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This study examined the relationships between oral contraceptive pill (OCP) use and measures of joint laxity. **Methods:** A menstrual history questionnaire identified 33 female athletes using OCPs. Separate stepwise linear regressions examined the extent to which OCP dosage (estradiol, progesterone) and SHBG% predicted measures of anterior knee laxity, general joint laxity and genu recurvatum. Secondary regression analyses examined the relationship between duration of OCP use and each joint laxity measure. **Results:** OCP dosage and %SHBG binding affinity were not predictors of joint laxity. Greater duration of OCP use positively predicted greater genu recurvatum (R^2 = 20.8%, *P*=0.008). **Conclusions:** While the variations in dosages of common OCPs were not found to influence measures of joint laxity, prolonged exposure to these synthetic hormones may increase genu recurvatum over time. Further work is needed to clarify the effects of OCPs on joint laxity, a potential risk factor for knee injury.

HORMONE STATUS AND MEASURES OF JOINT LAXITY

by

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Joint laxity has received considerable attention as an ACL injury risk factor based on its prevalence among females compared males (Larsson, Baum, & Mudholkar, 1987; Medrano & Smith, 2003; Scerpella, Stayer, & Makhuli, 2005; Seckin et al., 2005; Uhorchak et al., 2003), and its potential to modify ACL injury risk (Medrano & Smith, 2003; Ramesh, Von Arx, Azzopardi, & Schranz, 2005; Scerpella et al., 2005; Uhorchak et al., 2003). Joint laxity is defined as hypermobility of a joint which exceeds the normal range of motion with the main contributors being the static stabilizers of the joint (i.e., the joint capsule, ligaments, and tendons) (Grahame, 1999). Common measurements of joint laxity include anterior knee laxity (Scerpella et al., 2005; Uhorchak et al., 2003), general joint laxity (GJL) (Grahame, 1999; Jansson, Saartok, Werner, & Renstrom, 2004; Scerpella et al., 2005), and genu recurvatum (Loudon, Jenkins, & Loudon, 1996; Loudon, Goist, & Loudon, 1998). While anterior knee laxity (Beynnon et al., 2005; Pollard, Braun, & Hamill, 2006; Rozzi, Lephart, Gear, & Fu, 1999; Uhorchak et al., 2003) and general joint laxity (Scerpella et al., 2005; Uhorchak et al., 2003) have consistently been found to be greater in females compared to males, sex differences in genu recurvatum are conflicting (Loudon et al., 1996; Scerpella et al., 2005). Yet, as research supports that excessive values associated with each of these measures may be predictive

of ACL injury risk (Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003; Woodland-Rogers, Cyphert, & Denegar, 1994), it is prudent to understand why females may be at risk for increased joint laxity, and the physiological mechanisms by which knee laxity may be modulated.

The greater joint laxity observed in females compared to males appears to be at least in part due to sex differences in hormone concentrations (Shultz, Sander, Kirk, & Perrin, 2005). At the cellular level, the influences of sex hormones on soft tissue structures have come under examination (Liu, Al-Shaikh, Panossian, Finerman, & Lane, 1997; Yu, Panossian, Hatch, Liu, & Finerman, 2001). Estrogen and progesterone receptors have been localized on the human ACL, which has lead to speculation that these hormones may be influential in the structure and composition of ligaments (Lui et al., 1996; Lui et al., 1997). This in turn has led to studies investigating the relationship between changing hormone levels across the female menstrual cycle and their associated effects on anterior knee laxity (Belanger et al., 2004; Beynnon et al., 2005; Deie, Sakamaki, Sumen, Urabe, & Ikuta, 2002; Heitz, Eisenman, Beck, & Walker, 1999; Karageanes, Blackburn, & Vangelos, 2000; Romani, Patrie, Curl, & Flaws, 2003; Shultz, Kirk, Johnson, Sander, & Perrin, 2004; Shultz et al., 2005; Van Lunen, Roberts, Branch, & Dowling, 2003). While results are conflicting, studies that examined the daily changes in sex hormone concentrations with daily changes in knee laxity report knee laxity is greater in the early luteal phase while estrogen is at its highest peak and progesterone is on the rise (Shultz et al., 2004; Shultz et al., 2005). Further, this work indicates that the association between changes in knee laxity and hormones is stronger when all three

hormones (i.e. estrogen, progesterone, and testosterone) and their interactions are considered (Shultz et al., 2004). However, these relationships have primarily been studied in normal menstruating females to date, and have been limited to the measurement of anterior knee laxity.

Appreciating the relationships noted between sex hormones and anterior knee laxity, little attention has been directed towards our understanding of how oral contraceptive pills (OCPs) may modify these relationships. This is important, as OCPs greatly reduce endogenous hormone levels, and as many as 42% of intercollegiate athletic females use OCPs for one reason or another (Agel, Boris, Bershadsky, & Arendt, 2006). Estradiol, the most potent form of estrogen and progesterone compounds are the two major exogenous hormones combined in mainstream OCPs. Each OCP product contains different amounts of ethinyl estradiol and some form of progesterone. In today's market, there are several types of progesterone compounds including but not limited to levonorgestrel, norgestimate, drospirenone, and norethindrone (Frye, 2006). These synthetic hormones essentially have the same effect as their endogenous counterparts inhibiting follicle-stimulating hormone (FSH) and luetinizing hormone (LH) release of the pituitary and preventing ovulation. Therefore, if endogenous forms of estrogen and progesterone alter ligament composition and structure, it is likely that exogenous hormones from OCPs also may have some effect on ligamentous integrity and joint laxity.

Only two studies were found to date that have examined the relationship between OCPs and ligamentous laxity (Martineau, Al-Jassir, Lenczner, & Burman, 2004;

Pokorny, Smith, Calus, & Dennison, 2000). Pokorny et al. (2000) used self-reported measures of oral contraceptive use and investigated the changes in peripheral joint laxity. Their results indicated no association between OCP use with anterior knee laxity and finger joint laxity (i.e. fifth finger extensibility). Conversely, Martineau et al. (2004) identified significant decreases in anterior knee laxity in OCP users versus non-users. These conflicting results may be due to a number of factors, including the type of OCP's that subjects included in the study were using and different sample populations. While these limited studies reported the types of OCP used, these data were not included in their analyses when determining the relationship between OCP use and laxity.

Differing characteristics of common synthetic contraceptive formulations, in addition to their dosages, are their interaction with sex hormone-binding globulin (SHBG). SHBG is largely responsible for transporting biologically active androgens and estradiol in the blood, and regulates the free fraction concentration of testosterone that is available to the target tissues (Hammond, 2002). Specifically, circulating SHBG increases with increasing dosages of synthetic estradiol (Wiegratz et al., 2003), which can be counteracted by the progesterone compound used (Wiegratz et al., 2003, Hammond et al., 2003). While some synthetic progestins resemble testosterone-like compounds (e.g. norethindrone and levonorgestrel) which have some binding affinity to SHBG (Grow, 2002), others (e.g. norgestimate, drospirenone) do not bind with SHBG (Wiegratz et al., 2003; Hammond et al., 2003). Hence, contraceptive compounds that deliver higher estradiol levels and use a progesterone compound that does not bind with SHBG will result in greater increases in circulating SHBG, and ultimately greater reductions in the

free fraction of testosterone circulating in the blood (Wiegratz et al., 2003; Hammond et al., 2003). As previous research has found that the combined concentrations of estradiol, progesterone, and testosterone and their interactions are stronger predictors of changes in knee laxity (Shultz et al., 2004; Shultz, Gansneder, Sander, Kirk, & Perrin, 2006), it is plausible that variations in the dosages of synthetic estradiol and progesterone and the type of progesterone used in these OCPs may have differential effects on knee laxity.

Thus, the purpose of this study is to build on these studies, and determine the extent to which the type and dosage of exogenous estradiol and progesterone predict joint laxity. A secondary purpose was to determine if length of exposure to OCPs had any effect on joint laxity. The research hypothesis is greater dosages of exogenous estradiol, lower dosages of progesterone, and progesterone compounds that have a lower affinity for SHBG would predict greater anterior knee laxity, general joint laxity, genu recurvatum and in females using OCPs. Further, we expected that prolonged exposure to OCPs (thus suppressed endogenous hormone concentrations) would result in decreased joint laxity.

Statement of the Problem

While the prevalence of oral contraceptives is increasing every year, their effects on the soft tissue structures of the body remain relatively unknown. Of the limited research that has investigated the effects of exogenous hormones on joint laxity, results are conflicting (Pokorny et al., 2000; Martineau et al., 2004). Given evidence that increased joint laxity may increase the risk of ACL injury in females (Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003), and that cycling sex hormones across

the female menstrual cycle influence ligamentous laxity (Deie et al., 2002; Heitz et al., 1999; Scerpella et al., 2005; Shultz et al., 2004; Shultz et al., 2005; Uhorchak et al., 2003), understanding the modifying effects of OCPs on joint laxity may guide future research and preventative efforts. Hence, the proposed study aims to determine the influence of OCPs on measures of joint laxity.

Objectives

1. To determine the relationship between OCPs type and dose of exogenous estradiol and progesterone with general joint laxity, genu recurvatum and anterior knee laxity in a female athlete population.

   Hypothesis 1: Females taking OCPs with greater doses of exogenous estradiol, lower doses of progesterone, and progesterone compounds that have a lower affinity for SHBG will have greater general joint laxity, genu recurvatum and anterior knee laxity.

2. To determine whether length of time on OCPs will influence a female's general joint laxity, genu recurvatum, and anterior knee laxity in a female athlete population.

   Hypothesis 2: Females who are on OCPs for a greater length of time will have decrease general joint laxity, genu recurvatum, and anterior knee laxity.

Independent Variables

1. Dosage of ethinyl estradiol (μg/dose)

2. Progesterone dose (mg/dose)
3. Progesterone type: progesterone compounds will be categorized as having 1) 0 % binding to SHBG, 2) 36 % binding to SHBG, 3) 46 % of binding to SHBG.
4. Length of time on OCP, in months.

Dependent Variables

1. Anterior knee laxity: Measurement in millimeters (mm) of the amount of anterior tibial translation with respect to the femur using the KT-2000^{TM} knee arthrometer at a 133N applied anterior directed force.
2. General joint laxity (GJL): A composite score (0-9) of the presence of joint hypermobility at the fifth finger, thumb, elbow, knee and low back using Beighton and Horan's Joint Mobility Index (BHJMI).
3. Genu Recurvatum: The angle (degrees) of sagittal plane movement into hyperextension of the knee measured in supine during an active contraction of the quadriceps.

Limitations

1. Different examiners conducted each of the tests examined in this study. However, measures for each individual test were assessed by the same examiner for GJL, genu recurvatum, and anterior knee laxity.
2. Activity level was not controlled in all subjects prior to laxity testing due to the nature of screening.

3. Data collected as part of a mass, multi-station pre-season risk factor screening project creating an inability for current assay techniques to measure both endogenous and exogenous levels.
4. Serum was not collected in order to confirm actual hormone levels.
5. Results may be generalized to only healthy, college-aged female athletes and the OCPs used by these athletes.

Assumptions and Delimitations

1. All subjects were healthy, college-aged female athletes with at least a 3-month history of oral contraceptive pill use and no connective tissue disorder.
2. Subjects answered honestly and accurately on the self reported female hormone history questionnaire.
3. All measurements for joint laxity (GJL, genu recurvatum, and anterior knee laxity) were completed on the same day as the menstrual history questionnaire, which allowed for a gross estimate of time in the cycle for each subject.
4. Hormone concentrations are estimated based on manufacturer's prescribing information for each type of OCP.

Operational Definitions

Anterior knee laxity: The amount of anterior translation of the tibia with relation to the femur. Measured using KT-2000™ device to quantify in millimeters(mm), the translation based on a specific applied force in Newtons (N).

General joint laxity (GJL): The amount of joint mobility or laxity in various joints in the body. Non-pathological looseness of the soft tissue structures of the joint (skin, ligaments, fascia, and connective tissue).

Genu recurvatum: The sagittal angle (degrees) formed between the femur and the tibia with active contraction of the quadriceps muscles and extension of the knee.

Beighton and Horan Joint Mobility Index (BHJMI): Assessment which tests 5 specific joint of the body to denote any hypermobility. A composite score is obtained ranging from 0-9 based on the subject's ability to perform the action.

Sex Hormone Binding Globulin (SHBG): A carrier protein of sex hormones(mainly estradiol and testosterone), produced by the liver, which inhibits the function of the sex hormones as a result, the bioavailability of the sex hormones are influenced by the levels of SHBG.

Endogenous hormones: Hormones which are originated or produced naturally in the body. Examples include but not limited to estrogen, luetinizing hormone, and testosterone.

Exogenous hormones (Synthetic hormones): Hormones which are created via chemical processes outside of the body. OCPs contain synthetic hormones ethinyl estradiol and a type of progesterone (norgestimate, levonorgestrel, drospirenone).

CHAPTER II

LITERATURE REVIEW

Anterior cruciate ligament (ACL) injury is 2 to 8 times more likely in females than males who participate in similar sports with the same extrinsic factors such as playing conditions and footwear (Arendt, Agel, & Dick, 1999; Arendt, Bershadsky, & Agel, 2002; Bjordal, Arnly, Hannestad, & Strand, 1997; Ireland, 2002). Mechanisms of injuries to the ACL are classified into two categories: contact and noncontact. Approximately 70% of ACL injuries are non-contact in nature (Boden, Dean, Feagin, & Garrett, 2000). Potential risk factors for non-contact injuries may be attributed to four areas: environmental (extrinsic factors), anatomic (Q-angle, notch size, joint laxity), biomechanical (neuromuscular control, skill level), and hormonal (levels of estrogen or estradiol, testosterone, progesterone, luteinizing hormone [LH], and follicle stimulating hormone [FSH]) (Ireland, 2002). While the exact mechanism(s) for ACL injuries remains unknown to investigators (Griffin et al., 2000; Griffin et al., 2006), it is probable that a combination of factors may contribute to the higher prevalence of ACL injuries in females in comparison to males (Boden et al., 2000; Griffin et al., 2006; Ireland, 2002). By understanding possible gender differences in these risk factors, steps may be taken clinically to help control the increased incidence of female ACL injuries.

To that end, excessive joint laxity is thought to predispose an individual to injury (Harner, Paulos, Greenwald, & Rosenburg, 1994; Klemp & Chalton, 1989), and is defined as the amount of non-pathological looseness in the joint, with the main contributors being the static stabilizers (i.e., the joint capsule, ligaments, and tendons). Anterior knee laxity, GJL, and genu recurvatum are common measures of joint laxity, and have been found to be greater in females compared to males. When excessive, these measures of joint laxity have been associated with an increased risk of ACL injury, thus potentially contributing to the increased prevalence of ACL injury in females compared with males. This literature review will examine what is known about joint laxity and ACL injury, the gender differences that exist in joint laxity, and the role that endogenous and exogenous sex hormones may play in the relationship between sex, joint laxity, and ACL injury risk, in an effort to support the rationale and methods for the current study.

Joint Laxity and ACL Injury

In the orthopedic literature, joint laxity is most commonly characterized by measures of anterior knee laxity, GJL, and genu recurvatum. Each of these measures has been investigated for their influence on lower extremity injury and specifically, ACL injuries.

General Joint Laxity

The concept of GJL refers to the presence of joint hypermobility over multiple joints in the body. GJL is commonly used to identify hypermobile joints in individuals where the range of motion extends beyond the estimated standard, assuming the absence of specific diseases (Carter & Wilkinson, 1964; Grahame, 1999). The most well accepted measurement of GJL is the Beighton and Horan's Joint Mobility Index (BHJMI) (Decoster, Bernier, Lindsay, & Vailas, 1999; Grahame, 1999; Jansson et al., 2004; Klemp, Stevens, & Isaacs, 1984; Klemp, 1997; Scerpella et al., 2005; van der Giessen et al., 2001). This method was created by Carter and Wilkinson (1964) and was later adapted by Beighton, Horan and colleagues (Beighton & Horan, 1969; Beighton, Soloman, & Soskolne, 1973). The index score indicates the presence of hypermobility at four different joints in the upper and lower extremities bilaterally, and during forward trunk flexion. For each joint measured, a score of 1 is assigned if the criterion for a hypermobile joint is met, and a score of 0 is assigned if the subject can not meet the criterion. Each score is accumulative which creates an overall composite score between 0 and 9; with a score of 9 out of 9 indicating that each joint tested positively for hypermobility. The methods for determining GJL using these methods are time efficient, inexpensive, and simple for examiners to measure. In their original work, Carter and Wilkinson (1964) and Beighton et al. (1973) identified GJL when at least three of the five joints measured were positive for the criteria. While the literature indicates that a clear cut-off for determining hypermobility has yet to be established, scores greater to or equal

to 4 or 5 out of 9 seem to be the most consistent (Jansson et al., 2004; Klemp et al., 1984; Laurie, Nguyen, & Shultz, In Review; Seckin et al., 2005; van der Giessen et al., 2001).

GJL and Injury

Researchers have been investigating GJL for decades for its potential to increase the risk of lower extremity injury. GJL has been associated with both acute (Decoster et al., 1999; Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003) and chronic (Beighton & Horan, 1969) injury. Specifically, athletes with greater joint laxity have been found to suffer more joint sprains to the ankle and knee (Decoster et al., 1999), and particularly ACL injuries (Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003). Klemp & Chalton (1989) found a significantly higher rate of injury among dancers who scored 4/9 or higher, which also supports the conclusion that increased mobility or laxity in joints may be detrimental to athletes. But while these studies implicate GJL as a risk factor for lower extremity injury, the factors predisposing one to increased GJL is not well understood. Although retrospective studies show the relationship between GJL and ACL injury (Ramesh et al., 2005; Scerpella et al., 2005), the only prospective study (Uhorchak et al., 2003) shows the need for further research to establish whether GJL is a cause or effect of ACL injury.

