Study Protocol

Evaluation of rGH Therapy to Prevent Muscle Atrophy in Patients With ACL Tears

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University of Michigan Medical School IRB Protocol: HUM00087702

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Abstract and Hypothesis

Anterior cruciate ligament (ACL) tears are among the most frequent traumatic knee injuries that occur in athletes. Despite advances in minimally invasive surgical reconstruction techniques and aggressive rehabilitation, this atrophy and loss of strength can persist even after patients return to full activity and can place them at considerable risk for re-injury and developing osteoarthritis (OA). The design of new therapeutic interventions to prevent muscle atrophy is needed to advance the care of patients who suffer from ACL injuries. The growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis plays an important role in promoting muscle growth and protecting muscle from atrophy. While GH therapy has shown promise in protecting immobilized muscle from various models of disuse atrophy, it remains unknown whether GH can help to restore strength and protect against the loss in strength that occurs after ACL tear. Our purpose is to conduct a randomized, double-blind, pilot placebo-controlled study to gain additional insight into the safety and biological effects of GH on muscle size and strength in patients with ACL tears. ***We hypothesize that, compared to placebo treatment, a six week perioperative course of GH treatment will prevent muscle atrophy and weakness in patients undergoing ACL reconstruction, and cause the return of full quadriceps muscle strength by 6 months after surgery.*** If our hypothesis is supported, GH therapy may help to accelerate the safe return to play of patients that suffer ACL tears, and help to prevent the long-term OA and reduction in quality of life that occur after these traumatic knee injuries.

Background and Rationale

Epidemiology of ACL Tears, Muscle Weakness and Development of Osteoarthritis. ACL tears are among the most frequent knee injuries in physically active individuals, with tear rates in the US around 250,000 per year (Griffith 2005). Despite our best efforts in rehabilitation, many patients that suffer from ACL tears and undergo ACL reconstruction (ACL-R) have a persistent atrophy and weakness of their quadriceps muscles. In addition to reducing physical performance and increasing the susceptibility to repeated injuries, several studies have indicated this loss of strength can alter knee kinematics in a way that promotes the development of early-onset osteoarthritis (OA). For example, in a population of female soccer players who suffered ACL tears at an average age of 19 years, 12 years after the injury there was radiographic OA present in 51% of injured knees, while only 7% of uninjured contralateral knees showed signs of OA (Lohmander 2004). In male soccer players who suffered ACL tears, 14 years after the tear 75% of injured knees demonstrated signs of OA, compared to only 4% of uninjured, contralateral knees (von Porat 2004). Surgical reconstruction of torn ACLs, while helpful in restoring some joint kinematics and proprioception, does not appear to modify the likelihood of development of OA in ACL-R patients (Roos 2005).

Many studies have reported persistent weakness quadriceps muscles following ACL-R, typically around 30-40% at 6 months to one year following surgery (Ingersoll 2008). This weakness appears to occur for all types of ACL autografts and allografts. Muscle weakness following ACL tear is associated with the development of OA (Palmieri-Smith 2008, Keays 2010). This weakness likely plays two roles in the development of OA following ACL tear. First, muscle is an important dynamic stabilizer of the knee joint. Strength is required to precisely control the 3D motion of the knee during locomotion, and muscle weakness can lead to the development of pathological mechanics that cause greater wear of articular cartilage (Andriacchi 2004). Second, because muscle can transmit loads across the knee joint and function as a "shock absorber" during eccentric contractions (LaStayo 2003), greater strength allows the muscle to transmit forces throughout the kinetic chain and reduce loads on articular cartilage.

Developing strategies to prevent muscle atrophy following ACL tears will help reduce the rates and severity of knee OA and enhance the physical performance of athletes that undergo ACL-R.

While the vast majority of patients who suffer ACL tears are recreational athletes or "weekend warriors", ACL tears can have a significant negative impact on the playing careers for professional and elite athletes as well. In the NBA, almost a quarter of players who tear their ACLs do not return to playing professional basketball (Busfield 2009). NFL players that suffer ACL tears only have a 63% chance of returning to play, and for those athletes who do return to play in the NFL miss on average 78% of the regular season and require almost 11 calendar months to return to competition (Shah 2010). Improving the recovery of professional athletes with ACL tears will likely have an important short term impact on their playing careers, but also help to avoid long-term osteoarthritis and knee joint weakness that can occur a decade or more after retiring from professional sports.

