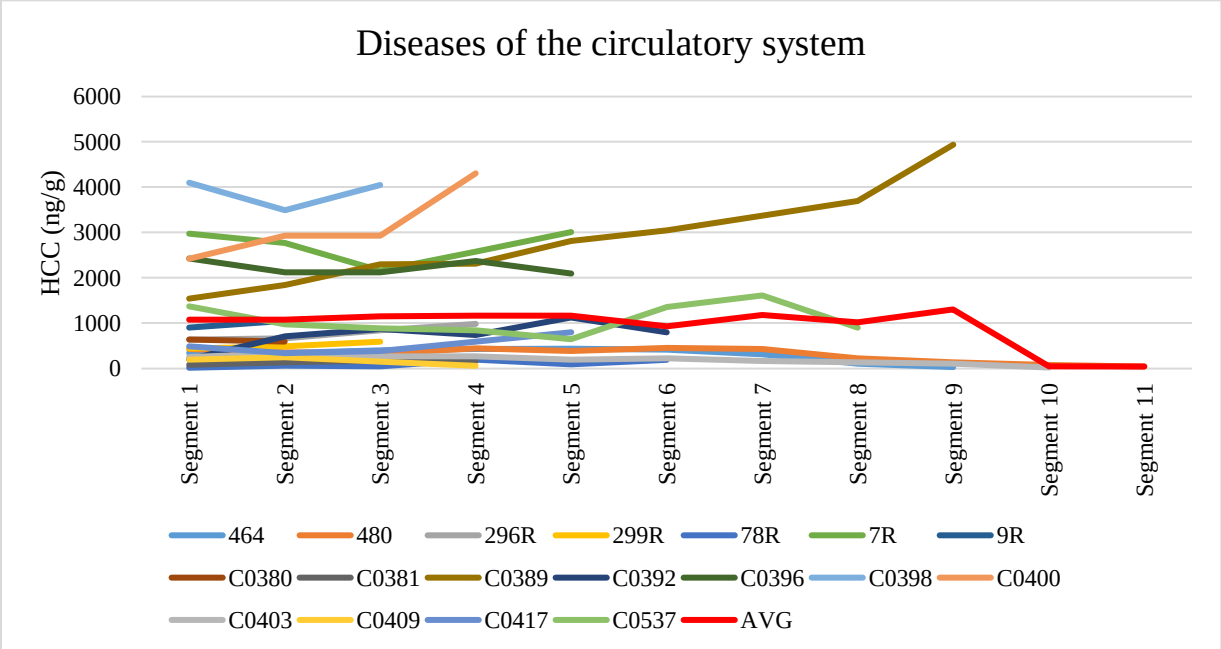

Figure E.3. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Diseases of the circulatory system' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

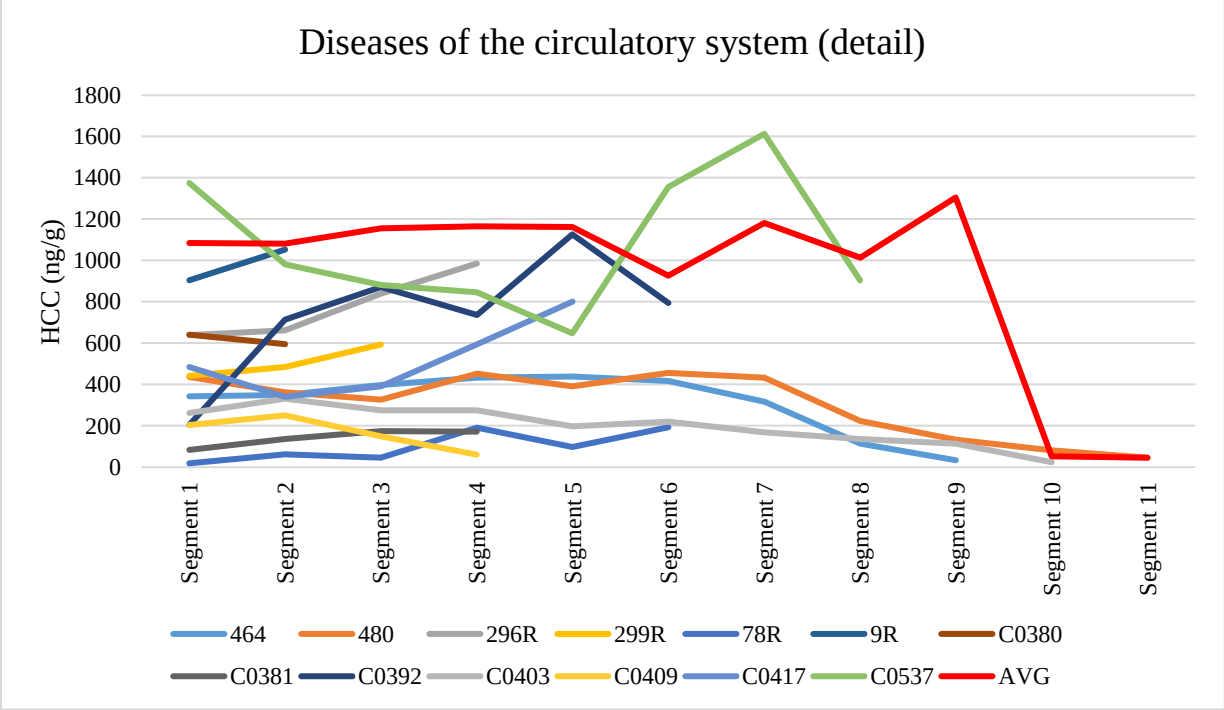

Figure E.4. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Diseases of the circulatory system' (with individuals with the highest HCC removed to show detail) in the Terry Collection and UCF Cadaver samples. Average curve is in red.