Anterior Knee Laxity

Anterior knee laxity is described as the amount of anterior displacement of the tibia relative to the femur. This anterior tibial translation is measured in a variety of ways depending on the setting and equipment available. In a clinical setting, anterior tibial

displacement is most commonly assessed using the Lachman's test and anterior drawer test. For more quantitative assessment, clinicians and researchers use the KT-1000™ or KT-2000™ knee arthrometers (MEDmetric® Corp; San Diego, CA), which place a specified amount of anterior directed force to the tibia (e.g. 89N, 133N) and calculates the anterior displacement of the tibia on the femur in millimeters.

Anterior Knee Laxity and ACL Injury

Greater anterior knee laxity has been associated with an increase in ACL injury rates (Scerpella et al., 2005; Uhorchak et al., 2003; Woodford-Rogers et al., 1994). Scerpella et al. (2005) and Woodford-Rogers et al. (1994) retrospectively examined the association between non-contact ACL injury and knee laxity, and determined that ACL injured athletes had greater anterior knee laxity than the uninjured group. Scerpella et al. (2005) also measured the uninjured side of ACL injured group to rule out possible bias from the actual injury or reconstruction. In agreement with these findings, Uhorchak et al. (2005) prospectively examined numerous risk factors for their ability to predict ACL injury, and found that higher than normal KT-2000™ athrometer values at 111N, 134N, 156N, and 178N were predictors of ACL injury risk when considered in combination with a BMI value higher than 1 SD or larger femoral notch width.

Genu Recurvatum

Genu recurvatum is a measurement of the degree of knee hyperextension. Genu recurvatum can be measured with the subject supine with a bolster placed under the heels to allow the knee to relax into full extension, or while standing in an erect posture while

the subject actively extends the knees into maximal extension (Loudon et al., 1996; Nguyen & Shultz, *In Press*; Ramesh et al., 2005; Scerpella et al., 2005). A goniometer is used to measure the angle based on the intersecting lines from the greater trochanter of the femur to the center of the lateral femoral condyle and from the center of the lateral femoral condyle to the lateral malleolus. To obtain an active knee extension measurement, the subject is instructed to contract their quads and straighten or push their knee down towards the table.

Genu Recurvatum and ACL Injury

Retrospective studies have reported an association between genu recurvatum and ACL injuries (Loudon et al., 1996; Ramesh et al., 2005; Scerpella et al., 2005). Loudon et al. (1996) classified subjects into 3 groups (normal vs. high vs. low sagittal knee position) and ACL-injured vs. non-injured were compared. Their results suggested a strong association between non-contact ACL injuries and a display of high standing genu recurvatum values. Using the supine method of assessment, Ramesh and colleagues (2005) reported a significant difference in the prevalence of knee hyperextension in subjects with (78%) and without (37%) an ACL injury. Similar findings were reported in athletes by Scerpella et al., (2005), noting athletes with ACL injuries had significantly greater knee hyperextension than their uninjured counterparts. Conversely, Trimble, Bishop, Buckley, Fields, & Rozea (2002), determined knee hyperextension was not correlated with tibial translation (i.e. increased knee laxity) and therefore concluded it was not a significant contributor to ACL injury. However, this study did not specifically

examine injury risk, and used statistical analyses to relate postural factors to tibial translation, given the association previously reported between knee laxity and ACL injuries. Based on the available evidence that have directly examined injury risk, there appears to be an association between excessive genu recurvatum and ACL injury.

Gender Differences in Joint Laxity

In an effort to explain the higher rate of ACL injuries in females compared to males, investigators have examined gender differences in joint laxity as an area of pertinent research. As such, sex differences in GJL, anterior knee laxity, and genu recurvatum have all been examined, and recognized to be more prevalent among females than males (Larsson et al., 1987; Nguyen & Shultz, In Press; Medrano & Smith, 2003; Rozzi et al., 1999; Scerpella et al., 2005; Seckin et al., 2005; Trimble et al., 2002; Uhorchak et al., 2003).

Investigators have shown that females have greater GJL compared to males across a variety of ages (Grahame, 1999; Grana & Moretz, 1978; Jansson et al., 2004; Scerpella et al., 2005), and in both sedentary (Grana & Moretz, 1978; Grahame, 1999; Jansson et al., 2004) and athletic populations (Scerpella et al., 2005; Uhorchak et al., 2003). When examining sex differences in the prevalence of hypermobility based on those scoring a (1) on at least 3 or more features, a greater ratio of females compared to males (5:1) were found to be hypermobile (Klemp et al., 1984; Larsson et al., 1987; Seckin et al., 2005). While Larsson et al. (1987) reported that fewer hypermobility characteristics were present with increasing age, the difference in gender remained.

Sex differences in anterior knee laxity have also been investigated and consistently have been determined to be greater in females compared to males (Beynnon et al., 2005; Rosene & Fogarty, 1999; Rozzi et al., 1999; Shultz et al., 2005; Uhorchak et al., 2005). Research on sex differences in genu recurvatum is more limited, and is somewhat conflicting (Nguyen & Shultz, In Press; Scerpella et al, 2005; Trimble et al, 1999). While two studies noted females had approximately 2- 4° greater hyperextension compared to males (Trimble et al, 1999; Nguyen and Shultz, In Press), one study found no sex differences in healthy athletes (Scerpella et al, 2005).

Endogenous Sex Hormones and Anterior Knee Laxity

One explanation for sex differences in knee laxity has been attributed to sex differences in hormone concentrations, and how these concentrations rise and fall during the female menstrual cycle (Heitz et al, 1999; Deie et al., 2002; Shultz et al., 2004, Shultz et al., 2005). Sex hormones concentrations changes across the female menstrual cycle are dependent on a complex association between the hypothalamus, pituitary gland and the ovaries, commonly referred to as the hypothalamus-pituitary axis (HPA) (Larsen, Kronenberg, & Melmed, 2003). The physiologic purpose of the HPA is the 1) formation and growth of the oocyte, and if necessary, 2) creation of an appropriate environment to support pregnancy, or 3) returning the body to physiologic homeostasis to begin the next cycle if no pregnancy is detected (Larsen et al., 2003).

The hypothalamus is responsible for releasing gonadotropin-releasing hormone (GnRH) which stimulates the anterior pituitary gland to release follicle-stimulating

hormone (FSH) and luteinizing hormone (LH) (Larsen et al., 2003). The concentration of ovarian hormones, estrogen and progesterone, are stimulated by the release of FSH and LH of the anterior pituitary (Guyton & Hall, 2000). Consequently, the secretion of GnRH, FSH, and LH are up or down regulated by the amounts of ovarian hormones circulating at any given time in the body (Guyton & Hall, 2000). As a result, the hormones which control the female menstrual cycle are dependent on one another such that the smallest of disruptions may alter the consistency of the cycle.

A 28-day cycle length for a complete menstrual cycle is considered to be the norm and consists of two phases: follicular and luteal, with ovulation acting as an event which divides the two (Larsen et al., 2003; Guyton & Hall, 2000). Each phase is distinguished by characteristic levels of various hormones (i.e. progesterone, estrogen, testosterone, LH, and FSH). The follicular phase (approximately days 1-9) is described when concentrations of estrogen and progesterone levels are relatively low. The initiation of ovulation occurs with a surge in estradiol and luteinizing hormone (LH), which typically is observed 14 days from the onset of menses (Karageanes et al., 2000). During ovulation (≈days 10-14), a spike in estradiol is observed while progesterone levels remain low. Following ovulation, progesterone then steadily rises and reaches its highest concentration in the luteal phase (≈days 15-end of cycle), while estrogen undergoes a second peak and then declines. The rise of progesterone antagonizes LH and therefore decreases this concentration until the next cycle. While these trends are generally similar between most individuals, the hormone concentrations may vary considerably within each phase, making it difficult to standardize subjects' stage in the cycle. Using the value

of 28 days as a mean cycle length represents a somewhat arbitrary value because the actual time (in days) may normally vary between and within subjects over a range of 26-32 days (Larsen et al., 2003). Because of this variability in the length and phasing of a female menstrual cycle, the mean length and days of cycle are often used in studies to represent all females, as they represent the days on average when key hormone changes occur. While understanding the hormones, phases, and details of the menstrual cycle gives insight to how the cycle may influence ligament structure and metabolism, and ultimately knee laxity, there is a need to appreciate the individual variability in these characteristics when studying these comparisons.

Effect of Sex Hormones on ACL Structure and Metabolism

The sex hormones, estrogen and progesterone, are representative of estrogens and progestins, respectively. Estradiol, estrone, and estriol are the three types of estrogens present in the female body, with the most potent and predominate estrogen secreted being estradiol (Guyton & Hall, 2000). Progesterone is the most significant type of progestin, with considerable amounts secreted in the middle stages of the luteal phase (Guyton & Hall, 2000).

Estrogen and progesterone have been suggested to have an influence on a variety of tissue in the body, including the growth and development of bone, muscle, and connective tissue (Lebrun, 1994, Liu et al., 1996, Yu et al., 2001). Based on findings by Liu et al. (1996), research also supports that progesterone and estrogen influence the physical properties of the ACL. They showed estradiol (the most potent form of

estrogen) and progesterone receptors were located on human ACL fibroblasts, which are the cells responsible for the synthesis and maintenance of all connective tissue fibers (Davis, 2001). Collagen is the primary connective tissue in the ACL, and provides the tensile strength for the ligament (Martini, Timmons, & Tallitsch, 2003). As a result, hormones are thought to play a role in the structure, composition, and physical properties of the ACL (Liu et al., 1996; Liu et al., 1997). Studies exposing ACL tissue to increased concentrations of estradiol resulted in decreased fibroblast proliferation and collagen formation (Lui et al., 1996, Slauterbeck, Clevenger, Lundberg, & Burchfield, 1999). Conversely, increased levels of progesterone have been associated with increased fibroblast proliferation and collagen formation (Lui et al., 1996). In addition, Wreje et al. (2000) implicated that OCP use decreases type I and III collagen synthesis. Slauterbeck et al. (1999) tested the tensile strength of ACLs' in rabbits with removed ovaries (ovariectomies) and compared those who were supplemented with excessive concentrations of estradiol to those who were not provided hormone supplementation. Their results revealed that rabbit ACLs' that received estrogen replacement had lower loads to failure when compared to the control group, further supporting estrogens potential impact on the properties of the ACL.

Although males produce testosterone more readily and in higher concentrations than females, the role of testosterone in females has many functions, including potential effects on soft tissue structures similar to that of estrogen and progesterone (Lovering & Romani, 2005). Lovering & Romani (2005) studied the effect of testosterone on the female anterior cruciate ligament, and identified androgen receptors and specifically

testosterone receptors on the ACL. They also associated higher concentrations of testosterone near ovulation with higher ACL stiffness (stiffness being defined as the change in force (45N) divided by displacement (mm)). This suggests that testosterone may also influence the remodeling and tensile strength of the ACL.

Relationship between Sex Hormones and Joint Laxity

Given the effects of sex hormones on collagen structure and metabolism, this has led to the suggestion that variations in concentrations of sex hormones may also influence the measurement of knee laxity. These relationships have been examined both within females across their cycle, and between males and females at various time points of the cycle.

Comparisons Within Females Across Their Cycle

Despite several attempts to link changes in sex hormones across phases of the menstrual cycle to changes in anterior knee laxity, there have been conflicting results. While some have observed changes in anterior knee laxity across the phases of the menstrual cycle (Deie et al., 2002; Heitz et al., 1999; Shultz et al., 2004; Shultz et al., 2005; Shultz et al., 2006), others have not (Beynnon et al., 2005; Belanger et al., 2004; Karageanes et al., 2000; Van Lunen et al., 2003). Although attempts to standardize the measurement time points in the cycle to known hormone levels via serum sampling, ovulation kits, or self-reported measures have been tried, each study used different methods in selecting the days to measure (thus the hormone concentration analyzed), which may explain the disparate results.

Of the studies that demonstrated a relationship between sex hormones and anterior knee laxity (Deie et al., 2002; Heitz et al., 1999 and Shultz et al., 2004, 2005, 2006), repeated measurements were obtained during critical phases of the menstrual cycle. Heitz et al. (1999) measured each subject's serum estrogen and progesterone levels at the onset of menses when the concentrations of estrogen and progesterone were low, then took three measurements during the corresponding estrogen rise (follicular phase) and three measurements during the luteal phase where progesterone concentrations peak. They reported the greatest anterior knee laxity to be associated with the luteal and follicular phase, when compared to the baseline (menstrual phase). Therefore, surges in estrogen and/ or progesterone concentrations were thought to influence ligament laxity. However, based on their methods and data collection time points, it was impossible to determine whether estrogen alone or the combination of estrogen and progesterone influenced changes in knee laxity. Deie et al. (2002) also demonstrated differences in anterior knee laxity across phases of the menstrual cycle within normal menstruating females, when repeated measures of knee laxity were pooled by phase based on hormone concentrations. Their results illustrated significant changes in knee laxity at 89N, using KT 2000™, between the follicular and luteal phase as well as at 134N between the follicular and ovulatory phase and follicular and luteal phase.

Shultz et al. (2004, 2005) expanded on these findings by measuring estradiol, progesterone, testosterone and knee laxity on a daily basis through one complete menstrual cycle in females. They found the relationships between sex hormones and knee laxity were strongest when all three hormones and their interactions were included,

and when a time delay was considered, with hormones accounting for 63% of the change in knee laxity across the cycle. The idea of a time delay is that any change observed in knee laxity in response to changes in hormone levels are not immediate, but may take a few days to occur. Further, the inclusion of testosterone in their analyses demonstrated contributions from testosterone that was independent of estrogen and progesterone in explaining changes in knee laxity.

In contrast, several studies have found no significant differences in knee laxity across the menstrual cycle, rejecting the hypothesis that hormones influence anterior knee laxity (Belanger et al., 2004; Beynnon et al., 2005; Karageanes et al., 1999; and Van Lunen et al., 2003). While two of these studies (Beynnon et al. 2005; Van Lunen et al. 2003) acquired blood samples to confirm the menstrual cycle phase of the subjects prior to testing knee laxity, they timed their knee laxity tests to days of the cycle, rather than actual peak and nadir hormone concentrations. Belanger et al. (2004) and Karageanes et al. (1999) defined the phase of the menstrual cycle by using the self-reported onset of menses and calculated the phase of interest from this point. While self-reports are often used to determine cycle phase due to availability and financial constraints of hormone measures, they have been shown to have poor reliability due to recall bias (Wojtys, Huston, Boynton, Spindler, Lindenfeld, 2002; Beynnon et al., 2005). Karageanes et al. (1999) also used 14-18 year old subjects which may be too young to have established consistent menstrual cycle patterns. While these methodological challenges may in part explain why these studies observed no changes in knee laxity across the cycle, further research is needed to clarify the relationships between sex hormones and knee laxity

changes across the cycle using actual hormone concentrations to accurately define the cycle phase and hormone environment for each female at the time of measurement.

Comparison of Females and Males Across the Female's Cycle

Sex differences in knee laxity have also been examined across the phases of the menstrual cycle (Beynnon et al., 2005; Pollard et al., 2006; Shultz et al., 2005). Although all studies observed females to have greater knee laxity values compared to males, the extent to which these sex differences changed across the menstrual cycle are conflicting.

In a cohort study, Beynnon et al. (2005), studied males and females over a one month time period (corresponding to one female menstrual cycle), to determine changes in ankle and knee laxity. Subjects were tested at five time points that were equivalent to a 28-day cycle and were chosen based on the hormone changes expected in females at these time points. While they reported significant differences between males and females overall in each testing period, there was no significance difference in sex by time point. Conversely, Shultz et al. (2005) aligned subject data based on actual hormone concentrations rather than day of the menstrual cycle and demonstrated sex differences that varied by day and phase. While females had greater values than males across all time points, sex differences were greatest in the first three days of the early luteal phase, where females experience an increase in knee laxity, post ovulation.

To date, no published studies were found that have investigated the influence of sex hormones on GJL or genu recurvatum. Since endogenous hormones affect many connective tissue structures throughout the body, it is likely that sex hormones may also influence the laxity of other soft tissues and joints, similar to anterior knee laxity. Future

research should consider if there are similar relationships between changes in sex hormones and these other laxity measures in the normal menstruating female.

Hormones and ACL Injury Risk

In addition to studies findings relationships between sex hormones and changes in knee joint laxity across the menstrual cycle, other studies have examined whether injury rates differ across phases of the menstrual cycle. (Beynnon et al., 2006; Slauterbeck et al., 2001; Saluterbeck et al., 2002; Wojtys, Huston, Lindenfeld, Hewett, & Greenfield, 1998; Wojtys et al., 2002). While most studies have noted a greater number of injuries than expected due to chance in the peri-ovulatory time of the cycle (Wojtys et al., 1998; Wojtys et al., 2002; Beynnon et al., 2006), another (Slauterbeck et al., 2002) determined that a greater incidence of ACL injuries occurred in the early follicular phase, within 1-2 days following the onset of menstruation. Although, there may seem to be some disparity among study findings, time delays and variations in hormones concentrations among females could be one possible explanation for the inconsistent findings. Collectively however, these studies appear to indicate that a higher proportion of ACL injuries seem to concentrate in the early and late follicular phases, which represent times in the cycle when sex hormone levels are dramatically falling and rising, respectively.

