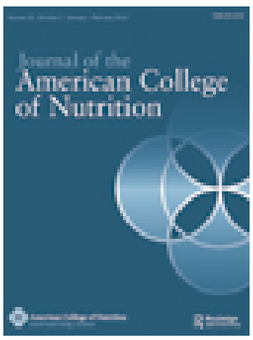

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Original Research

The Effects of Fortetropin Supplementation on Body Composition, Strength, and Power in Humans and Mechanism of Action in a Rodent Model

Matthew H. Sharp, MS, Ryan P. Lowery, MS, C. Brooks Mobley, MEd, Carlton D. Fox, BS, Eduardo O. de Souza, PhD, Kevin A. Shields, MS, James C. Healy, BS, Ned Q. Arick, MS, Richard M. Thompson, BS, Michael D. Roberts, PhD, Jacob M. Wilson, PhD

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Key words: supplements and functional foods, exercise, body composition, bioactive compounds, sports nutrition

Objective: The purpose of this study was to investigate the effects of Fortetropin on skeletal muscle growth and strength in resistance-trained individuals and to investigate the anabolic and catabolic signaling effects using human and rodent models.

Methods: In the rodent model, male Wistar rats (250 g) were gavage fed with either 1.2 ml of tap water control (CTL) or 0.26 g Fortetropin for 8 days. Then rats participated in a unilateral plantarflexion exercise bout. Nonexercised and exercised limbs were harvested at 180 minutes following and analyzed for gene and protein expression relative to mammalian target of rapamycin (mTOR) and ubiquitin signaling. For the human model, 45 (of whom 37 completed the study), resistance-trained college-aged males were divided equally into 3 groups receiving a placebo macronutrient matched control, 6.6 or 19.8 g of Fortetropin supplementation during 12 weeks of resistance training. Lean mass, muscle thickness, and lower and upper body strength were measured before and after 12 weeks of training.

Results: The human study results indicated a Group × Time effect ($p \leq 0.05$) for lean mass in which the 6.6 g (+1.7 kg) and 19.8 g (+1.68 kg) but not placebo (+0.6 kg) groups increased lean mass. Similarly, there was a Group × Time effect for muscle thickness ($p \leq 0.05$), which increased in the experimental groups only. All groups increased equally in bench press and leg press strength. In the rodent model, a main effect for exercise ($p \leq 0.05$) in which the control plus exercise but not Fortetropin plus exercise increased both ubiquitin monomer protein expression and polyubiquitination. mTOR signaling was elevated to a greater extent in the Fortetropin exercising conditions as indicated by greater phosphorylation status of 4EBP1, rp6, and p70S6K for both exercising conditions.

Conclusions: Fortetropin supplementation increases lean body mass (LBM) and decreases markers of protein breakdown while simultaneously increasing mTOR signaling.

INTRODUCTION

The molecular mechanisms that underpin skeletal muscle hypertrophy are complex and involve the interplay between anabolic and catabolic signaling pathways. One key variable that mediates skeletal muscle anabolism is activation of the mammalian target of rapamycin (mTOR) pathway [1]. Proteolysis in skeletal muscle appears to be mediated by ubiqutin–proteasomal degradation [2]. Myostatin is a major regulatory protein that impacts both mTOR and ubiquitin signaling [3]. Myostatin, also known as growth differentiation factor-8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily of growth and differentiation factors [4]. Once myostatin binds to its activin type II receptors, ActRIIA and ActRIIB [3], it initiates a signaling cascade through the transcription factors, Smad2 and 3, that results in an increase in protein breakdown and subsequent inhibition of protein synthesis. Myostatin knockout mice have shown a 2- to 3-fold greater muscle mass than their wild-type littermates and accumulate less fat [5]. Moreover, researchers have found that

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reduction of myostatin was associated with greater skeletal muscle hypertrophy and strength [6].

Inhibition of myostatin as a modality for preventing loss of skeletal muscle or increasing lean body mass (LBM) is being pursued by both therapeutics and nutrition scientists. One promising nutritional ingredient, which has clinically been shown to lower myostatin levels, is Fortetropin [7]. Fortetropin is an all-natural proteo-lipid complex made from fertilized egg yolk.

Although a previous clinical study showed that Fortetropin substantially reduced serum myostatin levels, its mechanism of action (MOA) and ability to induce skeletal muscle growth have yet to be evaluated [7]. Therefore the purpose of this study was 2-fold. First, we sought to investigate whether plausible mechanisms existed that could support the contention that Fortetropin supplementation could induce hypertrophy. If plausible mechanisms were identified in a preclinical rodent model, our second purpose was to evaluate its effect on skeletal muscle growth. This was conducted in a double-blind placebo-controlled clinical study using resistance-trained individuals.

MATERIALS AND METHODS

Preclinical Rodent Model Study

All experimental procedures described herein were approved by the Auburn Institutional Animal Care and Use Committee. Male Wistar rats (250 g) were purchased from Harlan Laboratories (Princeton, NJ, USA). Rats were allowed to acclimate in the animal quarters for 5 days prior to experimentation. Briefly, animal quarters were maintained on a 12-hour light : 12-hour dark cycle, at ambient room temperature, and water and standard rodent chow (18.6% protein, 44.2% carbohydrate, 6.2% fat; Teklad Global #2018 Diet, Harlan Laboratories) were provided to animals *ad libitum*.

Eight days prior to the acute resistance training experiment, rats were gavage-fed (force fed via tube) once daily with either 1.2 ml of tap water control (CTL) or 0.26 g of Fortetropin (equivalent to 0.39 g of Myo-X) dissolved in ∼1 ml of tap water. Feeding took place under light isoflurane anesthesia in order to reduce the daily repetitive stress of gavage feeding as previously described [8]. This dose of Fortetropin equaled a 2× human equivalent dose (13.2 g) per the species conversion calculations of Reagan-Shaw et al. [9] whereby the human body mass for an average male was assumed to be 80 kg. Due to resource limitations, we chose to employ a 2× human equivalent dose in rats because it was between the low- and high-dose values.

Exercise Procedure

The morning of the acute resistance training experiment, food was removed from home cages resulting in an approximately 5- to 6-hour fast. Rats were then transported to the Molecular and Applied Sciences Laboratory and allowed to acclimate for approximately 1–2 hours. Thereafter, rats were administered either 1.2 ml of tap water (CTL, $n = 10$) or 0.26 g Fortetropin ($n = 11$) dissolved in ∼1 ml of tap water via gavage feeding under light isoflurane anesthesia. Rats then remained under isoflurane anesthesia for an electrically stimulated lower body unilateral plantar flexion exercise per the modified methods of Baar and Esser [10].

Briefly, animals were fastened to an apparatus to allow the 2 hind limbs to move freely. Two subcutaneous electrodes connected to a Grass S48 Stimulator (Grass Medical Instruments, Quincy, MA) were placed parallel to the gastrocnemius in each rat's right leg. Four sets of 8 stimulations then occurred with the following settings: 70 mV, 100 Hz, 2000 ms train duration, 0.2 train rate per second (TPS), and 0.2 ms duration. Between sets rats were allowed 2 minutes of recovery. Following the electrically stimulated exercise bout, rats were allowed to recover for 180 minutes prior to being euthanized under CO_2 gas. Rats were injected with puromycin dihydrochloride 30 minutes prior to euthanasia (5.44 mg in 1 ml of diluted in phosphate buffered saline; Ameresco, Solon, OH) as a metabolic tracer in order to determine skeletal muscle protein synthesis via the surface sensing of translation (SUnSET) method described in detail elsewhere [11].