Molecular Regulation of Muscle Atrophy. At the molecular level, maintaining skeletal muscle mass is a balance between protein synthesis and protein degradation systems. Muscle atrophy can come about due to increased protein degradation or decreased protein synthesis. An overview of these pathways is presented in Figure 1. There are four main pathways that regulate skeletal muscle atrophy, the ubiquitin proteasome, autophagy/lysosome, caspase-apoptotic and calpain systems (reviewed in Sandri 2008 and Gumucio 2013). Myostatin is one of the most well-known atrophy-inducing cytokines, and plays a central role in the activation of all but the calpain proteolytic pathways. The potent effects of myostatin can be readily observed at the whole muscle level, as even small amounts of this cytokine leads to rapid and profound muscle atrophy and reductions in force production (Zimmers 2002, Li 2004, Mendias 2006, Mendias 2011). Since myostatin directs the major proteolytic pathways in skeletal muscle, it is an attractive therapeutic target for the prevention of muscle atrophy. Direct inhibitors of myostatin are currently in development and are likely at least several years away from when they might appear on the market. Employing an indirect way of inhibiting myostatin, though, can be accomplished with existing, approved drugs.

Growth occurs in two parts in skeletal muscle. Muscle fibers are multinucleated cells, and as fibers grow they rely on a pool of resident stem cells, called satellite cells, to provide new nuclei to growing fibers (Hawke 2001). Prolonged periods of muscle disuse can decrease the number of viable muscle stem cells, and this may result in a reduction in the number of cells able to regenerate the muscle as it recovers from inactivity and disuse (Favier 2008). Protein synthesis in muscle fibers takes place via activation of the Akt/mTOR pathway. As shown in Figure 1 (reviewed in Sandri 2008 and Gumucio 2013), growth hormone (GH) and insulin-like growth factor-1 (IGF-1) are potent inducers of muscle growth by directly activating the Akt/mTOR pathway and by promoting satellite cell proliferation (Vinciguerra 2010). Importantly, GH and IGF-1 can also directly inhibit the signaling activity of myostatin and other atrophy-inducing cytokines.

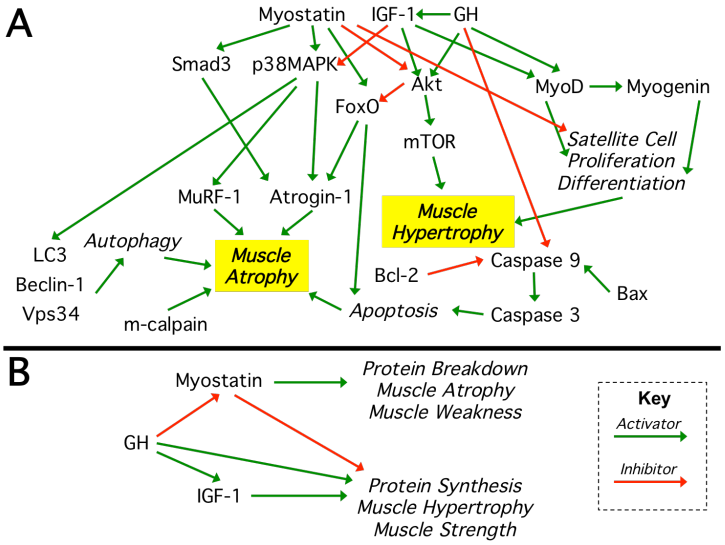

Figure 1. (A) Detailed overview of cellular signaling pathways that regulate muscle hypertrophy and atrophy. (B) Simplified overview demonstrating the role of GH, IGF-1 and myostatin in regulating muscle hypertrophy and atrophy.

Changes in Myostatin and Muscle Strength Over the Course of ACL Rehabilitation. To determine if circulating levels of myostatin changed over the course of ACL reconstruction and rehabilitation, we conducted a study of physically active patients who suffered an ACL tear (N=18, mean age 28±2 years; Mendias 2013). Patients were evaluated at 7 total visits, beginning one week prior to surgery, and seen at 6 post-operative visits until discharge at 26 weeks (Figure 2A). Blood was drawn at all study visits to measure circulating levels of myostatin and many other cytokines via ELISA or Luminex assay. Isokinetic knee extension strength (60º/second) was measured at the pre-op and last four post-op visits. All patients completed a supervised, accelerated rehabilitation protocol (Beynnon 2005a). As shown in Figure 2B, there was a significant elevation of circulating myostatin levels at the first and second post-op visits. Knee extension strength was normalized to the uninvolved side at each study visit, and as shown in Figure 2C, at the time of surgery patients had reduced strength, and by the time of discharge patients still had a 30% strength deficit. These results are in agreement with results from a rat model of ACL tears (Delfino 2013), in which increases in the expression of intramuscular myostatin, atrogin-1 and MuRF-1 corresponded with muscle atrophy after ACL transection.