Oral Contraceptive Hormones

OCPs are widely used by the female population, not only for their contraceptive benefits, but also for limiting pre-menstrual symptoms, regulating cycle phase and duration, and preventing ovarian cancer and acne (Murray & Sucato, 2003). In 1997, the prevalence of oral contraceptive pill (OCP) use among the US female population was estimated to be about 10 million and gaining popularity in specific populations such as athletes (Abma, Chandra, Musher, Peterson, & Piccinino, 1997). Since the introduction of low-dose OCPs, it is estimated that the use among athletes matches that of the general population and is increasing every year (Shawdon, 1999). However, Brynhildsen et al. (1997) indicated the prevalence of OCP use among elite female athletes was between 42%-52%, indicating this rate has held steady. Specific to intercollegiate athletes, the prevalence of OCP use in NCAA female athletes over the past decade has been reported to be between 30-35% per self report (Arendt et al., 2002; Agel et al., 2006). However, the actual use may vary by sport, as Agel et al. (2006) indicated that over 70% of female soccer athletes reported using OCPs. Yet despite the large number of OCP users in the athletic population, very little information is known regarding the effects of these exogenous hormones on soft tissue structures, more specifically ligaments.

Variations in Exogenous Hormone Preparations

OCPs contain synthetic steroids that differ in the types and amounts of estrogen and progesterone used. OCPs release synthetic estrogen and progesterone at higher concentrations than those observed during the normal menstrual cycle which creates a

down-regulation and destruction of the endogenous hormones by the liver (Guyton & Hall, 2000). Exogenous hormones decrease the bio-availability of the endogenous hormones by inhibiting the synthesis of estradiol through interference with the hypothalamus-pituitary-ovarian axis (Larsen et al., 2003). The most common synthetic estrogen used in OCPs is ethinyl estradiol, while the most conventional progestins used are norgestrel, norethynodrel, norgestimate, norethindrone, and levonorgestrel (Guyton & Hall, 2000). OCPs contain various combinations of estrogen and progesterone (both in type and dose), to control cycle length. The dosages of hormones can also be delivered in monophasic versus triphasic manner. A monophasic OCP delivers the same amounts of estrogen and progesterone throughout the three "on-pill" weeks of the cycle, while triphasic OCPs gradually increases the progesterone dosage over the three on-pill weeks of the cycle. Whether steady (monophasic) versus varying (triphasic) OCP dosages differentially influence soft tissue structures has not been studied, and should be considered when understanding the relationship between OCPs and ligamentous laxity.

Exogenous Hormones and Injury

Recently, Agel et al. (2006) studied 3150 NCAA female athletes from various institutions containing 1024 OCP users (≈ 33%) and 2026 non-users. They examined ACL and ankle injuries and used self-report recall as of means to report phase during the injury. Using these methods, they found no difference in injury prevalence by cycle phase. Further, there were no differences in injury rates between athletes using or not using OCPs. One limitation to this study is the sample size. Although, the sample size

appeared large, a power analysis suggested the total sample size of over 4000 subjects would be needed to determine a protective effect from injury. Further sufficiently powered, large scale longitudinal studies are needed to appropriately examine and compare the risk of ACL injury across the menstrual cycle in OCP users versus normal menstruating females.

Exogenous Hormones and Knee Laxity

Two studies have investigated the effect oral contraceptives on joint laxity. Pokorny et al. (2000) used a convenience sample to compare 30 low-dose OCP users and 25 non-users on anterior knee laxity and digit mobility. Knee laxity measurements were obtained with the KT-1000^{TM} knee arthrometer at 89N and 134N. A goniometer was used to measure digit joint motion as passive hyperextension of the fifth DIP and abduction and adduction of the fifth PIP. Measurements were taken at any point in the cycle except for during menses, since OCP users were not taking exogenous hormones during this time. Their results indicated no significant differences in knee laxity measurements at 89N or 134N between normal menstruating (6.2 $\pm$ 2.26mm and 7.9 $\pm$ 2.42mm) and OCP users (6.4 $\pm$ 2.47mm and 7.7 $\pm$ 2.76mm) respectively.

Similarly, Martineau et al. (2004) studied collegiate female athletes, 42 using low-dose OCP and 36 non-users, and measured A-P knee laxity (67N and 89N) using a KT-1000 ^{TM}. However, their results showed that OCP users had significantly less knee laxity and variability in comparison to non-users (3.88 $\pm$ 1.06 mm and 4.83 $\pm$ 1.82 mm respectively). While results were inconsistent between Pokorny et al. (2000) and Martineau et al. (2004), both used a self-reported questionnaire for inclusion criteria

which included identification of OCP use, type of OCP, history of menstrual cycle, pregnancy, and any underlying knee pathology. Further, both studies compared OCP users' ligamentous laxity with a control of normal menstruating subjects. One possible difference is the homogeneity of the subjects and study groups. Pokorny et al. (2000) studied a convenience sample while Martineau et al. (2004) studied female athletes. Also, Pokorny et al. (2000) had a smaller sample size and indicated there may have been insufficient statistical power to yield clinical differences. Another potential difference is that the types of OCP used by subjects in each study may have varied. For example, Pokorny et al.'s (2000) study included 13 subjects using Ortho-Tri-Cyclen® which contains norgestimate as the progestin, 10 OCPs with levonorgestrel, 4 OCPs containing norethindrone, and 3 with desogestrel. As previously discussed, there is considerable diversity among types of OCPs in regards to phasing (monophasic and triphasic) and type and dosages of estradiol and progesterone compounds, which may further confound findings.

Since OCPs alter the hormone concentrations at the cellular level and various OCPs contain different types and dosages of exogenous hormones, each OCP has the potential to influence ligamentous structures differently. As a result, stratifying the type and dosage of synthetic hormones may be beneficial in future studies to further clarify the effect of exogenous hormones on joint laxity. Further, examinations of the effects of OCPs have been limited to anterior knee laxity, and future research should determine the effects of these exogenous hormones on GJL and genu recurvatum as well. The high prevalence of OCP use in the athletic population (i.e. the population most at risk for ACL

injury), reinforces the need to understand effects of exogenous hormones on soft tissue structures promoting increased joint laxity.

CHAPTER III

METHODS

Participants

Thirty-three female Division I athletes ranging in age from 17-24 were identified as using OCP's as part of a pre-season physical examination and risk factor screening, and consented to participate. Inclusion criteria were: 1) no history of pregnancy, 2) a history of OCP for at least 3 months, 3) specific knowledge of the type of OCP they were using, 4) no medical conditions affecting the joints or connective tissue (e.g., Marfan's Syndrome or Ehlers-Danlos disease), and at least one healthy knee. A 3 month restriction on OCP use was chosen because research has shown that most women's cycles tend to regulate following three months of OCP use (Hatcher, Trussell, & Stewart, 1994). All participants gave informed consent prior to participation in the study.

Procedures

Once signing the informed consent form, the participants completed a menstrual history questionnaire to determine their inclusion in the study. The subjects then completed the pre-season risk factor screening, which included measures of GJL, anterior knee laxity, and genu recurvatum.

Menstrual History Questionnaire

Athletes completed a modified, self-report questionnaire of menstrual history / status and oral contraceptive use that was previously established and validated by Wojtys et al. (1998) (See Appendix C) Questions include the number of menses in past 12 months, the start date of the last and anticipated next menses, usage and length of oral contraceptive use, and the type or brand of OCP that they used. Modifications from the original questionnaire were minor, and included the replacement of questions regarding pregnancy with a more general question that documented missed periods, in an effort to capture both amenorrheic periods as well as pregnancies. While actual serum sampling is ideal for obtaining specific hormone concentrations to document cycle phase, this was not feasible due to expense and limitations associated with a mass screening. Further, current assays techniques are not able to measure the amount of exogenous hormones in the blood. Therefore, exogenous hormone concentrations were estimated using known hormone dosages and subsequent concentrations provided via manufacturer's physician's prescribing sheet.

General Joint Laxity

The Beighton and Horan's Joint Mobility Index (BHJMI) (Beighton, Soloman, Solokne, 1973) was used to measure GJL and assesses joint mobility at five different joints. This measurement has been found to be reliable and represents the most widely used method in previous studies to determine GJL and associated predisposing risk factors (Beighton & Horan, 1969; Decoster et al., 1999; Jansson et al., 2004; Klemp,

1997; Ramesh et al., 2005; Scerpella et al., 2005; Seckin et al., 2005; van der Giessen et al., 2001). Intratester reliability was established for the sole examiner using 11 subjects for GJL (ICC= 0.99; SEM=.0243). The criteria for each measurement were as follows:

Hyperextension of the 5th finger: The distal portion of the 5th metacarpal is stabilized with the thumb of the opposite hand while the tip of the 5th finger is extended as far as possible without pain by the subject using the index or middle finger. Hyperextension greater than 90º resulted in a score of 1, and hyperextension of 90º or less resulted in a score of 0. If the range of motion was close to 90º extension, goniometric measurements were taken with the fulcrum over the center of the metacarpophalageal joint, the distal arm along the length of the fifth finger, and the proximal arm along the fifth metacarpal to confirm the degree of extension.

Thumb opposition: The subject stabilized the distal portion of the forearm with the thumb of the opposite hand. The thumb being tested was then passively abducted by the fingers of the opposite hand toward the volar aspect of the forearm with the wrist in flexion. If the thumb touched the forearm, a score of 1 was recorded. Inability to touch the forearm resulted in a score of 0.

Elbow extension: With the subject's shoulder abducted to approximately 80º and the forearm supinated, the subject actively extended the elbow as far as possible. Hyperextension of the elbow greater than 10º resulted in a score of 1. Hyperextension of 10º or less resulted in a score of 0. If necessary, a goniometer was used to confirm extension angle, with the center of the fulcrum placed over the lateral epicondyle of the humerus, the distal arm positioned along the lateral midline of the forearm in line with

the radial styloid process, and the proximal arm positioned along the lateral midline of the subject's humerus.

Knee extension: Subjects stood in a bilateral stance with body weight equally distributed between each limb. Subjects were asked to stand naturally. Hyperextension of the knee greater than 10º resulted in a score of 1. Hyperextension of 10º or less resulted in a score of 0. If necessary, hyperextension angle was confirmed with a goniometer, with the center of the fulcrum placed over the lateral epicondyle of the femur, the proximal arm representing the midline of the femur referenced to the greater trochanter, and the distal arm representing the midline of the lower leg referenced to the lateral malleolus and fibular head.

Trunk Flexion: Subjects stood feet shoulder width apart while attempting to touch palms of hands to floor while keeping the knees fully extended. An ability to touch palms flat on the floor resulted in a score of 1. Inability to easily place the palms flat on the floor was scored as 0.

Anterior Knee Laxity

Anterior knee laxity is defined as the amount of anterior displacement of the tibia relative to the femur at a 133N anterior directed force using the KT-2000™ knee arthrometer (MEDmetric® Corp; San Diego, CA). One experienced examiner who had established excellent reliability on this measure ($ICC_{2,k}$ = .91; SEM = 0.44mm) performed all measures of anterior knee laxity. Manufacturer's guidelines were used to position the subject in supine with a thigh support placed proximal to the popliteal fossa to place the

knee in 25 degrees of flexion. The subjects' ankles were placed in the manufacturer's foot cradle, and a Velcro strap was placed on the thighs to control the amount of lower extremity rotation. Once positioned, the KT-2000™ was attached to the leg in proper alignment with the subject's joint line per manufacturer's instructions. With the participant relaxed, three posterior directed forces were applied to the anterior tibia to identify a stable neutral point. Then, an anterior directed force just over 133N was applied to the tibia. To insure a direct anterior pull was achieved, a bubble level affixed to the device was visually observed during the test. A practice trial was first performed to allow the subjects to become familiar with the test procedure and insure relaxation. Then, 3 measurements of anterior knee laxity were obtained, and recorded in millimeters (mm) of displacement. Prior to each measurement, the KT-2000™ arthrometer was reinitialized by zeroing the dial following three anterior to posterior directed loads at 89N, and proper extremity position was confirmed.

Genu Recurvatum

Genu recurvatum was recorded as the maximal, active range of motion of the knee into hyperextension. Intratester reliability was established with a single examiner using 11 subjects and repeated measures were taken 24-48 hours apart (ICC= 0.96; SEM= 0.785 degrees). With the subject supine on a table, a small bolster (4") was placed under the distal aspect of the tibia. The anterior and posterior portions of the lateral knee joint line were palpated and a mark placed at the midpoint in the sagittal plane. The most prominent aspect of the lateral malleolus and the greater trochanter were also palpated and marked. Subjects were then asked to actively contract their quadriceps and

maximally extend their knee while the investigator aligned the goniometer for measurement. The axis of the goniometer was positioned over the mark on the joint line, and the angle formed by a line from the lateral joint line to the greater trochanter, and a line from the lateral joint line to the lateral malleolus was measured to the nearest angle in degrees (^{o}).

Data Reduction

All measurements were performed bilaterally, and data from the right extremity data were used for analysis. For GJL, measurements of 5th finger extension, elbow extension, knee hyperextension, thumb opposition, and trunk flexion were scored bilaterally, yielding a total score from 0-9. This composite score was then recorded in SPSS and used for analysis. The average value of the three anterior knee laxity measurements was used for analyses. Three active genu recurvatum measurements were taken, and the average value of the three measurements was used for analyses. SHBG % binding affinities are determined based on previous findings of Schindler et al. (2003).

Statistical Analysis:

For each subject, OCP type (monophasic or triphasic), estradiol dosage, progesterone type and dosage, and length of use were entered along with each subjects GJL, anterior knee laxity and genu recurvatum measurements into SPSS (version 14.0, SPSS, Inc, Chicago, IL) for statistical analyses. Three separate stepwise linear regression analyses were used to predict the extent to which OCP type (monophasic, triphasic), estradiol dosage (μg/dose), progesterone type (0% SHBG binding

(drospirenone and norgestimate), low % SHBG binding (norethindrone), higher % SHBG binding (levonorgestrel)) and progesterone dosage (mg/dose) predicted anterior knee laxity, genu recurvatum and general joint laxity. Secondary linear regression analyses were used to predict the extent to which day of the cycle and length of continuous OCP use may influence each measure of joint laxity.

CHAPTER IV

JOURNAL - FORMATTED MANUSCRIPT

ABSTRACT

This study examined the relationships between oral contraceptive pill (OCP) use and measures of joint laxity. **Methods:** A menstrual history questionnaire identified 33 female athletes using OCPs. Separate stepwise linear regressions examined the extent to which OCP dosage (estradiol, progesterone) and SHBG% predicted measures of anterior knee laxity, general joint laxity and genu recurvatum. Secondary regression analyses examined the relationship between duration of OCP use and each joint laxity measure. **Results:** OCP dosage and %SHBG binding affinity were not predictors of joint laxity. Greater duration of OCP use positively predicted greater genu recurvatum (R^2 = 20.8%, *P*=0.008). **Conclusions:** While the variations in dosages of common OCPs were not found to influence measures of joint laxity, prolonged exposure to these synthetic hormones may increase genu recurvatum over time. Further work is needed to clarify the effects of OCPs on joint laxity, a potential risk factor for knee injury.

INTRODUCTION

Joint laxity has received considerable attention as an ACL injury risk factor based on its prevalence among females as compared to males (Larsson, Baum, & Mudholkar, 1987; Medrano & Smith, 2003; Scerpella, Stayer, & Makhuli, 2005; Seckin et al., 2005; Uhorchak et al., 2003), and its potential to modify ACL injury risk (Medrano & Smith, 2003; Ramesh, Von Arx, Azzopardi, & Schranz, 2005; Scerpella et al., 2005; Uhorchak et al., 2003). Joint laxity is defined as hypermobility of a joint which exceeds the normal range of motion with the main contributors being the static stabilizers of the joint (i.e., the joint capsule, ligaments, and tendons) (Grahame, 1999). Common clinical measurements of joint laxity include anterior knee laxity (Scerpella et al., 2005; Uhorchak et al., 2003), general joint laxity (GJL) (Grahame, 1999; Jansson, Saartok, Werner, & Renstrom, 2004; Scerpella et al., 2005) and genu recurvatum (Loudon, Jenkins, & Loudon, 1996; Loudon, Goist, & Loudon, 1998). While anterior knee laxity (Beynnon et al., 2005; Pollard, Braun, & Hamill, 2006; Rozzi, Lephart, Gear, & Fu, 1999; Uhorchak et al., 2003) and GJL (Scerpella et al., 2005; Uhorchak et al., 2003) have consistently been found to be greater in females compared to males, sex differences in genu recurvatum are conflicting (Loudon et al., 1996; Nguyen et al., In Press; Scerpella et al., 2005). As research supports that excessive values associated with each of these measures may be predictive of ACL injury risk (Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003; Woodland-Rogers, Cyphert, & Denegar, 1994), it is prudent to understand why females are more prone to greater joint laxity, and the underlying physiological mechanisms by which difference in knee laxity (both within and between sex) may be modulated.

The greater joint laxity observed in females compared to males appears to be at least in part due to sex differences in hormone concentrations (Shultz, Sander, Kirk, & Perrin, 2005). At the cellular level, the influences of sex hormones on soft tissue structures have come under examination (Liu, Al-Shaikh, Panossian, Finerman, & Lane, 1997; Yu, Panossian, Hatch, Liu, & Finerman, 2001). Estrogen and progesterone receptors have been localized on the human ACL, which has lead to speculation that these hormones may be influential in the structure and composition of ligaments (Lui et al., 1996; Lui et al., 1997). This in turn has led to studies investigating the relationship between changing hormone levels across the female menstrual cycle and their associated effects on anterior knee laxity (Belanger et al., 2004; Beynnon et al., 2005; Deie, Sakamaki, Sumen, Urabe, & Ikuta, 2002; Heitz, Eisenman, Beck, & Walker, 1999; Karageanes, Blackburn, & Vangelos, 2000; Romani, Patrie, Curl, & Flaws, 2003; Shultz, Kirk, Johnson, Sander, & Perrin, 2004; Shultz et al., 2005; Van Lunen, Roberts, Branch, & Dowling, 2003). While results are conflicting, work that examined the daily changes in sex hormone concentrations with daily changes in knee laxity report knee laxity is greater in the early luteal phase while estrogen is at its highest peak and progesterone is on the rise (Shultz et al., 2004; Shultz et al., 2005). Further, this work indicates that the association between changes in knee laxity and hormones is stronger when all three hormones (i.e. estrogen, progesterone, and testosterone) and their interactions are considered (Shultz et al., 2004). However, these relationships have primarily been studied in normal menstruating females to date, and have been limited to the measurement of anterior knee laxity.

