The Influence of Surgical Stress on Human Scalp Hair Fiber Dimensions

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Faculty of Environment

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or

b. advance approval of the animal care protocol from the University Animal Care Committee of Simon Fraser University;

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Abstract

Human scalp hair is an ideal medium for investigating the physiology and chemistry of an individual at the time of hair formation. Hair is taphonomically robust and, through its continuous growth, creates a chronological record of biochemical history. Changes to the physical characteristics of human scalp hair can therefore provide information on the presence and timing of antemortem acute physiological stress events.

Scalp hair samples were collected from males undergoing abdominal surgery for a variety of medical conditions. Surgery is a known and potent activator of the systemic stress response and the acute phase response, both of which require protein and lipid substrates for survival and wound healing. Hair samples were long enough to cover up to one month prior to surgery and one month following surgery. Methods for the assessment of hair fiber growth were compared for utility in stress analysis. Dimensions such as total fiber diameter, cuticle thickness, and cortex diameter were compared prior to and following surgery. This study was approved by all appropriate Ethics Review Boards.

Results of method comparison suggest that increased magnification from standard 400x to 1000x does not provide significantly different data. Measuring hair diameter digitally also does not provide data which differ significantly from diameter measured manually. Variables constructed from combined measurements do provide data more appropriate for detecting stress-related changes in the hair fiber than single dimension variables. Fiber dimensions analysed showed statistically significant differences between pre- and post-operative values, which returned to normal in the fourth post-operative week.

Keywords: Human scalp hair; stress; surgery
Acknowledgements

There are several people without whom data collection for this study would not have been possible. Dr. Carl Brown at St. Paul's Hospital in Vancouver, BC graciously allowed me to recruit his surgical patients as research participants. Dr. Nancy Baxter at St. Michael's Hospital in Toronto, ON not only greatly facilitated participant recruitment from her patients but offered additional support through a difficult period of data collection.

Thanks to Dr. Tammy Sage and Kathy Sault at the University of Toronto for their unending willingness to troubleshoot microscope problems. To Charles Victor of the Institute for Clinical Evaluative Sciences for providing reassurance about my statistical analysis. Special thanks to my committee advisors, Gail and Hugo, for their willingness to take on an orphaned graduate student.
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List of Acronyms

ACD       Average cortical diameter
ATD       Average total diameter
CMC       Cell membrane complex
HCC       Hair cortisol concentration
HLM       Hierarchical linear modeling (also LMM; linear mixed modeling)
HPA       Hypothalamus-pituitary-adrenal (axis)
KAP       Keratin-associated proteins
PCM       Protein calorie malnutrition
## Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anagen</strong></td>
<td>The active growth phase of the hair follicle</td>
</tr>
<tr>
<td><strong>Bulb</strong></td>
<td>The most proximal area of the hair follicle in which a proliferating cell matrix produces new hair fiber cells.</td>
</tr>
<tr>
<td><strong>Bulb Date</strong></td>
<td>“Any point along a hair shaft is defined as the number of days required for a cell to move from the hair bulb to the point” (Sims 1967:44).</td>
</tr>
<tr>
<td><strong>Bulge</strong></td>
<td>Area of the hair follicle outer root sheath located at approximately the insertion point of the arrector pili muscle containing a wide variety of cell types including those responsible for regeneration of the dermal papilla during early anagen.</td>
</tr>
<tr>
<td><strong>Catagen</strong></td>
<td>The phase during which the hair follicle transitions from actively growing to resting.</td>
</tr>
<tr>
<td><strong>Lanugo</strong></td>
<td>Very fine body hair, usually found on the fetus and shed immediately prior to or after birth.</td>
</tr>
<tr>
<td><strong>Micron (µ or µm)</strong></td>
<td>Unit of measure equal to $1 \times 10^{-6}$ of a meter, or 1/1000 of a millimeter, formally termed a “micrometer” by the International System of Units.</td>
</tr>
<tr>
<td><strong>Telogen</strong></td>
<td>The resting phase of the hair follicle during which a hair fiber is not growing.</td>
</tr>
<tr>
<td><strong>Terminal hair</strong></td>
<td>Long, normally heavily pigmented hairs found on the scalp and areas of secondary sexual development (face, chest, axillary and pubic regions).</td>
</tr>
<tr>
<td><strong>Vellus hair</strong></td>
<td>Short, normally lightly or non-pigmented hairs found on areas of the body without terminal hairs.</td>
</tr>
</tbody>
</table>
Chapter 1. Introduction

The study of stress has long been an important part of biological anthropology. Understanding how stress affects both individuals and populations provides insight into evolutionary processes, archaeological populations, culture change, and interpersonal violence. Recently, hair is playing an increasing role in the investigation of stress in both archaeological and modern populations (Bonnichsen, et al. 2001; Dettenborn, et al. 2012; Kirschbaum, et al. 2008; Sauve, et al. 2007; Stalder and Kirschbaum 2012; Steudte, et al. 2013; Webb, et al. 2011, 2013). Hair is taphonomically robust and provides a chronological record of an individual’s physical status at the time of growth. Changes to the physical characteristics of human scalp hair can therefore provide information on the presence and timing of acute antemortem stress events. The detection of acute stress events can improve our understanding of individual health and community behaviors in bioarchaeological contexts, and contribute to estimation of antemortem life events in forensic identification efforts.

Hair is an ideal medium for investigating the physiology and chemistry of an individual at the time the hair formed. Hair grows at a fairly constant rate and unlike bone and soft tissue, does not undergo any biogenic turnover. Hair follicle epithelium (the cells responsible for producing hair fibers) is one of the human body’s most rapidly proliferating tissue, second only to bone marrow, and hair fiber cells do not change once keratinized (Chang, et al. 2005; Tobin and Kauser 2005). In the last decade, multiple researchers have independently discovered that hair follicles exhibit a local functional equivalent to the hypothalamic-pituitary-adrenal (HPA) axis. This finding so significantly indicates the connection between stress and the hair follicle that it has given rise to a new field of study devoted to cutaneous neuroendocrinology (Arck and Paus 2006). Scalp hair provides a long-term history reflecting body chemistry which is collected non-invasively, making it an ideal resource for research applications (Gow, et al. 2010; Tobin and Kauser 2005).
Early research on human scalp hair began with a focus on indicators of malnutrition. Changes in the color and texture of scalp hair are now commonly accepted symptoms of chronic protein calorie malnutrition (PCM) (Thibaut, et al. 2005). Changes to the physical structure of hair have also been suggested to reflect the onset and duration of severe febrile illnesses, although the quantitative studies are few and their conclusions are not yet widely accepted by the scientific community. Psychological stress has often anecdotally been blamed for decreases in the amount and quality of scalp hair. Only a few studies have attempted to quantify these claims, with conflicting results.

In recent decades, chemical analysis of scalp hair has been used as an investigative tool for substance abuse, exposure to environmental toxins, and hormone levels, especially cortisol. Cortisol is a widely accepted biomarker of stress and an indicator of activation of the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol is deposited in the hair shaft from the hair follicle during growth. Immunoassay of hair can provide data on the average amount of stress an individual experiences on a monthly basis, and is increasingly used to study chronic stress.

The existing literature on the effect of stress on the growth and development of hair contains several weaknesses. To detect stress-related changes in hair fiber, it is first necessary to understand the nature and extent of normal, non-stress related variation. Previous studies mostly failed to address the amount of normal daily variation along the length of hair fibers. The few studies which did attempt to investigate normal variation did so on a very small, homogenous sample and did not specifically look into daily variation. Another major flaw in existing studies is the methods chosen to investigate stress-induced change. Most studies measure hair diameter in only one plane. If hair shaft cross-sections were circular, a single plane measurement would accurately reflect the volume of hair cells produced during growth. However, hair shafts are ovoid in cross-section, with the direction of the major axis twisting along the length of the hair shaft. Straight hair shows the least difference between “major” and “minor” axes and twists slowly, with curly hair showing the greatest difference between major and minor diameters with a tighter twist. Changes in a single diameter of the hair shaft are likely to reflect a change in perspective on the hair shaft (shifting from major to
minor axis) and not necessarily an actual reduction in the volume of the hair fiber at the location of measurement.

In addition, the influence of acute physiological stress events such as physical trauma has not been addressed. The investigation of malnutrition and psychological or emotional stress excludes the effect of sudden physical trauma on otherwise normally nourished or relatively physically healthy individuals. Chronic psychological stress, chronic malnutrition, and chronic pain have been investigated. However, large gaps remain in the literature regarding severe, sudden onset conditions such as injury. Hair cortisol concentrations are often used in research of chronic stress. The material necessities of cortisol immunoassay require approximately one month’s hair growth for analysis. This creates a window of approximately one month prior to hair sampling during which very little information is available about the acute stresses influencing an individual. Existing research on the influence of stress on hair has focused on nutritional stress or the use of cortisol to investigate chronic stress levels. Research on stress and hair has so far not been able to detect short-term, severe stress events such as injury.

In consideration of these issues, the current study addresses the following research questions:

1. What method should be used to assess changes in hair fiber physical characteristics?
2. What is the normal variation in fiber dimensions along the length of a hair fiber?
3. Do the dimensions of the hair shaft change in response to an acute physiological stress such as surgery?
Chapter 2. Background

Humans are the only mammals to exhibit a drastic reduction in body hair; while we maintain a similar number of hair follicles as non-human primates ours is significantly finer, lighter, and shorter (Lupi 2008; Sandel 2013). However, we also have scalp hair with an unusually long anagen cycle length. Why we have such an unusual hair growth pattern has been contested for years with only slight agreement regarding factors such as thermoregulation, parasite prevention and detection, and sexual/social communication (Robbins 1994, 2012; Sandel 2013). The many theories regarding our relative lack of body hair and abundance of scalp hair all agree that they result from unique selective pressures present during the course of human evolution (Tobin 2008). The scalp hair likely serves at least in part to protect the scalp from UV radiation during bipedality, especially in the equatorial African homeland of human evolution (Lupi 2008; Robbins 2012). Dark hair and skin is the ancestral state of humanity, as the high UV presence of early human savannah habitats necessitated protection. The photo-protective ability of melanin is much more effective in dark hair than in light hair (Robbins 2012) and recent genetic evidence suggests that the selective pressures against light hair mutations, which would have been quite high in early human evolution, were reduced in populations who migrated towards northern Europe (Tobin 2008). Selective pressures may have actually swung in the opposite direction to benefit vitamin D absorption in the low UV regions of northern Europe (Rees 2006; Rees and Harding 2012). Another theory considers the reliance on fish during early stages of human evolution and suggests the relatively long anagen stage of human scalp hair follicles to be a mechanism preventing the buildup of heavy metals, which are concentrated by many fish species. Binding toxic metals circulating in the bloodstream to melanin via a highly vascularized, rapidly and continuously proliferating tissue would trap the toxins in hair fibers thus segregating them away from the living tissues (Tobin 2008). Although the exact mechanism is still under debate, clearly some selective advantage to an extended anagen phase for the scalp follicles has persisted throughout human evolution.
The extended growth phase of human scalp hair provides a unique resource for assessing changes in individual body chemistry. The continuous growth of scalp hair records fluctuating hormone levels, nutritional status, exposure to intoxicants, and regional-specific isotope values over time. Hair not only provides a chronological record of an individual’s physiological status, it is a taphonomically robust bio-resource resistant to degradation in forensic and archaeological contexts. Scalp hair is non-invasive to collect and requires no special considerations in transport or storage.

2.1. Hair follicle and fiber

2.1.1. Hair follicle

Hair fibers are produced in the hair follicle, a sac originating in the subcutaneous tissue of the skin (Paus and Cotsarelis 1999). Human hair follicles begin developing between the eighth and twelfth weeks of gestation and by the time of birth, all the follicles a person will have throughout his or her life have been formed. Because of the finite number of follicles, the density of their distribution lessens throughout maturation as they spread further apart to accommodate increasingly larger areas of skin. Scalp hair follicles are more dense than other regions of the body in part because in comparison to the torso and limbs, the head increases in size less (Vogt, et al. 2008). Humans have roughly five million hair follicles over the body, the specific purpose of which (scalp, eyebrow, pubic, etc.) is established during formation of the follicle. Although the number of follicles is consistent, the size and types of hairs produced can change throughout life. During puberty, circulating androgens stimulate production of larger, thicker terminal hairs in follicles that once only produced fine, vellus hairs (axillary, pubic, and facial follicles) yet later in life the same hormones are responsible for the atrophy and miniaturization of scalp follicles leading to androgenic alopecia (“male pattern baldness”) (Paus and Cotsarelis 1999).

The follicle along with the adjacent sebaceous gland and the arrector pili muscle form the pilosebaceous unit (Vogt, et al. 2008) (Figure 2-1). In the scalp, several terminal and vellus hair follicles may share a single sebaceous gland and be encircled by a single arrector pili (Buffoli, et al. 2014). The anatomy of the pilosebaceous unit is
normally differentiated into a permanent, more superficial component and a cycling component which sits deeper within the surrounding tissues during active hair growth (Vogt, et al. 2008). The permanent region spans from the skin’s surface to just below the bulge region, while below the bulge region to the base of the follicle is the transient section of the follicle.

Figure 2-1  The pilosebaceous unit. (Modified with permission from Vogt, et al. 2008)

During fetal morphogenesis, hair follicle cells differentiate into multiple concentric layers responsible for the growth and production of the hair shaft (Figure 2-2). The layers are formed from both epithelial and mesenchymal cells, and include over 20 different cell populations responsible for the production of the hair shaft. At the base of the bulb sits the dermal papilla, which is composed of fibroblasts (specialized mesenchymal cells) and controls the number of matrix cells, thereby controlling hair growth (Paus and Cotsarelis 1999). A capillary loop provides nutrition to the papilla and overlying matrix cells of terminal hair follicles (Robbins 1994, 2012; Vogt, et al. 2008). Surrounding the dermal papilla sit the rapidly proliferating matrix keratinocytes
(sometimes called the basal layer), creating the cells that eventually become the hair fiber and the inner root sheath. Melanocytes produce the pigment granules to be incorporated into the hair fiber.

The inner root sheath (IRS) consists of three keratinized layers (the Henle, Huxley, and cuticle) and hardens prior to the keratinisation of the hair fiber itself (Thibaut, et al. 2005). The IRS cuticle abuts the hair fiber cuticle, anchoring the developing fiber in the follicle (Buffoli, et al. 2014). The dimensions and shape of the rigid inner root sheath contribute to the cross-sectional dimensions and shape of the final hair fiber (Buffoli, et al. 2014; Paus and Cotsarelis 1999; Powell and Rogers 1997; Vogt, et al. 2008).

Surrounding the inner root sheath is the outer root sheath (ORS), extending from the bulb up to where the duct from the sebaceous gland enters the follicle. The ORS is adjacent to the epidermis and is generally several cells thick, gradually thinning until only a single cell layer surrounds the follicle bulb. Unlike the other cell types, the generation and maintenance of the ORS appears to be independent of the follicle bulb proliferative cells (Powell and Rogers 1997). The outer root sheath contains multipotent stem cells (Buffoli, et al. 2014), which express a surprising variety of substrates distinct from those expressed by other epidermal cells including hormones, mediators, growth factor receptors, adhesion molecules, and keratins. These substrates contribute to wound healing by migrating out of the follicle into the surrounding epidermis after injury (Paus and Cotsarelis 1999; Vogt, et al. 2008).

The line of demarcation between the superior permanent part of the follicle and the inferior, cyclic region of the follicle is generally agreed to be at the inferior margin of the bulge region. The bulge region is an outward bulge in the outer root sheath just below the entrance of the sebaceous duct, at the location of the arrector pili muscle insertion. The bulge contains a cluster of biochemically distinct cells with the characteristic properties of epithelial stem cells. They are the longest-lived, but slowest-cycling epithelial cells of the pilosebaceous unit. In addition to epithelial and neuroectodermal stem cells, the richly innervated bulge contains populations of immature melanocytes, Langerhans cells (dendritic antigen-presenting cells), Merkel
cells (specialized neurosecretory cells), and mast cells (Paus and Cotsarelis 1999; Vogt, et al. 2008).
Superior to the bulge is the permanent region of the hair follicle, which can be further divided into subregions. From the bulge to the entrance of the sebaceous duct into the follicle is the isthmus (Buffoli, et al. 2014; Vogt, et al. 2008). The isthmus is relatively featureless, although this is the area where the normally thick, vitreous membrane between ectoderm and mesoderm is thinnest. The isthmus wall consists of two to three rows of flattened cells. The orientation or angle of these cells changes along the isthmus as the outer root sheath merges with skin epithelium (Vogt, et al. 2008). Above the isthmus, from the sebaceous duct opening to the skin’s surface is called the infundibulum (Vogt, et al. 2008). The infundibulum has two sub-regions, is funnel-shaped, and acts as a reservoir for sebum from the sebaceous gland and potentially for dermally applied substances (Buffoli, et al. 2014). The most superficial part, the acro-infundibulum, has well developed epidermal layers which become less differentiated inferiorly towards the infra-infundibulum (Vogt, et al. 2008).

2.1.2. Hair growth

Hair fiber production

The hair fiber passes through four distinct zones during its production. Hair cells are produced by the basal layer surrounding the dermal papilla in the base of the follicle. The lower or most proximal region surrounding the dermal papilla is referred to as the cell matrix, or zone of cell proliferation (Robbins 2012).

The germinative cells in the matrix undergo repeated mitotic cell division, but only within the follicle bulb up to “Auber’s line,” roughly across the widest part of the dermal papilla (Buffoli, et al. 2014). Below this critical level, the unusually high rate of cell proliferation creates a constant supply of undifferentiated new hair cells (Buffoli, et al. 2014; Powell and Rogers 1997). During synthesis of the hair cells, proteins are maintained in a “reduced” state with little to no disulfide cross-links enabling the cells to maintain pliability. As new cells are produced, they push the previously produced cells distally along the axis of the hair follicle into the zone of cell differentiation. In this region, pigment granules produced by melanocytes in the cell matrix are incorporated into the hair cells by a mechanism of phagocytosis (Robbins 2012) and the cells begin to align longitudinally (Buffoli, et al. 2014). Above this sits the cellular elongation region,
in which the bulb narrows forcing the hair cells to elongate. In this pre-keratogenous zone, fine microfibrils begin to form in the cortex (Buffoli, et al. 2014). In the zone of keratinisation, disulfide and iso-peptide cross-links are formed, the cells are hyalinised, dehydration occurs, and the hair keratins are stabilized into their final form (Buffoli, et al. 2014; Robbins 2012). Finally, the fully formed permanent hair fiber is pushed outwards and eventually exits the skin’s surface.

**Hair growth phase cycling**

Hair follicles cycle through three distinct phases. The anagen, or active growth, stage is a period of intense metabolic activity within the follicle bulb. Roughly 85% of scalp hair follicles are in anagen, lasting from two to five years; there is a large degree of individual variation. Roughly five percent of an individual’s hair follicles are in the transitioning catagen stage at any one time, when the follicular activity is reduced, hair growth slows down, and the follicle shifts from anagen to telogen. Ten to fifteen percent of follicles are in telogen phase, during which the follicular bulb is atrophied and no new growth occurs. The old hair fiber often stays in the follicle until pushed out by the next hair fiber growth, but can be shed through mechanical strain (pulling on the hair) or pathological factors (skin irritation, disease, environmental factors, etc.). After 4-8 weeks of resting, the follicle will re-enter anagen phase and begin producing a new hair fiber.

The timing of transition from anagen to catagen is genetically determined and varies according to location and type of follicle (vellus vs. terminal, etc.) (Vogt, et al. 2008). During catagen phase, the production of protein, hair cell, and pigment granules slows to a stop via a process of apoptosis (pre-programmed cell death) in the proliferative matrix. The follicle undergoes involution, with the lower transient region atrophying and the dermal papilla travelling upwards to rest underneath the bulge in a condensed state (Buffoli, et al. 2014; Messenger 1993; Paus and Cotsarelis 1999; Vogt, et al. 2008). The normally extensive extracellular matrix of the dermal papilla reduces in volume until practically non-existent during telogen, leaving only a condensed bundle of dormant cells resting below the epithelium adjacent to the bulge region. The capillary loops and innervations of the dermal papilla also atrophy and are non-existent by entry into the telogen phase (Messenger 1993; Vogt, et al. 2008).
After the follicle rests for one to two months, signals from the condensed dermal papilla bundle to the secondary germ cells in the bulge region initiate anagen growth. The stem cells in the bulge enter a proliferative state, giving rise to transient amplifying cells which travel, with the dermal papilla, down to the newly forming follicle bulb and become the new proliferative matrix (Messenger 1993; Robbins 2012). Thus while the number of hair follicles for each individual does not change throughout life, the follicle bulbs themselves are newly grown with each hair fiber production cycle. Transient amplifying cells are limited in their ability to divide and thus once exhausted, growth slows and the follicle enters catagen (Buffoli, et al. 2014; Messenger 1993). There is some debate regarding the controlling mechanism for anagen phase length. Some researchers have proposed a mechanism of an endogenous inhibiting substrate, which inhibits mitosis once a threshold limit has been reached. This hypothesis has yet to produce much evidential support however (Vogt, et al. 2008).

2.1.3. Hair fiber structure

Adult scalp hairs vary from 30 to 170 µ in diameter (Bost 1993; Robbins 2012) and grow at an average rate of 1 cm per month (Bhushan 2010; Robbins 2012). Individuals with European or North African/Middle Eastern ancestry have the finest hair, while individuals with Asian ancestry have the coarsest hair (Robbins 2012) although there is significant overlap of average hair diameters across biological groups (Bost 1993). Cross-sectional shape ranges from circular to ovoid, with triangular, pear- or kidney-shaped also observed (Das 1974). Asian populations tend towards more circular cross-sections and sub-Saharan African populations tend towards more elliptical cross-sections although, again, there is significant overlap among various populations (Robbins 2012). Cross-sectional shape is correlated with but not causal of hair curl. The very fine scalp hair of infants increases in diameter until 4-7 years of age. Hair diameter plateaus around this age, with an increase in diameter continuing but at a very gradual rate. Hair fiber diameter peaks for males in their late teens to early twenties, when diameter begins to gradually reduce. Women’s scalp hair diameter peaks during their late thirties to mid-forties, after which it also begins to decline (Robbins 2012). There is a direct correlation between hair fiber diameter and growth rate, regardless of ancestry, with thicker hairs growing faster (Saint Olive Baque, et al.
There is no evidence for correlation between hair diameter and sex, although some literature suggests women tend to have higher scalp hair density than men (Saint Olive Baque, et al. 2012). Taking into account high inter-individual variation in hair growth rates, Asian (Chinese) hair grows fastest (411µ ±43), European (French) hair grows approximately 367µ (+/-56) per day and African hair (South Africans or French from West/Central Africa) grows slowest at 28µ per day (+/-50). Difference in growth rates between individuals of Asian and African descent could lead to a difference of up to five centimeters of hair growth over the course of a year (Loussouarn, et al. 2005).