Molecular Analysis

Immediately following euthanasia, whole blood was removed via heart sticks using a 21-gauge needle and syringe, placed in serum separator tubes, and processed for serum extraction via centrifugation at 3500 × *g* for 5 minutes. Serum was aliquoted into multiple 1.7-ml microcentrifuge tubes for subsequent biochemical assays and then frozen at −20°C in a standard laboratory freezer for later analysis.

Approximately two 50 mg pieces of mixed gastrocnemius muscle were harvested using standard dissection techniques and placed in homogenizing buffer (Tris base; pH 8.0, NaCl, NP-40, sodium deoxycholate, SDS with added protease and phosphatase inhibitors; G Biosciences, St. Louis, MO) and Ribozol (Ameresco) for immunoblotting and mRNA analyses, respectively. Muscle samples placed in Tris base homogenizing buffer were homogenized using a 1.7-ml tube using a tight-fitting micropestle; insoluble proteins were removed with centrifugation at 500 × *g* for 5 minutes at 4°C, and supernatants were assayed for total protein content using a BCA Protein Assay Kit (Thermo Scientific, Waltham, MA) prior to immunoblotting sample preparation. Muscle samples placed in Ribozol were subjected to total RNA isolation according to manufacturer's instructions, and concentrations were performed using a NanoDrop Lite (Thermo Scientific, Waltham, MA, USA) prior to cDNA synthesis for mRNA analyses. Extra gastrocnemius muscle not processed during dissections was flash-frozen in liquid nitrogen and stored at −80°C for later potential analyses.

Directed Akt-mTOR Phosphoproteomics

The PathScan Akt Signaling Antibody Array Kit (Chemiluminescent Readout; Cell Signaling, Danvers, MA) containing glass slides spotted with antibodies was utilized to detect phosphorylated proteins predominantly belonging to the Akt-mTOR signaling network. Briefly, gastrocnemius homogenates were diluted to 1.0 μg/μl using cell lysis buffer provided by the kit and assayed according to the manufacturer's instructions. Slides were developed using an enhanced chemiluminescent reagent provided by the kit, and spot densitometry was performed through the use of a UVP Imager and associated densitometry software (UVP, LLC, Upland, CA). The calculation of each phosphorylated target was as follows: (Density value of the target − Negative control)/Summation of all density values for the sample

SUnSET Method for Muscle Protein Synthesis Determination

As mentioned previously, the SUnSET method was used in order to examine whether different dietary treatments with or without stimulated exercise differentially affected muscle protein synthesis (MPS). Briefly, 2 μg//micro;l gastrocnemius Western blotting preps were made using 4× Laemmli buffer. Thereafter, 30 μl of prepped samples was loaded onto 12% SDS–polyacrylamide gels and subjected to electrophoresis (200 V @ 75 μmin). Proteins were then transferred to polyvinylidene difluoride membranes (Whatman, Westran Clear Signal, St. Louis, MO, USA), and membranes were blocked for 1 hour at room temperature with 5% nonfat milk powder. Mouse anti-puromycin IgG (1:5,000; Millipore Cell Signaling, Danvers, MA, USA) was incubated with membranes overnight at 4°C in 5% bovine serum albumin, and the following day membranes were incubated with anti-mouse IgG secondary antibodies (1:2000, Cell Signaling) at room temperature for 1 hour prior to membrane development. Membrane development was performed using an enhanced chemiluminescent reagent (Amersham, Pittsburgh, PA), and band densitometry was performed through the use of a gel documentation system and associated densitometry software (UVP). Thereafter, membranes were incubated with Coomassie stain in order to visually verify equal protein loading between lanes.

Western Blotting

For determination of gastrocnemius phospho-SMAD2 (Ser465/467)/3 (Ser423/425), phospho-eEF2 (Thr56), ubiquitin monomer, and poly-ubiquinated protein levels 2 μg//micro;l gastrocnemius Western blotting preps were made using 4× Laemmli buffer. Thereafter, 30 μl of prepped samples was loaded onto 12% SDS–polyacrylamide gels and subjected to electrophoresis (200 V @ 75 min). Proteins were then transferred to polyvinylidene difluoride membranes (Whatman), and membranes were blocked for 1 hour at room temperature with 5% nonfat milk powder. Rabbit anti-phospho-SMAD2 (Ser465/467)/3 (Ser423/425) IgG (1:1000; Cell Signaling), rabbit anti-phospho-eEF2 (Thr56) IgG (1:1000; Cell Signaling), and mouse anti-ubiquitin (1:1000) were incubated with membranes overnight at 4°C in 5% bovine serum albumin, and the following day membranes were incubated with anti-rabbit (1:2000, Cell Signaling) or anti-mouse IgG secondary antibodies (1:2000; Cell Signaling) at room temperature for 1 hour prior to membrane development. Membrane development was performed using an enhanced chemiluminescent reagent (Amersham), and band densitometry was performed through the use of a gel documentation system and associated densitometry software (UVP). Thereafter, membranes were incubated with Coomassie stain in order to visually verify equal protein loading between lanes.

Real-Time Polymerase Chain Reaction

Furthermore, membranes were stripped and reprobed with rabbit anti-GAPDH IgG (1:3000; GeneTex) in order to visually ensure between-lane protein loading equality. RNA (1 μg) was reverse transcribed into cDNA for real-time polymerase chain reaction (PCR) analyses using a commercial cDNA synthesis kit (Quanta Biosciences, Gaithersburg, MD). Real-time PCR was performed using SYBR-green-based methods with gene-specific primers (Follistatin [Fstn], Activin IIB [ActRIIB], Atrogin-1, MuRF-1, beta-2 microglobulin [B2M; normalizer]) designed using primer designer software (Primer3Plus, Cambridge, MA). The forward and reverse primer sequences are as follows: (Fstn: forward primer 5′-TGAGAATGTG-GACTGTGGCC-3′, reverse primer 5′-CCAGTTCCGGCTGCTCTTTA-3′; ActRIIB: forward primer 5′-GAGATCAAGGCTCGAGGTCG-3′; reverse primer 5′-CAGGTTGGAGCCTCGTTTCT-3′; Atrogin-1: forward primer 5′-CTACGATGTTGCAGCCAAGA-3′, reverse primer 5′-GGCAGTCGAGAAGTCCAGTC-3′; MuRF-1: forward primer 5′-AGTCGCAGTTTCGAAGCAAT-3′, reverse primer 5′-AACGACCTCCAGACATGGAC-3′; B2M: forward primer 5′-CCCAAAGAGACAGTGGGTGT-3′, reverse primer 5′-CCCTACTCCCCTCAGTTTCC-3′). Melt curve analyses demonstrated that one PCR product was amplified per reaction.

Statistical Analysis

All data are presented in figures as means ± standard error. Of note, statistical outliers that were mean ± 2 SD from mean values were omitted from analyses in an *a priori* fashion prior to statistical analysis. Specifically, for MPS data, 1 rat from the CTL and 1 rat from the Fortetropin groups were removed because of an abnormally strong puromycin signal. For Western blotting, 2 Fortetropin rats were removed from the p-SMAD2/3 data for being positive outliers, 2 CTL rats were removed from the ubiquitin monomer data due to being poor

blots (low band development), and one Fortetropin rat was removed from the poly-ubiquitin data due to a positive outlier. For Chip data, one CTL rat was removed from p-Akt and 2 CTL rats were removed from p-70s6k due to being outliers. Finally, for PCR data, one Fortetropin rat was removed from all of the genes being interrogated due to a poor housekeeping gene value (i.e., poor cDNA prep). Two-way (exercise vs unexercised limb; CTL vs. Fortetropin) analyses of variance were performed to determine between-treatment significance using SPSS version 22.0 (IBM, Armonk, NY). Because there were only 2 treatment legs and conditions, significant main effects or interactions were followed with dependent and independent sample *t*-tests, respectively. Statistical significance was set at $p \leq 0.05$.