Appreciating the relationships noted between sex hormones and anterior knee laxity, little attention has been directed towards our understanding of how oral contraceptive pills (OCPs) may modify these relationships. This is important, as OCPs greatly reduce endogenous hormone levels, and as many as 42% of intercollegiate athletic females use OCPs for one reason or another (Agel, Boris, Bershadsky, & Arendt, 2006). Estradiol, the most potent form of estrogen, and progesterone are the two major exogenous hormones combined in mainstream OCPs. Each OCP product contains different amounts of ethinyl estradiol (usually ranging from 20-50 ng/mL) and various forms of progesterone compounds. In today's market, these various forms of progesterone include but are not limited to levonorgestrel, norgestimate, drospirenone, and norethindrone (Frye, 2006). But while OCPs generally suppress endogenous hormone synthesis, their values generally remain above menses levels. Further, these synthetic hormones essentially have the same effect as their endogenous counterparts; inhibiting follicle-stimulating hormone (FSH) and luetinizing hormone (LH) release of the pituitary and preventing ovulation. Therefore, if endogenous forms of estrogen and progesterone alter ligament composition and structure, it is likely that exogenous hormones from OCPs also may have some effect on ligamentous integrity and joint laxity.

Only two studies to date were found that have examined the relationship between OCPs and ligamentous laxity (Martineau, Al-Jassir, Lenczner, & Burman, 2004; Pokorny, Smith, Calus, & Dennison, 2000); yet the results were conflicting. Pokorny et al. (2000) used self-reported measures of oral contraceptive use and investigated the

changes in peripheral joint laxity. The results indicated no association between OCP use with anterior knee laxity and finger joint laxity (i.e. fifth finger extensibility). Conversely, Martineau et al. (2004), also with a self-reported questionnaire, identified significant decreases in anterior knee laxity in OCP users versus non-users. These conflicting results may be due to a number of factors, including differences in sample populations and size, large variations in length of OCP use among individuals between studies, and varying OCP types. While these limited studies reported the types of OCP used, these data were not included in their analyses when determining the relationship between OCP use and laxity.

Differing characteristics of common synthetic contraceptive formulations, in addition to their dosages, are their interaction with sex hormone-binding globulin (SHBG). SHBG is largely responsible for transporting biologically active androgens and estradiol in the blood, and regulates the free fraction concentration of testosterone that is available to the target tissues (Hammond, 2002). Specifically, circulating SHBG increases with increasing dosages of synthetic estradiol (Wiegratz et al., 2003), which can be counteracted by the progesterone compound used (Wiegratz et al., 2003, Hammond et al., 2003). While some synthetic progestins resemble testosterone-like compounds (e.g. norethindrone and levonorgestrel) which have some binding affinity to SHBG (Grow, 2002), others (e.g. norgestimate, drospirenone) do not bind with SHBG (Wiegratz et al., 2003; Hammond et al., 2003). Hence, contraceptive compounds that deliver higher estradiol levels and use a progesterone compound that does not bind with SHBG will result in greater increases in circulating SHBG, and ultimately greater reductions in the

free fraction of testosterone circulating in the blood (Wiegratz et al., 2003; Hammond et al., 2003). As previous research has found that the combined concentrations of estradiol, progesterone, and testosterone and their interactions are stronger predictors of changes in knee laxity (Shultz et al., 2004; Shultz, Gansneder, Sander, Kirk, & Perrin, 2006) it is plausible that variations in the dosages of synthetic estradiol and progesterone and the type of progesterone used in these OCPs may have differential effects on knee laxity.

Thus, the purpose of this study is to build on the previous literature, and determine the extent to which the type and dosage of exogenous estradiol and progesterone may predict the amount of joint laxity in female athletes using oral contraceptives. A secondary purpose was to determine if the length of exposure to OCPs has any effect on joint laxity. The research hypothesis is that greater dosages of exogenous estradiol, lower dosages of progesterone, and progesterone compounds that have a lower affinity for SHBG would predict greater general joint laxity, genu recurvatum and anterior knee laxity in females using OCPs. Further, we expected that longer exposure to OCPs (thus prolonged suppression of endogenous hormone concentrations) would result in decreased joint laxity.

METHODS

Design and Setting

Participants for this study were selected from a larger data base of NCAA Division I student-athletes from a single university who participated in a 18 station pre-season risk factor screening as part of their pre-participation physical examination. After reading and signing a consent form approved by the university's institutional review

board for the protection of human subjects, 92 female athletes participated in a multi-station, comprehensive pre-participation examination that included a general medical screening, orthopedic screening, psychological and menstrual history questionnaires, and measures of joint laxity, lower limb anatomic alignment, functional performance (hop and agility tests), balance, and kinematic analysis of landing. Athletes rotated through the test stations, with the starting station randomly assigned after first completing the medical and orthopedic screens. To identify females using oral contraceptives, we used a modification of the self-reported questionnaire of menstrual history / status that was previously established and validated by Wojtys et al. (1998). The questionnaire included information on the specific brand of OCP, day of last menstrual cycle, and length of time on OCPs. Modifications consisted of the replacement of questions on pregnancy with a question documenting any amenorrheic episodes (including specific time and length of absence of menses), which would account for pregnancy as well as menstrual dysfunctions.

Subjects

From a total sample of 92 female collegiate athletes examined, thirty-three (36%) (19.3 ± 1.4 years; 167.4 ± 7.1 cm; 64.1 ± 7.3 kg) were identified as currently using OCPs and were selected for the study. Inclusion criteria also included no previous history of pregnancy, knowledge of the brand of OCP used, and no history of medical conditions affecting the joints or connective tissue (Marfan's Syndrome, Ehlers Danlos).

Procedures

From the larger data set, variables of anterior knee laxity, general joint laxity, and genu recurvatum as measured on the right leg were extracted for this study. Each variable was measured by a single examiner who had been trained to perform the measure using standardized protocols that have been established in our laboratory with acceptable intertester and intratester reliability (Shultz, Nguyen, Windley, Kulas, Beynnon, 2006). The procedures for each of these measurements are as follows.

Anterior Knee Laxity

Anterior knee laxity was defined as the amount of anterior displacement of the tibia relative to the femur at a 133N anterior directed force using the KT-2000™ knee arthrometer (MEDmetric® Corp; San Diego, CA). The subject was positioned according to manufacturer's guidelines and a 133N anteriorly directed force was applied to the tibia. To insure a direct anterior pull was achieved, a bubble level affixed to the device was visually observed during the test. A practice trial was first performed to allow the subjects to become familiar with the test procedure and insure relaxation. Then, three measurements of anterior knee laxity were obtained, and recorded in millimeters (mm) of displacement. Prior to each measurement, the KT-2000™ arthrometer was reinitialized by zeroing the dial following three anterior to posterior directed loads at 89N, and proper extremity position was confirmed. All anterior knee laxity measures were performed by one trained examiner with prior establishment of excellent day to day reliability ($ICC_{2,k}$ = .91; SEM = 0.44mm).

General Joint Laxity

The Beighton and Horan's Joint Mobility Index (BHJMI) (Beighton, Soloman & Soskolne, 1973) was used to measure GJL which assesses joint mobility at five different joints (4 tested bilaterally): fifth finger extension, thumb opposition to the forearm, elbow hyperextension, knee hyperextension and forward flexion. Specific criteria was used for each joint to denote the absence (0) or presence (1) of hypermobility at that joint, and their score reflected a composite value, indicating the sum of scores from each joint which exceeded the criteria of hypermobility.

Hyperextension of the left and right fifth digits was performed by the distal portion of the 5^{th} metacarpal stabilized with the thumb of the opposite hand while the tip of the 5^{th} finger is extended as far as possible without pain by the subject using the index or middle finger. Extension of the joint greater than 90º received a score of 1, and extension equal to or less than 90º received a score of 0. To assess left and right thumb opposition, the thumb being tested was passively abducted by the thumb of the opposite hand toward the volar aspect of the forearm with the wrist in flexion and stabilized by the opposite hand and fingers. If the thumb touched the forearm, a score of 1 was recorded. Inability to touch the forearm resulted in a score of 0. With the shoulders abducted to 90^{o}, active hyperextension of the left and right elbows were assessed, with hyperextension greater than 10º resulting in a score of 1 while equal to or less than 10º scored a 0. Next, standing in a natural double leg natural stance, knee hyperextension was observed, and angles greater than 10º were scored a 1, while angles of 10º or less were score a 0. Lastly, trunk flexion was assessed as subjects stood with feet shoulder

width apart and attempting to touch palms of hands to floor while keeping the knees fully extended. Ability to touch palms flat on the floor resulted in a score of 1, while an inability to easily place the palms flat on the floor was scored as 0. All measurements close to the criteria were confirmed using a standard goniometer for consistency. A single trained examiner performed all measures of GJL, after establishing excellent test-retest intratester reliability on 11 subjects measured on two separate occasions, 24-48 hours apart (ICC= 0.99; SEM=.02).

Active Genu Recurvatum

Active genu recurvatum was recorded as the maximal, active range of motion of the knee into hyperextension, using a goniometer. The subject was positioned supine on a table with a bolster (4") placed under the distal aspect of the tibia. The anterior and posterior portions of the lateral knee joint line were palpated and a mark placed at the midpoint in the sagittal plane. The most prominent aspect of the lateral malleolus and the greater trochanter were also palpated and marked. Subjects were then asked to actively contract their quadriceps and maximally extend their knee while the investigator aligned the goniometer for measurement. The axis of the goniometer was positioned over the mark on the joint line, and the angle formed by a line from the lateral joint line to the greater trochanter, and a line from the lateral joint line to the lateral malleolus was measured to the nearest degree. Measurements were taken by the same trained examiner who measured GJL, and who also established excellent intratester reliability for this measure on the same 11 subjects (ICC= 0.96; SEM= 0.79 degrees).

Data Reduction and Analysis

From the menstrual history questionnaire, the brand of OCP and the length of time using OCPs (months) was determined. Maximum estradiol dosage, progesterone minimum and maximum dosage, progesterone type related to SHBG % binding affinity was estimated based on prescribing information provided by the manufacturer for each respective OCP brand as reported on the menstrual history questionnaire (Table 1). To confirm that the dosages across various hormones are relatively reflective of their serum concentrations, Table 1 also provides information on the pharmacokinetics reported for each OCP. These data indicate that as the dosage of estradiol and progesterone in the OCPs increased, the serum concentrations also increased. For the joint laxity measures, the composite score for GJL (0-9) and the average of 3 trials each for anterior knee laxity and genu recurvatum were used for analyses.

To test the first research hypothesis, three separate stepwise linear regression analyses were used to determine the extent to which estradiol dosage (μg/dose), progesterone dosage (mg/dose), and the %SHBG binding affinity of the progesterone compound [0 % SHBG binding (drospirenone and norgestimate), 36 % SHBG binding (norethindrone), 47 % SHBG binding (levonorgestrel)] predicted anterior knee laxity, general joint laxity and genu recurvatum. Values for SHBG % binding affinities for each progesterone compound were entered based on data reported by Schindler et al. (2003). To test the second research hypothesis, simple linear regression analyses were used to determine the extent to which length of continuous OCP predicted each value of joint

laxity (anterior knee laxity, GJL, and genu recurvatum). All statistical analyses were performed using SPSS (version 14.0, SPSS, Inc, Chicago, IL). The alpha level was set a-prior for all analyses at P=.05.

RESULTS

Table 2 lists information on the type of OCP used by each subject, as well as the length of use and individual laxity scores. The means and standard deviations values for each of the predictor variable [ethinyl estradiol dosage (Est_{max} Dose), minimum and maximum progesterone dosages ($Prog_{max}$Dose and $Prog_{min}$Dose) and SHBG % binding affinity for progesterone], and for each dependent variable [GJL, genu recurvatum (AGR), and anterior knee laxity (AKL)] are also represented (Table 2). Histograms displaying the distribution of values for each predictor and dependent variable for the sample population are presented in Figures 1-8.

Bivariate Pearson's correlations used for the primary stepwise linear regression analyses are provided in Table 3. Higher % binding affinity for SHBG was associated with lower minimum (-0.356, $P = 0.021$) and maximum (-0.381, $P = 0.014$) doses of progesterone. However, the stepwise linear regression analyses failed to identify any significant predictors for GJL, anterior knee laxity or genu recurvatum based on estradiol dose, minimum progesterone dose, maximum progesterone dose, and SHBG % binding affinity. For anterior knee laxity, SHBG% binding affinity was the only variable to enter the model, explaining only 1.9% of the variance ($P = 0.460$) (Table 4). Similar results were found for genu recurvatum, with SHBG% binding affinity being the only predictor

to enter the model and explaining 5.0% of the variance ($P = 0.210$) (Table 5). No variables entered the model for GJL.

Table 6 presents the simple linear regression model summary results when predicting each laxity measure based on length of time of OCP use. While no significant relationships were noted between length of OCP use and anterior knee laxity and GJL, length of time on OCPs was a positive predictor of genu recurvatum explaining 20.8% (Adjusted $R^2 = 0.182$, P=0.008) of the variance.

DISCUSSION

This study aimed to determine the extent to which type, dosage and length of exposure to OCPs predicted anterior knee laxity, GJL, and genu recurvatum. The primary findings were that the dosages of ethinyl estradiol and progesterone, and the types of progesterone compounds related to their SHBG % binding affinity showed little to no relationship with anterior knee laxity, GJL, and genu recurvatum. Hence, these findings did not support the research hypothesis that greater dosages of ethinyl estradiol, lower progesterone dosage, and lower % binding affinity to SHBG would predict increases in each of the laxity values. A secondary finding of this study revealed that increased length of time of OCP use was associated with greater genu recurvatum, but not GJL or anterior knee laxity.

Relationship of OCP Type and Dosage with Joint Laxity

Recently, it has been suggested that the use of OCPs may influence ligamentous laxity (Martineau et al., 2004), as the synthetic hormones in OCPs are known to be

biologically active. However, the relationship between OCP use and joint laxity is not well understood, based on two contrasting studies to date. While Martineau found OCP use to be related to lower knee laxity values, Pokorny et al. (2000) revealed no association between OCP use and joint laxity. One potential explanation for the discrepancy in these findings is the type and dosage of OCPs used by individual participants in each study. Therefore, the aim of the current study was to build on previous work, and determine the extent to which dosage characteristics of the individual OCPs used may explain these disparate findings.

OCPs drastically reduce the levels of endogenous hormone concentrations, and serve to stabilize the concentrations of estrogen and progesterone. Generally, OCPs provide a lower overall serum hormone concentration during the 7 days of menstruation when compared to normal menstruating females, due to the ingestion of placebo pills which deliver no synthetic hormones. For the remaining 21-days, hormone levels are elevated due to synthetic supplementation based on the unique combination of estrogen and progesterone in each type of OCP. As indicated in Table 1, mean exogenous estrogen concentrations of the majority of OCPs remain well above menses levels in normal menstruating females consistent with mean estrogen levels at the follicular phase as reported in previous studies (Beynnon et al., 2005; Shultz et al., 2004; Shultz et al., 2006). Thus, consistent with findings in normal menstruating females (Shultz et al., 2006), it was hypothesized that higher concentrations of exogenous estradiol, lower concentrations of progesterone, and progesterone compounds that have a lower affinity for SHBG would predict greater anterior knee laxity, general joint laxity, and genu

recurvatum. However, the results of the current study identified no relationship between the dosage and %SHBG binding affinity of OCPs and the various joint laxity measures.

When considering these findings it is important to first consider the distribution of values of the independent and dependent variables in the sample (See Figures 1-8), as sufficient spread in the data is necessary to identify linear relationships. The dosage distribution of estradiol does resemble a normal curve with the exception of a few outliers (0.050mg ethinyl estradiol), however there was no association shown between the estradiol dosage and anterior knee laxity, GJL, or genu recurvatum. In fact, the estradiol dosage did not enter as a predictor in any of the dependent measurements. But while these data resembled a normal distribution, it may be that the range of therapeutic dosages prescribed in the current cohort are not sufficient broad to have differential therapeutic effects on the soft tissue structures associated with joint laxity. While the dosages of ethinyl estradiol and progesterone compounds in Table 1 appear to be somewhat variable, the concentrations identified by the available pharmacokinetics of the OCPs used by subjects in this study were all low-dose OCPs, with estradiol levels typically ranging from 82-145pg/mL during the majority of the cycle. While one OCP contained doses of estradiol and progesterone that are consistent with minimum (menses) levels, higher estradiol doses yielded concentrations which would be considered on average more moderate concentration levels throughout the follicular and luteal phases compared to normal menstruating females (i.e. below pre-ovulation and luteal peak levels). Hence, the low dose OCPs used in this study appeared to have delivered only

moderate concentrations of estradiol and progesterone, which may have a limited effect on joint laxity.

Conversely, the distributions of minimum and maximum progesterone dosages within each type of OCP were highly skewed, with 20 of 33 subjects receiving between 0.0 – 0.5 mg/dose. Further, the distribution of OCPs with 0% SHBG binding affinity greatly outweighed the number of OCPs with some % binding affinity nearly 2:1 (Figure 4). So although progesterone dosage and SHBG % binding affinity had no association with anterior knee laxity, GJL, and genu recurvatum, these results may change if there were a more even and broader distribution of these values among subjects. This potential is further supported by the low but insignificant correlations between %SHBG binding affinity and each of the laxity values. Therefore, further work is needed in this area.