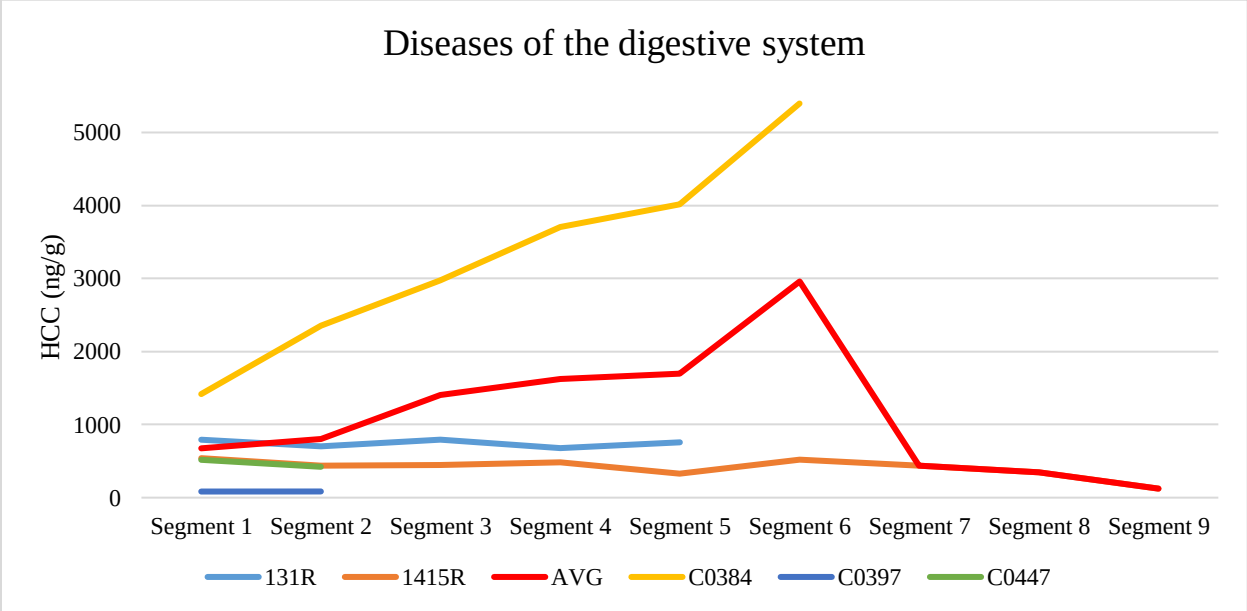

Figure E.5. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Diseases of the digestive system' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

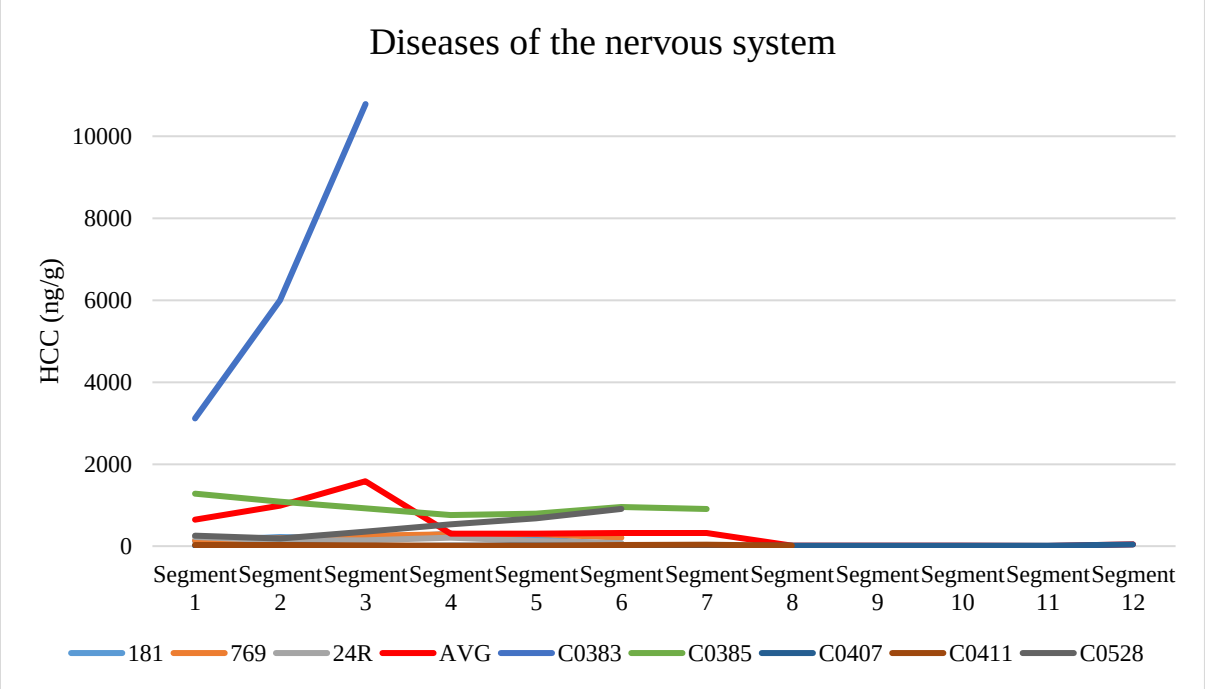

Figure E.6. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Diseases of the nervous system' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

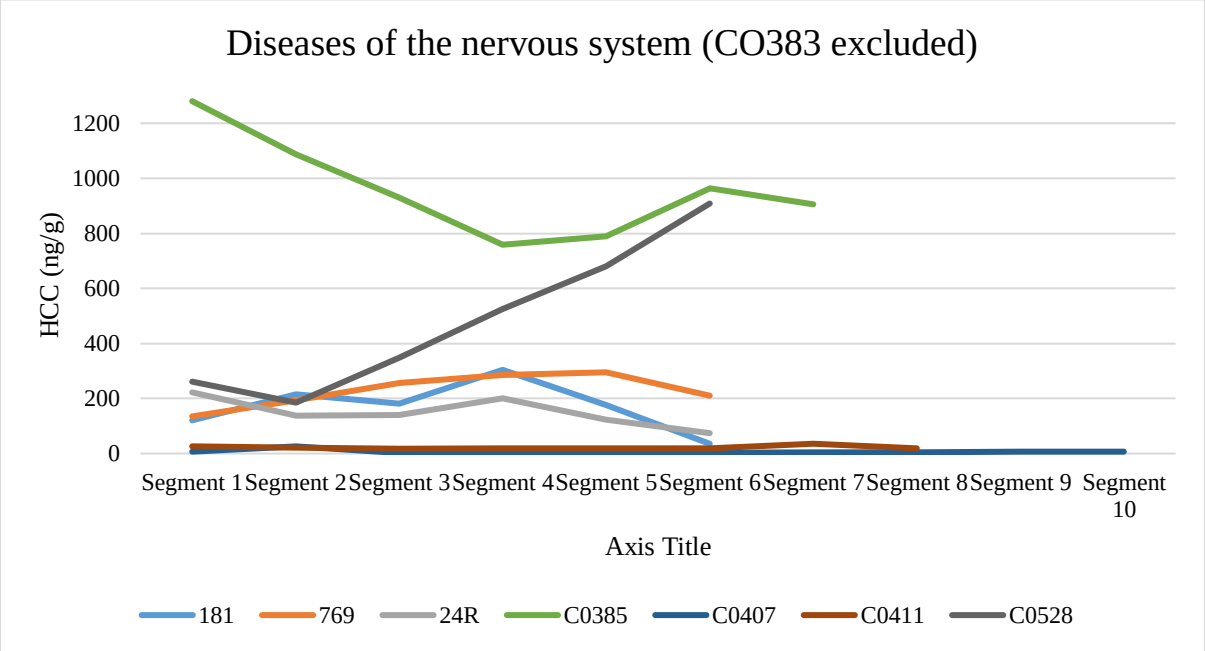

Figure E.7. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Diseases of the nervous system' (with CO383 and average curve excluded to show detail) in the Terry Collection and UCF Cadaver samples.