HUMAN CLINICAL TRIAL

Protocol Overview

This was a double-blind, placebo-controlled clinical trial designed to evaluate the effects and efficacy of Fortetropin in a resistance-trained male population (overall training history 29.6 ± 19.7 months and a bench press : body mass ratio of 1.21 ± 0.36). A familiarization phase took place prior to data collection. Data collection consisted of measurements of serum myostatin levels, muscle thickness, body composition, strength, and power. The training protocol was carried out over 12 weeks with baseline measurements occurring prior to beginning the trial. The same measurements were reassessed after 12 weeks for posttesting values. The primary endpoint was changes over time in muscle thickness measurements. The secondary endpoint was changes over time in body composition measurements. The tertiary endpoints were strength and power measurements as well as perceived recovery and soreness. The study was conducted according to the guidelines laid down in the Declaration of Helsinki.

Institutional Review and Approval

The University of Tampa Institutional Review Board approved all procedures involving human subjects. Written informed consent was obtained from all subjects.

Inclusion/Exclusion Criteria

Inclusion criteria for this trial were that subjects had to be male between the ages of 18 and 30 with a dual-energy x-ray absorptiometry (DXA) determined total body mass of no greater than 110 kg. Subjects had to have a minimum of 6 months' resistance training experience and be able to bench press and deadlift a minimum of 1–1.5 times their bodyweight. Subjects had to be free of musculoskeletal injury, be nonsmokers, have not taken supplements for at least 3 months prior to the beginning of the trial, and agree to not consume eggs for the duration of the study. Subjects were excluded if they had a history of arthritis, joint pain, inflammation, cardiovascular disease; were taking any other performance-enhancing supplement; had a history of drug or alcohol abuse; or had allergies to eggs.

Subjects

A total of 45 males ages 18–21 were recruited and enrolled in the study. Subjects were randomized into 3 groups; placebo, 6.6 g dose of Fortetropin (equivalent to one 10 g dose of MYO-X; ingredients Foretropin 6.6 g plus 4.4 g of fructose, dextrose, and vanilla flavoring) and 19.8 g dose of Fortetropin (equivalent to 30 g of MYO-X) daily. The groups were matched based on factors in the following order—muscle thickness, lean body mass, fat mass, and strength—and then randomly assigned to one of the conditions such that baseline values for these parameters were not statistically different (Table 1). Eight subjects dropped from the study due to scheduling conflicts and failure to follow criteria and protocols. The remaining 37 subjects completed the clinical trial. The number of subjects in each group was as follows: placebo: $n = 12$, 6.6 g dosage arm: $n = 13$, and 19.8 g dosage arm: $n = 12$.

Table 1. Baseline Subject Characteristics

Variable	Placebo ($n = 12$)	6.6 g ($n = 13$)	19.8 g ($n = 12$)
Age (years)	19.2 ± 0.71	20.2 ± 2.17	19.8 ± 1.53
Body mass (kg)	71.97 ± 12.71	69.3 ± 7.71	70.5 ± 9.79
Height (cm)	176.32 ± 5.78	174.28 ± 5.34	172.51 ± 5.13
Training history (months)	32.3 ± 20.3	27.8 ± 17.6	30 ± 21.6
Lean body mass (kg)	58.3 ± 9.95	56.89 ± 6.6	57.9 ± 7.67
Fat mass (kg)	13.68 ± 4.38	12.52 ± 3.08	12.65 ± 4.52
Muscle thickness (cm)	4.97 ± 0.73	4.78 ± 0.47	5.18 ± 0.73
Bench press 1RM (kg)	82.4 ± 18.69	86.88 ± 22.65	85.81 ± 27.52
Leg press 1RM (kg)	225.66 ± 66.22	228.89 ± 66.24	240.41 ± 73.59
Bench press : body mass ratio	1.14 ± 0.15	1.24 ± 0.25	1.21 ± 0.36

1RM = one repetition maximum.

Training Protocol

The training protocol consisted of an undulating periodized plan 2 days per week. One day per week consisted of muscle hypertrophy and the other day consisted of strength-oriented training. Rest time between sets was predetermined at 1 to 2 minutes for hypertrophy workouts and 3 to 5 minutes for strength workouts. Intensity increased weekly over 3 4-week blocks. At weeks 4, 8, and 12 subjects were reverted back to intensity from week 1. All subjects performed the same exercise order, with the same intensity, while completing the same number of sets and repetitions. All exercise sessions were monitored by a certified strength and conditioning specialist and supervised by trained laboratory technicians. Exercises selected were primarily compound and are summarized in Table 2. Prior to beginning the protocol, all individuals underwent a familiarization phase to learn each exercise.

Diet and Supplementation

Subjects worked individually with a dietician on a weekly basis. Subjects were prescribed an individually based diet. This entailed the dietician using the Mifflin St. Jeor equation for each subject to determine his caloric needs based on the lean body mass of the individual. The recommended diet consisted of 50% carbohydrates, 25% fat, and 25% protein. Total calories and macronutrients were the same among the groups. Poststudy analysis revealed that individuals consumed diets that resulted in 54% carbohydrates, 22% fat, 24% protein. There were no statistical differences in total calories or macronutrients between groups. Subjects were restricted from consuming eggs during the entire duration of the study. Subjects tracked their daily intakes using MyFitnessPal mobile application (MyFitnessPal, Inc.; San Francisco, CA, USA).

Subjects were required to come to the laboratory every morning and take their assigned supplement in front of the same investigator. In order to maintain control and blinded conditions, this investigator was the only person handling and distributing the supplementation to the subjects. This individual had no part in data collection, training, or poststudy analysis. Individuals were provided the supplement in the form of a shakes, which were labeled “A,” “B,” and “C.”

Body Composition Measures

A whole-body DXA (Hologic, Bedford, MA) scan was utilized to measure body composition. LBM and fat mass were determined for the total body with the subject lying in a supine position with the knee extended and instructed not to move for the entire duration of the scan (~10 minutes). Results from the each scan were uploaded and accessed on a computer directly connected to the DXA device. All DXA scans were conducted at baseline and after completion of the study after a 10-hour overnight fast. The coefficient of variation (CV) for body composition was 1.5%.

Muscle Thickness

Two-dimensional, B-mode ultrasonography (LoGIQ e, GE; Fairfield, CT, USA) was used to determine muscle thickness of the vastus lateralis (VL). Muscle thickness was measured at 50% femur length (the distance from the greater trochanter to the lateral epicondyle of the knee) along the lateral portion of the VL on the right leg. The specific spot was marked with a permanent marker and participants were instructed to keep their mark throughout the duration of the study in order to maintain consistency of the measurement site. An 8-MHz

Table 2. Resistance Training Protocol[a]

Tuesday (Hypertrophy) (65%, 70%, 75%, 77.5%) 1- to 2-Minute Rest		Thursday (Heavy) (80%, 85%, 90%, 92.5%) 3- to 5-Minute Rest	
Leg press	3 × (8–15)	Leg press	5 × (2–5)
Lunges	3 × (8–15)	Lunges	3 × (2–5)
Leg extension	3 × (8–15)	Bench	5 × (2–5)
(2) Leg curl	3 × (8–15)	Deadlift	5 × (2–5)
Bench	3 × (8–15)	Close grip bench	3 × (2–5)
Incline DB press	3 × (8–15)		
Deadlift	3 × (8–15)		
Lat pulldown	3 × (8–15)		
(2) DB shoulder press	3 × (8–15)		
DB flat row	3 × (8–15)		
BB bicep curl	3 × (8–15)		
(2) Tricep pullover	3 × (8–15)		
Preacher curl	3 × (8–15)		
(2) Skull crusher	3 × (8–15)		
Lateral raise	3 × (8–15)		

DB = dumbbell; BB = barbell.