The hair shaft consists of four zones or cell types. The cuticle is the thick protective covering of the hair fiber and is made up of flat, overlapping scales. The cortex contains spindle shaped cells which are aligned along the long axis of the fiber and consist mostly of fibrous proteins. The medulla is a region of loosely packed, hollow, spherical cells in the center of the hair shaft. The intercellular cement, or cell membrane complex (CMC), binds the cells together while forming pathways for diffusion of substances from the blood stream into the fiber.

**Cuticle**

The hair cuticle is the most chemically resistant region of the hair shaft and protects the hair fiber from taphonomic degradation through weathering and mechanical damage. The cuticle is a series of flat cells attached at the proximal end and overlapping on the distal end. The cuticle as a whole is usually 5-10 cells thick, with each cell being 0.5 to 1.0 µm thick and approximately 45 µm long. Only about 6-7 µm of each upper cuticle surface is exposed and not overlapped by the cell above it (Bhushan 2010; Robbins 1994, 2012; Wolfram 2003). This distance is sometimes referred to as the “scale interval” and may be an indication of growth rate (Takahashi, et al. 2006). The number and thickness of cuticle cell layers, as well as cuticle scale pattern, is not consistent throughout mammals and can be used to identify species in forensic examination (Robbins 2012). Cuticle cells emerge from the hair follicle with smooth edges overlapping in a gently undulating pattern. As the hair grows longer, weathering and mechanical damage chip the scale edges resulting in increasingly uneven, rough, broken scale edges closer to the tip of the hair. If degradation continues, the cuticle is degraded beyond the point of being able to protect the hair.
shaft and the cortex cell fibers begin to fray producing the “split ends” phenomenon (Robbins 2012).

Each cuticle cell contains five distinct zones. The epicuticle is the thin, outermost membrane, sometimes referred to as the F-layer. It is a 10-14 nm thick proteinaceous layer covered by a hydrophobic surface layer of strongly bound structural lipids. Beneath the epicuticle is the cystine rich A-layer (over 30% cystine), usually 50-100 nm thick. Continuing down, the exocuticle, or B-layer, is also rich in cystine (15-20%). The exocuticle is much more variable in thickness but approximately 150 nm. Also variable in thickness and below the exocuticle is the endocuticle, ranging from 50 to 300 nm in thickness and very low in cystine (only about 3%). The most inferior region of the cuticle cell is the cell membrane complex (CMC), or under-membrane. The CMC is similar in quality to the epicuticle and, along with an interlocking structure, binds overlapping cuticle cells together and cuticle cells to adjacent cortex cells (Bhushan 2010; Popescu and Hocker 2007; Robbins 1994, 2012).

**Cortex**

The cortex forms the majority of the bulk of the hair fiber, between 70% and 90% with finer hairs on the lower end of the range, and consists of two types of materials: the cortical cells themselves and the cell membrane complex. Human cortical cells are 50-100 µm long, up to 10 µm at their widest point, and are arranged longitudinally in an interdigitating pattern within a surrounding cell membrane complex. The “filament within matrix” organization of the cortical cells is mirrored in increasingly microscopic levels throughout the structure of the hair cell (Popescu and Hocker 2007). The majority of the cortical cell is comprised of 5-8 macrofibrils roughly 0.1-0.4 µm in diameter and surrounded by intermacrofibrillar matrix. The macrofibrils in turn contain 500-800 intermediate filaments (“microfibrils”) roughly 7.5 nm in diameter and surrounded by intermicrofibrillar matrix. Each intermediate filament (IF) contains 7 or 8 single or paired protofilaments organized in a double coiled rope fashion (Popescu and Hocker 2007; Robbins 2012; Wolfram 2003). While the intermediate filaments and macrofibrils mirror the longitudinal organization of the cortical cells themselves, the CMC matrix consists of keratin-associated proteins (KAPs) and is generally considered to be fairly amorphous (Popescu and Hocker 2007), although there is some early
evidence that some structural organization does exist (Robbins 2012). The filaments which make up the bulk of the hair fiber generally have a low sulfur content and helical proteins, while the matrix contains high sulfur content in an amorphous or crystalline organization (Wolfram 2003).

Cortical cells also contain pigment granules, which are oval or spherical in shape, 0.2-0.8 μm in size, and enter the cortical cells through a phagocytosis mechanism (Birbeck and Mercer 1957). According to Piper (1966), phagocytosis may also be responsible for an interlocking mechanism which links adjacent cortical and cuticle cells together (Robbins 2012). Melanosomes determine the color of the hair fiber through variation in the density and combination of oval-shaped, black to brown eumelanin and lamellar, yellow to red pheomelanin granules (Vogt, et al. 2008). The melanin granules are 70-500 nm in diameter, the size of which determines the amount of UV radiation absorbed and therefore the perceived color of the fiber (Popescu and Hocker 2007).

Many mammalian hair fibers contain two or three distinct types of cortical cells (orthocortex, paracortex, and mesocortex), the organization of which plays a part in determining hair curvature (Bhushan 2010; Powell and Rogers 1997; Robbins 2012; Wolfram 2003). Hair curl is partially determined by the arrangement of differing cortex cell types and partially determined by differential rates of cell production and keratinization on concave and convex sides of the hair follicle (Westgate, et al. 2013). Orthocortical cells tend to congregate on the outside or convex side of the curl, with paracortical cells on the inside or concave side of the curl (Thibaut, et al. 2007). The outer root sheath and connective tissues are thicker on the concave side of the follicle, while the proliferative matrix extends slightly above Auber’s line on the convex side of the follicle (Thibaut, et al. 2005). The structural and proliferative differences between curly and straight hair follicles and fibers are consistent across ancestral groups (Thibaut, et al. 2007; Thibaut, et al. 2005).

**Medulla**

The medulla occupies the center of the hair fiber, running longitudinally along its length. In human scalp hairs the presence, absence, and characteristics of the medulla
are highly variable, but coarser fibers such as beard hairs are more likely to have a continuous medulla (Robbins 1994, 2012). Human scalp hairs are less likely to be medullated than axillary or pubic hairs (Buffoli, et al. 2014). During hair fiber keratinization and dehydration, the loosely packed medullary cells shrivel to hollow, spherical vacuoles. These cells are organized in an approximate column with trabeculae of amorphous proteins and fine KAP filaments between them (Popescu and Hocker 2007; Robbins 1994, 2012). Unlike the KAP filaments of the cortex, within the medulla they do not run parallel to the long axis of the hair fiber (Clement, et al. 1982).

**Cell Membrane Complex**

The cell membrane complex (CMC - formerly called the intercellular cement) is an adhesive matrix of keratin fibers along with the membranes of the adjacent cells which it binds together. A central Delta layer approximately 15 nm thick is sandwiched between two lipid Beta layers, each approximately 5 nm thick (Robbins 1994, 2012; Wolfram 2003). There are slight variations between cuticle-cuticle CMC, cortex-cortex CMC, and the CMC between cuticle and cortical cells which exhibits characteristics of both other types. The external cuticle CMC is not a continuous membrane surrounding the hair fiber, but wraps each cuticle cell independently (Robbins 2012). The most external layer of the CMC surrounding the hair fiber is densely cross-linked and provides both stability and some degree of protection from mechanical damage (Wolfram 2003).

### 2.2. Hair research

#### 2.2.1. Hair in anthropology research

Human hair has been used to study diet, drug use, familial relationships, and migration in bioarchaeological contexts thousands of years old (Baez, et al. 2000; Bonnichsen, et al. 2001; Gilbert, et al. 2007; Roy, et al. 2005; Springfield, et al. 1993; White 1993). Hairs almost 10,000 years old have successfully produced reliable radiocarbon dates and aDNA sequences (Bonnichsen, et al. 2001). Additional studies have reported the utility of ancient hair for structural studies (Chang, et al. 2006; Lubec,
Hair is increasingly being used for biological anthropology research in contemporary populations as well (D’Ortenzio, et al. 2015).

Research into detecting glucocorticoid hormones began just over a decade ago, with several investigators developing liquid chromatography-mass spectrometry (LC-MS) methods for detecting corticosteroids in human scalp hair (Gow, et al. 2010). These methods were created primarily in response to the ability of athletes abusing performance enhancing substances to evade doping control with only a few days of abstinence, but have quickly been adopted by researchers for other purposes. Hair cortisol concentrations (HCC) have increasingly been used to study stress in clinical and archaeological contexts (Webb, et al. 2010); however, there are limitations to using HCC to evaluate acute stress events. Hair cortisol levels correlate to those found in 24-hour urine samples, but are not correlated with those found in saliva or blood (Sauve, et al. 2007). Hair cortisol levels thus reflect long term circulating cortisol and therefore chronic stress, but not short term blood chemistry levels resulting from acute stress events (Kalra, et al. 2007; Russell, et al. 2012; Sauve, et al. 2007). Circulating cortisol levels are influenced by age, sex (Purnell, et al. 2004; Takai, et al. 2007), menstrual cycle length (Nepomnaschy, et al. 2011), use of hormonal birth control (Derr, et al. 2006), and imbibed substances such as tobacco use (Rohleder and Kirschbaum 2006). Early data suggest that age and sex are confounding factors when it comes to hair cortisol levels, but these data are incomplete and inconclusive (Dettenborn 2012; Gow, et al. 2010). Hair cortisol concentrations specifically can be influenced by cosmetic hair treatments such as dyeing or bleaching (Sauve, et al. 2007), vigorous physical activity (Gerber, Jonsdottir, et al. 2013), adrenocortical function disorders such as Cushing’s syndrome or Addison’s disease (Stalder and Kirschbaum 2012), pregnancy (Kirschbaum, et al. 2008), and mental health disorders such as depression (Gerber, Kalak, et al. 2013; Staufenbiel, et al. 2013), PTSD (Staufenbiel, et al. 2013), or alcohol dependence (Stalder, et al. 2010). Since the mechanism for cortisol introduction into the hair fiber is poorly understood (Gow, et al. 2010; Kirschbaum, et al. 2008; Stalder and Kirschbaum 2012) and there is evidence suggesting a ‘wash-out effect’ of cortisol from more distal hair sample segments (Dettenborn 2012; Kirschbaum, et al. 2008; Russell, et al. 2012), there is still much to learn about the correlation between HCC and health status. Not only are drugs and hormones incorporated into the hair fiber via
multiple pathways, it is possible that pathways are utilized differently by different analytes (Kirschbaum, et al. 2008:33). Hair cortisol levels thus reflect chronic stress but not blood chemistry levels resulting from acute stress events (Kalra, et al. 2007; Ashley et al. 2011 cited in Russell, et al. 2012; Sauve, et al. 2007).

Hair stable isotope values have also been used to investigate nutritional stress in archaeological contexts (Mekota, et al. 2006, 2009; Neuberger, et al. 2013; O’Connell and Hedges 1999b; Webb, et al. 2015; White 1993). Although stable isotope analysis provides a wealth of information on diet and mobility over an individual’s lifetime, it is not without its own limitations. Wool fibers resting for long periods of time in warm, wet burial environments will undergo protein composition changes (specifically loss of hydrophilic amino acids) and increases in δ18O and δ2H values, while densely pigmented fibers in cold, wet burial environments will exhibit significant reductions in δ13C and δ15N values (von Holstein, et al. 2014). These taphonomic changes must be accounted for in stable isotope studies of archaeological hair fibers. The hair of individuals suffering chronic starvation will exhibit changes in the values of δ13C and δ15N in the hair. Carbon will mimic the changes to the body mass index (BMI) of the individual, with reduced values at low BMIs and an increase in values correlated with the improvement of BMI. The timing of change in hair carbon isotope values corresponds with the timing of increase of BMI in individuals being treated for anorexia nervosa (with a delay of at least two weeks for the hair fiber to leave the scalp and become available for sampling) (Mekota, et al. 2006). Low δ13C-values indicate insufficient energy intake and the body’s subsequent utilization of body fat deposits (Neuberger, et al. 2013). Nitrogen, on the other hand, will show a marked increase in two distinct contexts. Individuals suffering chronic starvation will have increased levels of δ15N, which drop suddenly when starvation ends and the metabolism returns to normal (Mekota, et al. 2009). Enriched δ15N-values illustrate the internal trophic effect resulting from the breakdown of the body’s existing muscle tissues for proteins (Neuberger, et al. 2013; Reitsema and McIlvaine 2014). In healthy individuals, rising δ15N-values simply reflect increased animal proteins (Mekota, et al. 2006, 2009; O’Connell and Hedges 1999a). However it is important to note the delay between change in diet and the resulting change in hair nitrogen isotopic values. Proteins released by normal catabolism into the bloodstream can be reabsorbed into keratin
during production of hair fiber cells, even after change to a new dietary pattern. Hair isotopic values may take 7-12 months to calibrate with and reflect isotopic values of a new diet (O’Connell and Hedges 1999a). Analysis of hair nitrogen isotope values without consideration of other isotopic values should be used cautiously.

Elemental studies comparing hair samples from ancient individuals to those of contemporary individuals suggest that burial matrix can have a significant effect on analysis results. Mansilla, et al. (2011) found that hairs of ancient Mexicans naturally mummified by arid burial conditions (912±30 BP) had higher levels of sodium and manganese. While they observed microscope adhesions of burial matrix on the hair fiber surfaces suggesting the excess elements originate from the exogenous environment, washing the hairs prior to analysis did not significantly alter the results. The mummified hairs also exhibited significantly lower levels of potassium and zinc than the contemporary samples (Mansilla, et al. 2011), however the authors do not hypothesize as to the cause. As these are elements often accused of being responsible for changes to hair fibers in cases of malnutrition (see section 2.3), the influence of external burial matrix on these levels cannot be ignored. Bertrand, et al. (2003) found that hairs from 2000 year old Greco-Roman period Egyptian mummies show a significant increase in calcium, zinc, lead, iron, as well as trace elements manganese, bromine, titanium, and strontium. The natron salts used during this time period to induce mummification included high amounts of calcium, strontium, manganese, bromine, and iron (Macke et al. 1997 cited in Bertrand, et al. 2003). Authors also observed adhesions on the external surface of the hair fibers, and found them to be mostly titanium and zinc. The authors conclude that the “abundant exogenous [elemental] atoms would have been fixed in a different environment from that of metabolic [elemental] atoms” (Bertrand, et al. 2003:390). Any research investigating diet or nutritional stress through elemental analysis of hair fibers should also perform analysis of the surrounding burial matrix to avoid conflating environmental influence with endogenous, metabolically available trace elements.

Both cortisol and stable isotope analysis require comparatively large samples for immunoassay processing (at least one centimeter weighing 8-50mg) (Gow, et al. 2010). Stable isotope analysis can be performed on a single strand of hair but to date the
minimum required for analysis is \(100\mu g\) for \(\delta^{15}N\), corresponding to a length of approximately 2 cm or two months of growth (Roy, et al. 2005). The authors suggest that the higher percentage by weight of carbon could reduce the minimum requirement of hair for productive \(\delta^{13}C\) analysis to \(50\mu g\) (Roy, et al. 2005) but this has not been tested either by the original authors or by anyone else. For now, the material requirements for both hair cortisol concentration and stable isotope analysis remain such that the time frame analysed covers at least a month’s worth of hair growth. Thus while important for research on chronic stresses, HCCs and stable isotope values are inadequate for the investigation of short term, but severe, acute stress.

2.2.2. Nutritional stress research

In 1946, Dr. William Hughes of the Colonial Medical Service wrote to the British Medical Journal describing “achromotrichia” in Nigerian children suffering from kwashiorkor (a severe form of calorically sufficient but protein-deficient malnutrition characterized by edema and loss of muscle). Hughes’ letter described changes observed in the scalp hair of several children under the age of four with the condition. Their hair was white or grey to light yellow, straight or wavy (in a child with normally tightly curled, dark hair), fine in texture, and easily broken off at the scalp with attempted epilation (Hughes 1946). When the children received protein- and fat-enriched diets, new hair growth came in “strong, dark, and curly” with an obvious and visible demarcation line (p.86). The length of time between the onset of dietary supplementation and changes seen in new hair growth was estimated to be a minimum of three weeks during which time the patients’ other symptoms began to improve. The amount of time between production of the hair fiber and its leaving the scalp and becoming externally visible is roughly two weeks (LeBeau, et al. 2011). Hughes’ letter to the editor instigated further letters relating similar observations (Castellani 1947; Roebuck 1946; Roy 1947) and decades of a general acceptance within the medical and nutritional research community that changes to the scalp hair are a common symptom of chronic malnutrition.

The 1960s continued with the trend of describing hair-related symptoms of malnutrition and began the shift towards more quantitative research of those symptoms.
During their study of kwashiorkor-related deaths among Guatemalan children, Scrimshaw and Behar (1961) concluded that protein-calorie malnutrition induced three types of alterations to the hair shaft: that curly hair becomes straight, hair quality is reduced (becoming dry, thin, and brittle), and the hair falls out spontaneously or is easily epilated. Color changes to the hair shaft were also observed, notably that alternating periods of adequate and inadequate nutrition produce alternating bands of normally pigmented and depigmented bands in the hair, which they suggested is a “flag sign” of malnutrition (Scrimshaw and Behar 1961). In his case report describing the symptoms of individuals suffering from “malabsorption syndrome,” Wells included “follicular changes” and “defective growth of hair and nails” to the list of symptoms (very similar to those seen in protein calorie malnutrition) and compared them to evidence of general “undernutrition” (specifically a lack of proteins and fatty acids) seen in Europe after World War II (Wells 1962:940).

In a study of 36 Zulu children between the ages of one and five, Sims (1968) noted that the hair of infants with kwashiorkor narrowed in diameter during illness while the hair of control infants maintained a consistent diameter. Hair of infants treated for kwashiorkor for one month exhibited a gradual increase in shaft diameter. During that time, the hair shaft did not reach and maintain a constant diameter, suggesting that the hair (and likely the patient in general) does not fully recover in such a short time frame (Sims 1968). Two researchers, Bradfield and Johnson, and their colleagues pushed for the use of hair roots to assess nutritional stress in the late 1960s to mid-1970s. Bradfield, et al. (1967) fed otherwise very healthy males in their mid- to late-20s calorically sufficient but protein deprived liquid diets for 15 days. Scalp hairs collected from participants at the end of the deprivation period exhibited severe root atrophy and decreased pigmentation of the hair bulb: bulb diameter was 0.025-0.075 mm after deprivation (vs 0.250-0.550 mm prior). While the authors conclude that 15 days was an inadequate period to induce a change in the anagen:telogen hair follicle ratio, atrophy of the bulbs was observed in 48-65% of anagen phase hairs and an increase of hair shaft breakage during epilation attempts from 41% to 77% was observed. They also observed a narrowing of the bulb neck (area immediately distal to the bulb) in many anagen hairs which did not atrophy (Bradfield, et al. 1967). In a later study of children with marasmus (severe caloric malnutrition of all nutrients characterized by wasting and
loss of both muscle and fat mass) and/or kwashiorkor, Bradfield concluded that the hair follicles of children suffering from the chronic undernutrition of marasmus adapted to the situation by an almost complete shift to telogen phase, while those suffering from kwashiorkor adapted by atrophy of anagen hair bulbs and a slight increase in the percentage of telogen follicles (Bradfield 1974). The reliability of hair root morphology to illustrate nutritional status, however, is questionable. Johnson, Latham, and Roe studied black Jamaican children in varying states of nutritional stress, and found a significant difference in the percentage of anagen and telogen hairs between well-nourished and severely malnourished children, (Johnson, et al. 1975, 1976) which agrees with Bradfield’s conclusions. The study results contradict those of Bradfield regarding hair root atrophy however and do not indicate a correlation between the degree of protein calorie malnutrition and the degree of hair root atrophy.

In their study of the effect of manual epilation on the hair root bulb, Maguire and Kligman found that in approximately 5,000 normal hairs digested out of (not pulled from) scalp specimens, none exhibited any dysplasia. Abnormal biopsy specimens (those following sub-epilating x-ray doses) were used as control, and showed a high proportion of deformed hair roots indicating that differences between normal and abnormal hair root specimens are easily observable. Further, normal hair roots pulled from scalp specimens according to normal hair sample epilation methods did exhibit root deformity. Authors concluded that it is the process of epilation itself which produces the distortions to the hair root misinterpreted by other researchers as resulting from malnutrition or illness. However, the dysplasia produced by plucking only happens in actively growing hairs, and therefore the anagen-telogen ratios are still accurately observable (Maguire Jr and Kligman 1964).

During the 1970s, research focus shifted towards quantifying differences between varying levels of malnutrition. Crounse, et al. (1970) were critical of the root morphology method, accusing it of being “subjective and only semi-quantitative” (p.465). They instead promoted the use of hair root volume, using displacement of water in a calibrated micropipette capillary tube to measure it. Crounse and colleagues (1970) hypothesized that the amount of protein deposited in the hair root correlates with the nutritional levels of protein circulating in the body and measured hair root volume as
an indicator of such. Institutionalized mental patients with histories of protein-insufficient diets and clinical evidence of malnutrition along with African adults and children with diagnosed kwashiorkor or marasmus showed very low values of hair root protein compared with control groups (Crounse, et al. 1970). They had good results with this method, although as with Bradfield’s method, examination of the hair root will only provide an estimation of what is happening at a single moment in time, while ironically also requiring a chronic condition to induce the changes sought.