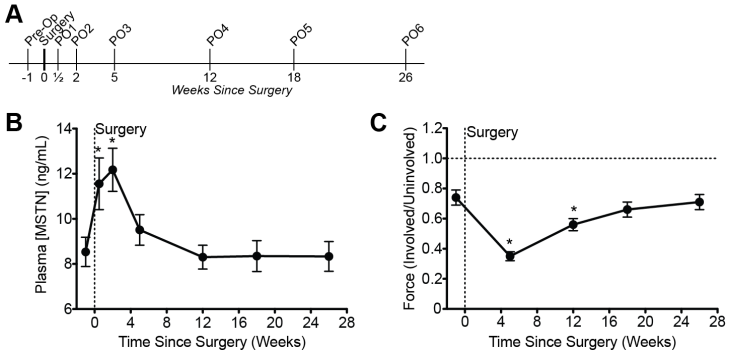

Figure 2. Changes in circulating myostatin and knee strength over the course of ACL reconstruction and rehabilitation. (A) Overview of study time points. Circulating (B) myostatin levels, and (C) knee isokinetic extension. Values are mean±SE, N=18. *, significantly different from pre-op value (one-way repeated measures ANOVA, Dunnett's post-hoc sorting).

GH to Counteract Muscle Atrophy and Weakness. GH has been well studied in several models of muscle atrophy and weakness. Although GH anecdotally has a reputation as a strong anabolic agent, several studies in humans have demonstrated that only at high doses does GH treatment induce appreciable muscle hypertrophy in otherwise healthy muscle (Chikani 2014). It is only when a

muscle is undergoing atrophy that lower, physiological doses of GH appear to be able to counteract the atrophy and prevent a loss in weakness. In both animal models of disuse atrophy (Linderman 1994, Lieber 1997, Dalla Libera 2004) and in humans with disuse atrophy (Hammarqvist 1992, Gullett 2010, Boesen 2013), GH administration was demonstrated to be a safe way to effectively counteract muscle wasting, and preserve strength, function and quality of life. While several studies have been conducted in frail patients with systemic diseases, a very striking recent study from the laboratory of Michael Kjaer in Copenhagen demonstrates the potential of GH to prevent muscle atrophy in younger, physically active individuals that are experimentally immobilized to simulate a scenario that might happen after an injury (Boesen 2013). In this study, patients were immobilized in a cast from hip to ankle on their dominant leg for two weeks, and then went through a period of rehabilitation training for six weeks. One group received GH while in the cast, and the other group served as a placebo control group. GH therapy very clearly protected both the size and strength of the quadriceps muscles from atrophy, as shown in Figure 4. GH also enhanced the expression of several genes related to muscle hypertrophy. While it remains to be determined if GH can protect a muscle from atrophy after joint injury and with a longer period of immobilization and limited activity, these results are promising for the potential use of GH to prevent atrophy after musculoskeletal injury in young, healthy individuals.