[a](2) Indicates that the exercise was paired with the previous exercise in a superset fashion. Supersets include leg extension and leg curl, lateral pulldown and DB shoulder press, BB bicep curl and tricep pullover, preacher curl and skull crusher.

scanning transducer was oriented perpendicular to muscle belly of the VL. Water-soluble transmission gel was applied to the scanning transducer provide to aid acoustic coupling and prevent direct contact with the skin that could pressurize and deform the underlying tissues. To obtain images, subjects laid supine with fully extended legs and their muscles relaxed. When a visible image was projected on the monitor, the image on the monitor was frozen. Muscle thickness measurements were extrapolated from the monitor screen by measuring the distance from the interface of the muscle tissue and subcutaneous fat to the surface of the femur. The same researcher performed all ultrasound assessments and was blinded to the treatment groups. Muscle thickness of the VL was assessed at baseline and completion of the study. The CV for VL muscle thickness measurements was 2%.

Muscle Strength Measures

Strength was assessed via one repetition maximum testing (1RM) in the leg press and bench press. A trained tester who was certified by the National Strength and Conditioning Association observed strength testing and loads were increased incrementally until maximal load or failure at a given load was reached. Briefly, subjects performed a general warmup and a specific warmup consisting of 2 sets. During the first set, subjects performed 10 repetitions with 50% of their predicted 1RM. For the second set, they performed 5 repetitions with 75% of the predicted 1RM. After the second warmup set, subjects rested for 3 minutes. Then, each subject had as many as 5 attempts to achieve their 1RM load with 3–5 minutes' rest between each attempt. Strong verbal encouragement was given throughout 1RM testing. The leg press was performed in a 45° leg press in which attempts had to reach at least a 90° knee flexion angle to be deemed successful. The bench press was performed on a standard flat bench using an Olympic barbell loaded with free weights. Subjects took the bar off the rest, with an assisted lift-off by request, with their thumbs wrapped around the bar and arms extended. The bar was lowered until it touched the chest where it was then pressed until the arms were fully extended. For the lift to be successful, subjects had to keep their feet flat on the floor, with their buttocks, shoulders, and head in contact with bench in all times throughout the lift. The CV combined for both methods of 1RM testing was 3.4%. Data were recorded as the weight lifted in kilograms. Strength measurements were conducted at baseline and completion of the study.

Muscle Power Measures

Anaerobic power was assessed via Monark Wingate cycle ergometry (Monark, Vansbro, Sweden). During the cycling test, the volunteer was instructed to cycle against a predetermined resistance (7.5% of body mass) as fast as possible for 10 seconds. The saddle height was adjusted for each individual in order to produce a 5°–10° knee flexion while the foot was in the low position of the central void. A standardized verbal stimulus was provided to the participant. Power output was recorded in real time by a computer connected to the Monark standard cycle ergometer (model 894e, Monark) during a 10-second sprint test. Wingate PP was recorded using Monark Anaerobic test software (Monark Anaerobic Wingate Software, Version 1.0, Monark). Power measurements were recorded in watts and were conducted at baseline and completion of the study. The CV for peak power was 4.0%.

Blood and Safety Measures

All blood samples were collected via venipuncture by a trained phlebotomist. Full safety and lipid panels were measured at baseline (prior to the onset of the study) and completion of the study. Measurements are provided in Table 3.

Serum Myostatin Level Measurements

Subjects reported to the laboratory after a 12-hour overnight fast prior to beginning the study and after completion of the study for blood draws. Each subject donated approximately 20 mL of fasted blood using venipuncture techniques of an antecubital vein in the forearm according to standard and sterile procedures. Blood was stored at −80°C until further analysis. A GDF-8 Myostatin Quantikine ELISA kit, catalog number DGDF80, from R&D Systems (Minneapols, MN, USA) was utilized to determine serum myostatin levels.

Perceptual Measures

The perceptual measures consisted of 2 visual analogue scales: rating of perceived recovery and rating of perceived soreness.

Table 3. Blood Safety and Lipid Profiles

Blood Markers	Placebo	6.6 g Dose	19.8 g Dose
Pre cholesterol (mg/dL)	175 ± 16	173 ± 14	184 ± 12
Post cholesterol (mg/dL)	174 ± 11	171 ± 13	184 ± 11
Pre HDL (mg/dL)	41 ± 5	42 ± 3	48 ± 4
Post HDL (mg/dL)	45 ± 6	46 ± 2	52 ± 4
Pre triglycerides (mg/dL)	73 ± 7	80 ± 7	80 ± 6
Post triglycerides (mg/dL)	75 ± 5	80 ± 7	82 ± 5
Pre AST (IU/L)	26 ± 2.3	27 ± 2.6	35 ± 6.0
Post AST (IU/L)	26 ± 1.6	27 ± 2.2	29 ± 2.4
Pre ALT (IU/L)	24 ± 2.8	25 ± 2.9	29 ± 5.0
Post ALT (IU/L)	26 ± 1.8	27 ± 3.0	27 ± 5.0
Pre glucose (mg/dL)	87 ±2.0	83 ± 4.0	84 ± 2.7
Post glucose (mg/dL)	90 ± 2.6	85 ± 1.9	87 ± 2.9
Pre BUN (mg/dL)	15 ±1.4	18 ± 1.1	15 ±0.8
Post BUN (mg/dL)	17 ± 1.5	20 ± 1.3	16 ± 0.7
Pre creatinine (mg/dL)	1.01 ± 0.04	1.10 ± 0.04	1.03 ± 0.04
Post creatinine (mg/dL)	1.03 ± 0.02	1.10 ± 0.04	1.12 ± 0.03
Pre BUN/Cr ratio	15 ± 1.3	17 ± 1.1	14 ± 0.8
Post BUN/Cr ratio	15 ± 0.9	16 ± 1.2	13 ± 0.4

HDL = high-density lipoprotein, AST = aspartate aminotransferase, BUN = blood urea nitrogen, Cr = creatinine.

Ratings of perceived recovery and soreness were recorded at weeks 0 and 12 on both training days. Both scales consisted of a measure from 0 to 10. Visual descriptors of *very poorly recovered* and *very well recovered* for perceived recovery and *no soreness* and *very, very sore* for perceived soreness were presented at numbers 0 and 10, respectively. Subjects were asked to identify their level of perceived recovery and perceived soreness after warming up, before performing the training protocol.

Statistical Analysis

A 3 × 2 (Condition × Time) repeated measures analysis of variances was performed using Statistica (StatSoft, Tulsa, OK) to determine differences in each dependent variable with an alpha level of ≤0.05. A Fisher's post hoc least significant difference test for pairwise comparisons was run in the event of a significant F-test.

RESULTS

Preclinical Rodent Model Study

As mentioned earlier, this research was undertaken to elucidate the MOA of Fortetropin. Three pathways involved in muscle balance were examined: myostatin pathway, ubiquitin pathway, and mTOR pathway. Based on previous research, we examined the 3-hour postexercise time point using a rodent model given that this time point has been shown to be the most robust with regards to increases in postexercise muscle protein synthesis and muscle proteolysis [12].