Length of Time on OCPs and Effects on Joint Laxity

A secondary finding was that subjects who used OCPs for a greater length of time had greater genu recurvatum values. No relationships were noted between length of OCP use and GJL or anterior knee laxity. These findings are in contrast to the original hypothesis that increased length of time on OCPs would predict lower measures of joint laxity. This hypothesis was based on earlier findings by Martineau et al. (2004), and previous research that suggests that OCP use decreases collagen synthesis and therefore would stabilize and reduce turnover of the collagen structure (i.e. ligaments and joint capsules) (Wreje et al., 2000). However, as previously noted, subjects using most of these preparations would have maintained moderately high levels of estradiol (i.e. well above menses levels, but below peak levels). Since higher estrogen concentrations have

been associated with increases in anterior knee laxity (Heitz et al., 1999; Shultz et al., 2004; 2006), it may be that prolonged exposure to moderately higher levels (i.e. without allowing these levels to return to menses levels) may in time result in higher genu recurvatum measures. In other words, even though differences in individual OCP dosages were not enough to result in changes in joint laxity, it is possible that the dosages of these OCPs stabilized hormone concentrations consistently at higher levels, with an overall effect of greater knee laxity values with prolonged exposure.

Of interest, most of the previous work noting relationships between sex hormones and joint laxity have measured anterior knee laxity, of which OCPs appeared to have no effect. Despite a significant relationship between length of time on OCPs and genu recurvatum, no relationship was identified for anterior knee laxity. This is not entirely surprising as low correlations were noted between these laxity measures (Table 3), which suggest they are measuring different aspects of joint laxity. Hence, these disparate findings may suggest that soft tissues other than ligament may be affected by these sex hormones. It is well accepted that the ACL is the primary restraint of anterior tibial translation, of which measures of anterior knee laxity in large part measures with the knee positioned in a relatively loose packed position. Conversely, genu recurvatum involved maximal extension of the knee joint, which would tend to tension the joint capsule and other soft tissue structures as well, suggesting the effect may more likely be on soft tissues other than ligament. More work is needed to separate out the effects of sex hormones on ligament, tendon and muscle.

No meaningful relationships between length of OCP use and GJL were identified. In fact, a trend toward decreased GJL was noted (P=.101), which is in contrast to the findings with genu recurvatum. Although standing knee hyperextension is also assessed with GJL, the measurements are performed entirely different. With the standing knee hyperextension measurement as part of GJL, the subject is instructed to stand naturally, without actively hyperextending the knee. Since supine active genu recurvatum is non-weight bearing and requires maximal straightening of the knee, it may be a more sensitive measurement of joint laxity. Still, since both tests (GJL and genu recurvatum) otherwise test maximal extension of joints, it is difficult to explain why extension would increase at the knee, but decrease at the thumb, elbow and 5th finger (extension tests of GJL). As the Beighton and Horan Joint Mobility Index is one of the most widely accepted and used test for the assessment of GJL and injury risk (Beighton & Horan, 1969; Decoster et al., 1999; Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003), future work should continue to explore the effects of sex hormones (both endogenous and exogenous) on this measure of joint laxity.

Another potential consideration as it relates to the significant findings between longer length of time on OCPs and greater genu recurvatum and not anterior knee laxity or GJL is the broader distribution of genu recurvatum values when compared to GJL and anterior knee laxity. Figures 5 – 8 shows the distribution of values for length of time on OCPs and genu recurvatum, with both resembling normal distribution curves. On the other hand, the spread of scores for anterior knee laxity and GJL are less than ideal. It appears that there is one subject with high (14.0mm) average value of anterior knee laxity

and only one subject obtained an average value below 5.0mm of anterior tibial displacement. Otherwise, 22 of the 33 values (66% of the sample) fell between 7-10mm which is a relatively small distribution. This is of importance because although the average values for this study is consistent with current literature (Pokorny et al., 2000), it is possible that a stronger relationship may have been noted with a more evenly distributed sample as was found with genu recurvatum. In effort to confirm the results of this study, a larger cohort with a more even and broad distribution of AKL and GJL scores are needed.

Clinical Implications

In summary, these findings indicate that joint laxity is not dependent on the dosage of individual OCP preparations, but that prolonged exposure to greater hormone concentration levels with sustained OCP may lead to higher genu recurvatum values. The greater values in genu recurvatum due to prolonged OCP use may have clinical implications, given previous research which has identified a relationship between genu recurvatum and ACL injury risk (Loudon et al., 1996; Ramesh et al., 2005; Scerpella et al., 2005; Uhorchak et al., 2003). Collectively, these studies agree, prospectively and retrospectively, that increased genu recurvatum may be associated with an increased risk of injury. Hence, these findings would not support the prevailing theory that OCP use would have a protective effect on ACL injury risk. In fact, recent research supports this notion, as no differences in injury risk have been noted between NCAA female athletes who are normal menstruating versus OCP users (Agel et al., 2006; Arendt et al., 2002).

Limitations and Future Directions

To our knowledge this is the first study which has examined specific OCP types in relation to joint laxity. However this study is not without limitations. One of the major limitations of this study is the nature in which data were collected. All data were collected as part of a mass, multi-station pre-season risk factor screening project. Because of this, as well the inability for current assay techniques to measure both endogenous and exogenous levels, serum was not collected in order to confirm actual hormone levels. Further, other aspects of this study, such as exercise prior to data collection, were not controlled. Given that subjects were randomly assigned to begin at different stations, some subjects may have participated in physical activity prior to laxity measurements which may have altered values of anterior knee laxity, GJL, and genu recurvatum. Further, as with all self-reported studies, some error or bias should be acknowledged for OCP information provided in the self-administered questionnaire. Better control of these factors in future studies would strengthen the study findings and may further elucidate the relationship between absolute hormone concentrations and joint laxity values. Additionally, the findings in this study are limited to a relatively small sample size from one university institution. Given the number of variables examined, and the limited distribution of some of the variables, a larger sample size may provide a more clear relationship between the variables and provide insight to the role of OCPs in functional joint motion. In 2000, Pokorny et al. indicated that OCP use is not related to joint laxity however, the authors determined the study was underpowered (0.09) due to the small sample size (n=55). In contrast, Martineau et al. (2004) showed OCP users had

significantly decreased ligamentous laxity compared to non-users with high power (0.93) for the comparison of the experimental OCP users and the controls (n=42 and n=36, respectively). The current study sample (n=33) is relatively small when considering using a regression with many variables.

In summary, this study provides additional evidence on how OCP type and length of time on OCPs may affect anterior knee laxity, GJL, and genu recurvatum, aiding clinicians and researchers towards a better understanding of the effects of OCP types on potential risk factors for injury. Future research is necessary to further clarify the influence that OCPs may have on measures of joint laxity, including the independent effects they may have on the various tissues involved (e.g. tendon, ligament and muscle). Ideally, serum hormone assays would allow accurate measurements of both endogenous and exogenous hormone levels when making these comparisons. While not a purpose of the current study, more research is also needed to determine how differences in hormone concentrations between OCP users and non users (e.g. normal menstruating, oligomenorrheic and ammenorrheic) may effect joint laxity and other measures of joint function.

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TABLES

Table 1 Mean Pharmacokinetics of Oral Contraceptives

		Estrogen			Progestin		
OCP Brand	**Cycle/Day**	**Compound**	**Dosage (mg)**	**C_{max} (pg/ml)**	**Compound**	**Dosage (mg)**	**C_{max} (ng/ml)**
Yaz®	1/21	Ethinyl Estradiol	0.020	45.1	Drospirenone	3.00	70.3
Avaine®	1/21	Ethinyl Estradiol	0.020	82.3	Levonorgestrel	0.10	6.0
Leios®	n/a	Ethinyl Estradiol	0.020	n/a	Levonorgestrel	0.10	n/a
Ortho Tri-Cyclen Lo®	3/21	Ethinyl Estradiol	0.025	95.9	Norelgestromin[A] Norgestrel[A]	0.18[A]	1.8 2.8
Yasmin®	1/21	Ethinyl Estradiol	0.030	92.1	Drospirenone	3.00	87.5
Seasonale®	1/1	Ethinyl Estradiol	0.030	145.0	Levonorgestrel	0.15	5.6
Valette®	n/a	Ethinyl Estradiol	0.030	n/a	Dienogest	2.00	n/a
Portia® / Levlen® / Levora®	n/a	Ethinyl Estradiol	0.030	n/a	Levonorgestrel	0.15	n/a
Estrostep® Fe	1/21	Ethinyl Estradiol	0.035	113.0	Norethindrone	1.00	12.7
Ortho Tri-Cyclen®	3/21	Ethinyl Estradiol	0.035	126.0	Norelgestromin[A] Norgestrel[A]	0.25[A]	2.7 3.7
Ovcon® 35	1/1	Ethinyl Estradiol	0.035	131.4	Norethindrone	0.40	4.2
Tri-Sprintec® / Tri-Previfem®	n/a	Ethinyl Estradiol	0.035	n/a	Norgestimate	0.18	n/a
Ortho-Novum 50® / Ovcon 50®	n/a	Ethinyl Estradiol	0.050	n/a	Norethindrone	1.0	n/a

[A] Norgestimate analytes: Norelgestromin and Norgestrel dosages; n/a No information available; C_{max} Maximum hormone concentration

Table 2. OCP User Descriptives of Each OCP

Subject	OCP Type	Progesterone Type	Est Dose (µg)	Prog Dose (mg)	SHBG % Binding[A]	Months on OCP	Mean AKL (mm)	GJL	Mean AGR (degree)
1	Valette	dienogest	30	2.00	0	12	5.0	0	4.3
2	Yaz	drospirenone	20	3.00	0	48	8.1	1	8.3
3	Yasmin	drospirenone	30	3.00	0	2	4.0	2	2.3
4	Yasmin	drospirenone	30	3.00	0	12	8.5	5	6.0
5	Yasmin	drospirenone	30	3.00	0	13	9.0	4	9.7
6	Yasmin	drospirenone	30	3.00	0	42	9.5	1	9.7
7	Yasmin	drospirenone	30	3.00	0	32	14.0	1	7.7
8	Yasmin	drospirenone	30	3.00	0	24	8.8	0	5.3
9	Yasmin	drospirenone	30	3.00	0	60	6.2	0	5.3
10	Aviane	levonorgestrel	20	0.10	47	5	9.5	3	1.7
11	Aviane	levonorgestrel	20	0.10	47	12	7.8	1	5.7
12	Leios	levonorgestrel	20	0.10	47	12	8.5	5	10.0
13	Portia	levonorgestrel	30	0.15	47	24	10.0	3	6.7
14	Levlen	levonorgestrel	30	0.15	47	36	6.0	1	4.3
15	Seasonale	levonorgestrel	30	0.15	47	24	6.0	2	8.0
16	Levora	levonorgestrel	30	0.15	47	9	8.3	0	2.0
17	Ovcon-35	norethindrone	35	0.40	36	4	8.7	0	6.3
18	Estrostep	norethindrone	35	1.00	36	16	8.5	0	4.3
19	Ortho-Novum 1/50	norethindrone	50	1.00	36	6	5.0	0	5.3
20	Ovocon-50	norethindrone	50	1.00	36	6	9.0	3	6.7
21	Orthotricyclin-Lo	norgestimate	25	0.18/0.25	0	18	8.0	1	7.0
22	Orthotricyclin-Lo	norgestimate	25	0.18/0.25	0	60	6.8	0	7.0
23	Orthotricyclin-Lo	norgestimate	25	0.18/0.25	0	13	9.5	1	5.7
24	Orthotricyclin-Lo	norgestimate	25	0.18/0.25	0	2	7.0	3	3.7
25	Orthotricyclin-Lo	norgestimate	25	0.18/0.25	0	7	9.0	0	8.7
26	Tri-Sprintec	norgestimate	35	0.18/0.25	0	20	9.5	3	3.7
27	Tri-Sprintec	norgestimate	35	0.18/0.25	0	24	9.0	1	4.7
28	Tri-Previfem	norgestimate	35	0.18/0.25	0	48	9.5	0	6.7
29	Orthotricyclin	norgestimate	35	0.15/0.25	0	48	8.7	1	8.7
30	Orthotricyclin	norgestimate	35	0.15/0.25	0	36	10.0	1	4.3
31	Orthotricyclin	norgestimate	35	0.15/0.25	0	60	9.3	1	11.7
32	Orthotricyclin	norgestimate	35	0.15/0.25	0	48	10.5	1	8.7
33	Orthotricyclin	norgestimate	35	0.15/0.25	0	36	7.0	4	7.7
		Mean	**30.7**	**1.0/1.0**	**14.3**	**24.8**	**8.3**	**1.5**	**6.3**
		SD	**7**	**1.2/1.2**	**20.8**	**18.4**	**1.9**	**1.5**	**2.4**

[A] Schindler et al., 2003; Est, estradiol maximum dosage; Prog, progesterone maximum/minimum dosages; AKL, anterior knee laxity; AGR, active genu recurvatum

Table 3. Bivariate Pearson Correlations for Stepwise Linear Regression Analyses

	AKL	GJL	AGR	Est_{Max}Dose	$Prog_{Max}$Dose	$Prog_{Min}$Dose	SHBG
AKL	1						
GJL	0.078	1					
AGR	0.303*	0.128	1				
Est_{Max}Dose	-0.035	-0.124	-0.001	1			
$Prog_{Max}$Dose	-0.037	0.006	0.068	-0.023	1		
$Prog_{Min}$Dose	-0.043	0.008	0.059	-0.019	1*	1	
SHBG Binding	-0.135	0.109	-0.224	0.007	-0.381*	-0.356*	1

Est_{Max}, maximum estradiol dosage (μg); $Prog_{Max}$, maximum progesterone dosage (mg); $Prog_{Min}$, minimum progesterone dosage (mg); SHBG Binding: (%)

* significance at $P \leq 0.05$

Table 4. Stepwise linear regression model summary when predicting anterior knee laxity from OCP dosages

				Standard	Change Statistics				
			Adjusted	Error of the	R^2	F			Significant
	R	R^2	R^2	Estimate	Change	Change	df1	df2	F Change
SHBG % Binding	0.135	0.019	-0.014	1.9104	0.019	0.573	1	31	0.455

Predictors: (Constant), SHBG

Table 5. Stepwise linear regression model summary when predicting active genu recurvatum from OCP dosages

				Standard	Change Statistics				
			Adjusted	Error of the	R^2	F			Significant
	R	R^2	R^2	Estimate	Change	Change	df1	df2	F Change
SHBG % Binding	0.224	0.050	0.019	2.385	0.050	1.635	1	31	0.210

Predictors: (Constant), SHBG

Table 6. Simple linear regression summary results when predicting each joint laxity measure (AKL, AGR, GJL) based on length of time of OCP use

				Standard	Change Statistics				
			Adjusted	Error of the	R^2	F			Significant
	R	R^2	R^2	Estimate	Change	Change	df1	df2	F Change
AKL	0.161	0.026	-0.006	1.903	0.026	0.823	1	31	0.371
GJL	-0.290	0.084	0.055	1.481	0.084	2.852	1	31	0.101
AGR	0.456*	0.208	0.182	2.178	0.208	8.128	1	31	0.008*

*significance at $P \leq 0.05$

FIGURES

Figure 1. Histogram noting the sample distribution for maximum estradiol dosage

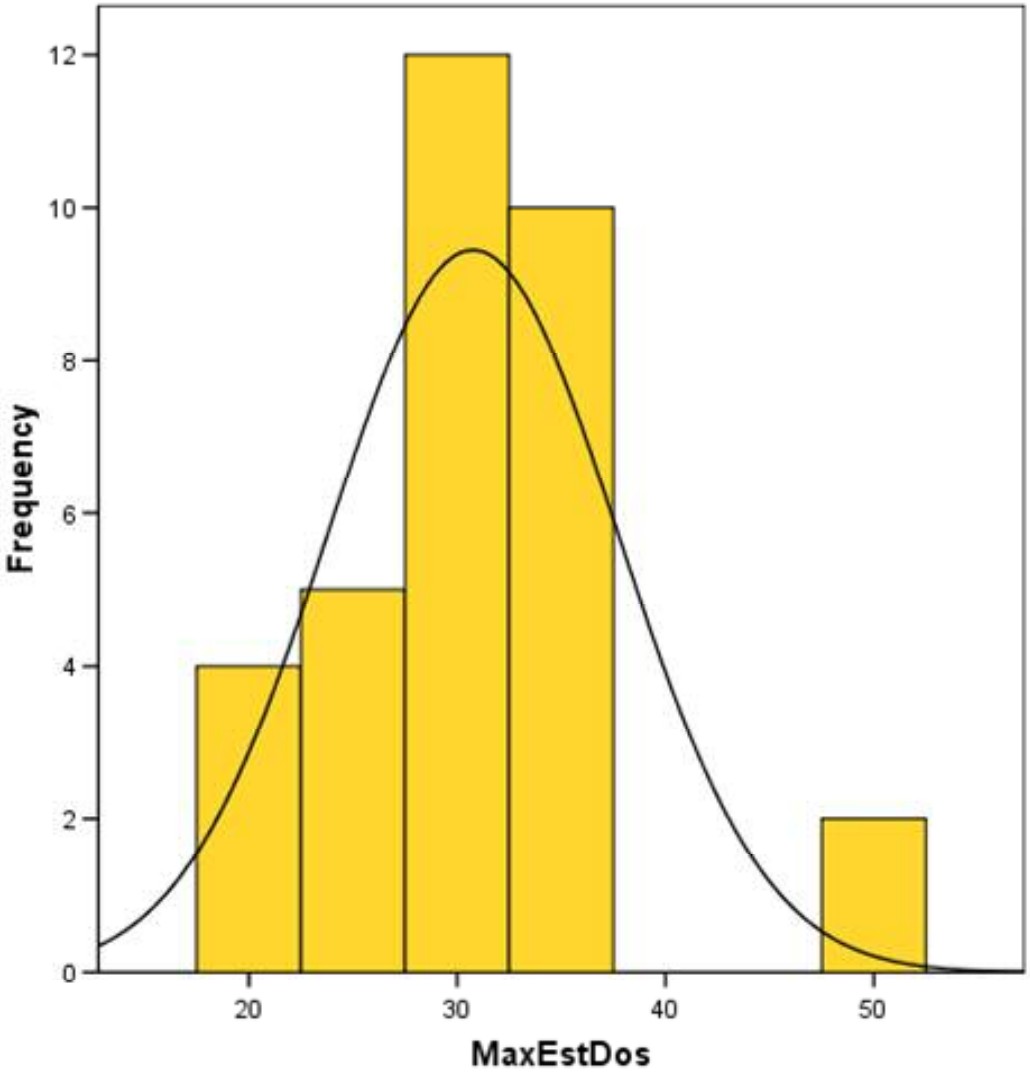


Figure 2. Histogram noting the sample distribution for maximum progesterone dosage