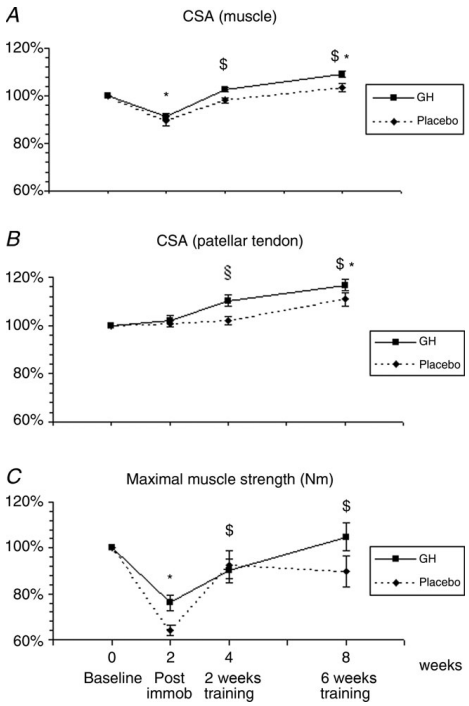

Figure 2. Changes in cross-sectional area (CSA) (muscle and patellar tendon) and maximal muscle strength during 2 weeks of immobilisation and 6 weeks of re-training in young males ($n = 20$) with either recombinant human growth hormone (rhGH) ($n = 10$) or placebo ($n = 10$)
A, CSA of the quadriceps muscle; time effect ($P < 0.01$) and group interaction ($P = 0.09$). *B*, CSA of the patellar tendon; time effect ($P < 0.01$) and group interaction ($P < 0.05$). *C*, maximal isometric muscle strength; time effect ($P < 0.01$) and group interaction ($P = 0.09$). *Time effect, $P < 0.05$, compared with baseline (both groups). $Time effect, $P < 0.05$, compared with Post immob (both groups). §Time effect, $P < 0.05$, baseline/Post immob. *vs.* 2 weeks of re-training (GH). Data are means ± SEM.

Figure 3. From Boesen 2013.

Anabolic steroids are another well-studied class of compounds that can also protect muscle from disuse atrophy (Orr 2004). However, there are several systemic side-effects of a prolonged course of anabolic steroids such as elevated cholesterol and liver enzymes, alopecia and gonadal hypoplasia (Orr 2004). While these drugs have the potential to prevent muscle atrophy after inactivity, they also have a well-described negative impact on tendon and ligament healing (Freeman 1995, Inhofe 1995, Orr 2004, Marqueti 2006). In the case of a patient recovering from an ACL-R, anabolic steroids could possibly harm the proper healing and integration of the ACL graft. While GH has not directly been studied in the context of ACL healing, GH appears to be beneficial in promoting the healing and regeneration of soft tissue injuries (Vestergaard 2012, Boesen 2014). We therefore anticipate GH to at the minimum not impair, but also potentially help to accelerate the repair and reintegration of the ACL graft.

Study Design and Experimental Methods

Overall Study Design. This is a prospective, double-blind placebo-controlled basic science-focused study to evaluate the ability of GH to prevent atrophy in patients undergoing surgical reconstruction of a torn ACL. The time points of the study were chosen based on our previous work in studies of ACL-R (Mendias 2013), and because they represent important hallmarks in the rehabilitation of patients undergoing rehab for an ACL-R (Beynnon 2005a). With the exception of growth hormone or placebo administration, blood draws, additional MRI scans, strength measures and surveys, the study does not deviate from the standard of care typically provided to patients undergoing ACL-R. An overview of the timeline of study visits is presented in Figure 4 and Table 1. This study is registered on Clinicaltrials.gov (NCT02420353) and was granted an IND exemption by the FDA (IND 123189).

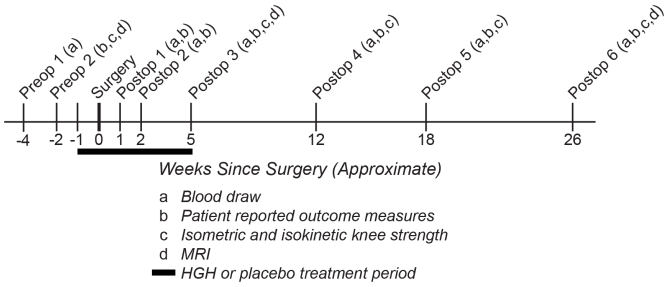

Figure 4. Overview of the study time points.

Study Visit	Measures Performed
1. Preop 1 (~4 wk prior to surgery)	History/vitals, blood draw, vital signs
2. Preop 2 (~2 wk prior to surgery)	Surveys, BioDex (isometric, isokinetic), MRI, medication instruction, vital signs
3. Postop 1 (~1 wk after surgery)	Surveys, blood draw, medication check, vital signs
4. Postop 2 (~2 wk after surgery)	Surveys, blood draw, medication check, vital signs
5. Postop 3 (~5 wk after surgery)	Surveys, BioDex (isometric, isokinetic), MRI, blood draw, medication check, vital signs
6. Postop 4 (~12 wk after surgery)	Surveys, BioDex (isometric, isokinetic, balance), blood draw, vital signs
7. Postop 5 (~18 wk after surgery)	Surveys, BioDex (isometric, isokinetic, balance), blood draw, vital signs
8. Postop 6 (~26 wk after surgery)	Surveys, BioDex (isometric, isokinetic, balance), MRI, blood draw, vital signs, patient discharged from the study

Table 1. Timeline of study visits and measures performed in this proposal.