As described below, the major findings of study one were that Fortetropin decreased Activin IIB receptor mRNA expression (Fig. 1b) while increasing and decreasing mTOR and ubiquitin signaling (Fig. 1e and 1f), respectively.

Myostatin Pathway

Fortetropin supplementation has been shown to lower the plasma levels of myostatin, though the exact MOA is not currently understood. It is known that interaction of myostatin with its receptor activin IIB (ActRIIB) initiates the signaling in this pathway, resulting in a decrease in protein synthesis. Thus, the muscle tissues from both the exercised control and the exercised plus Fortetropin arms were analyzed for gene expression of the myostatin pathway. In both the Fortetropin plus exercise and the control plus exercise, phospho-SMAD 2/3 mRNA was significantly

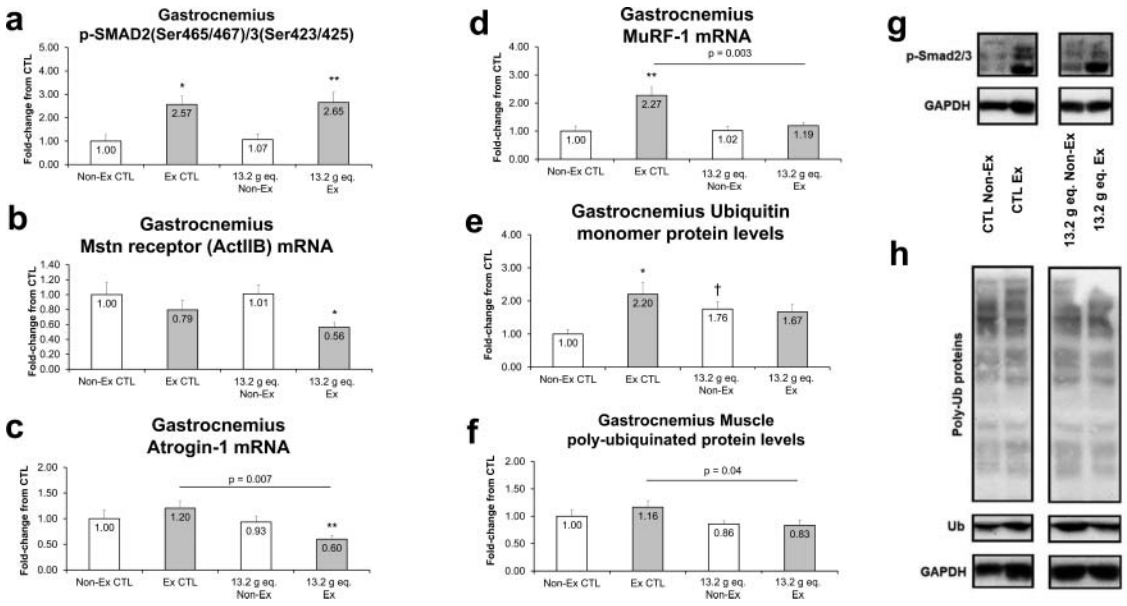

Fig. 1. Effects of 0.26 g Fortetropin (which is a human-equivalent dose of 13.2 g) on (a) phospho-Smad2 (Ser465/467)/3 (Ser423/425), (b) ActIIB receptor mRNA, (c) Atrogin-1, (d) MuRF-1, (e) ubiquitin monomer protein levels, and (f) poly-ubiquinated protein levels. Independent of Fortetropin feeding, acute resistance exercise increased phospho-Smad2/3 (*$p \leq 0.05$, **$p \leq 0.01$). Resistance exercise with Fortetropin feeding decreased Atrogin-1 mRNA expression patterns, whereas the control exercise condition did not present these changes (**$p \leq 0.01$). Resistance exercise with Fortetropin feeding also prevented the rise in MuRF-1 mRNA expression patterns, whereas the control exercise condition presented an increase in the expression of this mRNA postexercise (**$p \leq 0.01$). Baseline ubiquitin monomer levels were higher in the Foretropin feeding CTL versus non-Ex CTL muscle (†$p \leq 0.05$), though exercise increased ubiquitin monomer levels in the CTL condition and not the Foretropin condition (*$p \leq 0.05$). Finally, resistance exercise with Fortetropin feeding presented lower poly-ubiquinated protein levels compared to the control exercise condition ($p \leq 0.04$). (g) Representative image of phospho-Smad2/3 and (h) representative image of ubiquitin monomer and poly-ubiquinated protein levels.

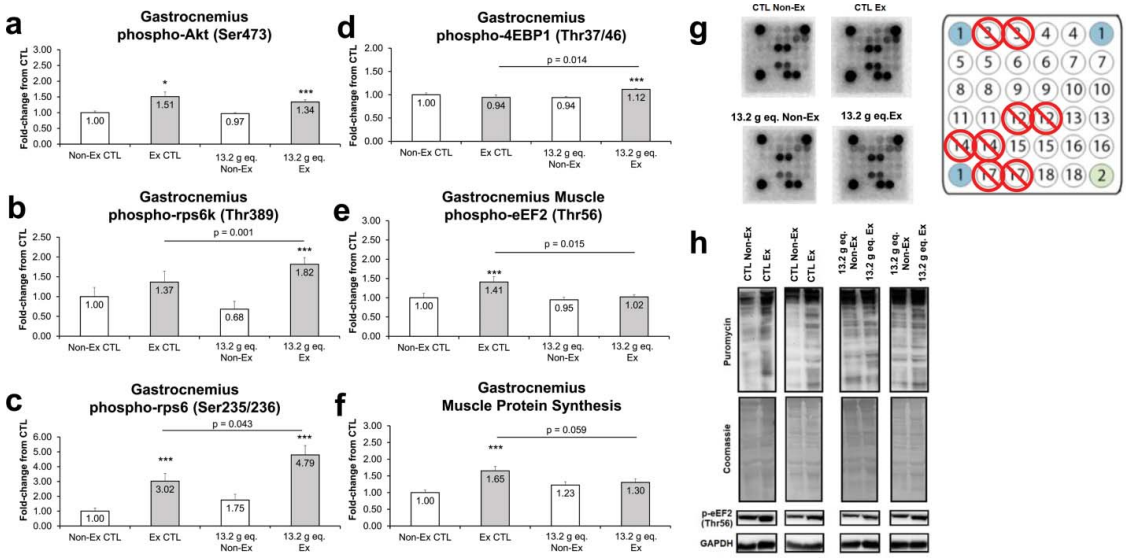

Fig. 2. Effects of 0.26 g Fortetropin (which is a human-equivalent dose of 13.2 g) on (a) phospho-Akt (Ser473), (b) phospho-rpS6k (Thr389), (c) phospho-rps6 (Ser2235/236), (d) phospho-4EBP1 (Thr37/46), (e) phospho-eEF2 (Thr56), and (f) muscle protein synthesis. Independent of Fortetropin feeding, acute resistance exercise increased phospho-Akt ($*p \leq 0.05$, $***p \leq 0.001$). Resistance exercise also increased phospho-rpS6k and phospho-rps6, though Fortetropin potentiated this effect. Resistance exercise with Fortetropin feeding increased phospho-4EBP1. Fortetropin feeding also prevented the increase in phospho-eEF2 and the control exercise condition increased from rest to exercise. (g) Representative image of the Akt-mTOR array along with a legend of the phosphor targets, which included 1: positive control, 2: negative control, 3: p-Akt (Thr 308), 4: p-Akt (Ser 473), 5: p-rps6 (Ser 235/236), 6: p-Ampk-α (Thr 172), 7: p-Pras40 (Thr 246), 8: p-mTOR (Ser 2481), 9: p-Gsk-3α (Ser 21), 10: p-Gsk-3β (Ser 9), 11: p-p70s6k (Thr 389), 12&14: p-p70s6k (Thr421/Ser 424), 13: p-Bad (Ser 112), 15: p-Pten (Ser 380), 16: p-Pdk1 (Ser 241), 17: p-Erk1/2 (Thr 202/Tyr204), 18: p-4ebp1 (Thr 37/46). Note that some targets were not included in the analyses due to poor and/or inconsistent signal (these targets are crossed out). (h) Representative SUnSET and blots for protein synthesis assessment phospho-eEF2.

increased (Fig. 1a), with no differences between conditions. Our results also indicated that the ActRIIB receptor mRNA was significantly lowered in the exercise plus Fortetropin group but not the exercise plus control group (Fig. 1b).