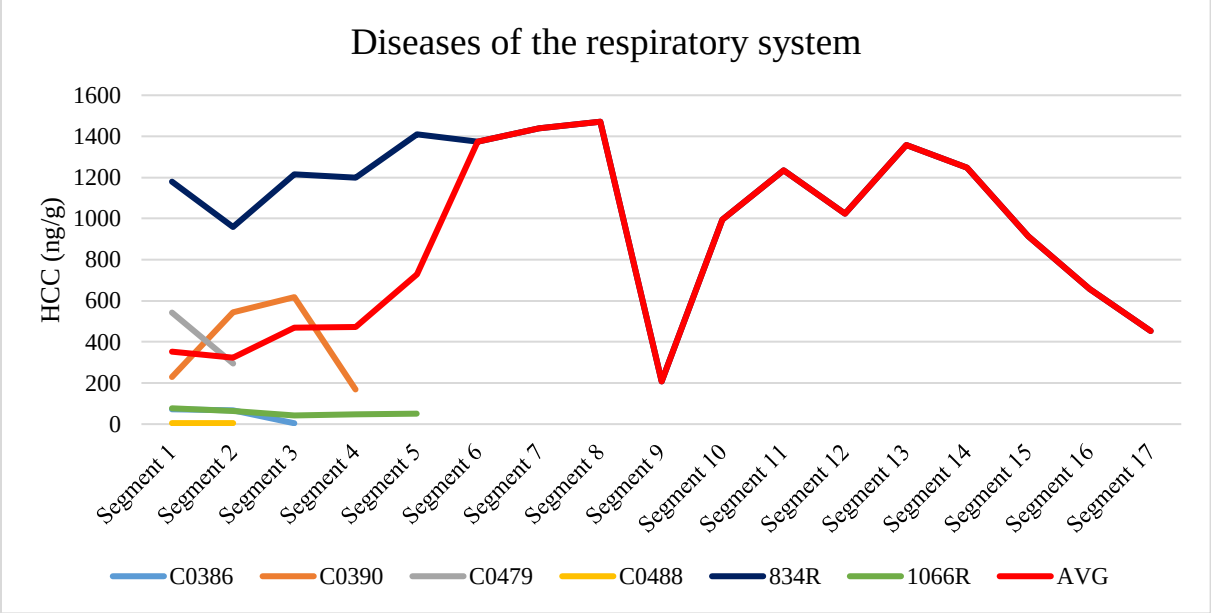

Figure E.8. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Diseases of the respiratory system' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

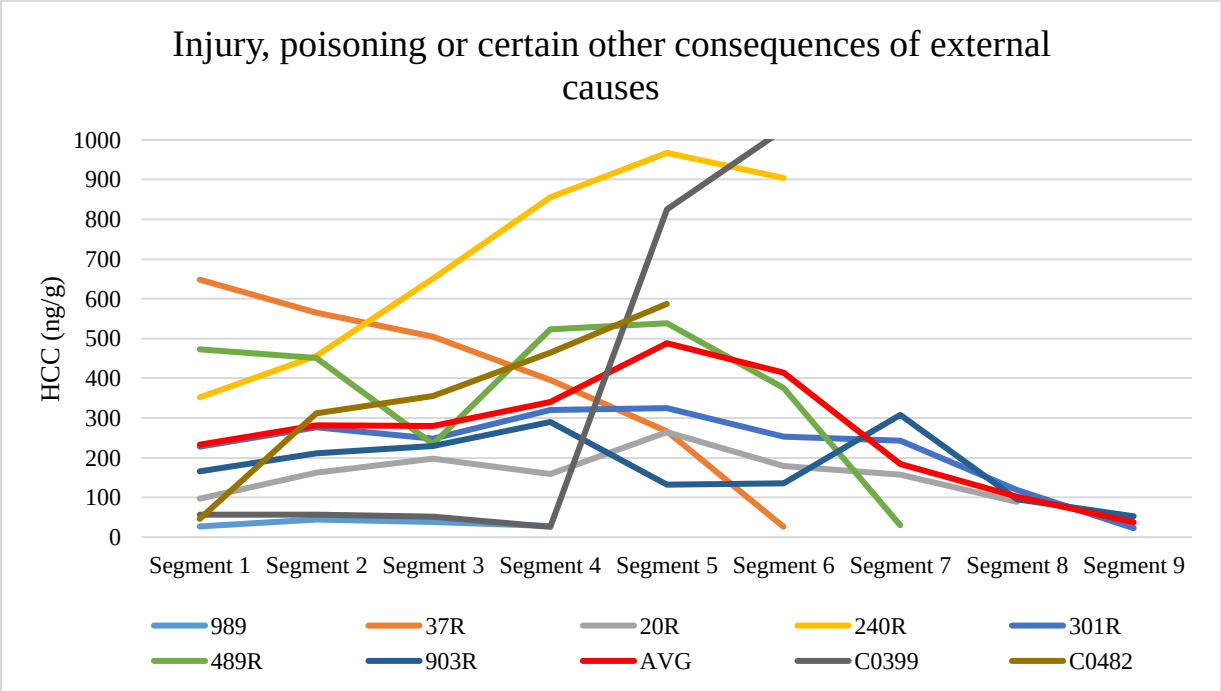

Figure E.9. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Injury, poisoning or certain other consequences of external causes' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