Patient Selection and Recruitment. This study is based on an already approved protocol by the University of Michigan Medical School Institutional Review Board (study # HUM00038126). All patients will provide informed consent prior to participating in the study.

Inclusion Criteria:

1. Males between the ages of 18 and 35
2. Have acute unilateral complete ACL tears with or without bucket handle medial meniscus tears that occurred within the past 6 months
3. Consent to undergo an ACL reconstruction by Dr. Bedi using a patellar tendon or hamstring autograft
4. Will be performing supervised post-operative rehabilitation at UMHS MedSport at Dominos Farms (4008 Ave Maria Dr, Ann Arbor, MI, 48106)

Exclusion Criteria:

1. Patients who are undergoing a revision ACL reconstruction
2. Had a previous injury to the involved knee
3. Have an allergy to recombinant GH
4. Have a BMI<20 or >35

5. Have a growth disorder of bones or connective tissue, type 1 diabetes mellitus, type 2 diabetes mellitus, or who have a history of carpal tunnel syndrome, trigger finger, myopathy, cancer, endocrine disorder, hypertension or rheumatologic disorder.
6. Systolic blood pressure >140mm Hg or diastolic blood pressure >90mm Hg, or with resting heart rate >110 BPM or <40 BPM at screening.
7. Additionally, because GH is currently listed as a banned substance by the World Anti-Doping Agency (WADA), National Collegiate Athletics Association (NCAA) and most professional sports agencies, we will exclude patients who are current collegiate, professional or elite athletes.

We will recruit up to 48 total patients (24 placebo control, 24 GH), recruited at a rate up to 3 to 6 patients per month. Interim data analysis will be performed, and actual sample sizes may be lower based on safety assessments and the effect of GH on strength.

We will exclude women from this study due to the consequences GH can have on reproductive cycles, and because GH can decrease the efficacy of birth control medications. Additionally, GH could have detrimental effects on a developing fetus. For these reasons, we are excluding women from participation in this study.

Screening Visit and Initial Study Measurements. Patients who consent to surgery and are interested in participating in the study will be seen at a screening visit. The screening visit will typically occur after a normal clinic visit, but may be scheduled at the convenience of the patient. The vital signs of patients will be measured, the history will be recorded, and patients will be asked to report to an M-Labs testing location for fasting blood work within the next 5 days. The labwork includes complete blood count with platelet differential, metabolic panel (glucose, urea nitrogen, creatinine, sodium, potassium, chloride, CO_2, calcium, total protein, albumin, total bilirubin, AST, ALT and alkaline phosphatase), lipid panel (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol), hemoglobin A_1C, high sensitivity CRP, creatine kinase, and IGF-1 (as described below). Patients who have normal values based upon M-Labs guidelines will be permitted to enroll in the study. Patients with minor deviations (typically less than 10%) from normal values may be allowed to participate based on consultation with Dr. Barkan and Dr. Bedi. Patients who do not pass screening will be provided with $25 compensation.

After assessing vitals and lab work, patients will be scheduled for an MRI, and for an appointment at MedSport where strength and stability measurements, completion of surveys and instruction on medication use will be performed. All screening and initial study measurements will be performed no more than one week before surgery.

Surgical Reconstruction and Postoperative Rehabilitation. Patients will have a thorough history and physical examination performed by Dr. Bedi. Examination under anesthesia will be performed to confirm functional instability and document the extent of meniscus tear. Surgical reconstruction of the ACL will be performed using autologous graft from the ipsilateral knee, and a partial meniscectomy or meniscal repair will be performed to repair the meniscal defect. A single-bundle, anatomic "center-center" ACL-R will be performed (Bedi 2009). Patients will follow a standard, supervised accelerated ACL rehabilitation protocol postoperatively (modified from Kim 2003, Beynnon 2005a). Rehabilitation begins with quad sets, straight leg raises, ankle pumps, heel slides, and partial weight bearing up to 25% in the first week. These exercises continue into the second and third weeks, with the addition of electrical stimulation for muscle reeducation, leg press, core and hip exercises, stationary biking, and progression to 50% weight bearing. From weeks 4 to 12, additional closed chain resistance exercises are added and patients progress to full weight bearing and open chain exercises. From weeks 12 to 20,

exercises increase in load and complexity, and patients progress to a jogging program. Agility and plyometric training begin at week 20, and continue in complexity and intensity based on passing functional tests demonstrating appropriate gait and motor control.