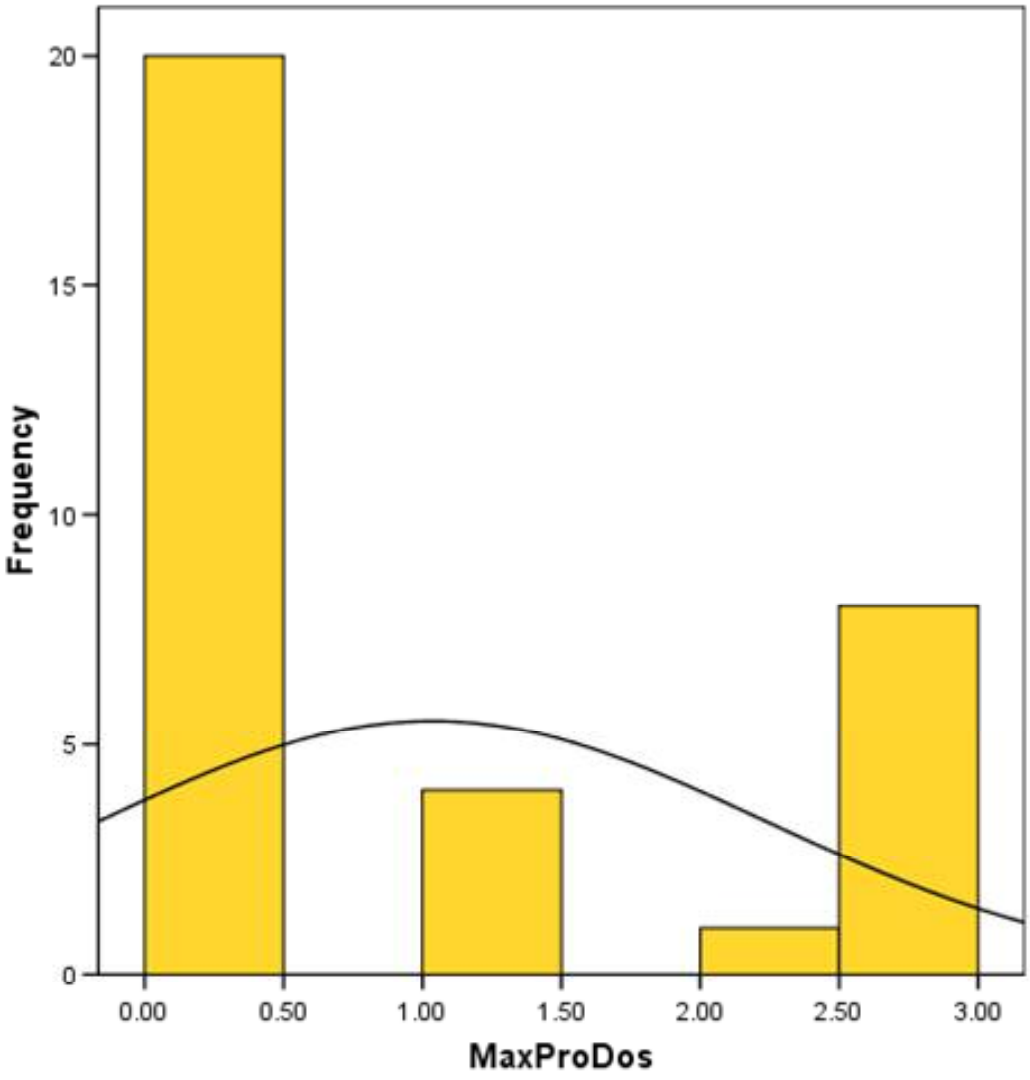


Figure 3. Histogram noting the sample distribution for minimum progesterone dosage

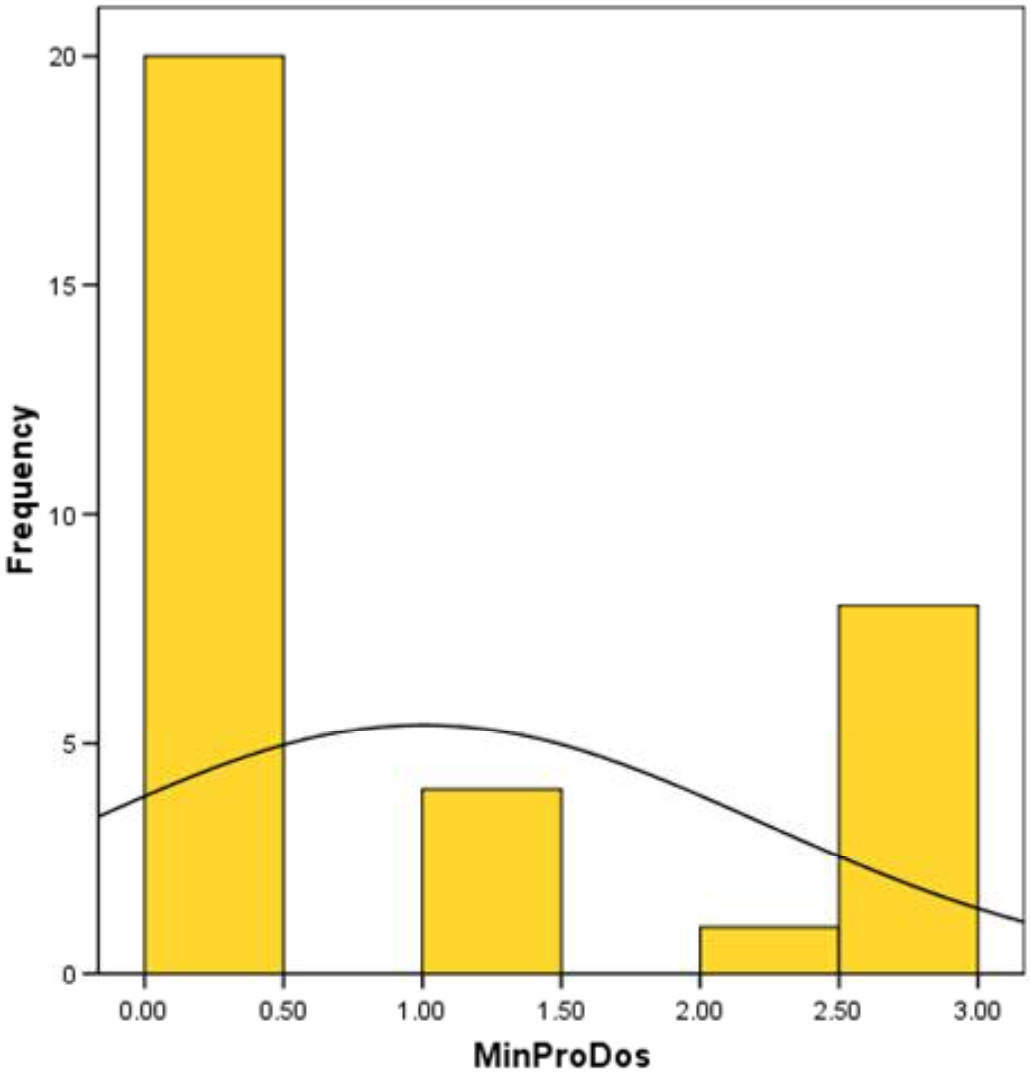


Figure 4. Histogram noting the sample distribution for % SHBG binding affinity

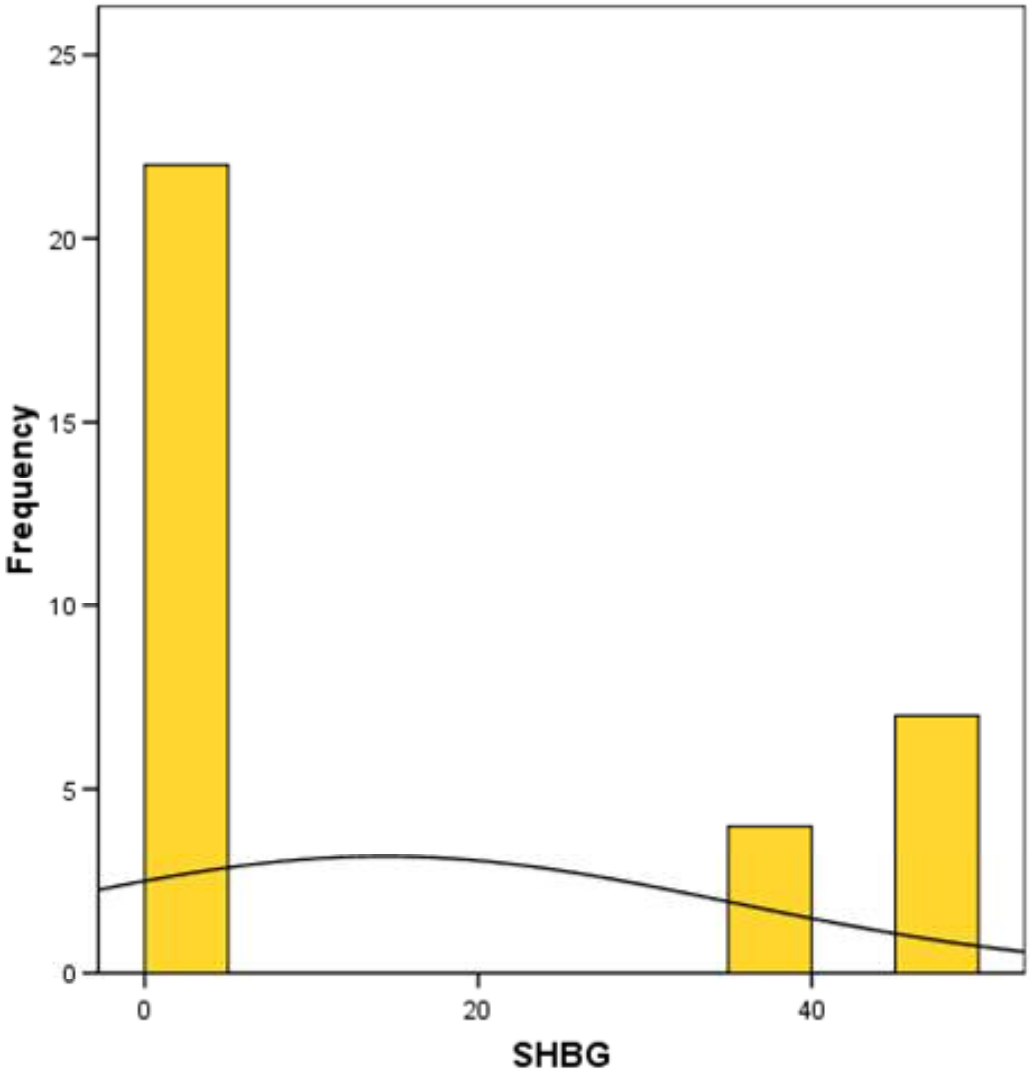


Figure 5. Histogram noting the sample distribution for genu recurvatum values

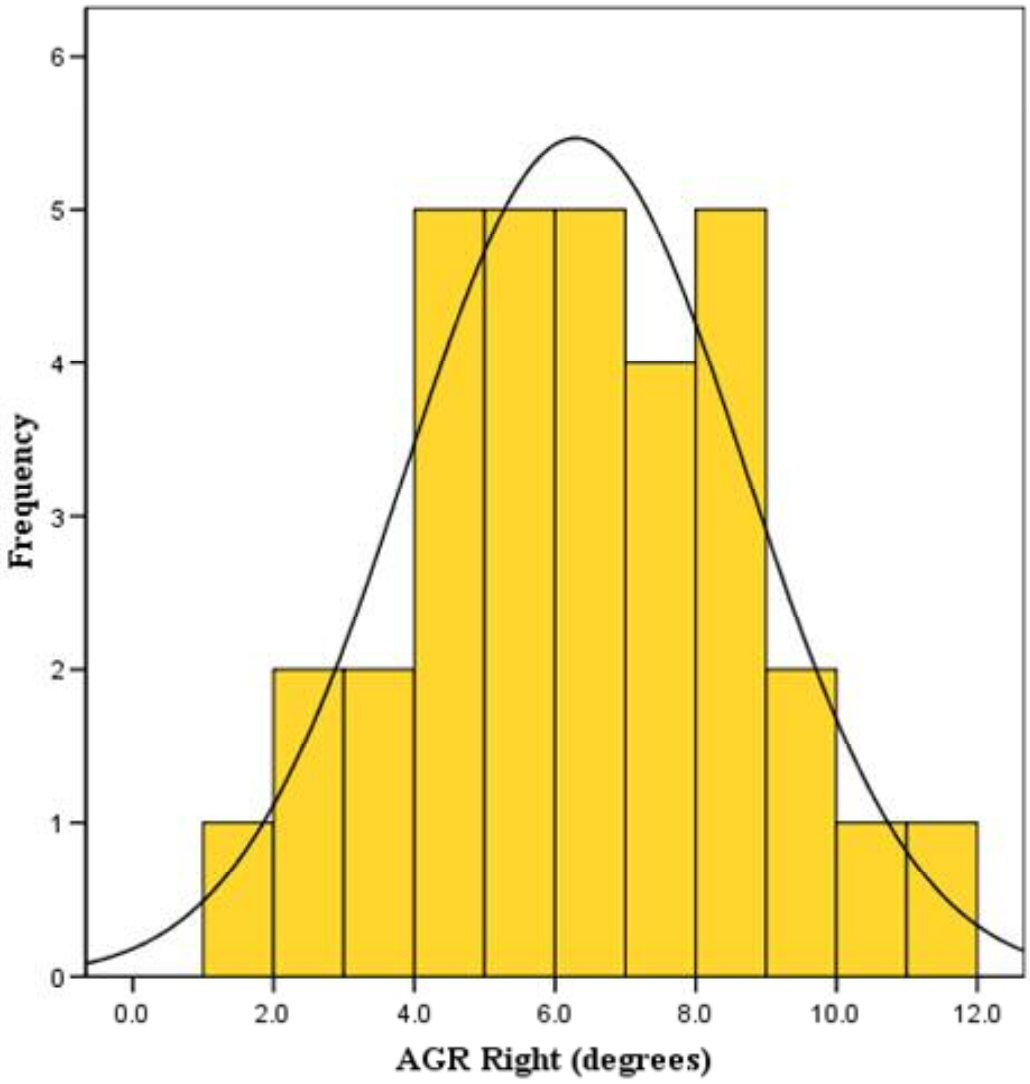


Figure 6. Histogram noting the sample distribution for anterior knee laxity measures