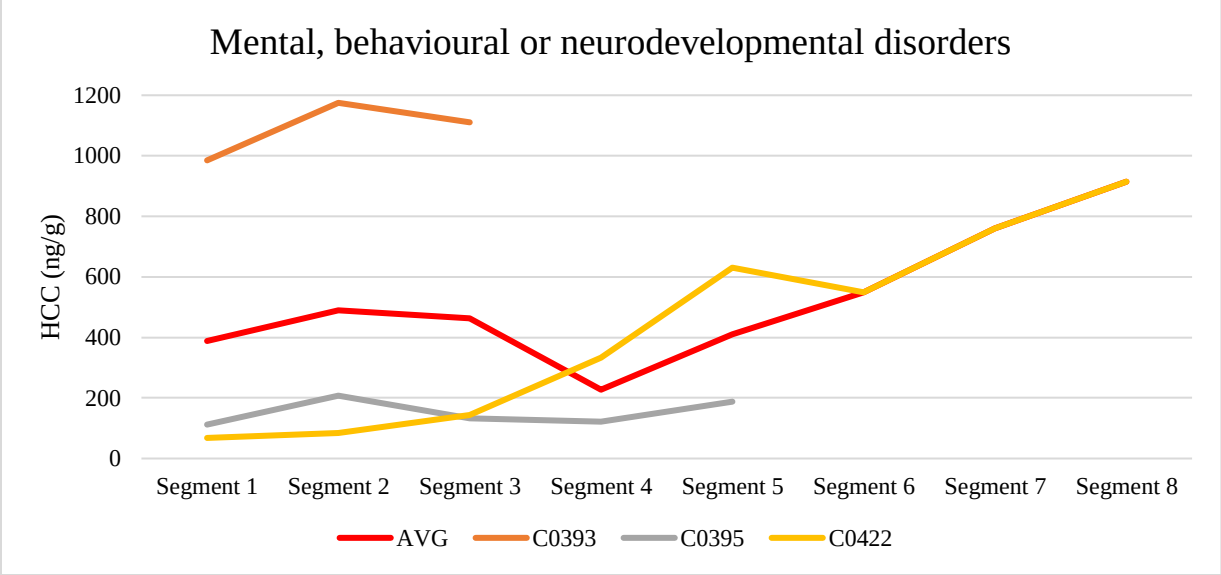

Figure E.10. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Mental, behavioural or neurodevelopmental disorders' in the UCF Cadaver sample. Average curve is in red.

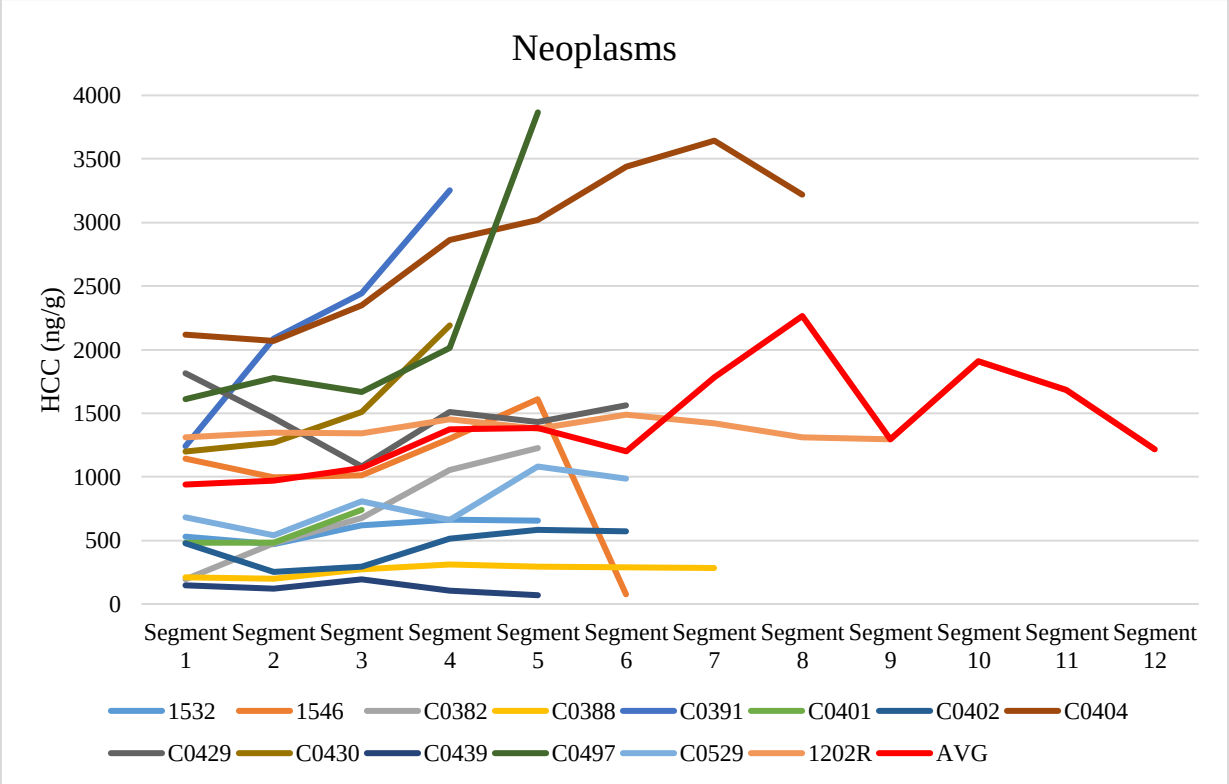

Figure E.11. Line graph of hair cortisol concentration (HCC) for individuals dying of 'Neoplasms' in the Terry Collection and UCF Cadaver samples. Average curve is in red.

E.2 DURATION OF DISEASE TEST RESULTS

Table E.16. Results of Independent-Samples Mann-Whitney U test comparing two-month average HCC in short-term and long-term conditions in the Terry Collection sample, where short-term and long-term are defined by duration of disease and differing duration cut offs are tested.

	Short-term		Long-term		
Cut off	Mean	Sample size	Mean	Sample size	P values
1 month	470.63	16	506.71	16	.184
3 months	454.35	18	533.94	14	.251
6 months	454.35	22	607.57	10	.704
9 months	435.80	23	651.13	9	.837
12 months	495.48	28	444.99	4	.934
15 months	495.48	28	444.99	4	.934
18 months	495.48	28	444.99	4	.934
21 months	490.32	29	478.01	3	.952

Notes: Total sample size = 32

E.3. HOSPITAL ENTRY AND DIAGNOSIS GRAPHS IN THE TERRY COLLECTION

Graphs in this section display monthly HCC values of those individuals in the Terry Collection who entered hospital more than two weeks before death. Blue dashed lines indicate diagnosis. Red solid lines indicate hospital entry. Changes in monthly HCC appear as disruptions in the direction of the HCC curve, such as a sudden, clear increase or decrease.