Surveys and Recording of Patient-Reported Adverse Events. Patients will complete patient reported outcome measure (PROM) surveys at each visit to measure pain, function and physical performance, including (i) the Veterans Rand-12 (VR-12), to measure overall health-related quality of life (Selim 2009); (ii) the International Knee Documentation Committee (IKDC) survey, to evaluate knee-specific sports function (Anderson 2006), and (iii) the Knee injury and Osteoarthritis Outcome Score (KOOS), to evaluate knee-specific general and sports function (Roos 2003). Patients will also be asked to report adverse events associated with the use of GH, as described in the GH Self Reporting Symptom Form.

BioDex Strength Testing and Ultrasound Muscle Cross-Sectional Area Measurements. Whole muscle isometric and isokinetic strength measurements will be performed in a BioDex System 3 dynamometer as described (Mendias 2013). Maximum isometric force will be measured at 45º and 90º of knee flexion. Maximum isokinetic flexion and extension will be measured over a 90º arc at a velocity of 60º/second. All measures will be performed bilaterally, and the highest force from a series of five trials from each side will be used.

MRI Muscle Volume Measurements. Bilateral MRI scans will be performed at the pre-operative, third post-op and sixth post-op visit. Scans will be performed in a 3.0T magnet and will extend from hip to knee. The volume of the quadriceps and hamstring muscle groups will be determined using quantitative MRI analysis software.

Blood Draw. Approximately 6-8mL of blood will be drawn from an antecubital vein and collected into either a K_2-EDTA tube for plasma preparation or a clot activator tube for serum preparation. A portion of the plasma or serum will be used for clinical labs performed by UMHS M-Labs and a portion will be stored at -80ºC for measurement of different proteins in Dr. Mendias' lab. The tests performed by M-Labs include a complete blood count with platelet differential, metabolic panel (glucose, urea nitrogen, creatinine, sodium, potassium, chloride, CO_2, calcium, total protein, albumin, total bilirubin, AST, ALT and alkaline phosphatase), lipid panel (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol), hemoglobin A_1C, high sensitivity CRP, creatine kinase, and IGF-1. For measurements performed in Dr. Mendias lab, the levels of myostatin (as a marker of muscle atrophy), MMP-3 (as a marker of cartilage catabolism), and hyaluronic acid (as a marker of cartilage catabolism) will be measured in duplicate using ELISAs as described (Mendias 2013).

Vital Signs. Patients' heart rate, temperature, blood pressure and respiratory rate will be recorded at each visit.

Wallet Card. Patients will be provided with a laminated card to carry in their wallet or purse in case of emergencies. The card will state that the patient is currently enrolled in a clinical trial evaluating GH, that they are taking either GH or placebo and the contact information for Dr. Mendias, Dr. Barkan, Dr. Bedi and the study research coordinator.

Administration of GH or Placebo. The UMHS Pharmacy IDS service will be used to assist with storing, preparing and dispensing drug. We will use Humatrope (somatropin of rDNA origin, Eli Lilly) as the test drug, and sterile bacteriostatic saline with no active hormone will serve as the control. We will use sterile insulin syringes to deliver drug or placebo subcutaneously. Patients will be provided with the manufacturer's instructions for the use of Humatrope, all supplies (needles, alcohol swabs, cartridges containing GH or placebo, disposal container) and will also receive personalized instruction on how to

prepare and deliver GH using a mock system by study personnel. Patients will be instructed to rotate injection sites to minimize discomfort.

GH dose will be calculated by nomogram estimation of body surface area, and will be administered via subcutaneous injection in the abdominal area twice per day (BID) at a dose of 0.5mg GH per body surface area (BSA) in m^2 (0.5mg/m^2). We will use the DuBois equation to predict BSA [$BSA(m^2) = 0.007184 \times Height(cm)^{0.725} \times Mass(kg)^{0.425}$], which is a commonly accepted equation in clinical medicine. This dose has previously been shown by Dr. Barkan to effectively increase systemic IGF-1 levels with minimal side effects (Surya 2009). Patients will begin GH treatment one week prior to surgery and will continue through 5 weeks after surgery. On the day of surgery, patients will not take GH, but otherwise patients will take GH every day over this 6 week period. Patients will be asked to keep a log detailing their injections, and will be asked to return all used and unused supplies for inventory.