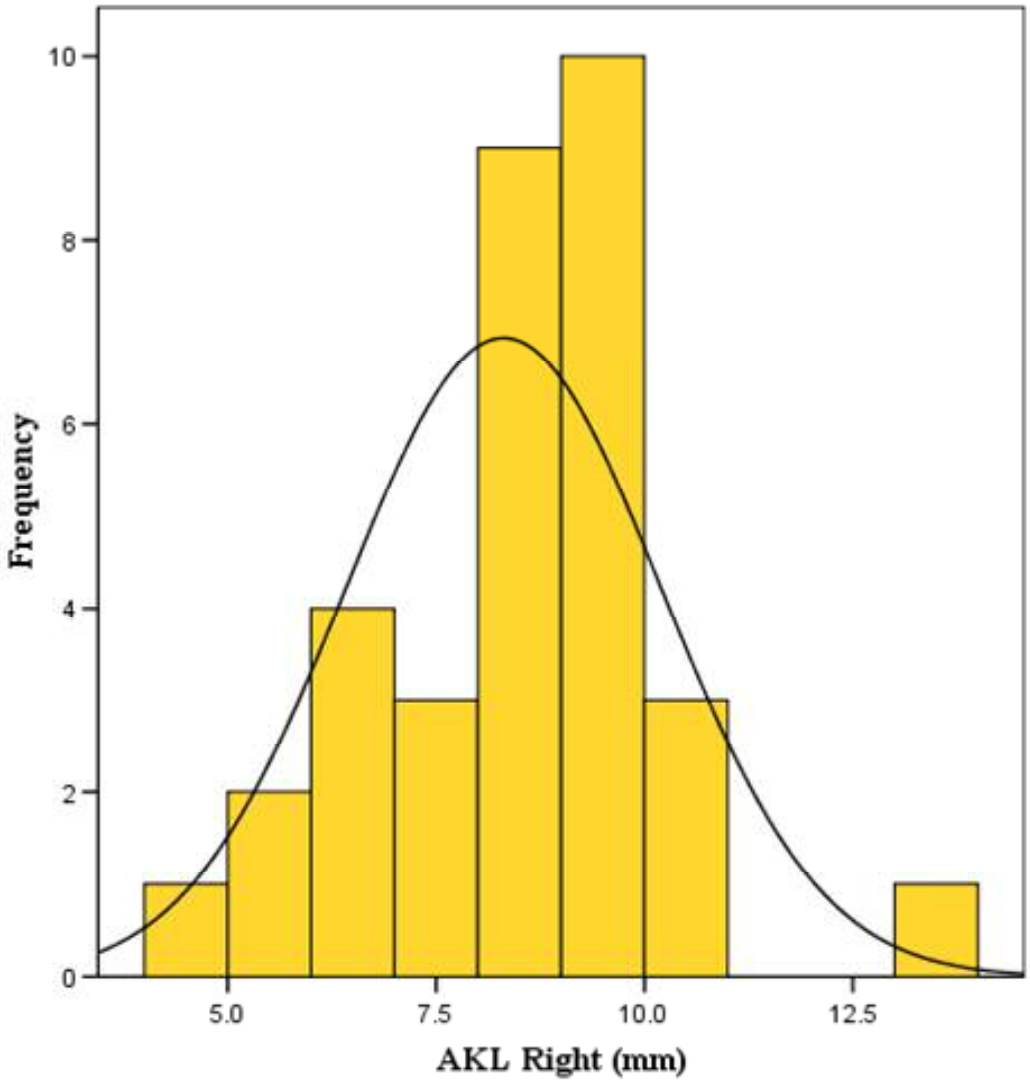


Figure 7. Histogram noting the sample distribution for composite GJL scores

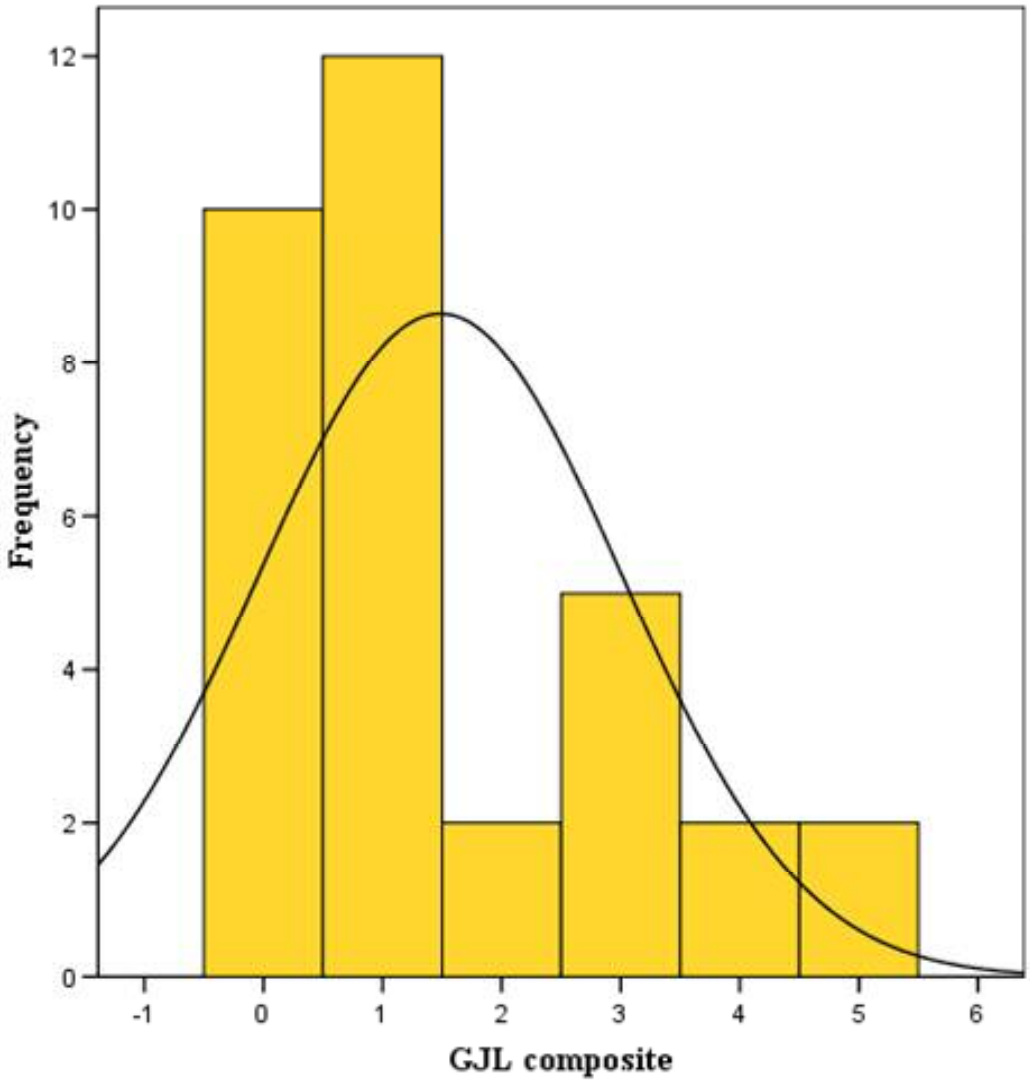
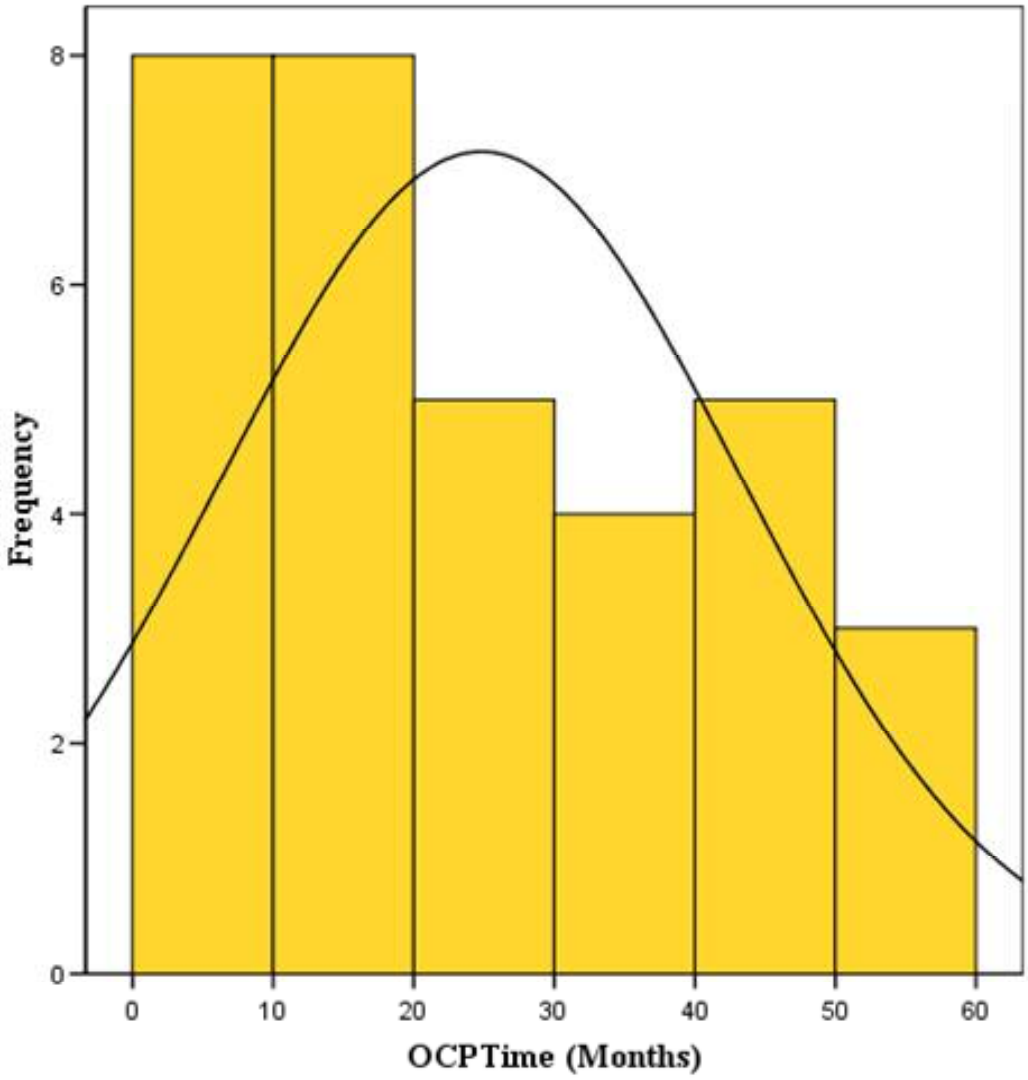


Figure 8. Histogram noting the sample distribution for subjects' length of time on OCPs



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Yu, W.D., Panossian, V., Hatch, J.D., Liu, S.H., & Finerman, G.A.M. (2001). Combined effects of estrogen and progesterone on the anterior cruciate ligament. *Clinical Orthopaedics and Related Research*, *383*, 268-281

APPENDIX A: IRB FORM

Review Process Log
Applications for the Use of Human Participants in Research
Principal Investigator: Complete the top section of this form only and submit it with the IRB checklist.

Researcher:	Randy Schmitz Sandy Shultz	Faculty Sponsor: N/A	
Original Date of Submission to Departmental Reviewer:	June 10, 2005	Projected Date of First Data Collection:	August 6, 2005

Departmental Reviewer:

IRB USE ONLY

Date of First Receipt by Departmental Reviewer:

First Review by Departmental Reviewer:

Disposition by Departmental Reviewer	Date	Notes
Returned complete application to PI		
Requested Major Revisions		
Requested Minor Revisions		
Forwarded to ORC		

Second Review by Departmental Reviewer:

Disposition by Departmental Reviewer	Date	Notes
Returned complete application to PI		
Requested Major Revisions		
Requested Minor Revisions		
Forwarded to ORC		

Third Review by Departmental Reviewer:

Disposition by Departmental Reviewer	Date	Notes
Returned complete application to PI		
Requested Major Revisions		
Requested Minor Revisions		

Forwarded to ORC		

Review by IRB Chair:

Disposition by IRB Chair	Date	Notes
Requested Major Revisions		
Requested Minor Revisions		
Forwarded to ORC		
Scheduled for Full Review		

Review Checklist
Applications for the Use of Human Participants in Research

Researcher:	Randy Schmitz Sandy Shultz	Faculty Sponsor N/A	
Submission Date:	June 10, 2005	Projected Date of First Data Collection:	August 6, 2005

Faculty and staff members should complete this checklist before they submit an application for their own research or when they serve as the faculty sponsor for a student's research. Please submit two complete copies of the application.
Top of Form

Review Criteria	Check by Researcher or Faculty Sponsor	Check by IRB Reviewer
Part A is complete.	x	
Evidence of training in the protection of human participants in research is attached for all principal investigators.	x	
If the principal investigator is a student, evidence of training in the protection of human participants in research is attached for the faculty sponsor.	x	
Part B: The researcher has answered questions 1-8 on separate paper. (DO NOT EXCEED THREE PAGES.)	x	
1. Goals for the project are clearly stated and suggest the need for human participants' consent.	x	
2. The protocol discusses:	☐	
a. data gathering procedures and tools (copies of tools must be attached to the application, unless the tool is well known).	x	
b. data recording procedures.	x	
c. the number of participants, justification for this	x	

Review Criteria	Check by Researcher or Faculty Sponsor	Check by IRB Reviewer
number, and procedures for selecting participants.		
d. the length of time for procedures.	x	
e. relationship between the researcher, participants, and participating institutions/agencies.	x	
f. any need for deception or less than full disclosure.	N/A	
g. if the research is conducted in class, what students who are not participating will do.	N/A	
h. copies of letters from any agencies involved with recruitment of participants or data collection.	N/A	
i. how consent will be obtained.	x	
j. provisions for providing copies of consent documents to participants.	x	
3. The protocol describes the benefits to individual participants AND society.	x	
4. The protocol addresses the risks to participants, including:		
a. the level of risk for participants (none, minimal, more than minimal).	x	
b. description of the risks to participants.	x	
c. precautions taken to minimize risks to participants	x	
d. how confidentiality will be maintained.	x	
e. how long data will be kept	x	
f. how data will eventually be destroyed.	x	
5. The protocol describes the participant population and justifies any decision to exclude persons on the basis of gender, race, or ethnicity.	x	
6. Materials to be used in recruiting participants are attached to the protocol.	x	
7. The CONFLICT OF INTEREST question is answered N/A, NO, or YES. (If the answer is YES, a completed Potential Conflict of Interest in Research form is attached.)	x	
8. The USE of PHI is answered NO or YES. (If the answer is YES, a completed Application to USE PHI in Research form is attached. If a waiver from the UNCG IRB is requested, a completed UNCG Request for Waiver of Authorization form is attached.)	x	
9. The researcher has indicated that s/he will keep	x	

Review Criteria	Check by Researcher or Faculty Sponsor	Check by IRB Reviewer
Confidentiality Certificates on file for all persons who assist with data collection or analysis during the research.		
Part C: The Consent Form includes:		
1. a clear explanation of the purpose of the research.	x	
2. a clear explanation of the procedures to be used.	x	
3. a description of the benefits to participants and/or society.	x	
4. the risks of participation. (If more than minimum risk is indicated, the Consent Form includes a statement regarding compensation, availability of treatment, and directions to contact Eric Allen.)	x	
5. the opportunity to ask questions.	x	
6. the opportunity to withdraw from the research without penalty.	x	
7. the amount of time required for participation.	x	
8. how confidentiality will be maintained.	x	
9. how long data will be kept.	x	
10. how data will eventually be destroyed.	x	
11. the researchers name and phone number for questions about the research.	x	
12. Eric Allen's name and phone number for questions about the rights of human participants in research.	x	
13. a place for the signature of a witness to the oral presentation, when the short form is used).	x	
14. a separate form for the assent of minors, if applicable.	x	

Your signature indicates that you have reviewed the IRB application and believe it to be in approvable formBottom of Form

Researcher's Signature Date

Researcher's Signature Date

IRB Initial Reviewer's Signature Date

THE UNIVERSITY OF NORTH CAROLINA
GREENSBORO
Instructions for Completing the Application for the Use of Human Participants in Research

All research with human participants conducted by students, faculty, or staff at UNCG must be reviewed initially by a member of the University's Institutional Review Board, whether or not requests for outside funding are involved. To initiate this review, the investigator/project director must complete this application and submit it to the IRB member in his/her college/school/department. The IRB member determines the category of review appropriate for the study and forwards it to the Office of Research Compliance. The University IRB meets if full committee review is necessary. Criteria for exempt, expedited, and full committee review are available at: <http://www.ohrp.osophs.dhhs.gov/polasur.htm>.

Please submit the original and one copy of this human participants application at least one month prior to the date you wish to initiate data collection. (You are advised to keep a copy for your records also.) YOU MAY NOT COLLECT DATA PRIOR TO RECEIVING AN APPROVAL FORM FROM THE IRB.

Faculty members will be informed by the IRB regarding the disposition of their applications and those of students they are sponsoring. Students do not receive direct notification of IRB disposition of proposals. Any changes in research protocol that affect human participants must be approved by the IRB prior to implementation unless the changes are necessary to eliminate apparent immediate hazards to the participant. Any unanticipated problems involving risks to participants or others must be promptly reported to the IRB.

COMPLETE PART A (On this Page) AND Numbers 1-8 ON PAGE 3. ATTACH THE APPROPRIATE CONSENT FORM INFORMATION. BE SURE TO SIGN THIS APPLICATION ON PAGE 3.

Part A

Date: 06 /02/2005

Project Title: Normative Data for Measures of Postural Alignment, Agility, and Strength in an Athletic Population.

Principal Investigator(s): Randy Schmitz PhD & Sandra J. Shultz, PhD, ATC

Email Address of Principal Investigator: rjschmit@uncg.edu & sjshultz@uncg.edu

Phone Number of Principal Investigator: (336) 334-3031 & (336) 334-3027

Address of Principal Investigator: 250 HHP Building

Relationship to the University (specify): Faculty ☒

If student, name of faculty sponsor:

Faculty sponsor's email addressN/A

School/College: HHP Department: Exercise and Sport Science

Funding Agency/Sponsor (if applicable):

Projected data collection dates*: From 08/06/2005 To 08/06/2006

Have the investigators attached certificates of completion of training in the use of humans in research? Yes

* Beginning date should be at least 1 month after submission of IRB application. Data collection cannot begin before IRB approval is received.

THIS PAGE IS FOR IRB USE ONLY
(IRB Representative: Indicate appropriate category of review: exempt, expedited, or full review. Note: the standard requirements for informed consent apply regardless of the type of review utilized by the IRB.)
Part B - Exempt
This proposed research is judged to be exempt from full committee review because it falls in one or more of the following categories (see 45 CFR 46, June 18, 1991, p. 5).
Check all that apply:

1. 46.101 (b)(1)
2. 46.101 (b)(2)
3. 46.101 (b)(3)
4. 46.101 (b)(4)
5. 46.101 (b)(5)
6. 46.101 (b)(6)

Part C - Expedited or Full Review
This proposed project has been reviewed and was found to require:
Expedited Review (63 FR 60364-60367, November 9, 1998)
Expedited category. Check all that apply:

1. (a)
1. (b)
2. (a)
2. (b)
3.
4.
5.
6.
7.
8. (a)
8. (b)
8. (c)
9.

Full IRB Review. Please explain_____

I certify that this project has been reviewed by me as an IRB member and that the research was not proposed by me or by a student working under my supervision.

_____ _____
IRB Signature Date

_____
Dept. /School

Send this application package to: IRB, Office of Research Compliance, 203 Foust Building.

Part D - IRB Action

Exempt Review (Date: / /)

Expedited Review (Date: / /)

RESPOND TO NUMBERS 1 THROUGH 8 ON SEPARATE PAPER. SUBMIT NO MORE THAN 3 PAGES FOR YOUR ANSWERS. Supporting materials (e.g. letters and consent forms) should be attached.