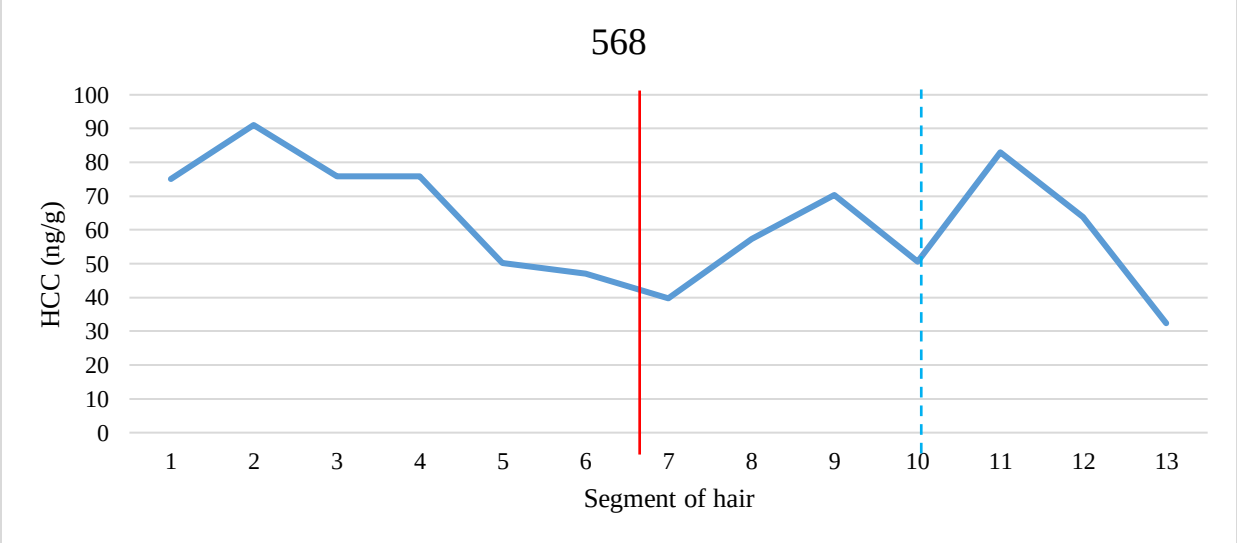

Figure E.12. Graph depicting response to hospital entry and disease diagnosis for individual 568 from the Terry Collection sample.

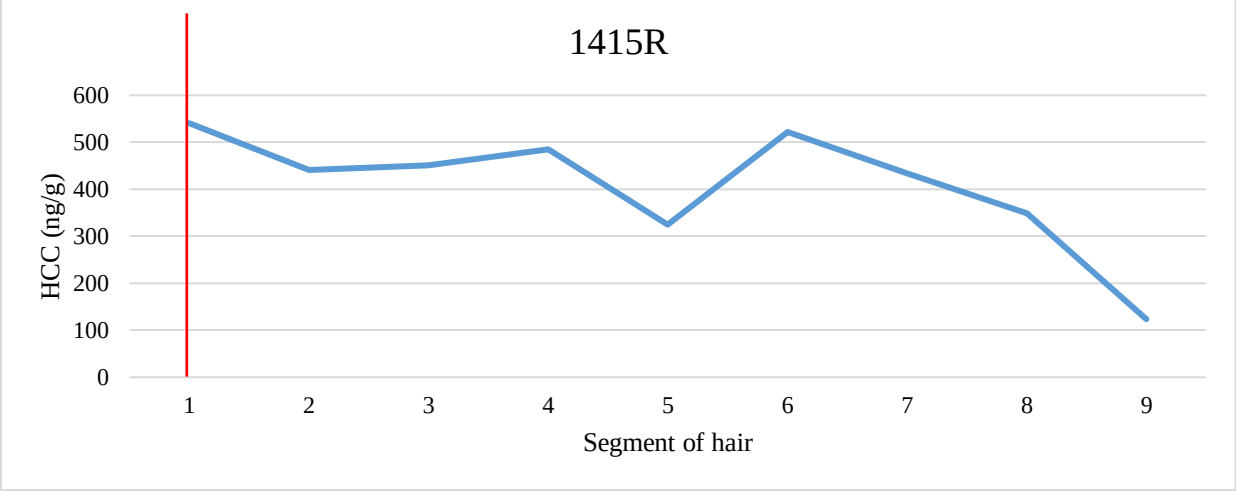

Figure E.13. Graph depicting response to hospital entry for individual 1415R from the Terry Collection sample.

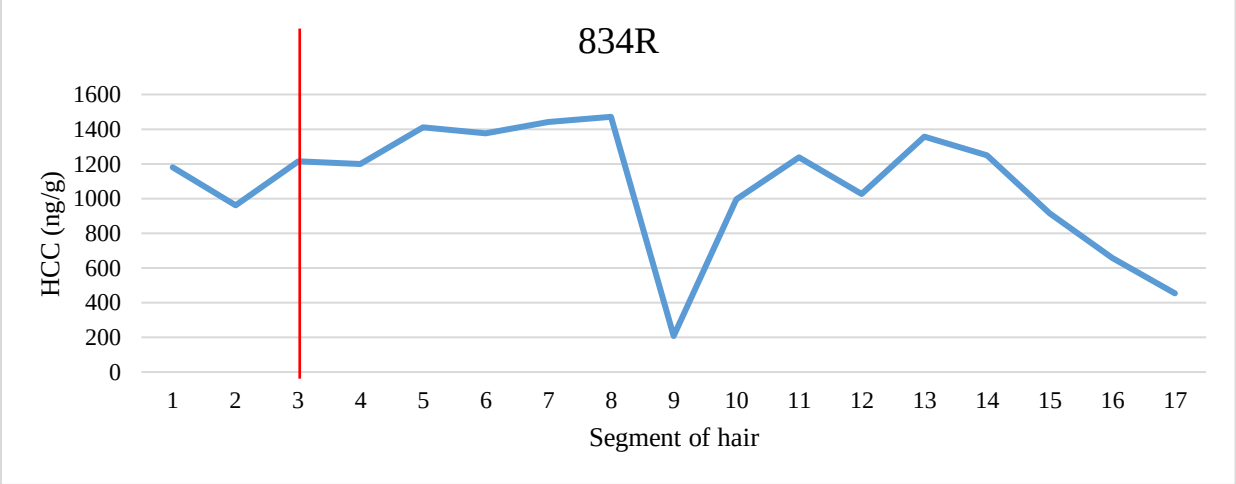

Figure E.14. Graph depicting response to hospital entry for individual 834R from the Terry Collection sample.