Ubiquitin Pathway

The ubiquitin pathway regulates the specific breakdown of skeletal muscle protein. The mRNA expression of the rate limiting E3 ligase Atrogin-1 was depressed in the Fortetropin plus exercise group compared to all other groups (Fig. 1c). Moreover, the mRNA expression of the rate-limiting E3 ligase MuRF-1 increased in the control plus exercise group compared to the Fortetropin plus exercise group (Fig. 1d). Finally, we found that in the control plus exercise group, but not in the Fortetropin plus exercise group, the expression of ubiquitin monomer protein was significantly increased (Fig. 1e). We also found that in the control plus exercise group, but not in the Fortetropin group, the expression of poly-ubiquitin protein increased. Independent *t*-test analysis indicated the increase in poly-ubiquitin protein expression in the control plus exercise vs Fortetropin plus exercise group to be statistically significant (Fig. 1f).

mTOR Pathway and Protein Synthesis

The mTOR pathway regulates skeletal muscle protein synthesis. mTOR itself regulates translation initiation through increasing the phosphorylation of eukaryotic initiation factor binding protein 4 (4EBP1), ribosomal protein S6 kinase (rps6k), and ribosomal protein S6 (rps6). Our results indicated an exercise effect for Akt (Ser473) but no Condition × Time effect (Fig. 2a). We also found that mTOR signaling was elevated as indicated by greater phosphorylation status of 4EBP1, rps6, and rps6k for both groups (Figs. 2b–2d). However, Fortetropin plus exercise resulted in greater elevations in these markers than the control plus exercise group (Figs. 2b–2d). Moreover, the Fortetropin plus exercise prevented the increase in the phosphorylation of eukaryotic elongation factor 2 (eEF2), which indicates greater elongation (Fig. 2e). Though mTOR signaling was elevated to a greater degree in the Fortetropin groups 3 hours following exercise, both groups increased protein synthesis equally (Fig. 2f).

Human Clinical Trial

The major findings from the human trial were that both Fortetropin supplement conditions significantly increased LBM and muscle thickness while significantly decreasing serum myostatin levels. There were no significant difference between the 6.6 and 19.8 g Fortetropin supplement groups among any variables tested.

There were no significant differences between the groups for mean age, height, body mass, training history (Table 1),

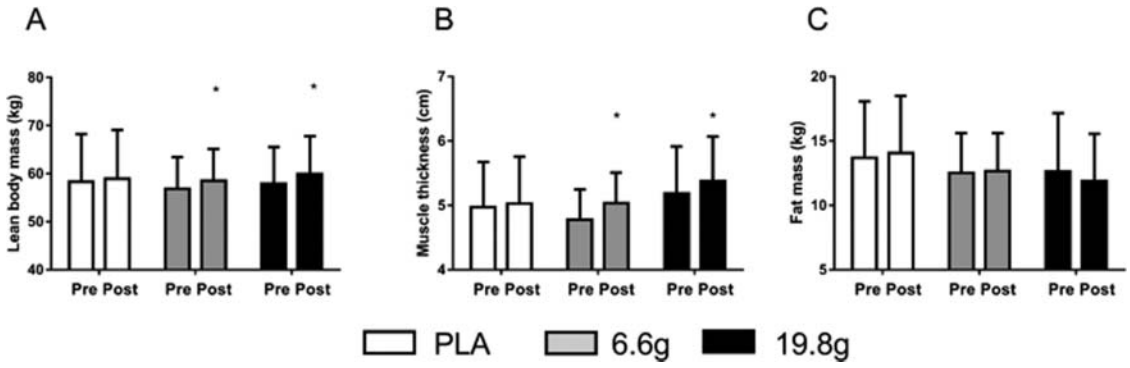

Fig. 3. Effects of a placebo (PLA) or 6.6 and 19.8 g of Fortetropin on body composition (A and C) and muscle thickness (B). *Within-group differences ($p \leq 0.05$).

body composition (Fig. 3), strength (Table 4), power (Table 4), or myostatin serum levels at baseline (Fig. 4).

There was a significant Group × Time interaction for LBM (Fig. 3a) and muscle thickness (Fig. 3b; $p < 0.04$). For LBM, the post hoc comparisons revealed that both Fortetropin arms increased from pre to post (6.6 g: 56.8 ± 6.6 kg to 58.5 ± 5.9 kg, $p < 0.0003$; 19.8 g: 57.9 ± 7.6 kg to 59.9 ± 7.9 kg, $p < 0.0001$) but the placebo condition did not (e.g., 58.2 ± 9.9 kg to 58.9 ± 10.1 kg, $p < 0.38$). For muscle thickness, the post hoc comparisons revealed that both Fortetropin arms increased from pre to post (6.6 g: 4.77 ± 0.46 cm to 5.03 ± 0.47 cm, $p < 0.0001$; 19.8 g: 5.18 ± 0.68 cm to 5.38 ± 0.73 cm, $p < 0.002$) but the placebo condition did not (e.g., 4.97 ± 0.70 cm to 5.02 ± 0.72 cm, $p < 0.84$). In addition, delta changes in muscle thickness were significantly greater in both Fortetropin arms compared to the placebo condition ($p \leq 0.05$). There were no time or Group × Time interactions for fat mass (Fig. 3c; $p > 0.05$). There were significant time effects in which all conditions increased from pre to post in bench press, leg press, and Wingate power (Table 4). However, there were no differences between conditions in any of these performance measures. There were no significant differences in any blood safety or lipid profile analyses between the groups or relative to baseline ($p > 0.05$; Table 3). Serum levels of myostatin were significantly decreased from baseline in both Fortetropin arms (6.6 g: 5.68 ± 2.48 ng/ml to 4.66 ± 2.74 ng/ml, $p < 0.01$; 19.8 g: 5.38 ± 1.90 ng/ml to 4.22 ± 0.98 ng/ml, $p < 0.006$) but the placebo condition did not (e.g., 5.97 ± 1.46 ng/ml to 5.42 ± 2.02 ng/ml, $p > 0.32$; Fig. 4). There were no time or Group × Time interactions for perceived recovery or perceived soreness.