Vandiviere et al. (1971) returned to hair shaft diameters to investigate the differences between five groups of Jamaican preschool children. They found that the hair shaft diameter distributions of the “normal” children were the same as the distribution from children with normal upper arm circumference measurements but less than 90% of normal weight-for-age ratios (termed “nutritional dwarfs”). Children allocated to the marasmus group (those less than 90% of normal for both measurements) had “slightly smaller” hair shaft diameters. Of the five nutritional groups, the normal, marasmus, and nutritional dwarf groups had large diameter distributions with almost complete overlap. The group of children clinically diagnosed with kwashiorkor had a narrower range with a smaller average diameter. The children who had been receiving treatment for kwashiorkor for six months with a diet known to be adequate in protein and calories also had a narrower distribution range, although the average was roughly the same as the normal children (those with expected upper arm circumference and weight-for-age ratio) (Vandiviere, et al. 1971). While researchers acknowledged the presence of major and minor hair shaft diameters and attempted to account for the difference, their methods had limitations. Vandiviere, et al. (1971) slide mounted hairs so the curl lay parallel to the microscope stage which, according to authors, ensured the minor axis of the hair shaft diameter was perpendicular to the line of microscope sight and therefore the axis measured. Despite their confidence in viewing the minor axis when the fiber is arranged in this manner, authors caution against the ease with which malalignment of the fiber produces significant error in measurements. Unfortunately, they don’t define what they considered this significant error to be, nor how they decided that particular arrangement would ensure an uninhibited view of specifically the minor axis. We know from recent research that the presence and degree of hair curl is determined at least in part by the proximal hair
fолlicle. The base of the hair follicle programs the degree of hair curl through the rate of cell division and differentiation, which occurs at different rates on convex and concave sides of the follicle base, and continues to do so when the hair follicle is removed from the surrounding dermal environment (Thibaut, et al. 2005). Since even straight hair can have a cross-sectional shape which is not completely circular, we must conclude that if there is any relationship between cross-sectional shape and hair curl, the former is a result of the latter, not the cause of it. During the data collection phase of this study, it became clear that slide mounting a hair so the curl is parallel to the microscope slide/stage does not ensure the minor axis is always perpendicular to the line of sight. Both curly and straight hairs exhibit a twisting of the major and minor diameter axes along the length of the hair shaft. The “extreme care” taken by Vandiviere, et al. (1971) in the slide mounting and analysis of hair samples was therefore inadequate to overcome the problems associated with using a single perspective diameter measurement to assess the dynamics of hair production in response to various physiological stresses. In fact, several years later, McCrone (1977) produced ellipticity ratios (major/minor diameter ratios) as well as a ratio for two diameters measured perpendicular to each other but oriented in an arbitrary direction. From this study, the authors concluded that the axis viewed perpendicular to the microscope line of sight is almost randomly oriented (McCrone 1977).

Johnson and colleagues (1975, 1976) also investigated hair shaft diameter in black Jamaican children aged 7 months to 5 years who were allocated into subgroups determined by their percentage of standard weight for age (from “well-nourished” at ≥91% to “severe PCM” at ≤60%). Researchers found a statistically significant difference in shaft diameter between the well-nourished and severely malnourished children. However, they found no significant difference between well-nourished and those with “mild to moderate” PCM. Nor did they observe a significant difference between the mild- to moderately-malnourished and the severely malnourished children. The children with severe PCM were the only group to show a significant increase in the percentage of telogen hairs. Hair bulbs were also examined, and while researchers claimed to observe a reduction in bulb diameter that positively correlates with severity of PCM, these observed differences did not meet statistical significance (Johnson, et al. 1975, 1976; Johnson and Roe 1975). From these studies, it appears that malnutrition
must become fairly severe to influence the hair shaft diameter and the ratio of anagen to telogen follicles.

During their evaluation of the hair root DNA volume of 335 malnourished and 48 normal children in Karachi, Zain, et al. (1977:1096) found a “progressive decrease in hair root DNA and protein with the decline in body weight.” A “dramatic fall” in root protein and DNA to 40-50% of normal was observed in all types of malnutrition (classified as: early malnutrition, marasmus, marasmic kwashiorkor, or kwashiorkor), with the greatest change seen in kwashiorkor. However, the serum total protein did not show any significant decrease until the malnutrition level reached Grade IV (body weight-for-age at less than 60% of normal), and this change only reached significance at the 1% level (Zain, et al. 1977).

Chase, et al. (1981) compared individuals with protein calorie malnutrition with otherwise healthy individuals undergoing elective surgery in a study of epilation force requirements. Results suggested that less force was required to pull hairs from the scalps of undernourished patients than from the scalps of control patients. Researchers found that epilation force correlated significantly with serum albumin levels and anthropometric measurements indicative of nutrition status, but not with the serum status of the seven vitamins tested (Chase, et al. 1981). This supports the hypothesis that hair follicle changes in response to malnutrition occur primarily because of protein and not vitamin deficit.

In addition to hair quality and texture, many of the early observational studies on the effects of nutritional stress on scalp hair growth include discussion of a change in hair color. Hypopigmentation, or loss of hair color, is currently a generally accepted symptom of chronic malnutrition (McKenzie, et al. 2007) but this variable is more difficult to quantify than the change in diameter, or decreased coarseness, of the hair fibers. In their atlas of hair characteristics, Ogle and Fox (1999) provided one of the few attempts to standardize assessment of hair color through the use of color photographs of example hairs illustrating each of their twelve different categories for hair color. While the intent to standardize the documentation of such a variable characteristic such as color is commendable, there is a great deal of subjectivity that could be alleviated by
providing examples of the boundaries between categories as opposed to the center of the category. However, any method of assessing color will be subject to inter-observer variation in color perception. McKenzie and colleagues (2007) avoided the problem of subjectivity in their color analysis by using liquid chromatography to measure the amount of melanin pigments in the hair of children being treated for malnutrition. They found a significant gradient in melanin content correlated with initiation of malnutrition treatment. This does not avoid the issue of intra-individual variation in hair fiber colors however. Birngruber and colleagues (2009) found during their search for validated, objective, technically supported methods significant intra-individual variability in the color of hairs pulled from a single individual.

### 2.2.3. Fever

As early as 1902, researchers were claiming to observe decreases in hair shaft in relation to febrile illness. From repeated measurements along the length of hair shafts from healthy and ill or recently dead individuals, Matsuura (1902) speculated that the duration of an illness could be estimated retrospectively from the length of affected hair. He concluded that 1) greater nutritional deficiencies produced greater decreases in diameter, 2) thicker hairs showed greater degrees of diameter decrease, 3) straighter hair showed greater decrease in diameter, and 4) hairs from individuals who were well-nourished before illness showed greater decrease than those from individuals who were already poorly nourished (Matsuura 1902). Pinkus similarly measured the hair shaft diameters of 116 hospitalized patients and concluded that pyrexial illnesses (those involving high fever) were associated with a reduction in the diameter (Pinkus 1928 cited in Sims 1967). Both Matsuura and Pinkus compared this phenomenon to Beau’s lines in the fingernails, although the causal relationship between systemic illnesses and Beau’s lines was poorly understood at the time (Sims 1967). By 1961, authors were commonly asserting as fact that “hair loss after febrile illnesses is the best known of the clinical varieties of telogen effluvium” (Kligman 1961:39).

During a 1980 outbreak of Dengue fever in China, of 510 victims observed, 99.6% manifested severe fever (≥39°C). Of the 344 patients followed up, 69% experienced hair loss starting approximately one month after illness and continuing for
over a month’s duration (Chen, *et al.* 1993). A retrospective case study of 250 victims of the 1987-1988 Dengue fever epidemic in southern Taiwan showed that while 98% of patients experienced fever lasting 3-6 days, only 45% experienced noticeable hair loss with onset approximately two months after recovery and lasting approximately one month (Harn 1989). Researchers of a small outbreak of typhoid fever in 1995 assert that temporary effluvium used to be a well-recognized clinical finding inherent to typhoid but is largely forgotten due to the introduction of anti-microbial agents for treatment (Haefeli, *et al.* 1995). Of 15 people exposed to typhoid in this particular outbreak, four tested positive for *Salmonella typhi* (including the source patient whose illness precipitated the investigation). All fifteen were given oral ciprofloxacin as a precaution until testing confirmed the diagnosis. All four who tested positive suffered a prolonged fever before treatment was initiated, and all four suffered spontaneous diffuse hair loss starting two to four weeks after the illness, and persisting for nine to sixteen weeks (Haefeli, *et al.* 1995). There are no reports of effluvium as a side effect of ciprofloxacin, and none of the eleven healthy individuals who received ciprofloxacin prophylactically experienced any increased telogen shedding. Authors of that study conclude that effluvium is a common consequence of viral and bacterial infections producing high fever such as influenza, pneumonia, scarlet fever, and typhoid fever but that the increased use of antipyretic and antibiotic medications has decreased observed incidences of fever-related hair loss (Haefeli, *et al.* 1995).

In 1994, a researcher in the Tai National Park, Cote d’Ivoire who contracted Ebola virus, including two weeks of severe fever, suffered reduction of hair quality and eventually diffuse effluvium which began approximately one month after onset of the disease and lasted approximately three months (Formenty, *et al.* 1999). The researcher did suffer an elevated fever for two weeks but also lost 6kg during her illness due to anorexia and diarrhea. The sudden and extreme weight loss could potentially contribute to the hair loss. However, if we accept other researchers’ assertions that in cases of malnutrition, hair loss is the result of more of a chronic rather than acute condition, then the observed effluvium may have been the result of the severe and prolonged fever rather than the sudden weight loss.
Nabavizadeh, et al. (2006) reported a case of a two-year-old boy who suffered immediate hair loss during an acute attack of Kawasaki disease (a rare and poorly understood inflammatory condition of the blood vessels and characterized by a high, persistent fever). The boy was well-nourished and healthy before the onset of the disease. The authors conclude that the hair loss was a result of the high fever as it developed suddenly on the sixth day of the fever and began reversal almost immediately after the fever was treated (Nabavizadeh, et al. 2006). These studies suggest that febrile illnesses do cause telogen effluvium. However, the research is inadequate and needs further examination before conclusions can be reliably drawn.

2.2.4. Psycho-emotional stress

Severe psychological stress is often listed as a common reason for telogen effluvium (Shrivastava 2009), although there are few clinical studies which quantify this anecdotal relationship between stress and effluvium (Atefi, et al. 2006). Conclusive evidence on the link between emotional stress and premature telogen effluvium is lacking (Arck, et al. 2001; Harrison and Sinclair 2002) and the often mentioned causal link between emotional stress and hair loss is becoming increasingly controversial (Harrison and Bergfeld 2009:362).

In 1961, Kligman claimed that telogen effluvium was common in psychiatric patients and young women burdened with school work, exams, migraines, and strenuous jobs. However he offered no evidence to support the theory that everyday stresses induce noticeable hair loss (Harrison and Sinclair 2002). York, et al. (1998) found that women aged 18-35 who experienced “high stress” (as determined by the Holmes and Rahe 1967 Social Readjustment Rating scale) were 11 times more likely to experience hair loss than women of similar demographics “not experiencing high stress” (York, et al. 1998:1045). This study is problematic though in two ways. Firstly, the participants who were categorized as having experienced hair loss were recruited from patrons of a beauty salon who self-volunteered a complaint of hair loss. This categorization was not validated by telogen follicle percentages and hence was determined entirely by self-perception, which may be influenced by research participants’ acceptance of the common folklore that “nerves” cause hair loss. Second,
there was no investigation into whether any observed hair loss could have been stimulated by other factors, such as behavioral changes (e.g. stress-related loss of appetite) or widespread breakage of hair fibers due to severe treatment-inflicted damage (from permanent waves, bleaching etc.).

Hair cortisol has been used in initial investigations into the interactions between hair formation and psychological stress. As of 2013, fourteen studies looked into the correlation between hair cortisol and psychological stress (in the absence of psychological comorbidities such as mood disorders) with disappointingly inconsistent results (Stalder and Kirschbaum 2012; Staufenbiel, et al. 2013). However, research on rhesus macaques (Davenport, et al. 2006) and vervet monkeys (Fairbanks, et al. 2011) provided early evidence suggesting that psychological stress can induce elevated hair cortisol concentrations. Recent research into hair cortisol concentrations of individuals with PTSD has suggested its utility in identifying psychological reactions to psychologically or emotionally traumatic events. Researchers found that although those suffering from PTSD initially had hair cortisol levels higher than similarly traumatized individuals not exhibiting clinical symptoms of PTSD, they eventually become hypocortisolemic (Steudte, et al. 2011). Hair cortisol levels are inversely proportional to frequency of trauma and number of traumatic events suggesting that the more traumatized an individual is, the less cortisol will be present in his or her hair (Steudte, et al. 2013). This shift needs to be taken into account when encountering higher or lower than normal expected hair cortisol levels in archaeological contexts. A study of forty adult premenopausal women in Tehran concluded that experiencing moderate or high levels of stress (determined with the Social Readjustment Rating Scale) was a significant risk factor in developing chronic diffuse hair loss (Atefi, et al. 2006). In their study of 25 otherwise healthy pregnant women, Kalra, et al. (2007) found a positive correlation between perceived chronic stress (as determined by the Perceived Stress Scale) and cortisol levels found along the length of the hair shaft. They concluded from these results that hair cortisol levels respond to chronic psychosocial and emotional stress (Kalra, et al. 2007). These studies are the first to show that the hair follicles are influenced by stress resulting from causes other than malnutrition or severe illnesses. Brittany, et al. (2007) found that individuals who suffer from severe chronic pain exhibit much higher hair cortisol concentrations compared to those in a control
group. Their chronic pain subjects also scored significantly higher on the Perceived Stress Scale questionnaire. While the data are insufficient to suggest that chronic pain is either the cause or the result of perceived stress, there is a correlation between the two.

The Miller, et al. (2007) recent meta-analysis on chronic stress and HPA-axis function suggests that features of both the stressor and the individual experiencing it affect HPA-axis function. External features such as whether the stressor threatens physical safety and is traumatic in nature contributes to the development of a PTSD-type cortisol profile (a flat, but elevated cortisol profile throughout the day which contrasts with a normal profile of robust fluctuation between highest levels in the morning and lowest levels in the evening). But the individual’s perception of the controllability of the stressor as well as their response to the stress shapes HPA activity. Increased cortisol output is correlated with the amount of subjective distress the individual experiences in response to the stressor (Miller, et al. 2007). The psychobiological approach to stress generally rests upon the theory that an individual’s response to a psycho-emotional stress is inherently one of anticipating the needs of a physiological stress, and therefore is dependent on the individual’s perception of a stimulus and/or environment, personality factors of appraisal and coping mechanisms, and individual variation in the hormonal response to perceived threat (Faraday 2005; Miller and Kraus 1996). An individual’s personality may contribute as much as 11% to the variance in their hormonal response to psycho-emotional stress (Childs, et al. 2014).

Recently, studies have begun to show the first indications of quantitative evidence to support the hypothesis that emotional stress is not only recorded by the follicle but affects hair growth itself. Arck, Peters, and colleagues have found that stress induces actively growing anagen phase follicles to shift to telogen resting phase in mice subjected to sensory stress (Arck, et al. 2003). They further hypothesize that psychoemotional stress modulates hair growth through the same neuroendocrine hormone pathways as physiological stress-induced activation of the HPA-axis (Peters, et al. 2006). Considering the interconnectedness of the hair follicle with the HPA-axis (Section 2.3.2), and the evidence that physiological response to psychological stress is
in some way mediated by personality factors (Delahaij, et al. 2010; Faraday 2005; Miller and Kraus 1996; Singley, et al. 2012), some individuals could then in theory experience change in hair follicle function resulting from emotional stress. No substantial evidence supporting or disproving this has yet been offered however.

2.2.5. “Metabolic” stress

Metabolic stress is another causal factor of hair change or hair loss often recited without any supporting evidence, but presumed to precede the onset of hair loss by approximately three months (Garcia Bartels and Blume-Peytavi 2008). Other than malnutrition studies (including malabsorption), the influence of these conditions on hair growth has not been properly examined. Research on the clinical manifestations of eating disorders is the only field which makes an attempt to link metabolic disturbances with hair change or loss. The observed correlation between restrictive eating disturbances and changes to hair growth are hypothesized by some researchers to be the result of a dysfunctional metabolic state: “The hypothyroidism most likely represents a compensatory response to the starved, hypercatabolic state, since the thyroid function normalizes with weight gain” (Gupta, et al. 1987:1387).

Research into the clinical manifestations of eating disorders, while originally undertaken in an effort to increase the efficiency of diagnosis and treatment, generally agrees with research regarding the effects of chronic malnutrition. The majority focuses on either premature telogen effluvium or the “quality” of hair (i.e. texture). Telogen effluvium is an increase in the anagen to telogen ratio to roughly 70:30 (normal ratio is approximately 90:10) and is a common side effect of malnutrition eating disorders (anorexia nervosa/bulimia nervosa resulting in extreme weight loss) along with a positive pull test (easy epilation) (Tyler, et al. 2002). In their study to document the dermatological markers for anorexia, Hediger, et al. (2000) found that 86% of their study participants noticed diffuse effluvium of scalp hair and 52% noticed dry hair during their weight loss. In 88%, these changes were observed when the BMI of the individual in question dropped below 16. The majority of patients noticed these changes four to twelve months after the onset of weight loss (Hediger, et al. 2000). Schulze, et al. (1999) observed diffuse effluvium in only 37% of children and adolescents with anorexia
or bulimia nervosa which occurred two to fourteen months after onset of their disease. Authors do not indicate whether they considered the point of “onset” to be the start of disordered eating behaviors or the start of significant weight loss. The severity and rapidity of onset of disordered behavior would determine the rapidity of weight loss and hence the onset of subsequent symptoms.

The hypothesis that production of hair shaft can be adversely affected by metabolic stress is explained by the differing needs of producing and non-producing hair follicles. Compared with follicles in telogen phase, anagen phase hair follicles have a much higher metabolic demand. Specifically, glucose utilization increases 200% over telogen phase, glycolysis 200%, pentose cycle activity 800%, “metabolism by other pathways” increases 150%, and ATP production via the respiratory chains 270% (adenosine triphosphate – ATP- transports energy within cells for metabolism) (Adachi, et al. 1970:907). It is unsurprising then, that in instances of metabolic disturbances or a decreased supply of resources, anagen follicles would shut down to conserve energy and resources. Considering the severe malnutrition suffered by individuals struggling with restrictive eating disorders, it’s impossible to exclude that as a causative or at least compounding factor. One must also take into account common restrictive behaviors of individuals with anorexia nervosa who tend to self-restrict access to protein foods, especially fish and animal products (Vaz, et al. 1998).

Malnutrition, febrile illnesses, and other significant metabolic disturbances have varying degrees of influence on hair growth. Protein calorie malnutrition induces a reduction in hair diameter and quality, while febrile illnesses apparently cause a shift towards a higher ratio of telogen follicles. Eating disorders, combining symptoms of both malnutrition and a pathologic metabolic state, generally cause both changes. The interaction between reduction in hair diameter and increased telogen is, at least in part, an artifact of the high resource requirements of the hair follicle.

2.2.6. High resource requirements of the hair follicle

Due to its constituent components, the hair shaft has a very high resource requirement and it follows logically that certain stresses (especially nutritional) would
adversely affect hair growth. Hair is approximately 65-95% proteins (depending on moisture content, which can be up to 32%). Water-free fibers are 90-97% proteins and roughly 2% lipids (both structural and free). The remainder of the hair fiber is pigment, nucleic acids, carbohydrates and inorganic substances (Popescu and Hocker 2007; Robbins 1994).

Elemental analysis shows the hair shaft to be approximately 50 wt% carbon, 22 wt% oxygen, 16 wt% nitrogen, 7 wt% hydrogen and 5 wt% sulfur. The percentages will vary slightly depending on the species, individual, or location of the hair but consistently stay around those values. The relatively high sulfur content results from the high cystine content. This characteristic is representative of α-keratin fibers and differentiates them from other protein fibers such as silk and collagen (Popescu and Hocker 2007). Less than 1% is made up of calcium, cadmium, zinc, chromium, copper, iron, arsenic, silicon, lead, and mercury. Most of these are incorporated into the hair via external sources and have been used to monitor exposure to environmental pollutants or toxins (Popescu and Hocker 2007).

The proteins that comprise the hair cortex are often referred to as keratin-associated proteins (KAP) and are classified into four classes based on the content of cystine and other amino acids. The low-sulfur proteins (LS-proteins) are approximately 50% by weight (wt%) of the total proteins and are generally considered to those of the intermediate filaments (IF). They are partly crystalline and presumed to form the α-helical components of the IF (Popescu and Hocker 2007). The intermediate filament-associated proteins (IFAPs) constitute the amorphous part of the fiber matrix and contain high-sulfur proteins constituting roughly 25 wt% of the total proteins (Popescu and Hocker 2007), which are 20-30% cystine. The ultra-high sulfur KAPs, another part of the IFAPs, are >30% cystine and 20% serine (Robbins 2012). Also making up the IFAPs are the tyrosine/glycine rich KAPs (sometimes called HGT-proteins or KAP6-8) (Robbins 2012), which account for approximately 10 wt% of total hair fiber proteins. The remaining 15 wt% of hair proteins are other high- and low-sulfur amorphous proteins found in the endocuticle, exocuticle, and cell membrane complex (Popescu and Hocker 2007).
Hair of individuals suffering from PCM has a lower cystine content, which is “probably caused by decreased synthesis of the sulfur-rich proteins” (Robbins 2012:158). PCM is correlated with low levels of cystine, arginine, and methionine. Methionine and cystine are the two sulfur-containing proteinogenic amino acids and are found in fish, meat, and some seeds and nuts. Arginine is a semi-essential or conditionally essential amino acid (infants cannot synthesize it) important for cell division, wound healing, immune function, hormone release, and removal of excess ammonia from the body. Arginine is also found in fish, meat, seeds and nuts, and additionally some dairy products (Robbins 1979, 1994). Animal products and other high-protein foods clearly provide amino acids essential for meeting the high demand of hair cell production. These are the same foods often unavailable to individuals suffering nutritional stress due to poverty, imprisonment, or geographic or political famine.

2.3. Utility of hair for stress research

2.3.1. Hair as a chronological record

The hair follicle is the only organ to permanently and consistently regenerate, even into adulthood and is the human body’s most rapidly proliferating tissue, second only to bone marrow (Tobin and Kauser 2005). With the beginning of each new hair growth phase, the follicle is regrown entirely from the condensed dormant dermal papilla (Powell and Rogers 1997; Robbins 2012). Unlike bone and soft tissue, which store and periodically give up their substrates, once hair is formed it experiences no further turnover of proteins or minerals. The hair fiber grows continuously and rapidly, but the resulting medium is permanent and no longer interacts with the body’s circulating biochemistry. The hair fiber thus records an ongoing history of the individual’s physiology at the moment of fiber production (Tobin and Kauser 2005). Hair has increasingly been used to investigate chronic stress specifically because of this ability to offer a history of circulating cortisol levels. One of the greatest obstacles to investigating trauma and trauma recovery is the inability to estimate pre-injury baseline values for variables such as cortisol, which hair provides a mechanism for overcoming (Walton, et al. 2013). While blood and urinary levels of cortisol, intoxicants, or toxins
reflect exposure only for a limited time, the consistent growth of hair provides a linear history of several months (Kirschbaum, et al. 2008; Raul, et al. 2004; Sauve, et al. 2007; Van Uum, et al. 2008; Yamada, et al. 2003).