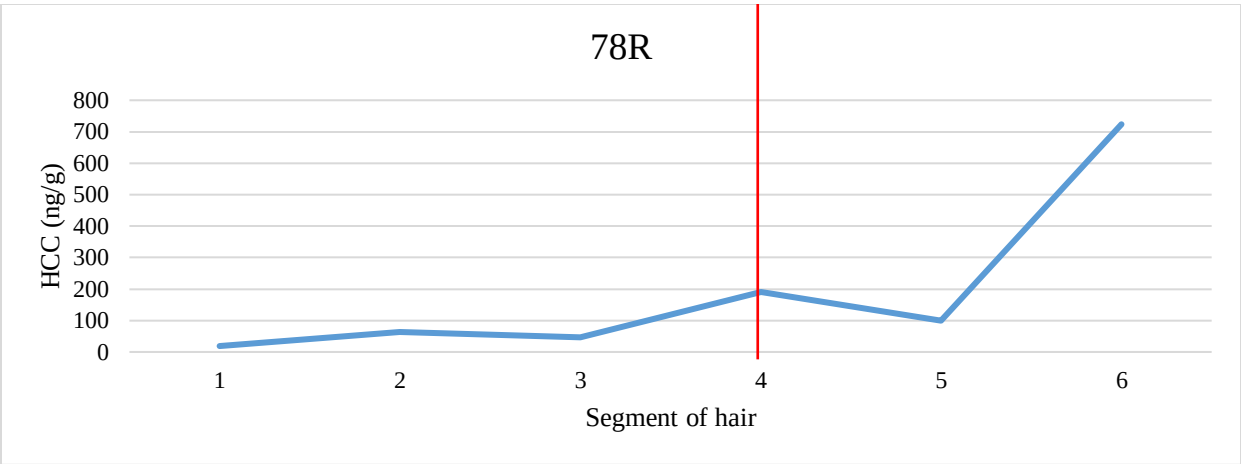

Figure E.15. Graph depicting response to hospital entry for individual 78R from the Terry Collection sample.

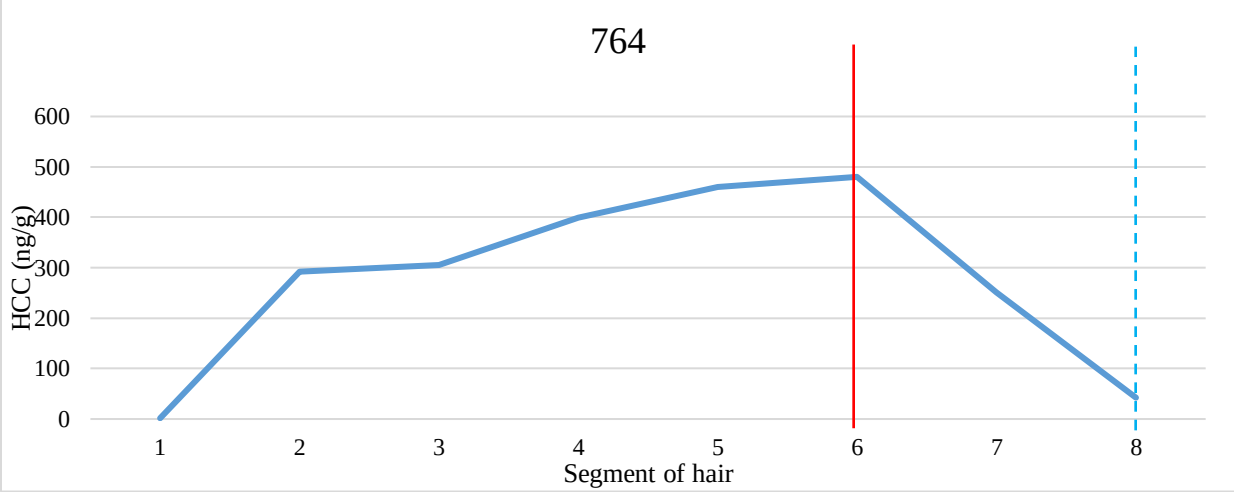

Figure E.16. Graph depicting response to hospital entry and disease diagnosis for individual 764 from the Terry Collection sample.

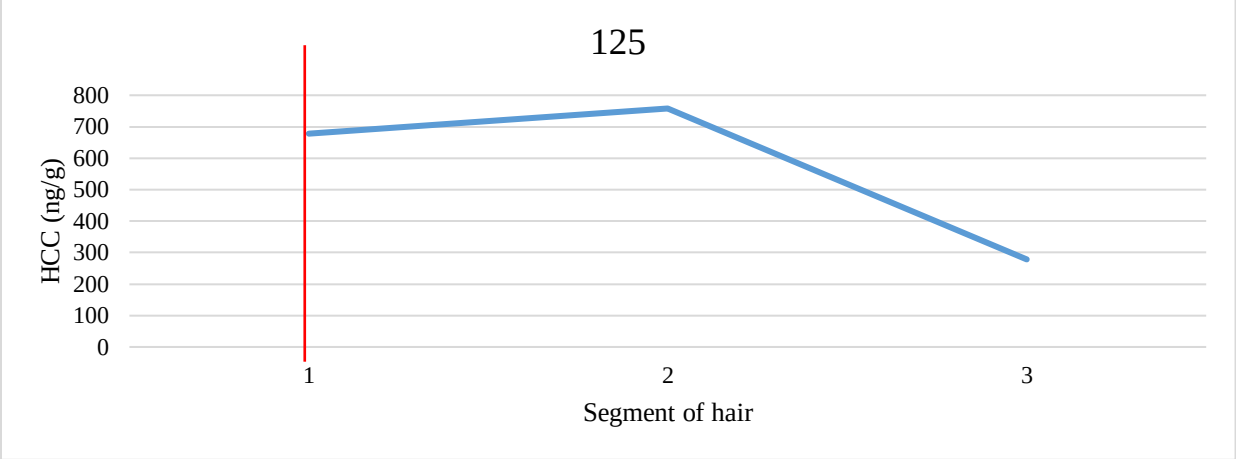

Figure E.17. Graph depicting response to hospital entry for individual 125 from the Terry Collection sample.