DISCUSSION

In this research, we explored the molecular efficacy of Fortetropin using a preclinical rat model to elucidate the MOA (Fig. 5) and then evaluated the efficacy of the supplement on resistance-trained males in a human model. Our rodent model study revealed several potential MOAs involving multiple pathways. For instance, Fortetropin significantly reduced mRNA expression of the myostatin receptor, ActRIIB, while

Table 4. Performance and Perceptual Measures

	Placebo		6.6g		19.8g	
Performance	Pre	Post	Pre	Post	Pre	Post
1RM leg-press (kg)	226.1±66.3	260.6±60.0	229.3±66.3*	252.9±53.2*	240.9±73.7	267.0±63.1*
1RM bench-press (kg)	82.5±18.7	89.1±17.3*	82.3±22.6	87.1±22.8*	85.9±27.5	92.8±26.8*
Wingate PP (Watts)	675.2±207.0	738.3±188.3*	681.7±157.5	768.6±161.4*	688.2±189.0	796.3±166.0*

Perceptual	**W0 D1**	**W0 D2**	**W12 D1**	**W12 D2**	**W0 D1**	**WO D2**	**W12 D1**	**W12 D2**	**W0 D1**	**WO D2**	**W12 D1**	**W12 D2**
PRS	8.75±0.89	6.25±1.16#	8.5±1.2	7.13±1.55	8.4±1.69	6.91±2.39#	8.45±1.86	7.44±1.57	8.2±1.31	6.7±1.77#	8.5±1.72	7.4±1.9
RPS	1.38±1.3	4.5±1.85#	2.13±1.36	3.38±2.07	1.09±0.94	4.18±2.35#	1.64±1.85	3.09±2.3	1.6±1.58	4.2±1.75#	1.9±2.18	3.1±1.59

1RM = one repetition maximum, PP = peak power, PRS = perceived recovery scale, RPS = rating of perceived soreness, W = week, D = day.

*$p \leq 0.05$ within-group comparisons.

#$p \leq 0.05$ within-group comparisons from W0 D1.

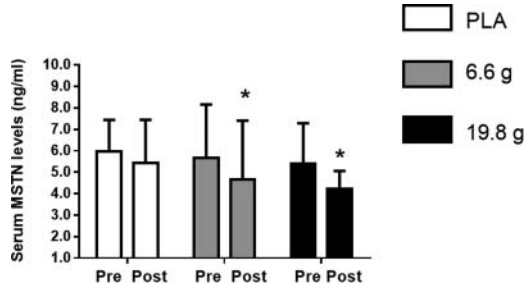

Fig. 4. Effects of a placebo (PLA) or 6.6 and 19.8 g of Fortetropin on serum myostatin levels. *Within-group differences ($p \leq 0.05$).

increasing anabolic (mTOR) signaling and decreasing catabolic (ubiquitin) signaling, respectively.

Our human clinical trial showed that Fortetropin supplementation significantly increased muscle thickness relative to a placebo matched control. The clinical study also found a Group × Time interaction in which Fortetropin supplementation significantly increased LBM and there were no differences in the placebo condition relative to baseline. Finally, Fortetropin supplementation resulted in a significant decrease in serum myostatin levels.

Human Relevancy of the Preclinical Model

The rat preclinical trial has obvious limitations that preclude the preclinical study from being directly applicable to humans. First and foremost are the potential species differences between human and rats. Though there is little doubt that rats have differences in whole-body metabolism relative to humans [13], acute rodent exercise and feeding models have been employed using rat hind limb skeletal muscle tissue. Importantly, a hallmark study by Baar and Esser [10] utilized a leg kicking protocol similar to ours and reported that acute alterations in muscle protein synthesis signaling occurs up to 6 hours postexercise. Likewise, acute protein feeding studies

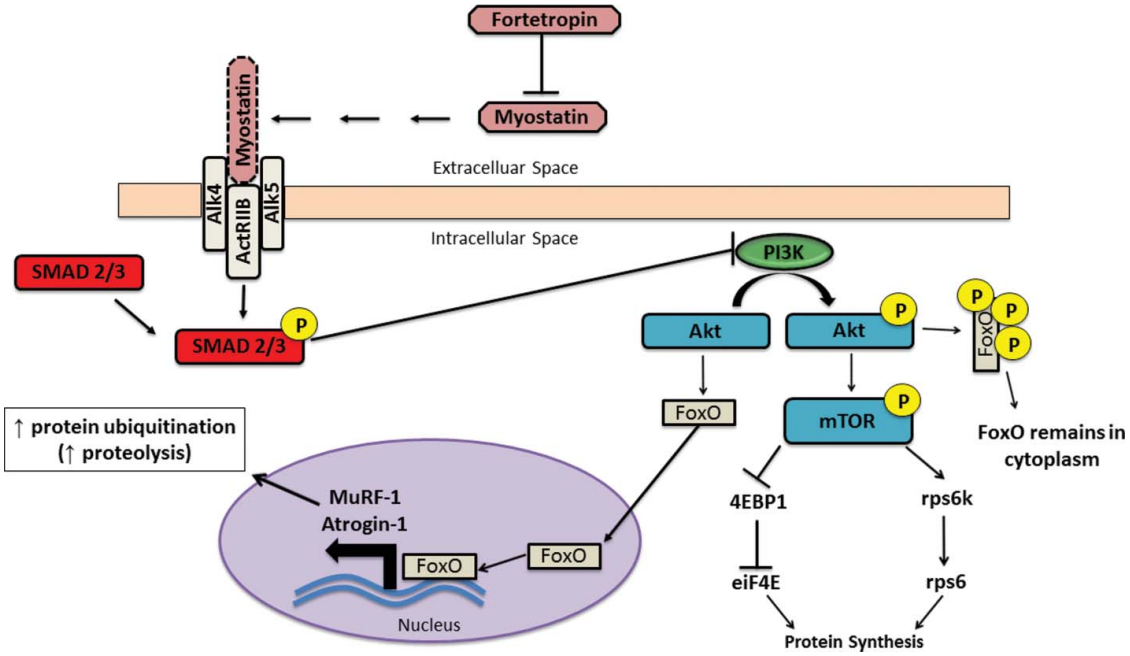

Fig. 5. Myostatin, when bound to Fortetropin, is left inactive in serum. Active myostatin binds to its receptors ActRIIA and ActRIIB (in our rodent model study we focused on the key molecular marker of the myostatin pathway, ActRIIB) and initiates a signaling cascade through the transcription factors Smad2 and 3 that results in inhibition of skeletal muscle protein synthesis and an increase in protein breakdown [3,6]. The current findings indicate that the ActRIIB receptor mRNA was significantly depressed in the exercise plus Fortetropin group but not in the exercise control group, indicating that Fortetropin downregulates the myostatin pathway via decreasing its receptor expression. A major anabolic pathway regulating protein synthesis in skeletal muscle is the Akt-mTOR pathway. mTOR signaling involves the regulation of translation initiation through increasing the phosphorylation status of eukaryotic initiation factor binding protein 4 (4EBP1), ribosomal protein S6 kinase (rps6k), and ribosomal protein S6 (rps6). The current research demonstrated that rats supplemented with Fortetropin increased the phosphorylation status of rps6, rps6k, and 4EBP1 following exercise, which are all strong indicators of enhanced mTOR signaling. One of the principal catabolic systems induced by the stress of training is the ubiquitin-proteasome pathway [16]. Proteins degraded by the ubiquitin-proteasome pathway are first covalently bound to ubiquitin, a process that is regulated by an enzyme cascade consisting of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) [17]. Two important muscle-specific ubiquitin E3 ligases, MuRF-1 and Atrogin-1, have been associated with various atrophic conditions [19] and are upregulated in many forms of muscle atrophy [16]. The current data showed that in the control plus exercise condition, but not in the Fortetropin plus exercise condition, the expression of both ubiquitin monomer protein and poly-ubiquitination increased. The data also demonstrated that the mRNA expression of the rate-limiting E3 ligases Atrogin-1 and MuRF-1 was depressed in the Fortetropin plus exercise group, whereas they increased in the control plus exercise group.

have also been performed by our group as well as others [14], both of which parallel human findings with regards to postprandial protein synthesis. Hence, though the preclinical study was performed on rats and this has inherent limitations, we contend that this is a good preclinical model to look at mechanisms that are paralleled in humans.