2.3.2. Hair follicle neuroimmunology

Exciting new research emerging in the last decade suggests hair follicles are much more intimately linked with the body’s stress systems than we previously thought. We now know that hair follicles do more than just record stress events in the chronological tissue of hair fibers; they play an active role in the systemic stress response. Skin is an ideal organ to interact with both physiological and immunological stressors, as it plays the unique role of the body’s boundary between internal and external environments. Considering skin is exposed to the body’s external environment at all times throughout an organism’s life, it has been called “the prototypic environmental interface organ of vertebrate life” (Arck, et al. 2006:1699). The strategic location covering the entirety of the body’s surface therefore plays a major role in providing sensory information necessary for maintaining internal homeostasis (Slominski, et al. 2007). The richly innervated skin transmits information on physical, chemical, or biological trauma, UV radiation, or other external stressors to the brain (Botchkarev 2003; Slominski, et al. 2000). The central nervous system then initiates general, systemic, or organ-specific responses to the threat (Botchkarev 2003). The skin is therefore more than a static barrier between the external environment and internal homeostasis; it maintains the latter in response to changes in the former (Slominski, et al. 2000). In fact, corticotrophin releasing hormone (CRH) and proopiomelanocortin peptides (major molecular components of mediating the systemic stress response), neurotransmitters, and cytokines are all expressed in the skin (Botchkarev 2003; Stamm and Safrit 1975).

In the last decade, several laboratories have independently discovered the extent of the link between the skin and the hypothalamic-pituitary-adrenal (HPA) axis regulated physiological stress response. Cyclic activity of the hair follicle (namely the anagen-catagen-telogen cycle) may be significantly influenced by neurohormones, neuropeptides, and neurotransmitters suggesting that the skin and hair follicles are
important targets for systemic and local stress responses (Botchkarev 2003). In their ground-breaking study Ito, Ito, and colleagues concluded that normal human scalp hair follicles respond to CRH not only by altering hair growth and pigmentation, but also by inducing cortisol production and activating the HPA-axis (Botchkarev 2003; Ito, Ito, Kromminga, et al. 2005). The finding that the hair follicle itself is capable of synthesizing cortisol supports the hypotheses that the hair follicle possesses a local functional equivalent to the HPA axis and that the follicle has the ability to both initiate and terminate the systemic stress response (Ito, Ito, Kromminga, et al. 2005; Ito, Ito and Paus 2005; Tobin and Kauser 2005).

Research by Sharpley, et al. (2009) offers a hint at the fine degree of response variation capable in hair follicles when responding to specific, localized stressors. After exposure to localized stress in the form of a Cold Pressor Test (CPT; cold water submersion of one hand), hairs of the exposed arm showed cortisol levels approximately 320% higher than normal. Hairs of the opposite leg, however, did not show any change in cortisol levels suggesting that hair cortisol response to the stressor was localized. The increased peripheral cortisol production (in limb hair) occurred independently of central HPA-axis cortisol production (as determined from saliva samples collected during the stress exposure) (Sharpley, et al. 2009). A repeat of the experiment measuring cortisol levels from hairs collected at varying distances from the localized stressor suggests that hair cortisol production sites within 250mm of each other act independently in the presence or absence of CPT or other pain stressors (Sharpley, et al. 2010).

2.4. Surgery as an acute physiological stress event

Surgery is a sharply demarcated stress event, which may be exclusive of other nutritional or physiological factors. Previous experimental studies tested nutritional stress apart from other physiological factors, (Section 2.2.2), but no research to date has tested the influence of only physiological stress on hair growth. There is some anecdotal literature suggesting surgical stress is capable of affecting hair fiber growth in the root. In their study of epilation force on malnourished vs. control patients, Chase and colleagues observed that otherwise healthy patients undergoing elective surgery
exhibited a drop in force required to pull scalp hairs three days following surgery, although the data did not reach statistical significance (Chase, et al. 1981).

Surgery allows for a known activation of the stress response while avoiding the ethical considerations of causing research participants unnecessary pain or discomfort. Laboratory stress protocols have been criticized for potentially lacking the naturalistic stress responses of field studies (Kudoh, Katagai, Takazawa, et al. 2001). Surgery is similar to field studies, wherein the stressor (surgery) would occur regardless of participant involvement in the study. Surgery is a known and potent activator of the systemic stress response. Although potentially modified by anaesthetic agents, the metabolic response to surgery follows the same general patterns as an unexpected injury (Frayn 1985). The physical trauma of surgery activates the systemic stress response, causing an almost immediate surge in HPA axis substrates, including cortisol (Giannoudis, et al. 2006; Vita, et al. 2006). Even less invasive surgical techniques such as laparoscopy are known activators of the HPA-axis cascade. However, the degree to which laparoscopic surgery triggers the systemic stress response is still under debate. Studies comparing the systemic stress responses to open and laparoscopic surgeries such as cholecystectomy have provided conflicting results (Nguyen, et al. 2002). However, the release of certain cytokines is reduced in laparoscopic surgery, suggesting the less invasive surgical technique triggers reduced neuroendocrine and acute phase responses and fosters faster recovery than open surgery (Grande, et al. 2002; Nguyen, et al. 2002). Some researchers have suggested a dose-response relationship between surgery and activation of the acute-phase cytokine cascade (Chernow, et al. 1987). Surgery has the added ethical benefit of studying injury without subjecting participants to the pain of injury. Modern anesthesia prevents surgical patients’ awareness of the procedure yet has little to no effect on the body’s cytokine response to the insult (Vita, et al. 2006). Surgery is a chronologically distinct event with a known mechanism, intensity, and duration. Thus surgery is an acceptable experimental substitute for studying physical trauma or acute physiological stress events.
2.5. Research Questions and Hypotheses

2.5.1. Research Question 1: What method should be used to assess changes in hair fiber physical characteristics?

In recent decades, technological advances have provided a range of new options for exploring biological materials at the microscopic level. In this increasingly digital, technological age, it may be tempting to rely on new technologies for data collection. Unbiased assessment of the benefits of new, more costly techniques must be performed.

Previous studies examining the diameter of the hair shaft used magnification as low as 10x and as high as 400x. Magnifications of 10x (Johnson, et al. 1975, 1976), 45x (Bradfield, et al. 1967), or 60x (Barman, et al. 1965) are not strong enough to provide a clear and distinct view of the hair margin for measurement at the micron level. Johnson (1975) examined hairs under 10x magnification and measured diameter to the nearest 10 microns, while Barman et al. examined hairs under 60x magnification and measured diameter using an ocular micrometer calibrated to 25 microns. In consideration of the range of normal hair diameters, some only 50 microns thick, the ability to adequately detect subtle changes along the hair shaft when measured to the nearest 25, or even 10, microns is questionable at best. Existing literature suggests a magnification of 400x is commonly used and considered the conventional choice (Hrdy 1973; Wynkoop 1929 cited in Saint Olive Baque, et al. 2012). Yet no one to date has tested whether increased magnification provides data which are significantly different from the conventional magnification. In addition to questioning the utility of greater magnification, we need to challenge our own assumptions about the superiority of digital analysis over manual analysis. With the increasing availability of tools for digital data collection, there is a risk of assuming those tools are preferable over traditional manual methods for data collection. However no studies have yet tested whether digital and manual methods provide significantly different data.

This study will address methodological questions by testing the assumptions that digital data collection provides data which varies significantly from that collected using manual methods. The utility of very high magnification examination will be
similarly tested by comparing data collected using 1000x magnification to that using 400x magnification. To answer Research Question 1, two null hypotheses will be tested:

\[ H_1: \text{Data collected using very high magnification (1000x) does not differ significantly from data collected using high magnification (400x).} \]
\[ (H_0: \text{H}_{1000x}=\text{H}_{400x}) \]

\[ H_2: \text{Data collected using digital measurement software does not differ significantly from data collected using manual measurement.} \]
\[ (H_0: \text{H}_{\text{digital}}=\text{H}_{\text{manual}}) \]

### 2.5.2. Research Question 2: What is the normal variation in fiber dimensions along the length of a hair fiber?

In 1967, Sims measured the hair of 10 individuals to assess normal variation in diameter along the length of the fiber. The fiber diameters were measured along two centimeters of hair, and found that the smallest diameter within that length was never less than 90% of the greatest diameter. From this, he concluded that any change in diameter equal to or greater than 10% of the maximum diameter was the result of a health “incident” (Sims 1967). There are several problems with Sims’ study. The first is the homogeneity of the control sample on which he bases his assumptions. All ten individuals were male undergraduate students of a single class at Cambridge University. One can assume that most, if not all, of the 10 males were young (early 20s), white, and relatively healthy (although he does not discuss any of these demographic factors explicitly). Also problematic is the lack of exploratory statistics. Sims did not present the means or standard deviations, or test the normality of individual data (or if he did, it was not reported). He also does not address the trend within his group of healthy males for individuals with relatively finer hairs to show less difference between minimum and maximum diameters than the individuals with relatively thicker hairs. The thickest hair in his control sample is 95µm, which is 10.5% thicker than the next larger hair in the sample, and is the only one to show the 10% difference between minimum and maximum diameters (actually 10.5%, which he rounded down). His entire sample rests on the smaller end of the spectrum (56-95µ) of potential hair diameters (50-180µ). In theory, a significantly thicker hair of 150µm may therefore have a range of daily variation which is greater than 10%.
A few years later, researchers began using statistical approaches to evaluate normal variation in hair fiber diameters. Sims and Knollmeyer (1970) measured the transverse diameters of ten hairs from each of 20 male, white, undergraduate students at the hair root and again 40mm distal. Both proximal and distal shaft diameters were found to be normally distributed with similar means and standard deviations (84.5µ ±15 and 85.0µ ±14) and a correlation coefficient of 0.847. Based on this and unreported experiments of the same nature on diameters 10, 20, and 30 mm from the root, the authors concluded that hair fiber diameters are reliably constant for at least 40mm from the hair bulb (Sims and Knollmeyer 1970).

The most problematic limitation of these early studies on variation is the reliance on a single diameter measurement to assess variation. Hair fibers are not perfectly circular in cross-section, but most instead are ovoid/elliptical, or even pear or kidney-shaped. In some individuals, the major axis diameter can be roughly twice that of the minor axis (Das 1974). Therefore their results may reflect a shift in perspective from major to minor axes as the hair fiber twists along the length of its shaft. Despite this significant problem in research design Sims and his colleagues are often cited in reference to normal hair variation, potentially due to the lack of other studies.

Before changes to the hair fiber characteristics caused by physiological stress events can be examined, it is first necessary to understand the degree to which hair fiber characteristics change in the absence of stress. Very little research to date has examined normal (non-stressed) variation along the length of hair fiber, and none has done so by examining daily values of multiple hair fiber dimensions. The goal of Research Question 2 is to address this knowledge gap by examining the inherent variability in the daily values of hair fiber dimensions.

### 2.5.3. Research Question 3: Do the dimensions of the hair shaft change in response to an acute physiological stress such as surgery?

The overwhelming evidence of the complex interactions between stress and the hair follicle suggest that non-nutritional physiological stresses would have a profound influence on the normal growth of hair, yet to date no exploratory studies have been
undertaken. Hair cortisol concentration studies illustrate that hair follicles register and record the changing stress-related hormonal environment of the body. The advent of cutaneous neuroimmunology as a new field of research has shown that hair follicles do much more than simply register stress events; they are in fact part of the systemic stress response itself. The increased sensitivity of hair follicles over other soft tissues to nutritional stress (McKenzie, et al. 2007; Roeder, et al. 1980) suggests that hair follicles are capable of registering short-lived but severe deviations from homeostasis.

Chronic nutritional stress causes a shift from normal anagen-telogen ratio to one in which there are an increased number of resting telogen follicles (Bradfield 1974; Johnson, et al. 1975, 1976; Johnson and Roe 1975), presumably either in response to a lack of available resources or as a mechanism to conserve those resources. Prior to this shift (or during the several weeks necessary to transition through catagen phase), active anagen follicles reduce the volume of hair fiber produced (either through production of fewer cells or of smaller cells and with fewer pigment granules). Anagen follicles may display atrophy of the root either in response to reduced substrate available or to conserve those substrates. Early researchers found that reduction in hair fiber diameters preceded other clinical manifestations of protein-calorie malnutrition, and without any associated decreases in serum proteins (Bradfield and Jelliffe 1974; Crounse, et al. 1970; MacKenzie, et al. 1995; Roeder, et al. 1980; Sims 1967). Similarly, recovery from malnutrition including both body weight gain and increase in hair fiber diameter, hair quality, and hair root protein levels occur without any measureable concurrent increase in serum albumin levels which had remained normal throughout the course of the disease (Crounse, et al. 1970; Roeder, et al. 1980; Sims 1967; Zain, et al. 1977). Clinical manifestations of protein calorie malnutrition most likely emerge only after the body’s protein reserve (skeletal muscle tissue) is depleted beyond the point of being able to maintain normal physiological and biochemical functions (Hoffer and Bistrian 2013). Thus normal serum levels are maintained at the expense of hair fiber production.

Hair follicles are likely just as sensitive to non-nutritional physiological stressors. Activation of the HPA-axis triggers a cascade of neurotransmitters and hormones which slow digestion, decrease sensitivity to pain, increase memory function, and mobilizes
Proteins from muscle catabolism and fats from storage cells provide the immediate energy needed for the “fight or flight” response to a dangerous situation (Barton 1985; Levine, et al. 2007; Staufenbiel, et al. 2013; Walton, et al. 2013). If injured during this stress event, then the acute phase response (along with the continued HPA-axis cascade) provides further cytokines, neurotransmitters, and hormones which preserve cardiac function, maintain vasopressure in the context of fluid loss, and stimulate further proteolysis to provide the necessary proteins for appropriate inflammatory and immune responses and tissue repair at the injury site (Barton 1985; Gabay and Kushner 1999; Hoffer and Bistrian 2013; Khovidhunkit, et al. 2000; Levine, et al. 2007). Some of these responses are proportional in degree to the severity of the injury, especially negative nitrogen balance (which reflects muscle breakdown and protein synthesis) and plasma cholesterol/lipid concentrations (Carpentier and Scruel 2002; Nguyen, et al. 2002).

The unusually high mitotic rate and significant protein and lipid resource demand of follicles creates a narrow margin of normal cell production, which is then at the mercy of available circulating substrates. Any biological process which alters or reduces the amount of available substrates would in turn reduce the synthesis of proteins in the hair follicle and therefore be reflected in the hair fiber produced during the deficit (Bradfield and Jelliffe 1974; Johnson, et al. 1976; Rushton 2002; Sims 1967; Sims and Knollmeyer 1970). Since early hair research, investigators have theorized that any stressor that interferes with normal rates of hair fiber cell synthesis, specifically anything which creates a disparity between resource requirements and resource availability, creates a suboptimal condition during which essential resources are prioritized for essential tissues (Bradfield 1974; Johnson, et al. 1976; Rushton 2002). Similarly, researchers of serum protein changes during the acute phase response to injury have suggested that the production of acute-phase proteins diverts amino acids away from production of those plasma proteins that are not required for the immediate immune response (Gabay and Kushner 1999). This likely includes the production of hair fiber proteins as well.

The normal physiological responses to acute stress and injury create an environment in which the hair follicles suffer functional malnutrition: i.e.- the substrate
resources may be available, but are diverted away from nonessential functions such as hair growth towards biological processes necessary for survival (e.g. maintenance of vasopressure, clotting, escape, etc.), healing (e.g. removal or destruction of infectious and pathogenic agents, angiogenesis, granulation, etc.), and an eventual return to pre-injury homeostasis (Barton 1985; Carpentier and Scruel 2002; Gabay and Kushner 1999; Neuberger, et al. 2013; Suffredini, et al. 1999). Thus the hair follicle would continue to produce hair fiber but at a significantly reduced rate. This response occurs immediately; in contrast, shifting hair follicles through catagen into telogen takes a matter of weeks. Reduction of hair fiber volume produced is a more rapid mechanism for reducing substrate demand (or responding to substrate unavailability) and one that can be initiated immediately and then reversed in less time than that required to shift anagen follicles to telogen. Quantitative characteristics such as fiber diameter or fiber segment volume should, in response to an acute physiological stress event, therefore show a change greater than that seen during normal healthy variation.

Major surgery and traumatic events are often listed as contributing factors of hair loss (Shrivastava 2009), although the etiology behind this is often not included in the discussion. The majority of research citing hair loss following surgery discusses localized hair loss resulting from pressure-related ischemia (restriction or loss of blood supply to tissues) to the scalp from resting in one position during extended periods of anesthesia (Ben-Amitai and Garty 1993; Bruce, et al. 2002; Hanly, et al. 1999; Lee, et al. 2012; Lypka, et al. 2008; Matsushita, et al. 2011; Patel and Henschel 1980). Diffuse hair loss following colorectal or bariatric surgery is assumed to result from the malnutrition of absorption disorders (either necessitating or resulting from the procedure) (Thompson 1989). There are suggestions that perioperative psychological stress (such as anxiety, adjustment/adaptation, or mood disorders) is a contributing factor to the development of postoperative alopecia areata (Khalaf, et al. 2004), although definitive evidence has not been presented. The relationship between psychological stress and telogen effluvium is poorly understood and needs to be researched independently (see Section 2.2.1.2) before we can understand the interplay of psychological and physiological stressors. Similarly, the influence of surgery as a physiological stress also needs to be better understood. Although there is significant
literature suggesting that physiological stress would negatively influence hair growth, experimental studies are lacking. The current study will address this inadequacy.

Multiple early studies into malnutrition have suggested that PCM leads to a reduction in the amount of hair fiber produced by the follicle (Section 2.2.2). These studies are generally several decades old and have some research design flaws. A more recent and commonly held opinion is that a period of dramatic hair loss usually follows a triggering event such as severe febrile illness (Section 2.2.3), hemorrhage, accidental trauma, or surgical operations (Section 2.4) by one to three months (Atifi, et al. 2006; Paus and Cotsarelis 1999) or chronic stress (Section 2.2.4). However, to date no studies have specifically explored the relationship between a reduction in hair fiber cell production and telogen effluvium.

Research Question 3 will be addressed by comparing pre-surgical to post-surgical values for several hair fiber dimensions. If the stress response triggered by surgery initiates a re-allocation of resources away from non-essential functions such as hair production towards essential functions such as wound healing, the hair should show a significant reduction in these dimensions.
Chapter 3. Materials and Data Collection

This chapter will discuss the materials used for the study and methods for collecting those materials. Recruitment of and inclusion/exclusion criteria for research participants, sample collection procedures, and data collection from samples are described. The methods and materials discussed in this chapter are the same for all research questions discussed in subsequent chapters.

3.1. Participant Recruitment

Participants were recruited in accordance with standard Research Ethics requirements for protecting the rights and privacy of research participants. Males between the ages of 20 and 75 scheduled for abdominal surgery at St. Paul’s Hospital, Vancouver, BC (Phase I) or St. Michael’s Hospital, Toronto, ON (Phase II) were eligible for participation in the study. The recruitment of only abdominal surgery patients was not intentional but was a byproduct of surgeon willingness to collaborate. Individuals who had received chemotherapy and/or radiation therapy within one month prior to surgery were excluded due to the effect of these treatments on the growth and quality of patients’ hair. Patients on steroid or other medications which affect cortisol levels were also excluded. Patients with no hair or hair too short to include at least one month’s growth prior to surgery (at least 2cm) were excluded. Twenty-one participants provided informed consent and joined the study. Two participants voluntarily withdrew before their surgery. Samples from one participant could not be used as all collected hairs lacked bulbs. Data were collected from the remaining eighteen participants (Table 3.1).

Potential participants were identified by the local PI (collaborating surgeon). A research assistant (Phase I), the collaborating surgeon, or her administrative assistant (Phase II) first informed the patient of his eligibility of the study. If he gave permission,
the author would either meet him after his surgical follow-up appointment or phone him directly. During this phone conversation, the nature of the study was explained as was the extent of required involvement. If he were interested to hear more, a face-to-face meeting time with the potential participant was scheduled. These meetings occurred between two and six weeks after their surgery. During the personal meeting (whether organized over the phone or in clinic after his appointment) full details of the study, the requirements for participation, and their rights as study participants were discussed. Opportunity for questions was provided. Potential participants were not obligated to participate and could decline for any reason without affecting their medical care. Those who agreed to participate then signed informed consent forms (Appendix C). A hair sample was collected at that time and the extent of their participation in the study concluded. This study was approved by the Office of Research Ethics at SFU (Phases I & II), Providence Health Care Research Ethics Board for St. Paul’s Hospital (Phase I), and the Research Ethics Office at St. Michael's Hospital (Phase II).
<table>
<thead>
<tr>
<th>Participant Number</th>
<th>Age</th>
<th>Procedure</th>
<th>Method of Surgery</th>
<th>Surgery Time (~hrs)</th>
<th>Days in Hospital</th>
<th>Sample Days*</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>21</td>
<td>Right Hemicolectomy</td>
<td>Laparoscopic</td>
<td>2.5</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>103</td>
<td>55</td>
<td>Right Hemicolectomy</td>
<td>Laparoscopic</td>
<td>2.5</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>105</td>
<td>70</td>
<td>Right Hemicolectomy</td>
<td>Laparoscopic</td>
<td>2.5</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>109</td>
<td>65</td>
<td>Reversal of loop ileostomy/pelvic pouch reconstruction</td>
<td>Open</td>
<td>1+</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>112</td>
<td>56</td>
<td>Reversal of loop ileostomy</td>
<td>Open</td>
<td>1+</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>113</td>
<td>34</td>
<td>Subtotal colectomy w/end ileostomy</td>
<td>Open</td>
<td>2.5</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>147</td>
<td>55</td>
<td>Reversal of loop ileostomy</td>
<td>Open</td>
<td>2+</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>184</td>
<td>65</td>
<td>Bariatric (stomach resection)</td>
<td>Laparoscopic</td>
<td>3</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>236</td>
<td>49</td>
<td>Rectosigmoid Resection w/ Diverting Loop ileostomy</td>
<td>Open</td>
<td>4</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>240</td>
<td>54</td>
<td>Cholecystectomy</td>
<td>Laparoscopic</td>
<td>2</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>244</td>
<td>75</td>
<td>Laparotomy w/lysis of congenital adhesion</td>
<td>Laparoscopic</td>
<td>1</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>255</td>
<td>51</td>
<td>Sigmoid Resection</td>
<td>Lap to Open</td>
<td>3.5</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>257</td>
<td>56</td>
<td>Reversal of (Hartmann’s w/diverting) loop ileostomy</td>
<td>Laparoscopic</td>
<td>1+</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>268</td>
<td>65</td>
<td>Low anterior resection w/ diverting loop ileostomy</td>
<td>Open</td>
<td>3.5</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>271</td>
<td>61</td>
<td>Low anterior resection w/ diverting loop ileostomy</td>
<td>Open</td>
<td>3.5</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>288</td>
<td>20</td>
<td>Reversal of loop ileostomy</td>
<td>Open</td>
<td>2</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>295</td>
<td>70</td>
<td>Revision of ileostomy</td>
<td>Open</td>
<td>2</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>297</td>
<td>60</td>
<td>Cholecystectomy</td>
<td>Laparoscopic</td>
<td>2</td>
<td>1</td>
<td>22</td>
</tr>
</tbody>
</table>

*Number of days between surgery and sample collection.
3.2. Hair Samples

Scalp hairs were collected from participants between 10 and 52 days following their surgery. Samples were collected from Phase I participants 10 days after their surgery (except for participant 113 which was collected 20 days after surgery). Early observations from this subset led the author to believe 10 days was not an adequate length of time to observe a return to pre-operative conditions. Therefore the elapsed time between surgery and sample collection was increased to a goal of 30 days for Phase II. Actual post-operative interval for Phase II participants depended on participant availability and willingness to meet for sample collection, and therefore ranged from 16 to 41 days.