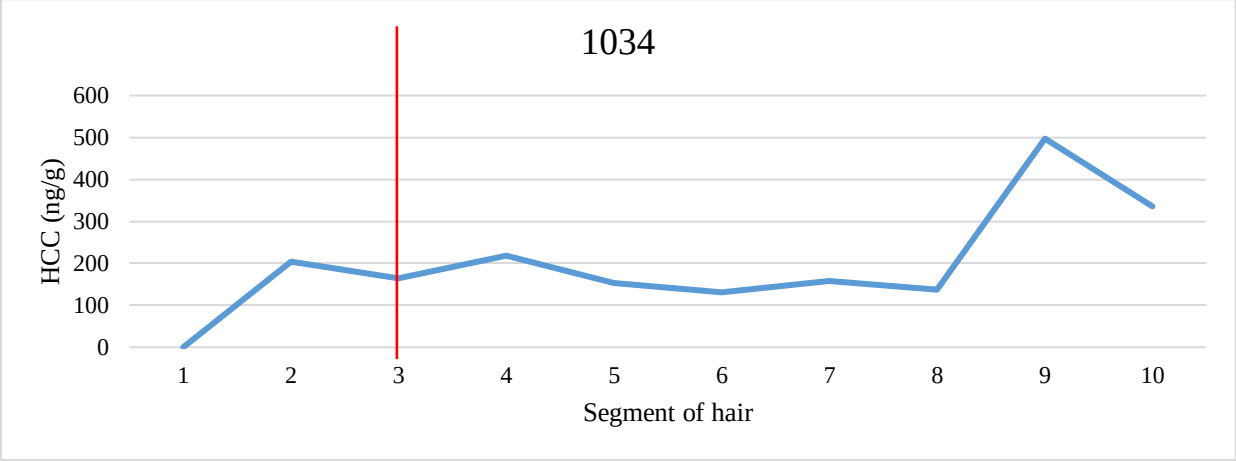

Figure E.18. Graph depicting response to hospital entry for individual 1034 from the Terry Collection sample.

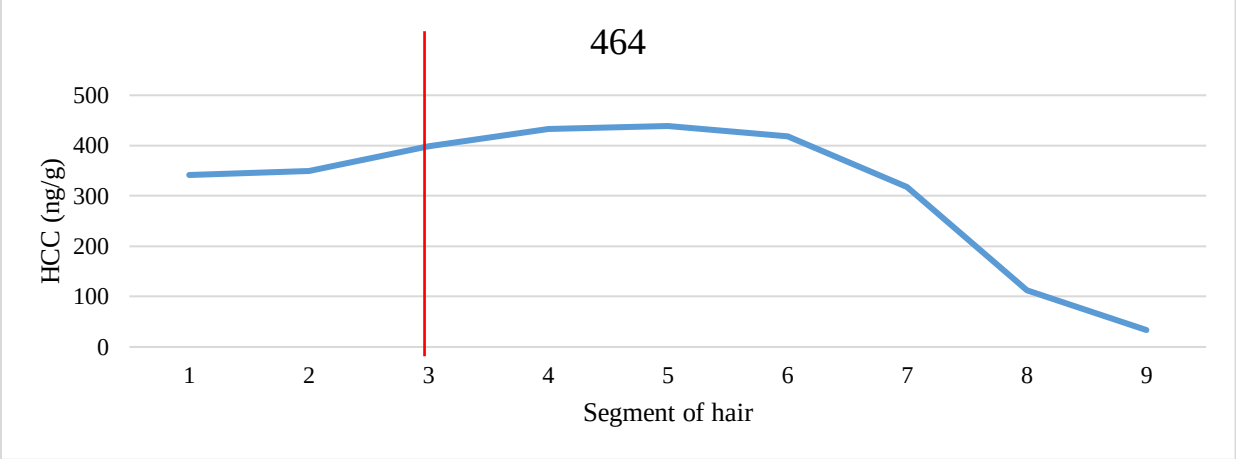

Figure E.19. Graph depicting response to hospital entry for individual 464 from the Terry Collection sample.

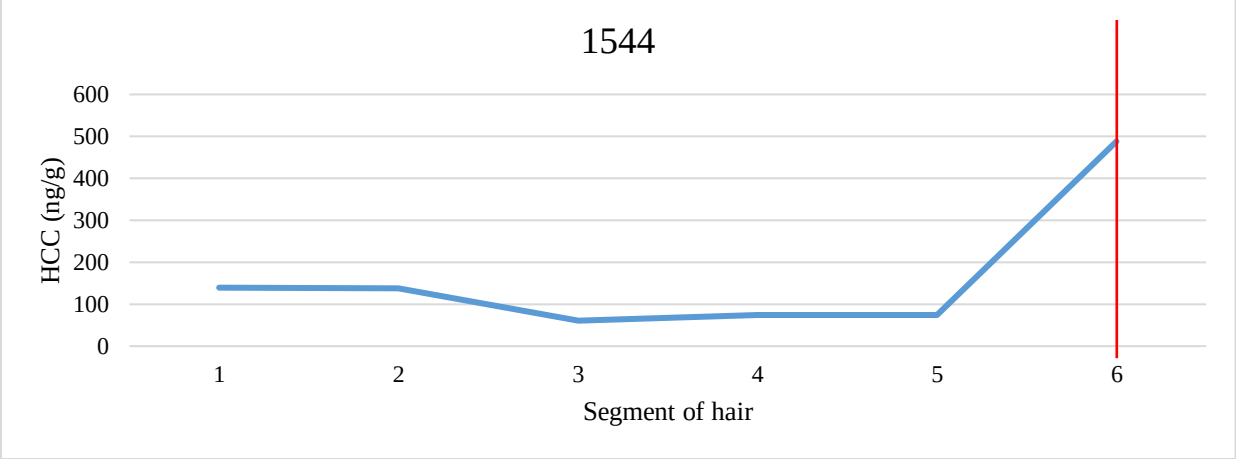

Figure E.20. Graph depicting response to hospital entry for individual 1544 from the Terry Collection sample.

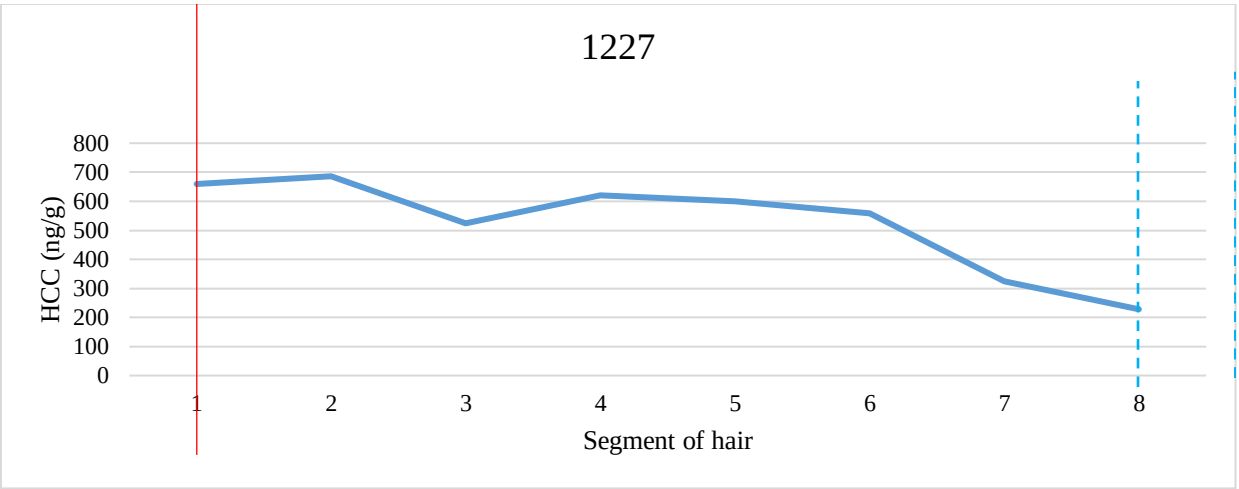

Figure E.21. Graph depicting response to hospital entry for individual 1227 from the Terry Collection sample.