While GH is currently not indicated for orthopaedic muscle atrophy, GH does have an FDA indicated use for the treatment of HIV-associated muscle atrophy and wasting. Although the FDA approved label of Humatrope does not specifically indicate an approved use for treating HIV-associated muscle atrophy and wasting, there is a form of somatropin, Serostim, that is FDA approved to be marketed specifically for the of HIV-associated muscle wasting. Other than slight differences in preservatives, Humatrope and Serostim are identical forms of somatropin. The FDA approved dose of somatropin for the treatment of HIV-associated muscle wasting and cachexia is 0.1mg/kg subcutaneously daily, up to 6mg total per day. Several clinical trials have shown these doses to be generally safe, even in immune compromised HIV+ individuals, as well as effective at preventing cachexia, although there is an attenuation of protection against atrophy after 12 weeks (reviewed in Gelato 2007). Based on an individual who has a BSA of 2.25m^2 (188cm tall and 100kg in mass), which is our estimate of the high end of subjects in this study, the dose of somatropin would not differ substantially from levels used to prevent muscle atrophy in patients with atrophy and cachexia caused by HIV infection. There are likely similar mechanisms behind ACL tear-related and HIV-related muscle atrophy, as an elevation in myostatin has been identified in both groups of symptomatic patients (Gonzales-Cadavid 1998, Mendias 2013), and GH can downregulate myostatin expression both directly and indirectly through the activity of IGF-1 (Liu 2003, Morissette 2009). The total dose of GH is also similar to that of Boesen (2013), who used a scheme of 33-50µg/kg/day (for a 100kg subject, this would be approximately 3.3-5mg/day) in otherwise young, healthy subjects who underwent lower limb cast immobilization to simulate muscle atrophy. In this study, subjects in the treatment group (N=10) received an initial dose of 33µg/kg/day for one week followed by 50µg/kg/day for 7 weeks, while the control group (N=10) received placebo injections. Of the 10 subjects who received GH, only 3 reported adverse events (carpal tunnel syndrome, and one of these also reported trigger finger) at the 50µg/kg/day dose. When the dose was adjusted down to 33µg/kg/day the symptoms resolved and the dose was then increased back to a tolerable dose that approached 50µg/kg/day. As the dose of GH used in the proposed study is less than that of Bosen (2013), and symptoms only appeared at 50µg/kg/day, we anticipate that the dose of GH in the current study will not result in side effects in our study population.

Adjustment of Dose for Musculoskeletal or Other Minor Side Effects. For subjects who develop minor musculoskeletal side effects, such as carpal tunnel syndrome or trigger finger, or other minor side effects such as mild hypoglycemia or hyperglycemia (glucose lower than 65 mg/dL or greater than 100 mg/dL) mild headaches, fluid retention, peripheral swelling, and joint pain in the unoperated leg or other area of the body (these symptoms are likely to be present in the operated limb as a result of surgery) the dose of GH or placebo will be reduced to 0.25mg per BSA BID. For patients

with carpal tunnel or trigger finger, they will also receive treatment consisting of ice (15-20 minutes twice daily) and range of motion exercises, which are the standard care for the onset of carpal tunnel or trigger finger. If the symptoms/abnormal values resolve in 7-10 days, the dose will then be titrated as tolerated at increments of 0.05mg per BSA BID every 7 days until reaching a dose at which symptoms do not appear/abnormal values return to normal, or upon reaching the normal study dose of 0.5mg per BSA BID. If symptoms/abnormal values persist at the dose of 0.25 mg per BSA BID greater than 7-10 days, the dose will be decreased to 0.1 mg per BSA BID and titrated up at increments of 0.05mg per BSA BID every 7 days until reaching a dose at which symptoms do not appear/abnormal values return to normal levels. If symptoms/abnormal values do not resolve after 7-10 days at a dose of 0.1mg per BSA BID, the subjects will be discontinued from the study.

Randomization and Assignment of Groups. Patients will be assigned to placebo or GH treatment groups using a block randomized assignment schedule that maintains equal group sizes. All included patients will be assigned unique identification numbers by Christopher Robbins, clinical research coordinator in Dr. Gagnier's lab, with no involvement in this study design or interpretation of any resultant data. Dr. Robbins will then assign identification numbers in a consecutive fashion to either group "maize" (randomly designated by Dr. Robbins as either the placebo or treatment group) or group "blue" (the group not selected to be represented as group A). Dr. Robbins will keep all patient names and assigned identifier lists private in a sealed envelope in a locked cabinet as well as on two separate passcode protected hard drives. He will make the unblinded group identities available to Dr. Barkan, relevant members of the UMHS Pharmacy IDS and safety board members if necessary. Therefore, the patients, investigators, attending physicians, those measuring outcome will be unaware of the allocation of the patients and thus will be blinded.