Five hairs were pulled from the scalp from each participant using clean, flat-head, non-serrated forceps. Hairs were pulled rather than cut; including the root allows for more accurate determination of elapsed time since hair cell formation and avoids the generally suggested two week delay before newly formed hair exits the hair follicle at the scalp level (LeBeau, et al. 2011). Samples were pulled from the vertex posterior aspect of the sagittal plane from each participant (approximately the halfway point between bregma and opisthocranion cranial landmarks). Each hair sample was taken from the same location to avoid potential variation in hair due to location on the scalp, and because the hair at that location generally has the most consistent growth rate (LeBeau, et al. 2011; Wennig 2000). Collected hairs were stored in polyethylene sealable bags until examination. Season of scalp hair collection should not be an affecting variable. Research by Randall and Ebling (1991) showed that although the number of hair follicles in anagen phase fluctuates seasonally in males in correlation with fluctuations in testosterone, the diameter of growing scalp hairs does not exhibit significant seasonal fluctuations. According to Sims (1967) early research into hair diameter, when collecting three hairs from a participant the chance of getting zero anagen hairs is less than five percent. Only one consented participant had to be excluded due to a lack of anagen hair roots. None of the five hairs collected from this patient had any visible roots attached; most likely the hairs were fragile and broke under the strain of epilation. This could be due to epilation method, or to poor hair quality resulting from surgery and/or subsequent nutritional stress.
3.3. Data Collection

A single anagen hair from each participant was slide mounted for microscopic examination. The two centimeters closest to the scalp were mounted on a standard glass slide using Permount mounting medium and a cover slip. Permount was chosen because its refractive index is almost identical to that of hair (1.543-1.554) (Ogle and Fox 1999) and therefore encourages, rather than hinders, visibility of internal and external hair shaft features (Ogle and Fox 1999; Petraco 2004; Roe, et al. 1991). The section length includes hair grown at least one month prior to surgery and the time elapsed between surgery and sample collection. After allowing the mounting medium to cure, the hair was examined using an Olympus AX-70 with a 10x ocular objective. Hair was examined at 1000x magnification using a 100x oil immersion objective. The hair was examined, measured, and photographed first at the root bulb and moving distally along the shaft. A new digital photograph was taken every 350µm, the generally accepted approximation of a day’s worth of growth (LeBeau, et al. 2011; Russell, et al. 2012; Saint Olive Baque, et al. 2012; Wilson, Janaway, et al. 2001). This produced between 40 and 60 time points for each examined hair, depending on the amount of time between surgery and sample collection. An ocular micrometer was calibrated to the nearest micron using a 2mm stage micrometer with 0.01mm divisions. The calibrated ocular micrometer was then used to measure the total diameter of the hair shaft, the thickness of the cuticle, and the diameter of the cortex perpendicular to the microscope line of sight (“transverse” plane; Figure 3-1) while looking through the ocular. By calibrating the tic marks on the fine focus knob with a slide cover slip of known thickness and focusing on the “top” and “bottom” margins of the cortex, an approximate cortex diameter was measured parallel to the microscope line of sight (“longitudinal” plane diameter) (Harris 1985; Korkmaz and Tumkaya 1997; Petraco 2004). All dimensions were manually measured and recorded to the nearest whole micron (µ) excepting the cuticle, which was estimated to the nearest half micron. The longitudinal cortex diameter was measured instead of a longitudinal total diameter because it is the presence of pigment granules in the cortex which enables the visualization of the cortex boundary. Hair fiber cuticle generally lacks pigment granules and are therefore translucent in longitudinal perspective. Both transverse and longitudinal cortex diameters were measured to account for the elliptical cross-section
of hair fibers. The total diameter, cuticle thickness, and cortex diameter were digitally measured from each photograph using Image Pro Plus 6.0 software calibrated with the stage micrometer. After calibration, the Image Pro Plus native tool for measuring linear distances on digital photographs was used to measure dimensions to the nearest 1/10th micron (0.1µ). Each slide-mounted hair segment was again measured manually, photographed, and measured digitally under 400x magnification using the 10x ocular objective, a 40x objective, and the ocular micrometer calibrated for the lower magnification. At 400x magnification, only the transverse total diameter was recorded as the margin between cortex and cuticle was difficult to reliably visualize. It was not possible to measure some segments photographed under 1000x magnification due to problems visualizing fiber or cortex margins. This occurred most often in thicker hairs and resulted in fewer data points for some participants.

![Figure 3-1](image)

**Figure 3-1.** Measurements recorded from each location along the length of the hair fiber (in µ); A-Total diameter, B-Cuticle thickness, C-Transverse cortex diameter, D-Longitudinal cortex diameter. (Photomicrograph taken at 1000x magnification.)
3.4. Statistical Analysis

3.4.1. Variables used in analysis

The methods described above produced nine single-measurement variables for the statistical comparison of data collection modality (Research Question 1), which are summarized in Table 3-2. Total hair fiber diameter (including cortex and cuticle) at each location was measured manually and digitally at both 1000x and 400x magnifications. Cuticle thickness and transverse cortex diameter were measured manually and digitally at 1000x magnification. Longitudinal cortex diameter could only be measured manually at 1000x magnification.

<table>
<thead>
<tr>
<th>Measurement Description</th>
<th>SPSS Variable Name</th>
<th>Magnification</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Diameter (cortex and cuticle)</td>
<td>Diam_1000_Dig</td>
<td>1000x</td>
<td>Digital</td>
</tr>
<tr>
<td>Total Diameter</td>
<td>Diam_1000_Man</td>
<td>1000x</td>
<td>Manual</td>
</tr>
<tr>
<td>Total Diameter</td>
<td>Diam_400_Man</td>
<td>400x</td>
<td>Manual</td>
</tr>
<tr>
<td>Total Diameter</td>
<td>Diam_400_Dig</td>
<td>400x</td>
<td>Digital</td>
</tr>
<tr>
<td>Longitudinal Cortex Diameter</td>
<td>Cortex_Long</td>
<td>1000x</td>
<td>Manual</td>
</tr>
<tr>
<td>Transverse Cortex Diameter</td>
<td>Cortex_Trans_Man</td>
<td>1000x</td>
<td>Digital</td>
</tr>
<tr>
<td>Transverse Cortex Diameter</td>
<td>Cortex_Trans_Dig</td>
<td>1000x</td>
<td>Manual</td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>Cuticle_Man</td>
<td>1000x</td>
<td>Manual</td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>Cuticle_Dig</td>
<td>1000x</td>
<td>Digital</td>
</tr>
</tbody>
</table>

Research into the influence of stress (whether nutritional, pyrexial, or physiological) has mostly used the diameter of the hair shaft measured from a single perspective (perpendicular to the line of microscope view). Measuring diameter in a single plane negates any confidence we have that a change in diameter observed along the length of a hair represents an actual change in the amount of hair content produced by the bulb. For example, on first glance at the transverse cortex values of the hair from an individual (in this case Participant 271) the fiber appears reduced following surgery (Figure 3-2). However, if the hair was instead measured perpendicular to the first set of observations, the fiber would instead appear to significantly increase after
surgery (Figure 3-3). The discrepancy between the two perpendicular measurements illustrates the dangers of relying on a single measurement taken in a single plane to assess change along the length of the hair fiber. Most hair shafts are elliptical or ovoid in cross-section, with a “maximum” and “minimum” diameter at any given location of measurement. To avoid confusion, I will hereafter refer to these as the “major” and “minor” diameters. “Total diameter” will be used to refer to the diameter of the hair fiber containing both cortex and cuticle. Average cortex diameter (ACD) refers exclusively to the average of the cortex diameters measured parallel and perpendicular to the line of microscope sight, and the same applies for average total diameter (ATD). “Mean” will be used to denote the arithmetic or statistical average of sample population values. To adequately represent the amount of hair fiber being produced on any given day, variables comprised of multiple measurements which reflect the inconsistent cross-sectional shape of the hair fiber must be used.

![Figure 3-2. Transverse cortex diameter by days leading up to and following surgery of Participant 271 (surgery occurred on Day 0).]
Each measurement locus was treated as an imaginary “slice” of the hair shaft at that location. Transverse and longitudinal cortex diameters (along with cuticle thickness) were used to determine the surface area of each “slice.” Surface area was then used to determine the volume of each measured segment of hair (based on 350 microns of growth between each measurement locus). Transverse and longitudinal cortex diameters were also used to create an ellipticity ratio (McCrone 1977) of major to minor axes. Variables are described in Table 3-3. Participants 184, 240, and 295 had completely colorless (gray) hair. The lack of pigment granules in their hair fibers prevented the visualization of cortical margins in the longitudinal plane. These three participants were therefore not included in assessment of combined measurement variables.

Any variable used to assess normal variation or stress-induced changes should include at least major and minor fiber diameters. Ideally, the length of fiber grown during a determined time period should be included in the analysis of total hair growth. Unfortunately in the current study, the measurement of individual growth rates was not possible. Since volume is calculated as the product of surface area and length,
examination of fiber segment volume is only useful in cases where each segment potentially has a different length. As individual pre- and post-operative growth rates were not measured, that is not the case here. Therefore surface area relates the same information as volume in this particular dataset. Surface area is calculated as $A = \pi r^2$, or in the case of hair fiber cross-sections which are not perfectly circular, $A = \pi r_{major}r_{minor}$. The surface area of the segment “slice” and the ellipticity ratio of major:minor diameters were compared to the average total diameter to assess whether they convey the same information as ATD.

Comparison of average total diameter and surface area of the segment “slice” using the Pearson’s product-moment correlation coefficient suggests a very strong, positive correlation between average total diameter and the surface area of the fiber segment slice ($R=0.986; p<0.0005$). Average total diameter conveys approximately the same information on hair fiber growth as surface area, but is easier to record and analyze without extraneous data conversion. Therefore average total diameter was used as representative of the amount of hair fiber produced during a given growth period (daily) to assess normal daily variation. Average total diameter, average cortex diameter, and cuticle thickness were used to assess the influence of surgical stress on hair production. Cortex and cuticle were examined to assess whether post-operative reduction in diameter occurs primarily in the cortex, the cuticle, or equally in both regions. All single measurement variables (total diameter, cuticle, cortex diameters) and combined measurement variables (average total diameter, average cortex diameter, surface area, volume) are dependent variables of analysis. Independent variables are participant, magnification (1000x vs 400x), method (manual vs digital), and the timing groups (pre- and post-operative months, 10-day blocks, and weeks). Fixed independent variables are magnification, method, and the various timing groups. Participant was treated as a random independent variable.
### Table 3-3.  New variables based on combining multiple measurements*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Cortex (AC)</td>
<td>The average of transverse and longitudinal cortex diameters;</td>
</tr>
<tr>
<td></td>
<td>(Transverse Cortex + Longitudinal Cortex)/2</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td>The average of transverse and longitudinal total diameters;</td>
</tr>
<tr>
<td>(ATD)</td>
<td>(Transverse total diameter + (Longitudinal Cortex + (Cuticle Thickness*2))/2</td>
</tr>
<tr>
<td>Surface Area_Total</td>
<td>The surface area of the section “slice” based on the Average Total Diameter;</td>
</tr>
<tr>
<td>(SAT)</td>
<td>Π(Average Total/2)^2</td>
</tr>
<tr>
<td>Volume (VOL)</td>
<td>The estimated volume of the section “slice” based on average growth rate of</td>
</tr>
<tr>
<td></td>
<td>350µ/day;</td>
</tr>
<tr>
<td></td>
<td>Surface Area_Total*350</td>
</tr>
<tr>
<td>Ratio</td>
<td>Major axis: Minor axis</td>
</tr>
</tbody>
</table>

*All taken at 1000x magnification

3.4.2. **IntraClass Correlation Coefficients**

Intraclass correlation coefficients (ICC) were produced to evaluate any systematic differences between manual and digital methods of measuring hair fiber physical properties and between high and very high magnifications. The ICC is a measure of reliability that reflects both the variability between subjects and the inherent error of instrumentation, observer, etc. (Weir 2005). ICC can be used as a measure of inter-rater reliability; in this case the “raters” would be the measurement methods being compared. Intraclass correlation coefficients were used here to investigate systemic variation between magnifications (1000x vs 400x) and modalities (manual vs digital). When calculating an ICC a choice must be made between two models and two types. When testing measurement methods (magnification and modality), the two-way mixed model was used as measurement methods are fixed and participants are random. The “absolute agreement” type was used because systematic differences between the measurement methods are relevant to the research question. The strength of a correlation coefficient was based upon both the informal interpretation of the value and the coefficient of determination, which indicates the percentage of shared variance. The informal interpretation of the coefficient often used by statisticians stipulates that a correlation coefficient of 0.4-0.6 indicates a moderate relationship, 0.6 to 0.8 a strong one, and 0.8 to 1.0 a very strong relationship (Salkind 2004).
3.4.3. Mixed Models Analysis

To assess change over time and differences between measurement techniques, the data were analyzed using hierarchical linear modeling (HLM; also known as linear mixed effects models (LMM), random-effects models, and multi-level or nested models). While Repeated Measures ANOVA is capable of examining changes in variables over time in longitudinal studies, it is not the most appropriate statistical test for these data. Repeated Measures ANOVA has strict data requirements, especially those concerning homogeneity of variance and the necessity of all individual units (research participants) to match in the nature and number of observations points (Krueger and Tian 2004; Shin 2009). On the other hand, mixed models accommodate for a more flexible data point schedule (Keselman, et al. 2001; Krueger and Tian 2004; Shin 2009) which is beneficial for this study since not all participants entered the study the same number of days after their surgery or had long enough hair to include the same number of days prior to surgery. Unlike ANOVA, HLM also has the ability to treat time either continuously (number of days prior to or after surgery) or categorically (pre- vs post-surgery) (Krueger and Tian 2004).

HLM has a few advantages over ANOVA that are especially beneficial to longitudinal studies in which there may be significant variation between individuals in their natural state (pre-intervention). While Repeated Measures ANOVA focuses on group changes between time points, HLM examines change patterns in individuals, and uses the variation in patterns between individuals to estimate the group’s average change trajectory. HLM also examines the variation of each individual from that average trajectory (Hernández-Llreda, et al. 2003). HLM not only reports whether a difference exists between groups, but also how much of a difference exists. HLM acknowledges and characterizes differences and patterns of change at both the individual and the group levels. Since there is quite a lot of variation in hair thickness between individuals, this study requires a statistical model which emphasizes individual changes over time. Mixed models accommodate this, while Repeated Measures ANOVA simply focuses on group changes between time points (Krueger and Tian 2004; Shin 2009). In consideration of the structure and nature of the study data,
Hierarchical Linear Modeling is the most appropriate method for analysis of hair diameter over time. SPSS Version 22 was used for all statistical analysis.

3.4.4. Statistical Approach to the Research Questions

Research Question 1: What method should be used to assess changes in hair fiber physical characteristics?

Hypothesis 1: Data collected using very high magnification (1000x) do not differ significantly from data collected using high magnification (400x).

Existing hair fiber studies rarely justify their decisions in regards to magnification and methods. Magnification of 400x is considered the standard (Saint Olive Baque, et al. 2012), and we need to assess whether increased magnification provides consistently different results. Similarly, digital methods for data collection are increasingly relied upon, but the methods need to be assessed for increased accuracy and precision over manual data collection.

Hierarchical Linear Modeling was used for pair-wise comparison of measurement values to test for differences between levels of magnification. Total diameter measured manually with 400x magnification was compared to total diameter measured manually at 1000x magnification to assess differences in values based on the effect of magnification. The diameter value was treated as the dependent variable, magnification as a fixed, independent variable and participant as a random, independent variable. The null hypothesis of this model was thus no difference between measurements taken at 1000x magnification and those taken with 400x magnification ($H_0:1000\times_{\text{Manual}} = 400\times_{\text{Manual}}$). Significance was set at $\alpha=0.05$. The test was repeated with total diameter measured digitally at 400x and 1000x magnifications ($H_0:1000\times_{\text{Digital}} = 400\times_{\text{Digital}}$).

Hypothesis 2: Data collected using digital measurement software do not differ significantly from data collected using manual measurement.

Single-measurement variables were similarly assessed for differences in data collected manually and digitally. Total diameter measured manually at 400x magnification was compared to total diameter measured digitally at 400x. In this model,
the diameter values are also treated as the dependent variable, while measurement modality was treated as a fixed, independent variable. Participant remained a random, independent variable. The null hypothesis was no difference between total diameter values measured manually or digitally when using 400x magnification ($H_0:400x_{Manual}=400x_{Digital}$). This model testing was repeated with total diameter measured with 1000x magnification, transverse cortex diameter at 1000x, and cuticle thickness at 1000x ($H_0:1000x_{Manual}=1000x_{Digital}$).

**Research Question 2: What is the normal variation in fiber dimensions along the length of a hair fiber?**

**Intra-individual, intra-hair variation**

Applying Sims (1967) technique on the data from the current study produced results far different from his. Sims based his assessment on only one hair fiber variable: the transverse hair diameter. As discussed in Chapter 2, the major and minor diameters of a hair fiber twist along the length of its axis. Therefore Sims’ method for assessing change was based upon incomplete and potentially misleading data. To avoid this and using the most optimistic data possible, his technique was applied to the major diameter (the larger of the two axes) at all loci along the length of the fiber. In this study’s dataset, the smallest value is always greater than 10% difference from the largest value of the major diameter (Table 5-2). When using the same method, the current study population exhibits smallest major diameters on average 18.4% smaller than largest major diameters (12-24%). In Sims’ sample, the greatest (10.5%) difference occurred in the hair with the largest diameter at 95µ. The smallest difference between largest and smallest values in the current study population is 12%, which is only present in only two participants with diameters of 90µ and 120µ. Sims’ 10% boundary is less definite than he suggests, it may result from the relatively thin diameters of his sample, and it has not been properly tested or reproduced since the original publication.
Table 3-4.  Largest and smallest values of major diameters (in µ) of current sample population (pre-surgical data) and the percent difference between them (Sims (1967) method).

<table>
<thead>
<tr>
<th>Participant</th>
<th>Largest Diameter</th>
<th>Smallest Diameter</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>271</td>
<td>121.5</td>
<td>98.0</td>
<td>19</td>
</tr>
<tr>
<td>113</td>
<td>120.9</td>
<td>99.1</td>
<td>18</td>
</tr>
<tr>
<td>102</td>
<td>106.0</td>
<td>93.3</td>
<td>12</td>
</tr>
<tr>
<td>268</td>
<td>98.9</td>
<td>77.3</td>
<td>22</td>
</tr>
<tr>
<td>109</td>
<td>97.0</td>
<td>76.0</td>
<td>22</td>
</tr>
<tr>
<td>297</td>
<td>96.6</td>
<td>73.9</td>
<td>24</td>
</tr>
<tr>
<td>112</td>
<td>92.4</td>
<td>74.4</td>
<td>19</td>
</tr>
<tr>
<td>147</td>
<td>90.2</td>
<td>79.4</td>
<td>12</td>
</tr>
<tr>
<td>103</td>
<td>90.0</td>
<td>75.0</td>
<td>17</td>
</tr>
<tr>
<td>244</td>
<td>88.5</td>
<td>67.0</td>
<td>24</td>
</tr>
<tr>
<td>255</td>
<td>85.8</td>
<td>72.3</td>
<td>16</td>
</tr>
<tr>
<td>236</td>
<td>85.7</td>
<td>71.0</td>
<td>17</td>
</tr>
<tr>
<td>105</td>
<td>84.0</td>
<td>69.7</td>
<td>17</td>
</tr>
<tr>
<td>257</td>
<td>82.7</td>
<td>66.7</td>
<td>19</td>
</tr>
<tr>
<td>288</td>
<td>82.7</td>
<td>66.9</td>
<td>19</td>
</tr>
</tbody>
</table>

Das-Chaudhuri and Chopra (1984) examined the diameters of 25 hairs from each of 387 individuals. The authors conclude that using the mean value of multiple observations on an individual’s hair diameter is representative of that individual (Das-Chaudhuri and Chopra 1984). The mean of multiple observations for each individual was therefore used as a starting point for assessing normal daily variation in the current study. Daily ATD values (pre-operative days only) of one hair from each participant was used to investigate normal intra-individual, intra-hair variation. The distances of each individual’s daily ATD values from his pre-operative mean were used to establish individual ranges of normal variation.
Intra-individual, inter-hair variation

Two hair fibers (designated Sample 1 and Sample 2) from five participants were compared to assess intra-individual, inter-hair variation. Distances of individual daily ATD values from individual pre-operative means were determined for each of the two hairs and compared. Mixed model analysis was also used to assess differences between hair samples from the same individual. In this model, average total diameter was treated as the dependent variable. The sample number was treated as a fixed, independent variable and participant as a random, independent variable. Time (pre- and post-operative months) was treated as a covariant. The null hypothesis for this model was no significant difference between samples 1 and 2 for each participant ($H_0$: $ATD_{Sample1} = ATD_{Sample2}$)

**Research Question 3: Do the dimensions of the hair shaft change in response to an acute physiological stress such as surgery?**

Hierarchical Linear Modeling (HLM) was used to look for changes in post-operative hair values following surgery. Models 1, 2, and 3 compare all pre-operative data to post-operative data pooled into month, 10-day, and 7-day blocks (respectively). The first model tests for an overall change in hair fiber diameter resulting from surgery, while models 2 and 3 are designed to establish a rough time frame for estimating the time necessary for hair dimensions to return to pre-surgical normal. Models 4 and 5 compare all post-operative data to pre-operative data pooled into 10- and 7-day blocks. These models specifically test for gradual changes in fiber dimensions prior to surgery. In each model, the dependent variable was the value of the measurement (average total diameter, average cortex diameter, cuticle thickness). Individual participants are considered random effects. The time frame being compared are fixed effects (i.e. those are pre-chosen by the investigator and hence are not random).