1. BRIEF STATEMENT OF PROJECT GOALS

2. PROTOCOL: Procedures: what will be done? How long will subjects require to complete procedures?

Name and description of data gathering tool (if not well known, attach a copy)

How will data be recorded? (audiotapes, videotapes, written records)

Number of participants, respondents, or participants. From where will participants be obtained?

What, if any, relationship exists between the researcher and the participants, and between the researcher and agencies (e.g., schools, hospitals) participating in data collection? (Example: Is researcher employed at the agency?)

Any special situations (Example: Deception used because full disclosure prior to procedure would bias data.)

If data collection is done in class, explain what students who do not participate will be doing.

Attach statement of approval from any agencies (e.g., schools, hospitals) that will be involved with recruitment of participants or data collection.

3. BENEFITS: Describe the benefits to individual participants and to society.

4. RISKS: Describe the risks to the participants and precautions that will be taken to minimize them. This includes physical, psychological, and sociological risks.

How will confidentiality of data be maintained? Attach signed confidentiality agreements (form attached) for members of research team who will have access to personal data on human research participants.

Final disposition of data (What will be done with questionnaires, inventories, videotapes, and/or audiotapes? How long will they be stored, and how will they be destroyed?)

How would you describe the level of risk for participants taking part in this project?

☐ No risks ☒ Minimal risks ☐ More than minimal risks

5. POPULATION: Briefly describe your participant population. Will you exclude persons on the basis of gender, race, color, or any other demographic characteristic? If so, justify.

6. PARTICIPANT CONSENT: Describe how and where participants will be informed of their rights and how informed consent will be obtained and documented. Attach a copy of consent form, oral presentation (if used), and any materials to be used in recruitment (e.g. fliers, advertisements). See next page for details on content of Consent Forms.

Note: Signed consent forms must be retained in a secure location, for a minimum of three (3) years, after completion and available for IRB review.

7. CONFLICT OF INTEREST: At any time will any members of the research team or their immediate family members have financial interest in, receive personal compensation from, or hold a position in an industry sponsoring this study, or otherwise have potential conflict of interest regarding conduct of this study?

☒ N/A no industry sponsors ☐ NO ☐ YES If yes, attach Potential Conflict of Interest in Research form.

8. PHI: Personally identifiable health information (PHI) is defined by HIPAA to include data on a person's physical or mental heath, health care, or payment for health care. As part of this study, will you obtain PHI from a hospital, health care provider, or other HIPAA-defined Covered Entity? (If unsure, read the Application to Use PHI in Research.)

☒ NO ☐ YES If yes, attach the Application to Use PHI in Research (available from ORC website.)

I certify that the statements made herein are accurate and complete. I agree to inform the Board in writing of any emergent problems or proposed procedural changes. Should changes be made, I further agree not to proceed with the research until the Board has reviewed and approved the changes that I propose to make in the protocol.

_____

Principal Investigator Date

_____

Faculty Sponsor (for student investigators) Date

1. BRIEF STATEMENT OF PROJECT GOALS
The purpose of the study is to establish normative, baseline data for measures of lower extremity postural alignment, agility, and strength in an athletic population.
2. PROTOCOL:
Procedures and Instrumentation: Height and mass will be measured and recorded manually. Age and sex, will be subjectively reported by the subject and manually recorded by the examiner. Then 13, non-invasive lower extremity alignment variables will be measured:
1. Anterior/Posterior Pelvic Angle
2. Hamstring Extensibility
3. Thomas-Kendal test (hip flexor tightness)
4. Standing Quadriceps (Q) Angle (thigh muscle angle in standing)
5. Tibiofemoral Angle (knee angle in standing)
6. Femur (thigh) Length
7. Tibia (lower leg) Length
8. Navicular Drop (foot pronation)
9. Knee Laxity
10. Genu Recurvatum (knee hyperextension)
11. General laxity tests
12. Hip Anteversion (hip rotation)
13. Tibial Torsion (lower leg rotation)

Measures 1,2,4-10,12-13 have been used in a previous approved project. Measures 3 and 11 will be added to this protocol. All measures will be recorded to the nearest degree or millimeter on protocol sheets and later in a computer database program. All standing measures will be taken with the subject in a relaxed stance, with feet placed shoulder width apart and their toes pointing forward. Each measure will be taken three times during the session. The lower extremity postural alignments will be measured using one of the following instruments: an inclinometer, a standard goniometer, a caliper, a straight ruler, or a KT 2000 Knee Arthrometer (Medmetric corporation, San Diego, CA)..

Functional, agility testing will happen after the alignment testing in the following order: Single Leg Balance (BESS), Double Leg Drop Landing and Jump , Single Leg hop, T test, Agility Square, and Vertical Jump. All testing will be preceded by adequate practice time to ensure the tasks are performed correctly and safely.

Balance Tests (BESS) - The BESS comprises 6 conditions: double-leg, single-leg, and tandem stances on both a firm surface and foam surface. Each of the 6 conditions are performed for 20 seconds, with the number of balance errors recorded.
Double Leg Drop Landing and Jump- Subjects will perform 3 landings from a height of a 0.45 m (1.5 ft.) box onto the ground. When landing, subjects will be instructed to place their hands on their hips and to drop down onto the ground with both feet while

immediately jumping directly up into the air and then returning to the ground. Subjects will complete 3 landings while being videotaped.
Single Leg Hop for distance- Subjects will stand on one leg and will perform 3 maximal hops forward with the same leg. Subjects will complete this 2 times and attempt to hop as far as possible.
T test for time- Subjects will sprint forward 10 yards, touch the tip of the cone, shuffle to the left 5 yards, touch a cone, shuffle to the right 10 yards, touch a cone, shuffle to the left 5 yards, touch a cone, and complete the task by backpedaling 10 yards. Subjects will complete this 2 times as fast as possible.
Agility Square for time - The subject sprints forward 10 yards, touches the tip of the cone, shuffles to the left 10yards, touches a cone, back-pedals 10 yards, touches the cone, and then completes the test by backpedaling 10 yards. Subjects will complete this 2 times as fast as possible.
Vertical Jump - Subjects will reach as high as they with one arm can to determine their standing reach. Subjects will them be instructed to jump as high as possible off both feet, reaching up as far as possible. Subjects will complete 3 jumps

Strength Assessment will include bilateral Isokinetic (constant velocity) testing of the knee extensors and flexors at 60 and 180 degrees per second. Five repetitions of both flexion and extensions will occur at each testing velocity.
Total time for all testing will be approximately 2 hours.

Name and description of data gathering tool (if not well known, attach a copy)
Instruments that will be used to take these anatomical measures include an inclinometer, a standard goniometer, a caliper, a straight ruler and ligament testing device. All of these devices are routinely used for these measures, and have been utilized in previously approved protocols.
Functional and Agility testing will be measured with a stop watch or measuring tape.
Strength Assessments will be performed on a Biodex System 3 Dynamometer that is routinely used for both clinical and laboratory measurement and has been utilized in previously approved protocols.
How will data be recorded? (Audiotapes, videotapes, written records)
Data will be obtained and maintained in electronic and written format.
Demographic, non-invasive lower extremity alignment measures, and agility and strength measures recorded manually (BESS, Drop Landing, Single Leg Hop, T-Test, Agility Square, and Vertical Jump) while strength assessment measures will be collect through a computer program. Additionally all BESS and Drop landing Testing will be videotaped. All data will be reduced and entered into a computer database for storage and later analysis. All data will then be transferred to computer storage disks for later offline analyses. Data will be stored in a locked room, identified by subject code number, accessible only to the investigators. Electronic and Video data will be maintained for 2 years after all manuscripts have been published then data will be permanently destroyed.

Number of subjects, respondents, or participants.

175 recreationally active and apparently healthy college aged subjects ranging in age between 17-25 yrs will be recruited. Participants will include both males and females.
From where will subjects be obtained?
Subjects will be recruited from the university athletic teams. (See attached flyer)
What, if any, relationship exists between the researcher and the subjects?
Some participants may be students in ESS department.
What, if any, relationship exists between the researcher and agencies (e.g., schools, hospitals) participating in data collection? (Example: Is researcher employed at the agency? In what capacity?) N/A
Any special situations (Example: Deception - Full disclosure prior to procedure is not feasible because biased data will result.) Non
If data collection is done in class, explain what students who do not participate will be doing. N/A

Attach statement of approval from any agencies (e.g., schools, hospitals) that will be involved with recruitment of subjects/participants or data collection. N/A
3. BENEFITS: Describe the benefits to individual participants and to society.

The individual will receive no direct benefit for participating in this study. This study will establish normative data for lower extremity postural alignment, agility, and strength in an athletic population. .

4. RISKS: Describe the risks to the subjects/participants and precautions that will be taken to minimize them. This includes physical, psychological, and sociological risks.

How would you describe the level of risk for participants taking part in this project?

☐ No risks ☒ Minimal risks ☐ More than minimal risks

There is minimal risk to participating in this study. These anatomical, agility, and strength measures are commonly used in the clinical, athletic, and research settings. There is a very small chance of muscle or joint injury during the agility and strength tests. Subjects will be given adequate practice and warm-up to help minimize these risks. In the extremely rare circumstance that a minor injury would occur, the certified athletic trainer that is assigned to the athlete's respective UNCG team will be available to care for the injury as would normally be done for any injury sustained by a UNCG athlete.

How will confidentiality of data be maintained?
Code numbers will be assigned to the data. The list linking the names to the code numbers will be kept in a locked file, accessible only to the investigators.
Final disposition of data (What will be done with questionnaires, inventories, videotapes, and/or audiotapes? How long will they be stored, and how will they be destroyed?)
Data will be stored on a PC hard drive or on video tapes in the ANRL in a locked office. Data will be retained for two years following publication of manuscripts.
How would you describe the level of risk for subjects participating in this project?

No risks X Minimal risks More than minimal risks

5. Population: Briefly describe your subject population. Will you exclude persons on the basis of gender, race, color, or any other demographic characteristic? If so, justify. Current UNCG athletes ranging in age from 17-25 will participate in the experiment. Subjects must be a member of a UNCG athletic team with no current history of injury to the lower extremity, or any previous history that would affect their ability to perform measures of agility and strength. Subjects will not be excluded on basis of gender, race, color, or any other demographic characteristic.

6. Subject Consent: Describe how subjects will be informed of their rights and how informed consent will be obtained and documented. Attach a copy of consent form, oral presentation (if used), and any materials to be used in recruitment (e.g. fliers, advertisements). See next pages for details on content of Consent Forms.

7. CONFLICT OF INTEREST: At any time will any members of the research team or their immediate family members have financial interest in, receive personal compensation from, or hold a position in an industry sponsoring this study, or otherwise have potential conflict of interest regarding conduct of this study?
☒ N/A no industry sponsors ☐ NO ☐ YES If yes, attach Potential Conflict of Interest in Research form.

8. PHI: Personally identifiable health information (PHI) is defined by HIPAA to include data on a person's physical or mental heath, health care, or payment for health care. As part of this study, will you obtain PHI from a hospital, health care provider, or other HIPAA-defined Covered Entity? (If unsure, read the Application to Use PHI in Research.)
☒ NO ☐ YES If yes, attach the Application to Use PHI in Research (available from ORC website.)

I certify that the statements made herein are accurate and complete. I agree to inform the Board in writing of any emergent problems or proposed procedural changes. Should changes be made, I further agree not to proceed with the research until the Board has reviewed and approved the changes that I propose to make in the protocol.

_____

Principal Investigator Date

_____

Faculty Sponsor (for student investigators) Date

APPENDIX B: CONSENT FORM

THE UNIVERSITY OF NORTH CAROLINA

GREENSBORO

CONSENT TO ACT AS A HUMAN PARTICIPANT: LONG FORM

Project Title: Normative Data for Measures of Postural Alignment, Agility, and Strength in an Athletic Population.

Project Directors: Randy Schmitz PhD & Sandra J. Shultz PhD, ATC

Participant's Name: _____

DESCRIPTION AND EXPLANATION OF PROCEDURES:
The purpose of the study is to establish normative, baseline data measures of lower extremity postural alignment, agility, and strength in an athletic population. In order to qualify for this investigation, you must be a member of a UNCG athletic team with no current history of injury to the lower extremity, or any previous history that would affect your ability to perform measures of agility and strength. If you meet these criteria, you will be asked to attend one, 2 hr testing session. During the test session, we will record your height, weight, and age, and we will take measures of your hip, knee and foot alignment using standard measurement devices (ruler, goniometer, etc). Knee laxity (the amount of movement at the knee joint when a small force is applied to the lower leg) will be recorded using a commercially available ligament testing device.

Functional, agility testing will happen after the alignment testing in the following order: Single Leg Balance (BESS), Double Leg Drop Landing and Jump, Single Leg hop, T test, Agility Square, and Vertical Jump. All testing will be preceded by adequate practice time to ensure the tasks are performed correctly and safely.

1. Balance Tests (BESS) - The BESS comprises 6 conditions: In a random order you will be asked to balance for 20 seconds using a single leg stance, double leg stance, or heel to toe stance on firm and foam surfaces. Each of the 6 conditions will be performed for 20 seconds.
2. Double Leg Drop Landing and Jump- You will perform 3 landings from a height of a 0.45 m (1.5 ft.) box onto the ground. When landing, you will be instructed to place your hands on your hips and to drop down onto the ground with both feet while immediately jumping directly back up into the air and then returning to the ground. .
3. Single Leg Hop - You will stand on one leg and will perform 3 maximal hops forward with the same leg. You will complete this 2 times on each the right and left leg.
4. T test - You will sprint forward 10 yards, touch the tip of the cone, shuffle to the left 5 yards, touch a cone, shuffle to the right 10 yards, touch a cone, shuffle to the left 5 yards, touch a cone, and complete the task by backpedaling 10 yards. You will complete this 2 times as quickly as possible.
5. Agility Square - You will sprint forward 10 yards, touch the tip of the cone; shuffle to the left 10 yards, touch a cone; backpedal 10 yards, touch a cone; and then complete the test by backpedaling 10 yards. You will complete this 2 times as quickly as possible.
6. Vertical Jump - You will reach up as high as you can with one arm to determine your standing reach. You will then be instructed to jump as high as possible off both feet, reaching up as far as possible. You will complete 3 jumps

Strength Assessment will include bilateral Isokinetic (constant velocity) testing of the knee extensors and flexors at 60 (slower) and 180 degrees per second (faster). You will be seated in a strength testing device and your trunk, thigh and lower leg will be affixed to the device with Velcro straps. After submaximal practice repetitions you will complete five repetitions of both flexion and extension at 60 degrees per second (slower). The practice repetitions and five repetitions of both flexion and extension will them be repeated for the 180 degrees per second (faster) condition. Total time for all testing will be approximately 2 hours.

RISKS AND DISCOMFORTS:
There is an extremely minimal risk of muscle or joint injury from the agility and strength testing. If at anytime the testing causes you any discomfort or concern, please notify the examiner immediately.

POTENTIAL BENEFITS:
There are no direct benefits to you from participating in this study.

COMPENSATION/TREATMENT FOR INJURY: In the extremely rare circumstance that a minor injury would occur, the certified athletic trainer that is assigned to your UNCG team will be available to care for the injury as would normally be done for any injury sustained by a UNCG athlete. If you have a question about any injury incurred from the study please contact Mr. Eric Allen at (336) 256-1482

CONSENT:
By signing this consent form, you agree that you understand the procedures and any risks and benefits involved in this research. You are free to refuse to participate or to withdraw your consent to participate in this research at any time without penalty or prejudice; your participation is entirely voluntary. Your privacy will be protected because you will not be identified by name as a participant in this project.

The research and this consent form have been approved by the University of North Carolina at Greensboro Institutional Review Board, which insures that research involving people follows federal regulations. Questions regarding your rights as a participant in this project can be answered by calling Mr. Eric Allen at (336) 256-1482. Questions regarding the research itself will be answered by Randy Schmitz by calling (336) 334-3031 or Sandra Shultz by calling (336) 334-3027. Any new information that develops during the project will be provided to you if the information might affect your willingness to continue participation in the project. You will receive a copy of this consent form.

By signing this form, you are agreeing to participate in the project described to you by Randy Schmitz and/or Sandy Shultz.

_____ _____
Participant's Signature* Date

*If participant is a minor, complete the following:
Participant is _____ years old.

_____ _____
Custodial Parent(s)/Guardian Signature(s) Date

APPENDIX C: FEMALE HORMONE HISTORY QUESTIONNAIRE

This questionnaire asks questions about your menstrual cycle. As a reminder, this information is strictly confidential. None of this information will be shared with anyone besides the study investigators. Your survey uses a coded identification number in substitution for your name. If you have any questions, or do not understand any of the questions, please let us know.

Subject Code:_____ Date:_____ Age:_____

1. How old were you when you started your menstrual periods? _____Years

2. When was the first day of your last period (month/day)?_____

3. On average, how many days are there between your menstrual periods (i.e. 21 days, 28 days, 32 days, etc.)?_____

4. How many menstrual periods have you had in the last 12 months?_____

5. Have you missed any menstrual periods within the last 12 months? ____YES ____NO

6. Since starting your menstrual periods, has there ever been an extended time where you did not have a menstrual period? ___YES ___NO If YES, when and how long? _____

7. Do you know when your next menstrual periods will start? ____YES ____NO
When will this be (month/day)? _____

8. Do you exhibit premenstrual symptoms? ____YES ____NO

On a scale of 0-10, please indicate the severity: **0_____5_____10**
no symptoms severe symptoms

Check all that apply:

Bloating: _____	Mood Swings: _____	Spotting: _____
Irritability: _____	Food Cravings:_____	Other: _____

9a. Are you currently using estrogen therapy/birth control medicine for any reason?_____YES_____NO

_____Birth control pills: Brand Name: _____
_____Birth control patch
_____Injections
_____Other Please specify: _____

9b. How long? (months/ years) _____