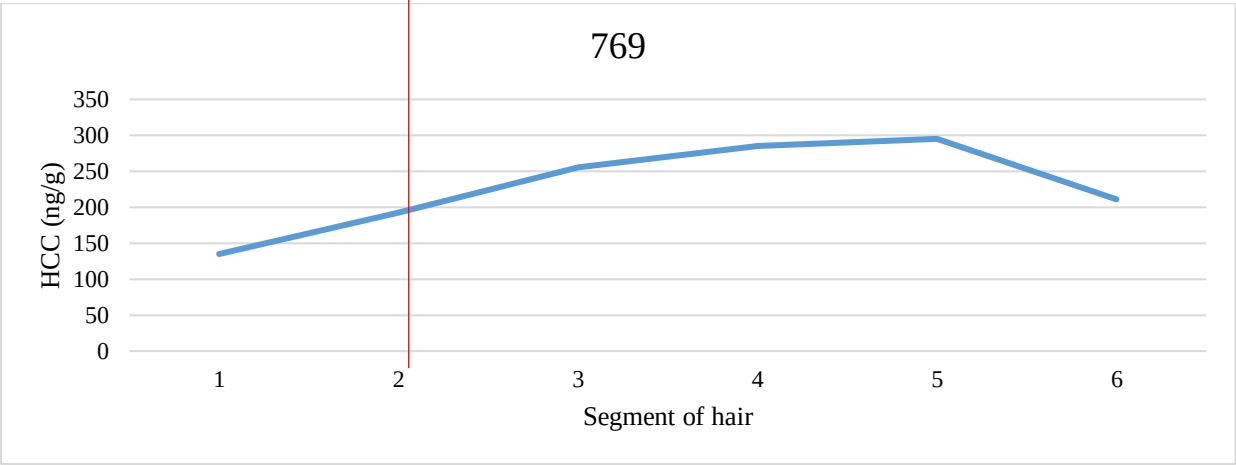

Figure E.22. Graph depicting response to hospital entry for individual 769 from the Terry Collection sample.

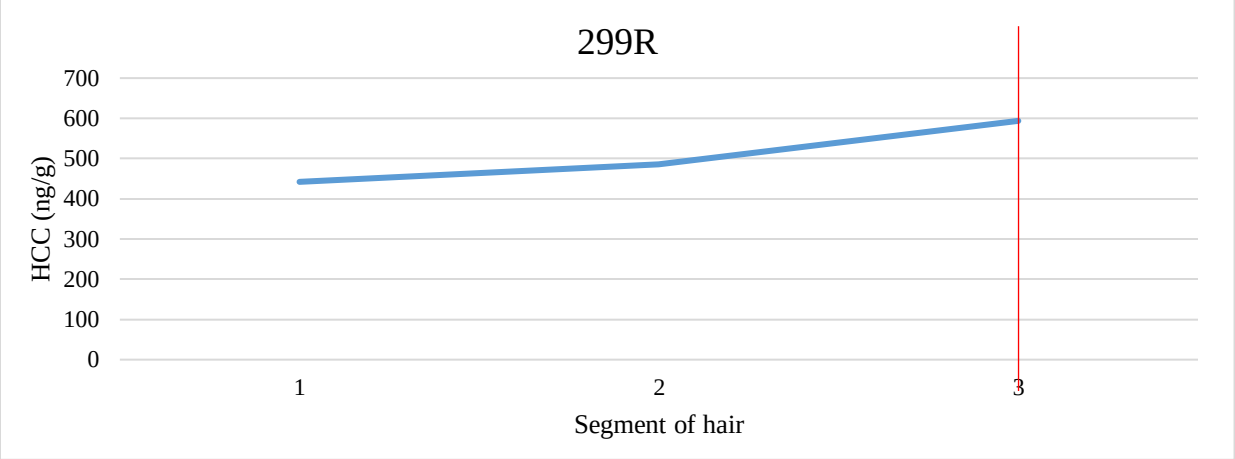

Figure E.23. Graph depicting response to hospital entry for individual 299R from the Terry Collection sample.

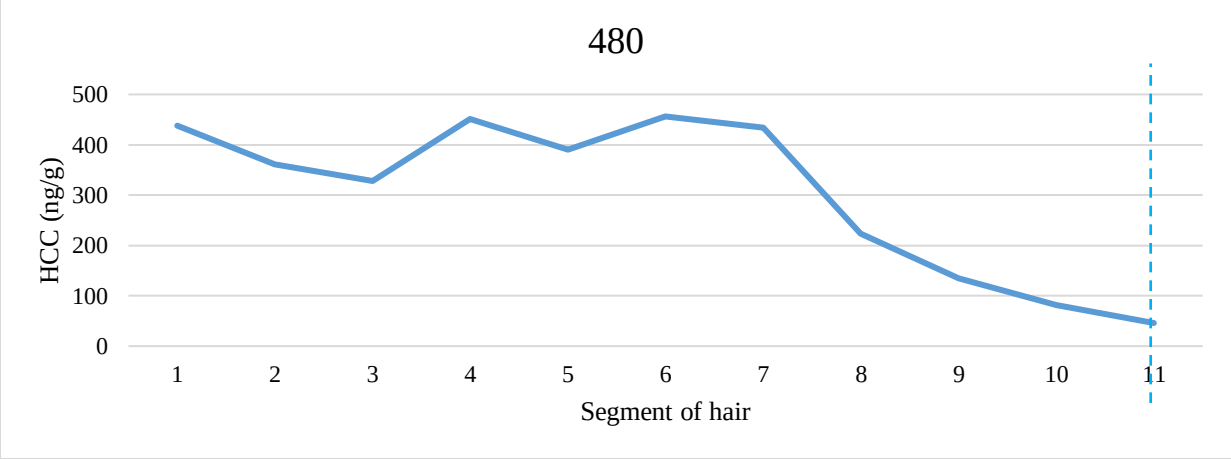

Figure E.24. Graph depicting response to disease diagnosis for individual 480 from the Terry Collection sample.