Model 1 compares the entire pre-operative period (month) to the entire post-operative period. The entire pre-operative period was chosen to represent the average of pre-operative values in the way Das-Chaudhuri and Chopra argued that the mean of multiple values appropriately represents that individual (1984). Model 2 compares the entire pre-operative period to post-operative 10-day blocks. Model 3 compares the entire pre-operative period to post-operative weeks. Models 4 and 5 compare pre-
operative blocks (10-day and week, respectively) to the entire post-operative period. Models 4 and 5 were tested to look for changes in the hair shaft which occurred prior to surgery.

**Group Trends**

Mixed model analysis was performed on distances of post-operative total average diameter values from individual pre-operative means to explore subgroup trends. Research participants were divided into two groups based on whether their operations were performed open or laparoscopically. Eight participants underwent laparoscopic surgery, while ten had open surgery. The one participant whose surgery was initiated laparoscopically and then converted to open was included with the other open surgeries. Participants were similarly divided into subgroups based on the length of surgery (≤2hrs, n=9; >2hrs, n=9) and the length of their hospital stay (≤3 days, n=8; >3 days, n=10). In this model, the dependent variable is the post-operative distance from the pre-operative mean. Surgical modality, length of surgery, and length of hospital stay in days were treated as additional fixed effects.
Chapter 4. Statistical Results

4.1. Research Question 1: What is the best method for assessing change in hair?

4.1.1. Hypothesis 1: Data collected using very high magnification (1000x) does not differ significantly from data collected using high magnification (400x).

The means, standard deviations, and coefficients of variation for diameter at 1000x and diameter at 400x are very similar whether measured manually or digitally (Table 4.2). The Intraclass Correlation Coefficients (for absolute agreement type) for digital and manual diameter measurements are very high at 0.966 and 0.967 respectively, suggesting that magnification is responsible for less than 4% of the variation between data collected using 1000x magnification and the data collected using 400x magnification. A scatterplot of total diameter measurements taken manually at 1000x and 400x magnification illustrates the correlation between the two in Figure 4-1. Figure 4-2 illustrates the correlation between the total diameter values measured digitally at 1000x and 400x magnifications.

Mixed Modeling was used to further test the null hypothesis of no difference between the two magnifications within a significance of p<0.05 (H₀:1000x = 400x). When the diameters were compared using the magnification as the fixed effect in the model and participants as the random effect, digital methods failed to reject the null hypothesis. When total diameter was measured digitally, the greater magnification (1000x) produced values 0.29 microns larger than those produced by the lower magnification (400x) but with a p-value of 0.659 this difference is not statistically significant. Manual measurements of total diameters, however, rejected the null hypothesis. The lower magnification gave values 1.67 microns larger than the higher magnification (p=0.012).
Table 4-1. Statistical Analysis of 1000x vs 400x magnification (in µ).

<table>
<thead>
<tr>
<th>Total Diameter</th>
<th>1000x</th>
<th></th>
<th>400x</th>
<th></th>
<th>Intraclass Correlation Coefficients</th>
<th>Mixed Model Paired t-test (Significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>s</td>
<td>CV</td>
<td>Mean</td>
<td>s</td>
<td>CV</td>
</tr>
<tr>
<td>Digital</td>
<td>66.47</td>
<td>14.31</td>
<td>0.2268</td>
<td>66.17</td>
<td>13.31</td>
<td>0.2011</td>
</tr>
<tr>
<td>N=839</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manual</td>
<td>67.15</td>
<td>14.01</td>
<td>0.2087</td>
<td>65.48</td>
<td>13.43</td>
<td>0.2051</td>
</tr>
<tr>
<td>N=827</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CV = coefficient of variation; $s$ = standard deviation

Figure 4-1. Total diameter (in µ) measured manually when viewed at 400x and 1000x magnification (blue line represents the ideal fit line; n=827).
4.1.2. **Hypothesis 2:** Data collected using digital measurement software does not differ significantly from data collected using manual measurement.

Hair shaft diameter at both 1000x and 400x magnification, cortex diameter at 1000x magnification, and cuticle thickness at 1000x magnification were all recorded both manually and digitally. Descriptive statistics suggest preliminary similarities among pairs (Table 4-2). Means, standard deviations, and coefficients of variation are very similar within cortex and diameter pairs. Intraclass correlation coefficients are very
high (≥0.982) for the cortex and total diameter pairs, suggesting near perfect agreement. Correlation between total diameter measured manually and digitally is illustrated in Figure 4-3, while correlation between cortex diameter measured manually and digitally is illustrated in Figure 4-4.

**Table 4-2. Exploratory Statistics and Model Testing of Measuring Manually vs. Digitally (in µ).**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Manual</th>
<th>Digital</th>
<th>Intraclass Correlation Coefficient</th>
<th>Mixed Model Paired t-test (Significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>s</td>
<td>CV</td>
<td>Mean</td>
</tr>
<tr>
<td>Cortex a) N=808</td>
<td>61.65</td>
<td>13.98</td>
<td>0.2268</td>
<td>60.73</td>
</tr>
<tr>
<td>Cuticle a) N=782</td>
<td>2.79</td>
<td>0.40</td>
<td>0.1434</td>
<td>2.96</td>
</tr>
<tr>
<td>Total Diameter a) N=934</td>
<td>67.15</td>
<td>14.01</td>
<td>0.2087</td>
<td>66.47</td>
</tr>
<tr>
<td>Total Diameter b) N=1016</td>
<td>65.48</td>
<td>13.43</td>
<td>0.2051</td>
<td>66.17</td>
</tr>
</tbody>
</table>

a)@1000x magnification  
b)@400x magnification  
CV=coefficient of variation; s = standard deviation
Figure 4-3. Total fiber diameter (in µ) when measured manually and digitally at 400x magnification (blue line represents the ideal fit line; n=934).
Mixed model t-tests were again used to test the null hypothesis of no difference between manual and digital methods (H₀: Manual = Digital) for each of the three measurements (cortex, cuticle, and total diameter). At 1000x magnification, total diameters were less than one micron greater (0.68) when measured manually, although this difference is not statistically significant (p=0.327; Table 4-2). At 400x, a similar trend is observable with digital measurements 0.70 micron greater than manual measurements but not reaching statistical significance (p=0.275). Manual measurements of the hair fiber cortex were barely greater than digital measurements.
(0.09µ) and this difference also did not approach significance (p=0.172). Cuticle measurements were 0.17µ greater when measured digitally (p<0.0005).

The cuticle thickness is the exception to this trend. Means and standard deviations in the manual and digital measures of cuticle thickness are similar, but not to the degree seen in other pairings. The coefficient of variation for the manual cuticle measurements is slightly higher than that for the digital measurements (0.14 vs 0.11). The Intraclass Correlation Coefficient is strong 0.694, but this is noticeably lower than those seen for other pairings, which are all greater than 0.982. Correlation of manual and digital cuticle measurements at 1000x magnification is illustrated in Figure 4-5. Mixed model t-test shows that manual measurements of the cuticle are 0.17 microns less than digital measurements (p<0.000).
Figure 4-5. Cuticle thickness (in µ) when measured manually and digitally at 1000x magnification (blue line represents the ideal fit line; n=782).
4.2. **Research Question 2: What is the normal variation in fiber dimensions along the length of a hair fiber?**

4.2.1. **Intra-individual, intra-hair variation**

The distances of individual pre-operative ATD values from individual pre-operative means were examined to evaluate normal daily variation along the length of a hair fiber. Figure 4-6 illustrates that the majority of ATD measurements cluster within approximately 5 microns of the mean, regardless of the relative coarseness of the fiber. The only participant whose average total diameter varies to a greater degree than that is #113, who stands out as an outlier (discussed in section 5.4) and therefore was excluded. This method is preferable for two reasons. Firstly, major and minor cortex diameters are combined into a single value representative of the overall cross-sectional surface area of each measurement location. Secondly, the variation from mean is more consistent throughout the sample data than using Sims’ method of evaluating the narrowest value as a percentage of the largest value. While the percentages vary from 12 to 24%, the absolute values don’t vary much beyond 10 microns around the individual mean. Ten microns may be roughly 8% for a hair fiber of approximately 120µ, but for a hair fiber of only 40µ it is 25%.
4.2.2. Intra-individual, inter-hair variation

Two hair samples (designated sample 1 and sample 2) from five participants were compared to assess intra-individual variation. Despite Sims’ (1967) assertion that collecting five hairs from each participant assures adequate anagen hair fibers, in this study hair samples from only five participants included more than one anagen hair. Linear mixed model comparison of the two samples (compared pairwise for each participant) suggests that the average cortex diameters of sample 1 hairs were less than one micron larger than those of sample 2 (0.664μ) but that this difference is not statistically significant (p=0.072). Due to the small size of the comparative sample, an actual significant difference cannot be excluded (a Type II error of the analysis results). Scatterplots of samples 1 and 2 from each of the five individual illustrate that pre-operative total diameter values cluster within five micron on either side of the total diameter pre-operative mean (Figures 4-7 through 4-11). This is consistent with the original samples from the same individuals. Despite the small sample size, these data suggest that multiple hairs taken from an individual exhibit consistent daily variation within a specific range in the absence of a stress event.

Figure 4-6. Distance of daily average total diameter values (in μ) from individual mean during the 30 days prior to surgery (all participants; n=615).
Figure 4-7. Distribution around the mean of average total diameter values (in μ) for samples 1 and 2 from participant 147 (pre-operative values only).
Figure 4-8. Distribution around the mean of average total diameter (in μ) for samples 1 and 2 from participant 255 (pre-operative values only).

Figure 4-9. Distribution around the mean of average total diameter (in μ) for samples 1 and 2 from participant 268 (pre-operative values only).
Figure 4-10. Distribution around the mean of average total diameter (in µ) for samples 1 and 2 from participant 288 (pre-operative values only).
Figure 4-11. Distribution around the mean of average total diameter (in µ) for samples 1 and 2 from participant 297 (pre-operative values only).

4.3. Research Question 3: Do the dimensions of the hair shaft change in response to an acute physiological stress such as surgery?

4.3.1. Descriptive statistics

The means and standard deviations for each of the three key measurements are presented for each participant in Table 4-3. Standard deviations are very similar for participants despite differences in coarseness of the hair fiber. The two youngest participants have the largest standard deviations. This could potentially be a normal difference between the hair of younger and older individuals. One thing to note, however, is that the largest standard deviation is seen in participant #113, who has the coarsest hair of the sample group, so the greater standard deviation could also be a factor of diameter. Cuticle thicknesses and cuticle standard deviations are fairly consistent across participants, regardless of the coarseness of the hair fiber. When examined on an individual level, as expected most of the measurement data are
normally distributed. The cuticle thicknesses of three individuals are not normally distributed (participants 113, 147, and 240). Diameters for two participants are not normally distributed (113, 147), for one of those participants cortex diameters are also not normally distributed (147). Distributions of pre- and post-operative average total diameter values for each participant are illustrated in Figure 4-12.
Table 4-3. Descriptive statistics for key (single measurement) variables by participant (in µ).

<table>
<thead>
<tr>
<th>Participant (variables)</th>
<th>Pre-operative</th>
<th>Post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>102</td>
<td>15</td>
<td>84.51</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>77.59</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>3.46</td>
</tr>
<tr>
<td>103</td>
<td>30</td>
<td>72.69</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>66.78</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>2.96</td>
</tr>
<tr>
<td>105</td>
<td>27</td>
<td>63.97</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>58.33</td>
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<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>2.82</td>
</tr>
<tr>
<td>109</td>
<td>30</td>
<td>71.07</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>64.68</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>3.19</td>
</tr>
<tr>
<td>112</td>
<td>30</td>
<td>66.57</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>59.63</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>3.47</td>
</tr>
<tr>
<td>113</td>
<td>24</td>
<td>95.29</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>89.19</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>3.05</td>
</tr>
<tr>
<td>147</td>
<td>29</td>
<td>70.26</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>64.66</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>2.80</td>
</tr>
<tr>
<td>Participant (variables)</td>
<td>Pre-operative</td>
<td>Post-operative</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>184*</td>
<td>24</td>
<td>3.05</td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>236</td>
<td>28</td>
<td>71.26</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>3.13</td>
<td>0.23</td>
</tr>
<tr>
<td>240*</td>
<td>22</td>
<td>3.34</td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>244</td>
<td>30</td>
<td>59.84</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>2.84</td>
<td>0.28</td>
</tr>
<tr>
<td>255</td>
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<td>65.53</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>2.98</td>
<td>0.20</td>
</tr>
<tr>
<td>257</td>
<td>23</td>
<td>57.86</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>2.50</td>
<td>0.32</td>
</tr>
<tr>
<td>268</td>
<td>20</td>
<td>69.65</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>3.15</td>
<td>0.19</td>
</tr>
<tr>
<td>271</td>
<td>20</td>
<td>77.77</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td>3.15</td>
<td>0.22</td>
</tr>
<tr>
<td>Participant (variables)</td>
<td>Pre-operative</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>288</td>
<td>30</td>
<td>67.59</td>
</tr>
<tr>
<td>Average Total Diameter</td>
<td></td>
<td>61.23</td>
</tr>
<tr>
<td>295*</td>
<td>25</td>
<td>2.53</td>
</tr>
<tr>
<td>Cuticle Thickness</td>
<td></td>
<td>68.51</td>
</tr>
<tr>
<td>297</td>
<td>30</td>
<td>62.74</td>
</tr>
<tr>
<td>Average Cortex Diameter</td>
<td></td>
<td>2.89</td>
</tr>
</tbody>
</table>

*Hairs for these individuals are colorless; longitudinal measurements could not be recorded and therefore average total diameter and average cortex diameter could not be generated for these individuals.

N = number; $\bar{x}$ = mean; s = standard deviation
Figure 4-12. Boxplots of average total diameter before and after surgery for each individual participant (in µ).
4.3.2. Model 1

Pooled pre-surgical values (up to 30 days) were compared to pooled post-surgical values (up to 30 days). Descriptive statistics of average total diameter, average cortex diameter, and cuticle thickness post-operative values are shown in Table 4-4. All three variables show a statistically significant difference between pre-operative and post-operative months (Table 4-5). Total average diameters are -2.574µ smaller after surgery than pre-surgical values (p<0.0005). Average cortex diameters show a similar reduction of 2.304µ after surgery (p<0.0005) and cuticle thicknesses show a reduction of 0.149µ (p<0.0005).

Table 4-4. Descriptive statistics for key variables for Model 1 (pre-operative month vs post-operative month).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Operative Month</th>
<th>Post-Operative Month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>$\bar{x}$</td>
</tr>
<tr>
<td>Average Total</td>
<td>395</td>
<td>70.08</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>411</td>
<td>64.17</td>
</tr>
<tr>
<td>Cuticle</td>
<td>475</td>
<td>3.02</td>
</tr>
</tbody>
</table>

N = number; $\bar{x}$ = mean; $s$ = standard deviation

Table 4-5. Linear Mixed Model analysis of Model 1 (pre-operative month vs post-operative month).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1: Post-Operative Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Total</td>
<td>-2.574 (&lt;0.0005)</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>-2.304 (&lt;0.0005)</td>
</tr>
<tr>
<td>Cuticle</td>
<td>-0.149 (&lt;0.0005)</td>
</tr>
</tbody>
</table>

4.3.3. Model 2

Post-operative 10-day blocks were also compared against pooled pre-operative data. Descriptive statistics for post-operative 10-day blocks are shown in Table 4-6; results of Linear Mixed Model analysis are summarized in Table 4-7. The average total diameters are significantly narrower for the first and second 10-day periods (-3.458µ, p<0.0005 and -2.402µ, p<0.0005 respectively). The difference between pre-operative
values and those 20-30 days after surgery are not significantly different (0.300µ, p=0.599). In the first 10 days after surgery, the difference in average cortex values is -3.141µ (p<0.0005), but by the 10-20 days block the difference is only -2.113µ (p<0.0005). The cortex values during third post-operative 10 day block (days 20-30) are not significantly different from pre-operative cortex values (0.809µ; p=0.136). Unlike the average total diameter and average cortex diameter, the cuticle thickness is still statistically significantly thinner throughout the entire post-operative month. The difference between pre-surgical cuticle thickness and post-surgical cuticle thickness actually increases from -0.097µ in the first 10 days following surgery to -0.202µ in the 21-30 days following surgery (p<0.0005).

Table 4-6. Descriptive statistics for key variables for Model 2 (pre-operative period vs post-operative 10-day blocks).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Operative Period</th>
<th>Post-Operative 10-day Blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 1-10</td>
<td>Days 11-20</td>
</tr>
<tr>
<td>N</td>
<td>395</td>
<td>146</td>
</tr>
<tr>
<td>Average Total</td>
<td>70.08</td>
<td>67.37</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>64.17</td>
<td>7.46</td>
</tr>
<tr>
<td>Cuticle</td>
<td>475</td>
<td>3.02</td>
</tr>
</tbody>
</table>

N = number; \( \bar{x} \) = mean; \( s \) = standard deviation

Table 4-7. Linear Mixed Model analysis of Model 2 (pre-operative period vs post-operative 10-day blocks).

| Variable   | 1-10 | 11-20 | 21-30
|------------|------|-------|------|
| Average Total | -3.458 (<0.0005) | -2.402 (<0.0005) | 0.300 (0.599)
| Average Cortex | -3.141 (<0.0005) | -2.113 (<0.0005) | 0.809 (0.136)
| Cuticle     | -0.097 (<0.0005) | -0.190 (<0.0005) | -0.202 (<0.0005)

4.3.4. Model 3

Pre-surgical average diameter values were compared to first, second, third, and fourth post-operative week values (descriptive statistics in Table 4-8). Hair fiber average
total diameters are 3.311µ and 3.555µ narrower than pre-operative values in the first and second weeks after surgery (p<0.0005; Table 4-9). Average total diameter values during the third post-operative week were only one micron narrower than prior to surgery (p=0.036). By the fourth week following surgery ATD values were not significantly different from values before surgery (0.348µ, p=0.598). Cortex and cuticle values produced similar results. Cortex average diameters were 3.105µ and 3.320 narrower in the first and second weeks after surgery (p<0.0005) with a one micron difference in the third week which approaches significance (0.900µ, p=0.056). By the fourth post-operative week, values were no longer significantly different (p=0.128). The cuticle also exhibits significantly reduced values after the surgical stress. However, unlike the cortex the cuticle does not begin to recover in the third week after surgery. On the contrary, the differences between pre- and post-operative values appear to increase throughout the post-operative month. In the first week after surgery the cuticle is 0.097µ narrower (p<0.0005) but by the fourth week after surgery the cuticle is 0.212µ narrower (p<0.0005). Results of LMM by week are summarized in Table 4-9.
### Table 4-8. Descriptive statistics for key variables by participant for Model 3 (pre-operative period vs post-operative weeks).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Operative Period</th>
<th>Post-Operative Weeks</th>
<th>Days 1-7</th>
<th>Days 8-14</th>
<th>Days 15-21</th>
<th>Days 22-28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>(\bar{x})</td>
<td>s</td>
<td>N</td>
<td>(\bar{x})</td>
<td>s</td>
</tr>
<tr>
<td>Average Total</td>
<td>395</td>
<td>70.08</td>
<td>8.85</td>
<td>115</td>
<td>67.39</td>
<td>7.36</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>411</td>
<td>64.17</td>
<td>8.87</td>
<td>119</td>
<td>61.61</td>
<td>7.19</td>
</tr>
<tr>
<td>Cuticle</td>
<td>475</td>
<td>3.02</td>
<td>0.35</td>
<td>135</td>
<td>2.94</td>
<td>0.34</td>
</tr>
</tbody>
</table>

N = number; \(\bar{x}\) = mean; s = standard deviation

### Table 4-9. Linear Mixed Model analysis of Model 3 (pre-operative period vs post-operative weeks).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 3: Post-Operative Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 1-7</td>
</tr>
<tr>
<td>Average Total</td>
<td>-3.311 (&lt;0.0005)</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>-3.105 (&lt;0.0005)</td>
</tr>
<tr>
<td>Cuticle</td>
<td>-0.097 (&lt;0.0005)</td>
</tr>
</tbody>
</table>
4.3.5. Model 4

Pre-operative data were analyzed in 10-day blocks and compared to pooled post-operative data (descriptive statistics are summarized in Table 4-14). Table 4-10 shows that all three 10-day blocks for all three variables show a difference in values when compared to post-operative pooled data with all differences being statistically significant at \( p<0.0005 \). The ATD is approximately 2.6µ larger before surgery (2.589-2.622). Average cortex values range from 2.168µ (10 days prior to surgery) to 2.508µ (11-20 days prior to surgery) larger than post-surgical average cortex diameters (\( p<0.0005 \)). Cuticle values prior to surgery are 0.104µ (11-20 days prior to surgery) to 0.161µ (10 days prior to surgery) larger than post-surgical values (Table 4-11).