Risks of Participation. There are some potential risks of GH that will be closely monitored. The risks include: glucose intolerance or reduced insulin sensitivity, fluid retention and peripheral swelling, joint pain, headache, numbness or fatigue, tissue atrophy, pancreatitis, intracranial hypertension, injection site lipoatrophy, and local or systemic allergic reactions. The injection of GH might infrequently cause some slight discomfort and there is a rare risk of infection from injections. There are minimal risks associated with the blood draws -- the needle stick may infrequently hurt and cause bruising, and a rare risk of local infection. There are no known harmful effects from the strong magnetic field used for MRI, although any loose metal object has the risk of causing damage or injury if it gets pulled toward the strong magnet. Strength measurements might infrequently cause some soreness that would last less than a day.

Benefits of Participation. If our hypothesis is supported, patients that receive GH will have improved recovery after ACL-R.

Payment to Subjects. Patients will also receive $1000 for participating in the study. Patients who drop out prior to completion will receive a pro-rated amount ($125 per visit).

Outcome Measures. The overall objectives of the study are to evaluate if GH is safely tolerated in closely monitored patients undergoing ACL-R, and if so, to collect pilot and early efficacy data to determine if subsequent larger clinical trials are warranted. The primary outcome measure of the study is isokinetic knee extension strength at 26 weeks. Secondary outcome measures include isokinetic strength at other time points, MRI measurement of muscle size, PROMs, and circulating protein biomarkers (IGF-1, myostatin, MMP-3, and hyaluronic acid).

Sample Size and Statistical Analyses. We base the sample size for this study on maximum isokinetic knee extension values at the time of discharge from our previous study (Mendias 2013). To detect a 30% difference in isokinetic knee strength between the placebo and GH group, with a power of

80% and α=0.05, requires N=17 per group. To account for any patient attrition, we will aim to recruit 7 additional patients for each group into the study for a total of N=24 per group or N=48 for the entire study. We will use a mixed-effects linear regression model (Zhang 2010) to look at the changes in individual parameters between the placebo and GH groups at multiple time points over the course of the study, as well as the associations between changes of multiple parameters. We will also perform interim data analysis to determine if there are significant differences in strength and other outcomes between groups.

Data and Safety Monitoring Plan. We will have a committee to oversee the safety and adverse events that may come up in this study. The committee will consist of Dr. Ariel Barkan (endocrinologist), Dr. James Carpenter (sports medicine orthopaedic surgeon), and Dr. Tariq Awan (primary care/sports medicine). All lab reports will be sent to Dr. Barkan as they occur, and the full committee will review outcomes and patient (surveys, strength measurements, M-Labs blood work reports, injuries, etc.) every 3 months (or as needed) and review. If an excessive number of adverse events or infrequent but serious events happen, the committee will have the ability to halt the study or unenroll patients from the study. The committee may also stop the trial if there are any concerns that GH is causing harm. These potentially serious events that would cause the patient to unenroll from the study include any vital signs rising to levels above those described in ths exclusion criteria, or signs of intracranial hypertension, severe fluid retention, severe hyperglycemia. These are potentially serious side effects described from previous clinical trials of GH, and although we cannot predict all potentially serious side effects, we will rely on the clinical expertise of the safety monitoring team to assess any other potentially serious but unexpected risks. In these situations the random code will be broken for that patient and any required care for them will then be initiated. Unblinding for all study team members will occur after the last patient has been discharged from care.

At the conclusion of the study, original data will be archived in the UM Department of Orthopaedic Surgery following FDA guidelines.

Adverse Event Reporting Timetable. We will follow the standard IRB adverse events reporting timeline. Adverse events that are expected and non-serious will be reported to the safety committee at their regular meetings, and to the IRB as part of the scheduled continuation application. Serious adverse events will be reported to the safety committee immediately, and to the IRB within 7 days, and to the FDA as per the regulations.

Regulatory Affairs Coordinator. Jaimee Gauthier, clinical research manager in the Department of Orthopaedic Surgery, will coordinate safety committee meetings and ensure the study maintains regulatory compliance with federal and state laws, and UM policies.

References

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