Table 4-10. Descriptive statistics for key variables by participant for Model 4 (pre-operative 10-day blocks vs post-operative month).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Operative 10-day Blocks</th>
<th>Post-Operative Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 1-10</td>
<td>Days 11-20</td>
</tr>
<tr>
<td></td>
<td>( N )</td>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>Average Total</td>
<td>147</td>
<td>70.71</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>149</td>
<td>64.45</td>
</tr>
<tr>
<td>Cuticle</td>
<td>170</td>
<td>3.05</td>
</tr>
</tbody>
</table>

N = number; \( \bar{x} \) = mean; \( s \) = standard deviation

Table 4-11. Linear Mixed Model analysis of Model 4 (pre-operative 10-day blocks vs post-operative month).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 2: Pre-Operative 10-day Blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
</tr>
<tr>
<td>Average Total</td>
<td>2.601 (&lt;0.0005)</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>2.168 (&lt;0.0005)</td>
</tr>
<tr>
<td>Cuticle</td>
<td>0.161 (&lt;0.0005)</td>
</tr>
</tbody>
</table>
4.3.6. Model 5

Average total diameter, average cortex diameter, and cuticle thickness data were pooled into pre-operative 7-day periods and compared to pooled post-operative data. Table 4-12 presents descriptive statistics. Similar to the 10-day blocks, all pre-surgical weeks showed a statistically significant (p<0.0005) difference over post-surgical values for all variables (summarized in Table 4-13). The average total diameter ranged from 2.450µ (the week immediately preceding surgery) to 2.766µ (the third week prior to surgery) larger than post-operative average total diameter. In the second, third, and fourth weeks prior to surgery average cortex values were 2.488-2.719µ larger than cortex values following surgery. While statistically significant (p<0.0005), average cortex diameters the week immediately before surgery were only 1.980µ larger than those after surgery. Pre-operative cuticle thicknesses ranged from 0.105µ to 0.175µ larger than post-operative cuticle thicknesses.
Table 4-12. Descriptive statistics for key variables by participant for Model 5 (pre-operative weeks vs post-operative month).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Operative Weeks</th>
<th>Post-Operative Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 1-7</td>
<td>Days 8-14</td>
</tr>
<tr>
<td>Average Total</td>
<td>102</td>
<td>70.54</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>105</td>
<td>64.32</td>
</tr>
<tr>
<td>Cuticle</td>
<td>118</td>
<td>3.07</td>
</tr>
</tbody>
</table>

\( N = \) number; \( \bar{x} = \) mean; \( s = \) standard deviation

Table 4-13. Linear Mixed Model analysis of Model 5 (pre-operative weeks vs post-operative month).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 3: Pre-Operative Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 1-7</td>
</tr>
<tr>
<td>Average Total</td>
<td>2.450 (&lt;0.0005)</td>
</tr>
<tr>
<td>Average Cortex</td>
<td>1.980 (&lt;0.0005)</td>
</tr>
<tr>
<td>Cuticle</td>
<td>0.175 (&lt;0.0005)</td>
</tr>
</tbody>
</table>
4.3.7. Group Trends

Subgroups based on surgical modality, surgical length, and length of hospital stay were included in HLM analysis as fixed effects in addition to pre- and post-operative timing (descriptive statistics are presented in Table 4-14). There was a very small difference in response to surgical stress between the two groups, with the average total diameter of participants’ hair who underwent laparoscopic surgery changing less than those who underwent open surgery by approximately one micron (-0.94; p=0.011). Participants were divided into subgroups based on the length of their surgery. Linear Mixed Model analysis suggests no difference in response to surgical stress between individuals whose surgery was accomplished in two hours or less versus those whose surgeries lasted longer than two hours (0.44; p=0.279). Longer hospitalized individuals showed a decrease in hair diameter which exceeded shorter hospitalized individuals by 1.69µ (p<0.0005).

Table 4-14. Descriptive statistics for average total diameter (in µ) in subgroup analysis.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Pre-Operative Period</th>
<th>Post-Operative Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td><strong>Modality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>155</td>
<td>-0.0005</td>
</tr>
<tr>
<td>Open</td>
<td>216</td>
<td>0.0012</td>
</tr>
<tr>
<td><strong>Length of Surgery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 hours</td>
<td>202</td>
<td>0.0004</td>
</tr>
<tr>
<td>&gt;2 hours</td>
<td>169</td>
<td>0.0006</td>
</tr>
<tr>
<td><strong>Length of Hospitalization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3 days</td>
<td>169</td>
<td>0.0016</td>
</tr>
<tr>
<td>&gt;3 days</td>
<td>202</td>
<td>-0.0005</td>
</tr>
</tbody>
</table>

N = number; \(\bar{x}\) = mean; s = standard deviation
Chapter 5. Discussion

5.1. Research Question 1: What method should be used to assess changes in hair fiber physical characteristics?

5.1.1. Hypothesis 1: Data collected using very high magnification (1000x) do not differ significantly from data collected using high magnification (400x).

Very high Intraclass Correlation Coefficients and the results of linear mixed model analysis suggest that there are no statistically significant differences between the values derived from digital measurement using a 100x objective and those derived using a 40x objective (in combination with a 10x ocular). There was a small difference between magnifications when measurements were recorded manually. Manual measurement of total diameter produced values 1.67µ larger when 400x magnification was used (p=0.012). Future studies need to explore this difference in more depth, specifically in regards to accuracy of the two methods. Examination of values recorded using 1000x magnification plotted against those recorded using 400x magnification (Figure 4-2) illustrates how the scatter is weighted above the ideal fit line (towards the lower magnification). This may be an artifact of lower precision when measuring manually using the lower magnification, but this conclusion cannot be supported until precision is specifically tested for data collected using both measurements. It should be noted that the majority of points located outside of the main cluster (both above and below the cluster) represent a single participant hair. This sample may have been particular difficult to measure, as sometimes inconsistencies in mounting medium impaired visualization of distinct fiber margins. A very high Pearson’s correlation coefficient (0.945; p<0.0005) suggests the data collected manually at 1000x and 400x magnification may both reflect stress-related changes in the hair fiber dimensions, but this too needs to be tested in a more formal study designed specifically to explore these questions. This study detected a slight, but insignificant, difference between 1000x and
400x magnifications when measured digitally (0.29; p=0.659). Examination of digital measurement values of the two magnifications plotted against each other (Figure 4-3) shows that the points cluster around the ideal fit but skew slightly towards the higher magnification. The points which fall well outside the main cluster again represent the same participant hair as those exceeding the cluster in Figure 4-2.

5.1.2. **Hypothesis 2: Data collected using digital measurement software do not differ significantly from data collected using manual measurement.**

Total diameter at 1000x magnification, total diameter at 400x magnification, and cortex diameter at 1000x magnification all showed no significant difference between values measured manually and those measured digitally. Only the cuticle thickness showed a difference between measurements collected manually and digitally, with digital values 0.17 microns larger than manual values (p<0.0005). Visual examination of manual values plotted against digital values (Figure 4-6) illustrates that the scatter of values is not only quite broad, but mostly falls under the ideal fit line (skewed towards larger digital values). This may in some way be an artifact of the very thin nature of the cuticle, which is only roughly three microns thick ($\bar{x} = 2.96\mu$). When measuring the thickness manually, the ocular micrometer could be calibrated to the nearest micron, and measurements could be estimated to the nearest half micron. This resulted in measurements which are less precise than those recorded digitally. The thicker structure of the cortex (and therefore of the total diameter as well) may have masked this phenomenon as “rounding” measurements may produce a value which varies from the true value by more than the standard deviation of the sample data (0.35µ). Future studies testing accuracy and precision need to specifically address the unique requirements of measuring such a small structure. If nothing more, effort should be made to utilize a stage micrometer which will enable more precise calibration than to the nearest whole micron.

There are some benefits to digital measuring, specifically the ease of data collection, image capture, and image annotation. Digital photomicrography software includes the capability of exporting recorded measurement data directly into spreadsheets, which can then easily be imported directly into statistical analysis.
software. There is no doubt about the benefit of instant and accurate data entry. However, the results of the comparisons performed here illustrate that the data collected manually do not differ significantly from data collected digitally (except for the cuticle). However in contexts where digital data collection is not logistically feasible, manual collection is not an inferior method.

5.1.3. Concluding Remarks on Research Question 1

It is important that researchers question their own methods for measurement, especially when the study depends on the ability to observe change. Here, the methods generally considered standard have been evaluated and more appropriate variables for evaluating change in hair shaft growth have been suggested. The results of mixed model analysis suggest that there is no significant difference between high and very high levels of magnification to measure hair fiber total diameter. Similarly, there are no differences between manual and digital modalities for measuring hair shaft characteristics, excepting the cuticle. Internal hair fiber structures such as medulla, ovoid bodies, pigment granules, etc. are more easily visualized at 1000x magnification (Ogle and Fox 1999) which may aid in measurement. If these structures are a necessary part of the research study, higher magnification and digital measurement are recommended. However, for most common hair analysis needs based on total diameter, manual analysis at 400x magnification will not only give the same results, but at a fraction of the cost and with reduced infrastructure requirements.

Univariate measures of hair shaft production should be avoided. Hair shaft diameter measured in only a single plane offers a perspective on hair growth that is incomplete at best and misleading at worst. There is no evidence to support claims that any single plane of measurement is capable of ensuring either the major or minor diameter of the shaft consistently. In contrast, it appears the major and minor diameters of the shaft twist longitudinally along the length of the shaft. Both major and minor axes of hair diameter should be used when assessing the amount of hair produced during a given time period. Ideally, the length of growth during that time should be combined with major and minor diameters to estimate the volume of hair fiber produced during said period. Care should be taken to observe whether growth rates change in response to
acute stresses. Future studies should incorporate methods to assess not only individual
daily growth rates, but changing individual daily growth rates to ensure the most
accurate estimation possible of hair fiber volume. In the absence of resources or ability
to estimate growth rate and therefore volume, average total diameter is the next best
option for estimating the amount of hair fiber produced daily growth periods.

5.2. Research Question 2: What is the normal variation in fiber dimensions along the length of a hair fiber?

The few previous studies to address hair fiber intra-individual and intra-hair
variation suggested a degree of variation which was significantly lower than that
observed during this research study. These studies based their analysis of variation on
only a single diameter measurement (perpendicular to the microscope line of sight)
(Das-Chaudhuri and Chopra 1984; Sims 1967; Sims and Knollmeyer 1970). The pre-
operative variation observed along the hair fiber length in this study was much larger
than that suggested by previous studies such as Sims (1967). However, many
participants in this study were scheduled for surgery due to pre-existing health issues
such as colon cancer or ulcerative colitis. While all were stable and relatively
healthy (not experiencing active symptoms), the influence of comorbidities cannot be excluded.
Individuals with symptomatic illnesses that affect digestion and absorption (ulcerative
colitis, Crohn’s disease, irritable bowel disease, bowel lesions, etc.) may exhibit less of a
difference between pre- and post-operative values if their hair is already exhibiting the
effects of chronically inadequate substrate resources. Alternatively, hair growth may be
maintained at normal levels (along with other bodily functions), but at the cost of
maintaining an adequate substrate pool circulating in the blood stream. In that case, the
protein and lipid requirements of responding to an acute physiological insult such as
surgery would immediately deplete the pool, and the hair would exhibit a greater
difference between pre-and post-operative values. This study does not specifically
address this question and so cannot offer an informed answer. Future studies will need
to assess the normal daily variation in hair fiber characteristics using a wider
demographic variety of participants and specifically those without pre-existing health
conditions. Additionally, future studies need to address intra-individual, inter-hair variation in more depth with analysis of multiple hairs from multiple participants.

5.3. Research Question 3: Do the dimensions of the hair shaft change in response to an acute physiological stress such as surgery?

The results of Linear Mixed Model analysis indicate that hair fibers do reflect the influence of surgical stress in their production. Cortex diameters are significantly narrower in the post-surgical month than the pre-surgical month. Results of model 2 suggest the hair fibers begin their return to normal during post-operative days 11-20. Hair fiber average total diameters are roughly 3.5μ narrower in the first 10 days following surgery, but only 2.4μ narrower in the second 10-day block after surgery. Model 3 results, however, suggest the hair dimensions are consistently reduced for the first two weeks after surgery (3.3μ and 3.6μ narrower). The fibers do not begin returning to normal until the third post-operative week (only approximately one micron narrower). Therefore the transition back to normal pre-surgical values occurs approximately between post-operative days 14-20. This suggests that recovery from a single physiological stress may require roughly three weeks even if, like surgery, this event lasts only a few hours and is followed by good recovery care. In contexts of limited or no access to pain management or infection prophylaxis and in contexts of compromised immunity, very advanced age, very young age, or poor health prior to the event we can reasonably expect extended recovery time.

Cuticle thicknesses, however, are significantly thinner for the entire 30 days following surgery. This may be a result of the higher lipid content of the cuticle, and the significant changes in serum lipoproteins during the post-surgical acute phase response. Increased plasma lipid concentrations have been theorized to contribute to defense against exogenous microbes introduced during injury as well as to healing of damaged membranes (Carpentier and Scruel 2002; Gabay and Kushner 1999; Khovidhunkit, et al. 2000; Pruzanski, et al. 2000). Future studies should incorporate a longer interval between triggering stress event and hair sample collection in the hopes of seeing when
cuticle values return to pre-stress normal and how long the trend of increasing cortex diameter continues.

Analysis of pre-surgical blocks compared to post-surgical pooled data shows that there is less of a difference between pre- and post-operative values during the week immediately preceding surgery (results of model 5). This could reflect a difference in growth rates, in which the reduction in diameter actually did occur post-operatively but a stringent use of the 350µ per day average growth rate obscured the actual timing of the reaction. Another option is that emotional or psychological stress and anxiety about upcoming surgery caused reduced appetite or reduced absorption of nutrients in the days leading up to surgery. This study was not designed to specifically address the influence of psychological or emotional stress related to surgery. Patients commonly feel nervous anticipating an upcoming surgery, and this emotional stress can lead to integrated plasma cortisol levels on the day prior to surgery which are double that of normal days (Wheler, et al. 2006). Complications, pain, and reduced independence during the healing process may cause similar psycho-emotional stress following surgery. However, the influence of psychological stress itself on hair fiber growth hasn't been properly examined to the extent of ruling it out as a causative or at least confounding factor. The differences in fiber reduction following surgery among participants in this study may be partially related to psychological or emotional factors. Future studies should attempt to specifically examine the influence of psychological and emotional state on the response to and recovery from surgery using assessment questionnaires such as the Cohen, et al. (1983) Perceived Stress Scale.

The results of this analysis are consistent with the few existing studies specifically investigating narrowing of hair diameter in response to non-nutritional illness or hospitalization (although illness and injury cannot be fully segregated from nutritional factors) (Matsuura 1902; Pinkus 1928; Sims 1967). However, these studies are severely outdated, inadequately reported, and utilized questionable methods. The current study was undertaken in part as an attempt to remedy the poorly designed or poorly reported study methods previously used. This study does not entirely accomplish that goal, but is a first step of many required to formally quantify and validate methods for investigating
change in hair fiber dimensions resulting from acute physiological stress events which are not primarily nutritional stresses.

5.3.1. Discussion of individuals

**Participant 102**

Participant 102 is a 21 year old male who underwent a right side hemicolectomy to remove a bowel lesion. His surgery lasted approximately 2.5 hours and was performed laparoscopically. He was ambulatory the following morning and was discharged four days after the operation. His average total diameter shows a reduction of 5-8µ following surgery (6-10% of the pre-surgical mean; Figure 8-1). Although participant 102 was young and otherwise healthy, the bowel lesion was likely interfering with his digestion and nutrient absorption. This may have created a situation in which his biological reserves of proteins and lipids were already depleted or sub-optimal. The below-mean values in the few days prior to surgery could reflect psychological stress and anxiety about the upcoming procedure or recurring symptoms of the condition necessitating his surgery. They may also reflect inaccuracy in the growth rate of his hair. Being young and having thicker hair fibers are two factors correlated to a faster growth rate. In this case, those values may actually represent post-operative growth of reduced diameter. If so, the pre-surgical mean may be higher than estimated.
Figure 5-1. Distance of participant 102 average total diameter daily values from average total diameter pre-operative mean (84.5µ).

\textbf{Participant 103}

Participant 103 is a 55-year old male who underwent a right hemicolectomy to remove a colon polyp. His surgery was performed laparoscopically and lasted approximately 2.5 hours. He was discharged after six days of hospitalization. The post-operative average diameter of this participant’s hair appears to be minimally affected by the surgical stress, if at all. Pre-operative values vary from the mean less than five microns (approximately 7% of the pre-operative mean), and post-operative values mostly stay within this same range. Only on the seventh post-operative day is the average total diameter six microns thinner than the pre-operative mean. Colon polyps typically do not interfere with digestion and absorption unless they are large enough to cause a colon blockage. This participant’s hair may not show a post-operative decrease in average total diameter because he was nutritionally healthy and had adequate reserves of circulating substrates to manage the survival and recovery needs of the surgical stress. However, without testing multiple hairs, there is no way to exclude the possibility of the sample hair transitioning into or through catagen at the time of collection.
Participant 105

Participant 105 is a 70 year old male who underwent a right hemicolecctomy to remove hepatic flexure cancer. His laparoscopic surgery lasted approximately 2.5 hours and he was discharged six days later. His average total diameter was dispersed around the mean ≤4µ (5.5% of pre-operative mean). His post-operative values were less than the pre-operative mean, but did not exceed a five micron range of variation until the seventh and eighth post-operative days. The below-mean values for the post-operative week suggest a hair follicle reaction to the surgical stress, but without daily values beyond the first week no conclusions can be drawn on the full extent of stress-related change nor on the length of time before returning to the range of normal pre-surgical variation.

Figure 5-2. Distance of participant 103 average total diameter daily values from average total diameter pre-operative mean (72.7µ).
Participant 109

Participant 109 is a 65-year old male. He underwent a loop ileostomy closure following a pelvic pouch reconstruction. His surgery was open, and lasted just over an hour. He was discharged after three days of hospitalization. This participant’s pre-operative values varied within three microns on either side of the mean (approximately 7% of the mean). The average total diameter values did not exceed this range of variation following surgery. Similar to participant 103 participant 109 was either healthy and well-nourished enough to physiologically cope with the insult of surgery or the examined hair had begun to slow active growth in preparation for transitioning to telogen phase.
Participant 112

Participant 112 is a 56-year old male who underwent a loop ileostomy closure. His open surgery lasted approximately one hour and he was discharged from the hospital after three days. His pre-operative average total diameter values fall within three microns greater than to three microns less than the mean (4.5% on either side). Immediately after surgery his values drop below that threshold for the duration of the post-operative week, reaching approximately eight microns less than the pre-operative mean (approximately 12% of the mean). This participant underwent the same surgery, for the same length of time, and was discharged the same number of days as participant 109, who did not show any change in hair diameter values. The difference in hair fiber reactions highlights the influence of individual characteristics in the reaction to surgical stress.
Figure 5-5. Distance of participant 112 average total diameter daily values from average total diameter pre-operative mean (66.6µ).

**Participant 113**

Participant 113 was a 34-year old male who underwent a subtotal colectomy with creation of a loop ileostomy to treat ulcerative colitis. His open surgery lasted approximately 2.5 hours. He responded poorly to the surgery and on post-operative day four was moved to the intensive care unit (ICU) where he remained until post-operative day ten. He was discharged from the hospital 35 days after his surgery. Prior to surgery his ATD values varied from roughly ten microns over the mean to ten microns below the mean. However, Figure 5-5 shows that during pre-operative days 20-26, his diameter values were much larger. In addition, during pre-operative days 1-4, the diameter was smaller. There are several potential explanations for this trend. The early larger diameter values of pre-operative days 20-26 could represent his true “healthy” hair. Around the twentieth pre-operative day, he may have experienced a resurgence of symptoms of his ulcerative colitis, causing significant digestive problems. The reduced diameter in the weeks leading up to surgery and the significantly reduced diameter around the time of his surgery may be the result of this. Following surgery, the average total diameter showed a reduction of over twenty microns below the pre-operative mean.
(roughly 21% of the mean). This is the largest decrease in diameter following surgery of the study sample population.

Figure 5-6. Distance of participant 113 average total diameter daily values from average total diameter pre-operative mean (95.3µ).

Participant 113 looked and behaved the most unwell during pre-operative meetings for study recruitment. Unlike the other participants, he appeared pale, weak, fatigued, and visibly ill. His recovery from surgery was the longest and most complicated of the study participants, necessitating six days in ICU. He most likely had the fastest growth rate of all research participants. Saint Olive Baque, et al. (2012) found a direct relationship between diameter and growth rate. Even within the same scalp, thicker hairs grow faster (80µ fibre would exhibit growth rate of 0.38mm per day). Participant 113 would (according to their results) grow at a rate of approximately 430µ per day, which is consistent with the results found by Loussouarn et al. (2005). The reduced ATD values in pre-operative days 1-3 may actually reflect post-operative changes in the case of a growth rate faster than the average 350µ daily rate used in this study. The significant post-operative reduction in diameter illustrates the significant impact of an acute physiological stress event on homeostasis when the individual is already functioning well below optimum conditions.
Participant 147

Participant 147 was a 55-year old male. He underwent a loop ileostomy closure (open surgery) that lasted just over two hours and he was discharged two days later. The pre-operative ATD values do not vary outside of a five micron range (7%) on either side of the mean except for a brief dip in values approximately seven microns below the mean on the 25th pre-operative day (Figure 5-7). During the recruitment and consent discussions, the participant did not mention any specific health or psychological events and therefore the author cannot speculate as to the cause of this temporary reduction in diameter. In the absence of this event the pre-surgical mean may have been slightly larger than estimated, and the associated normal daily variation would probably not exceed three microns on either side of the mean. In that context, the post-operative reduction in average total diameter would have approached ten microns below the pre-operative mean. Without excluding the diameter reduction at 25 days prior to surgery the post-operative reduction in diameter plateaus around seven microns less than the pre-operative mean. The average total diameter values of participant 147 did not recover during the time period reflected in the hair fiber.

Figure 5-7. Distance of participant 147 average total diameter daily values from average total diameter pre-operative mean (70.26µ).
**Participant 236**

Participant 236 was a male, 49 years old. He underwent an open rectosigmoid resection and creation of a diverting loop ileostomy. The procedure lasted approximately four hours and he was discharged seven days later. His pre-surgical average total diameters are distributed ≤4µ on either side of the pre-surgical mean (5.6%), and drop to almost 8µ below the mean (approximately 11%) following surgery for approximately 8 days.

![Figure 5-8](image)

**Figure 5-8.** Distance of participant 236 average total diameter daily values from average total diameter pre-operative mean (71.3µ).

**Participant 244**

Participant 244 is a 75-year old male who underwent a laparotomy (laparoscopic exploratory surgery) during which a congenital adhesion was found to be restricting the intestine and was removed. The surgery lasted approximately one hour and he was discharged five days later. Eight days prior to the laparotomy, participant 244 had undergone a minimally invasive surgery to repair an abdominal hernia. Three days prior to the laparotomy he was hospitalized due to pain and a sudden deterioration in digestive function, which eventually initiated the surgery. His average total diameter
values showed a slight increase following the hernia surgery; this is surprising as hernias don’t typically have a direct influence on digestive function. However, general discomfort may have interfered with his appetite, in which case he may have been less adequately nourished than normal. Following the laparotomy, the diameters reduce to approximately 8µ below the pre-operative mean which is approximately 13% of the mean.

![Graph](image)

**Figure 5-9.** Distance of participant 244 average total diameter daily values from average total diameter pre-operative mean (60µ).

**Participant 255**

Participant 255 is a 51 year old white male who received a sigmoid resection. The surgery was initiated laparoscopically and converted to open during the procedure which lasted approximately 3.5 hours. He was discharged nine days later. His average total diameter values show a slow and steady increase from approximately five microns below pre-surgical mean around 30 days prior to surgery to approximately five microns above pre-surgical mean roughly 30 days after surgery. Five microns represents 7.6% of his pre-operative mean of 65.5µ.
Participant 257

Participant 257 was a 56-year old male who received a reversal of his Hartmann's procedure (which is essentially a high anterior resection) and the associated loop ileostomy. The open procedure required approximately one hour and he was discharged after six days of hospitalization. Prior to surgery, the ATD values vary within three microns on either side of the mean, except for the twelfth pre-operative day on which the ATD is approximately four microns larger than the mean. Three microns represents approximately 5% of the mean average total diameter of 59 microns. Following surgery the ATD values mostly fall below the mean but within four microns except for the sixteenth post-operative day. On this the day the ATD value was roughly eleven microns narrower than the pre-operative mean. This value appears to be an outlier, possibly due to measurement error. If the value accurately reflects a sudden decrease in average total diameter lasting only one day, the cause for this is not clear.
Participant 268

Participant 268 was a 65 year old male. He underwent an open low anterior resection with a diverting loop ileostomy which lasted approximately 3.5 hours. He experienced a post-operative urinary tract infection following surgery. An associated febrile reaction began a couple of days after surgery and lasted approximately three days. He recovered and was discharged six days after his surgery. Prior to surgery his normal range of variation encompassed roughly four microns on either side of his mean. Participant 268’s average total diameter drops to roughly 7µ (10%) below the pre-operative mean on post-operative day five (Figure 5-12) and hovers around five microns less than the mean for fifteen days. There is visible increase in the values by day 20, but by day 30 the values have not yet returned to pre-operative normal. There are not enough data to determine if the overall reduction in hair diameter is caused by the fever, the intestinal resection (and resulting reduction in digestive absorption), psycho-emotional stress over the complications, or a combination of these factors. It appears that the surgery and resulting complications initiated a decrease in average total diameter that had not resolved by the time of hair sample collection.

Figure 5-11. Distance of participant 257 average total diameter daily values from average total diameter pre-operative mean (59µ).
Figure 5-12. Distance of participant 268 average total diameter daily values from average total diameter pre-operative mean (69.7µ).

**Participant 271**

Participant 271 was a 61 year old male. He underwent a 3.5 hour low anterior resection with a diverting loop ileostomy to remove a rectal cancer (open). He was discharged three days after his procedure. His pre-operative diameter ranged within approximately six microns of his mean (7.7% of the mean). Following surgery, the values continue to fall within this range. This may reflect a healthy and robust substrate reserve prior to surgery which allowed his body to function normally during the healing process without influence on hair growth. It is unexpected, however, given that a section of intestine was removed and this generally affects digestive absorption.
**Participant 288**

Participant 288 was the youngest participant at 20 years old (male). He underwent a closure of his loop ileostomy which required two hours. He was discharged after two days. Prior to his procedure, his average total diameter fell within five microns (7.4%) on either side of the pre-operative mean. Following the surgery, the ATD values do not vary outside of this range. He most likely was in good health at the time of surgery and had adequate physiological reserves to withstand a minimally invasive procedure with very little strain on his circulating bank of proteins and other substrates. Young adults tend to have more reserves available for injury survival and healing than elderly adults, so this is not surprising.
Figure 5-14. Distance of participant 288 average total diameter daily values from average total diameter pre-operative mean (67.6µ).

**Participant 297**

Participant 297 was a 60 year old male. He underwent a laparoscopic cholecystectomy which lasted approximately two hours. He was discharged the next day. His range of variation prior to surgery encompassed approximately four microns on either side of the pre-operative mean (7.3%). His post-operative average total diameter values did not drop below 5% less than his pre-operative mean (Figure 5-15). On the contrary, the values appear to increase slightly around his surgery as all post-operative values are larger than the pre-operative mean. The conditions necessitating a cholecystectomy (cholecystitis or cholelithiasis) generally produce severe nausea, sometimes leading to vomiting. The slight increase in post-operative average total diameter values may represent an improvement in the digestion and nutrition of participant 297 or it may simply reflect normal variation along the length of the hair fiber.
5.3.2. Discussion of Group Trends

Subgroups were compared to investigate the influence of other factors on participant reaction to surgical stress. The reaction of participant 113 was so extreme he was excluded from subgroup analysis to prevent his outlier values from influencing the subgroup trends (Walton, et al. 2013).

Surgical modality

The changes in ATD values following surgery in regards to surgical modality were explored by dividing participants into two groups based on whether their operation was performed open or laparoscopically. Results of HLM analysis suggest that participants who underwent open surgery saw a greater reduction in their post-operative ATD values, which is illustrated in Figure 5-16. Laparoscopic surgeries are less invasive than open, but colorectal surgeries inherently involve digestion-related comorbidities.

Although many researchers agree that less invasive surgeries lessen surgical stress via reduced tissue damage, inflammatory response, and post-operative pain
(Giannoudis, et al. 2006), cytokine production during the acute phase response and associated reallocation of serum proteins and lipids for healing and recovery is activated to a similar degree (Desborough 2000; Giannoudis, et al. 2006). However, this study was not designed to address these issues and more directed research should be undertaken before any conclusions can be reliably drawn. Future studies on hair fiber response to surgical stress should attempt to recruit participants who are otherwise healthy, such as orthopaedic surgery patients.

![Graph showing scatterplot of individual distances from individual average total diameter means (in µ) by surgical modality.](image)

**Figure 5-16.** Scatterplot of individual distances from individual average total diameter means (in µ) by surgical modality.

**Length of surgery**

Hierarchical model analysis showed no statistically significant difference in the post-operative change in hair diameter between participants whose surgeries lasted longer than two hours and those whose surgeries lasted two hours or less. Visual inspection of ATD distances from individual means of the two groups gives the impression that the difference should be significant (Figure 5-17). Either the sample size is not adequate to fully test the difference between the two subgroups or the scatterplot is simply misleading. The choice of two hours as a cutoff point between the two groups was an arbitrary one designed to produce two groups of roughly equal size for
comparison. The length of operations to which individuals respond differently may well be significantly shorter or longer than two hours but if so, this sample size is not adequate to evaluate those differences. On the other hand, the main influencing factor in response to surgery may be unrelated to or completely overshadow the influence of surgical length.

![Figure 5-17. Scatterplot of individual distances (in µ) from individual average total diameter means by length of surgery.](image)

**Length of hospital stay**

Linear Mixed Model analysis suggests that those individuals who were hospitalized for longer than three days following their surgery showed a greater decrease in average total diameter. Longer hospitalized individuals showed a decrease in hair diameter which exceeded shorter hospitalized individuals by 1.69µ (p<0.0005). Just as with length of surgery, the use of three days as a discriminating point was chosen to create two subgroups of roughly equal size. The length of hospital stay as a proxy for healing time may provide even more meaningful data if the subgroups are divided differently.
An important factor when considering length of hospitalization following surgery is how patients are assessed for discharge. Following colorectal surgeries, patients are often not released from the hospital until the intestines are functional again (i.e.- bowel movements are easily passed). A patient may be suffering no adverse reactions to the surgery and still not be released. For example, participant 255 underwent a sigmoid resection and was not discharged from the hospital for nine days. However, his ATD values show no visible reduction following his surgery. Participant 102 was ambulatory the morning after his surgery, but was not released for another three days. A greater understanding of the nuances of post-surgical care and assessment would better inform research designs when investigating surgical stress.

![Figure 5-18. Scatterplot of individual distances from individual average total diameter means (in µ) by length of hospitalization following surgery.](image)

5.4. Benefits/Limitations of study

5.4.1. Benefits

This study suggests a method for analysis of hair which would provide information on stress-related changes in the hair fiber dimensions. The examination of
hair dimensions allows for the observations of changes on a daily to weekly basis which is not only significantly more precise than cortisol levels but avoids some of the problems with hormonal analysis of fibers from archaeological contexts. Analysis of 3cm serial segments of hair fiber showed a sharp decrease in cortisol levels for the first 12 weeks, after which the cortisol stabilizes at a level significantly lower than that of the proximal segment (Kirschbaum, et al. 2008). The authors suggest this is due to the leaching or “washout effect” of multiple, repeated hair washes over the months preceding hair sample collection (pg. 36). Using the method proposed by the current study avoids this complication. When examining an intact hair sample of the same length, even if the cuticle is damaged, the cortex average diameter is still an effective method for evaluating stress-induced changes along the length of the hair shaft, although cuticle appears to take longer to recover than cortex. The ability of this technique to compare each individual to themselves before and after injury alleviates one of the barriers to our understanding of individual response to and recovery from an acute physiological stress event (Walton, et al. 2013).

From a purely logistic standpoint, there are benefits to using hair instead of other biological tissues (LeBeau, et al. 2011). Unlike blood, saliva, or urine hair requires no refrigerated storage or transport which means hair samples can be stored without electricity and transported without dry ice or complicated packaging and licensing issues. Hair as a biological medium has significantly less associated risk of pathogen transmission than blood or soft tissue samples. Although many researchers are using mass spectrometry to test for drugs, toxins, hormone substrates, or stable isotopes this research study is using quantitative/metric microscopic examination which requires significantly less expensive equipment and less stringent laboratory procedures. Both of these factors make hair ideal for field examination or examination in laboratories with less funding resources. Collection of hair samples is a minimally invasive procedure; creating less discomfort than blood/serum draws and intruding less into daily life than repeated urine or saliva collections (Wilson, Taylor, et al. 2007). A single hair sample collection event can provide information on months’ worth of growth, where a similar history would require repeated collections of serum, urine, or saliva. Chemical analysis of hair requires a section of hair to be cut from the scalp, resulting in a potentially noticeable missing section of hair which may be distressing to some participants.
Microscopic analysis, while requiring the hair to be pulled from the scalp at the root, only requires a few hairs from each individual.

5.4.2. Limitations

Study population

Each aspect of a research study must be designed to maximize the utility of the data gathered while minimizing the cost of collecting such data. Decisions regarding the study population are often difficult to balance. Population sampling assumes that the group chosen for a study is a representative sample of the larger population as a whole, and therefore results generated from the sample population are applicable to the whole population. However, designing a research protocol must consider the effects of social and biological heterogeneity. Age- and sex-related differences in brain function, stress hormone responses, and disease vulnerability are becoming increasingly apparent (Giannoudis, et al. 2006; Kudoh, Katagai and Takazawa 2001). Stress research must control for such differences either by restricting the study population or by allowing for these differences when analyzing results. The problem with the former approach is the inability to apply results from a narrow demographic to a population of broader age, ancestry and sex (Kudoh, Katagai and Takazawa 2001). The problem with the latter approach is the large amount of resources required for a study population large enough to include an adequate number of individuals representing each available subgroup. This study lacked the resources necessary to accommodate all aspects of human demographic variation and therefore some were excluded. Homogeneity of sample demographics is beneficial to the statistical analysis of an exploratory, proof-of-concept study such as this one. However, future studies should specifically address excluded demographic groups such as women and younger adults. More complex demographic categories with recognized influence on health and stress should be explored, especially the influence of socio-economic status and social marginalization. Dietary differences of omnivores, vegetarians, and vegans should be considered as well.
Logistics (i.e. lessons learned)

Individual variation in growth rates is a potentially confounding factor when examining changes to hair growth in response to stressors. Measuring individual growth rates would produce the most accurate results by accounting for the length of hair grown during a time period, not just the diameter of hair grown. Several suggestions have been made for how to effectively measure hair growth rates in individuals. While shaving a portion of the scalp a set time period prior to sampling will provide a definitive measure of growth during that period (Hayashi, et al. 1991), a less invasive method may be to bleach or dye a very small section of hair (Barth 1986; LeBeau, et al. 2011)

Analysis of hair samples presented its own unique learning experiences. Using a stereoscopic microscope was relatively easy when measuring hair fiber diameters in a plane perpendicular to the axis of the microscope line of sight. In order to measure diameter parallel to the microscope axis, the ocular fine tuning knob had to be calibrated against a slide mounted object of known thickness. There is a small degree of instability built into the fine focus knob movement necessitating that to get an accurate depth measurement; the knob can only be turned in one direction. Going against gravity is recommended (Harris 1985). In addition, since the human hair cuticle contains no (or very few) pigment granules, longitudinal diameter could only be measured for the cortex. The longitudinal maximum diameter then had to be reconstructed from average cortex diameters and average cuticle diameters. To increase the precision and accuracy of measurements, future research of this nature could take advantage of confocal microscopy for longitudinal and transverse diameter measurements.

5.5. Potential Applications

5.5.1. Anthropology/Bioarchaeology

Webb, et al. 2010; Webb, et al. 2011, 2013; Wilson, et al. 2013), Europe (Boudin 2014), Africa (Schwarcz and White 2004), and Asia (Chang, et al. 2008; Chang, et al. 2006). Keratin structure has been observed preserved down to the scale of microscopically visible hair cortex cells after two thousand years of storage in an arid environment with no exposure to light or humidity, although the lipid fraction of hair matrix was lost during that time (Bertrand, et al. 2003). Hair fibers pulled from the scalps of corpses (as soon as four days following death) suggest that keratin fibers present in the hair root are more resistant to enzymatic digestion than surrounding soft tissues and therefore are also more resistant to decompositional changes (similar to fingernails). Both archaeological and experimental data suggest the postmortem depositional environment is a more influential factor in the state of hair fibers than elapsed time since death (Chang, et al. 2005; Chang, et al. 2006; Wilson, Dodson, et al. 2007; Wilson, et al. 1999). This is consistent with the postmortem alteration to soft tissues during decomposition, and therefore should be of no surprise to bioarchaeology and forensic researchers. The cuticle plays a major role in protecting the cortical hair fiber structures from postmortem taphonomic changes (Chang, et al. 2006; Gilbert, et al. 2007). The ability of the cuticle to protect the hair cortex from damaging elements is also subject to the nature of the depositional environment; electron microscope examination of the Daejeon mummies showed neither holes in the medulla of hair fibers nor detachment or loss of cuticle scales or cortical layers (Chang, et al. 2006). After 500 years of burial, the hair fibers were better preserved than hairs intentionally buried by the same researchers for only 25 years. The combination of hair fiber’s inherent taphonomic robusticity along with its chronological recording of an individual’s biochemistry presents a unique resource in archaeological research which is limited only by the new applications developed for it. Once verified, the method presented here will enable researchers to determine the presence and timing of acute stress events to the day. In the context of sensitive archaeological remains, this can be achieved with a minimum of destructive analysis. The limitations lie only in the length of hair present for examination and the burial environment, both of which are also true for existing methods of hair analysis. Although future studies should specifically address taphonomic influences on hair fibers and whether burial contexts or post-mortem interval elicit changes to hair diameter. The ability to detect acute stress events (as opposed to chronic stress conditions) can
provide information on individual health and community behaviors related to both health and violence.

5.5.2. **Forensic contexts**

The ability to detect the occurrence and timing of injuries or acute stress events has potential applications for forensic investigations. Toxicological hair analysis is already used for forensic investigation of drug-facilitated crimes when elapsed time prevents detection in serum or urine (Frison, *et al.* 2003; Kintz, *et al.* 2005; Kintz 2007; Negrusz, *et al.* 2002; Scott 2009; Villain, *et al.* 2004). Stable isotope analysis has been successfully used to reconstruct life histories in contexts of criminal neglect and abuse by malnutrition (Neuberger, *et al.* 2013). Child and domestic abuse may be hard to verify once soft tissue injuries heal. If hair fibers register and record traumatic injuries, abuse events can be verified weeks, or even months, after the event occurred. The detection of a period of acute physiological stress resulting from abuse can similarly be sought using hair fiber measurements after soft tissue injuries have healed. Detection of stress events can be used to validate allegations of abuse. Evidence of stress events in post-mortem hair samples can be cross-referenced against information on surgeries or injuries provided by families of missing persons. This could potentially be used to exclude matching individuals during the identification process without the use of expensive and time consuming DNA comparisons.

5.5.3. **Clinical contexts**

There may be some clinical applications to the results of this study and the new hair fiber examination method in development. Further research is necessary to better understand why cortex and cuticle thicknesses do not return to pre-stress normal values at the same rate. If this difference is the result of differing protein and lipid requirements of cuticle and cortex, this may reflect the substrate requirements for post-injury healing. These results can potentially inform future clinical studies in improving recovery from surgery or acute injuries.
5.6. Future Research Directions

Future research will address the gaps in this exploratory study population while also investigating the new research questions spurred by the results. Age and sex differences in individual reaction to and recovery from acute stress events and injuries must be incorporated into future research designs and evaluated. Now that early data suggest that the hair follicle does react to injury with a temporary reduction in hair cell production, the limits of this reaction need to be explored in more detail. Specifically, the amount of time between event and hair sample collection should be increased to more adequately investigate recovery time after a stress event. The participants of this study were not critically ill in the sense of suffering an immediate threat to life. However, they all were living with illnesses which necessitated surgery, mostly colorectal malignancies and ulcerative colitis. This will obviously have an influence on functioning of the gastrointestinal tract and therefore on the absorption of nutrition.

Future studies should focus on individuals who are relatively healthy but experience a short term, severe stress such as an accidental injury, non-emergency orthopedic surgery, or elective surgery. This will provide more focused data on the reaction to and time required to recover from injuries in individuals who are not suffering any other threats to bodily allostasis. The inclusion of a sample population of individuals who are recently diagnosed with health problems due to symptomatic presentation may also provide very useful information on the body’s reaction to more chronic conditions. Similarly, individuals who are mostly healthy but suffer repeated bouts of recurring illnesses would also provide unique data.

Considering the lack of concrete evidence on the effect of psychological or emotional stress on hair growth, this is a potential area for future research. A sample population of individuals of mixed age and sex who have recently experienced a psychologically stressful event but who are otherwise healthy would be ideal. For example, undergraduate students often feel “stressed” around end-of-term exams, but is this stressful enough to induce changes in hair growth? This would likely be partially determined by individual personalities and their psychological “robustness” (Childs, et al. 2014; Faraday 2005; Kobasa 1979; Miller, et al. 2007; Miller and Kraus 1996). The
question of “How stressed is stressed enough?” is both academically interesting and clinically applicable in health and stress research, but it is also a complicated and difficult question to answer. The influence of physical illness on the psyche and vice-versa makes testing one while excluding the other almost impossible. The method proposed in this study could be useful in addressing those questions, if a large enough sample size of individuals experiencing various stressors could be gathered.

More research is needed to replicate and verify the results of this study. Specifically the observation that prior to investigative intervention (surgery), the hair fiber diameter of most participants fluctuated roughly within five microns on either side of personal mean, regardless of the coarseness of the hair fiber. A significantly larger sample size of multiple hairs from each individual will be necessary to gain a full understanding of intra-individual variation in hair diameters.

The utility of various methods for data collection needs to be continually re-assessed. Results of this study suggest there is little difference in the data provided by various measuring modalities, but considering the differences in a few of the method comparisons (1000x vs 400x manual diameter and manual vs digital cuticle measurements), the next step will be to formally assess the precision and accuracy of these methods. Repeatability, replicability, and inter-observer reliability of manual and digital data collection all need to be formally tested as well. Different methods for measuring the longitudinal diameter of a hair fiber should be critically compared.

6.1.1. Research Question 1

This study explored the methods previously used to assess hair fiber change in response to stress and found them inadequate. Most relied on diameters measured only perpendicular to the microscope axis and therefore reaching conclusions on only half of the data available regarding hair fiber cross-sectional volume. The method proposed in this study includes diameter measured both perpendicular and parallel to the microscope line of sight. This allows for an approximation of the volume of hair fiber produced during each day’s growth. In ideal situations individual growth rates should be determined to produce the most accurate measurements of fiber volume produced each day. Differences between magnifications and methods for collecting measurement data.
from the hair shaft were examined. Results suggest there is no statistically significant difference between digital measurements obtained through the generally accepted level of magnification (400x) and those from a greater magnification (1000x). Average total diameter data collected manually at 1000x magnification did vary from data collected at 400x magnification. There appears to be no statistically significant difference between average total diameter data collected via manual and digital measurement. While measuring hair shaft diameters digitally and at 1000x magnification may produce more precise data, the results of this study suggest they do not necessarily produce more accurate data. Future research should focus specifically on testing both the precision and accuracy of these methods. In contexts where only manual measurement at 400x magnification is possible, the data produced are capable of reflecting changes. Cuticle measurements, however, did show a significant difference between manual and digital measurements. Therefore the use of greater magnification and digital measurement, however, is suggested in contexts where researchers need to examine the demarcation line between cortex and cuticle or visualize and measure other hair fiber sub-structures.

6.1.2. Research Question 2

The normal daily variation in hair fiber cross-sectional dimensions was also explored. In this sample, the average total diameter fluctuates within 5µ on either side of the individual mean, regardless of coarseness of the fiber. In this particular sample, five microns represents 5.2-8.5% of individual pre-surgical means. For this method to be used to assess stress-related changes in hair diameter, intra-individual variation must be accounted for. Individual pre-stress values should be plotted against individual means to determine approximate individual boundaries for normal daily variation. Using the distance from mean is an easy and effective way to establish limits expected to be surpassed by stress-induced changes.

6.1.3. Research Question 3

The influences of surgical stress on the characteristics of the hair fiber were examined using Linear Mixed Modeling. Analysis showed that the volume of hair fiber produced on a daily basis is reduced in response to surgical stress. Hair fiber total and
cortical diameters begin to return to normal in the third week following surgery and are consistent with pre-operative values by the fourth post-operative week. The cuticle, however, continued to decrease in thickness with subsequent post-operative weeks. The difference between pre-operative and post-operative values were different at the level of p=0.05, and that difference increased every week after surgery for the four weeks measured.

6.2. Final Remarks

Human scalp hair has been increasingly utilized in the past few decades to explore stress, diet, mobility, and the use of intoxicants in archaeological populations. Hair is taphonomically far more robust than soft tissue, while also providing a more linear chronology than hard tissues. This research study has shown that microscopic examination and measurement of hair fibers can be used to investigate changes along the length of the fiber. A new method has been suggested which produces consistent results at moderate and high magnifications, and whether the measurements are performed manually with a calibrated ocular micrometer or digitally with measurement software. Studies requiring examination of fiber structures, however, are recommended to utilize higher magnification and digital measurement. This new method has been used to assess the normal variation in volume of hair fiber produced per day. Additional steps should be taken towards incorporating individual growth rates into this analysis, and that includes assessing changes in individual growth rates resulting from stress events. Finally, this method was used to investigate changes in hair fibers resulting from an acute stress event; in this case, surgery. The scalp hair of participants showed a statistically significant narrowing in diameter immediately after surgery, and continuing for approximately two weeks post-operatively. This exploratory proof of concept study was not able to illuminate whether these changes result from physiological processes of the acute phase response or from temporary functional malnutrition. The results of this study have generated important research questions and objectives to be addressed in future studies.
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