It should also be noted why we chose to analyze the gastrocnemius muscle in the preclinical trial. The rat gastrocnemius is made up of a heterogeneous pool of slow-twitch and fast-twitch fibers that is more similar to human locomotor muscles [15]. Alternatively, the plantaris and soleus muscles are predominantly fast-twitch and slow-twitch muscles, respectively [15]. Thus, we chose not to analyze the latter 2 muscle groups given that their proportions are less human relevant. It should be finally noted that human research has shown that muscles with stark differences in fiber type do not appreciably differ with regards to muscle protein synthesis rates (i.e., ∼7% difference in synthesis rates between soleus and vastus lateralis tissue samples). Thus, again, though there are limitations to the rat preclinical model, we contend that the employed methodologies make this acute exercise model relatively translatable to humans.

Fortetropin Reduces Ubiquitin Pathway Markers in Rat Skeletal Muscle Following Exercise

One of the principal catabolic systems induced by the stress of training is the ubiquitin–proteasome pathway [16]. Proteins degraded by the ubiquitin–proteasome pathway are first covalently bound to ubiquitin, a process that is regulated by an enzyme cascade consisting of ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) [17]. The resulting ubiquitinated proteins are then degraded by the 26S proteasome complex in an adenosine triphosphate–dependent process [18]. Two important muscle-specific ubiquitin E3 ligases, MuRF-1 and Atrogin-1, have been associated with various atrophic conditions [19] and are upregulated in many forms of muscle atrophy [16].

Our data showed that in the control plus exercise condition, but not in the Fortetropin plus exercise condition, the expression of both ubiquitin monomer protein and polyubiquitination increased. Our data also demonstrated that the mRNA expression of the rate-limiting E3 ligases Atrogin-1 and MuRF-1 were depressed in the Fortetropin plus exercise group, whereas they increased in the control plus exercise group. These interesting findings provide evidence that Fortetropin has anticatabolic effects via suppression of the ubiquitin pathway. Future research should examine Fortetropin as an intervention for muscle atrophy associated with aging and other diseases that are also related to elevated expression of the ubiquitin pathway.

Fortetropin Increases mTOR Pathway Markers in Rat Skeletal Muscle Following Exercise

A major anabolic pathway regulating protein synthesis in skeletal muscle is the Akt-mTOR pathway. mTOR signaling involves the regulation of translation initiation through increasing the phosphorylation status of eukaryotic initiation factor binding protein 4 (4EBP1), ribosomal protein S6 kinase (rps6k), and ribosomal protein S6 (rps6). Our research demonstrated that rats supplemented with Fortetropin increased the phosphorylation status of rps6, rps6k, and 4EBP1 following exercise, which are all strong indicators of enhanced mTOR signaling. However, these findings were not accompanied by differences in postexercise protein synthesis control and supplemented groups. It is possible that the nondetection of elevations in protein synthesis is explained by our sampling at the 3-hour time point. Interrogating the 3-hour postexercise sampling time point was based upon prior human studies literature that commonly measure this time point for muscle protein synthesis in fasting [12] and protein feeding studies [20]. However, Wilson et al. [21] have demonstrated that protein synthesis peaks 90 minutes after a nutrition intervention but returns to baseline 180 minutes after feeding irrespective of the remaining rise in mTOR signaling. Thus, if Fortetropin does enhance MPS, it remains plausible that rises in postexercise MPS could have occurred sooner than the time point measured in the current study (i.e., 90–120 minutes). Therefore, with our limited preclinical data we are unable to firmly ascertain whether Fortetropin affects muscle protein synthesis prior to or following the 3-hour postexercise time point. In this regard, future research should analyze earlier time points in order to determine whether Fortetropin supplementation enhances postexercise muscle protein synthesis.

Fortetropin Decreases the mRNA Expression of ActIIB in Rat Skeletal Muscle Following Exercise

Myostatin was discovered by the research team of Lee and McPherron [3,22], who showed it to be a negative regulator of skeletal muscle mass. In skeletal muscle tissue, myostatin binds to its receptors, ActRIIA and ActRIIB, and initiates a signaling cascade through the transcription factors Smad2 and 3 that results in inhibition of skeletal muscle protein synthesis and an increase in protein breakdown [3,6]. Studies have shown that during periods of skeletal muscle anabolism, myostatin is decreased allowing for improved skeletal muscle protein balance [23] as well as a 2- to 3-fold increase in hypertrophy [5].

In our rodent model study we focused on the key molecular marker of the myostatin pathway, ActRIIB. Our findings indicate that ActRIIB receptor mRNA was significantly depressed in the exercise plus Fortetropin group but not the exercise control group, indicating that Fortetropin downregulates the myostatin pathway

via decreasing its receptor expression. Though exercise increased phosphorylated SMAD 2/3, a downstream target of myostatin, there were no differences between supplemented and nonsupplemented conditions. Collectively these findings provide preliminary insight into Fortetropin's mechanism of action. However, the incongruence between depression of the myostatin receptor and SMAD 2/3 signaling requires further exploration before concluding that the myostatin pathway mediates Fortetropin's impact on protein balance.

Human Clinical Trial

Muscle tissue is increasingly recognized as a key marker for overall health and wellness. The development of skeletal muscle mass and preservation of muscle quality is considered central to optimizing human performance. As such, we conducted a 12-week resistance training style investigating the dose-dependent effects of Fortetropin on muscle mass and function.

Fortetropin Reduces Serum Myostatin Levels

Myostatin is a key regulator of muscle balance. Myostatin acts locally in skeletal muscle as a negative regulator of satellite stem cell differentiation and growth [3]. When myostatin binds to its receptors, ActRIIA and ActRIIB, it initiates a signaling cascade that results in an increase in protein breakdown and subsequent inhibition of protein synthesis. In this clinical study, we took blood samples at baseline and again at 12 weeks at completion of the study to determine the impact of Fortetropin supplementation on steady-state levels of serum myostatin levels. Our results showed that myostatin serum levels at the end of the study decreased significantly from baseline in both Fortetropin arms but not in the placebo arm.

Impact of Fortetropin on Muscle Thickness and Lean Body Mass

Our human trial demonstrated that muscle thickness and LBM significantly increased in both Fortetropin arms but not the placebo arm. Moreover, delta changes in muscle thickness were greater in the Fortetropin arms compared to the placebo arm. Based on the results from our rodent model, these increases in muscle thickness and LBM strongly appear to be the result of positive changes in protein balance, which favor accretion of lean tissue. Specifically, our rodent model indicated that this could be the result of a chronic reduction in postexercise proteasome activity, a chronic elevation in postexercise muscle protein synthesis, or a chronic reduction in serum myostatin levels.

Impact of Fortetropin on Muscle Strength and Power

Though our study demonstrated greater increases in muscle thickness in the active arms compared to the placebo, all groups showed similar increases in strength and power. Though this does not line up directionally with the hypertrophy data, it appears to make physiological sense. Research by Sale [24] demonstrated that resistance training–induced increases in strength in novice subjects are primarily driven by neural adaptations (>80% of the variance). This is evident because the placebo group in this study made robust increases in strength without demonstrating changes in hypertrophy. It could be hypothesized that in a population of highly experienced resistance-trained athletes, where neural adaptations have plateaued, strength and power gains may favor the Fortetropin-supplemented groups. However, future research will need to investigate this scenario.

Impact of Fortetropin on Blood Chemistry and Serum Lipid Profile

Comprehensive blood safety panels at baseline and the end of the trial did not show any changes on the blood chemistry of the study participants. Although there are numerous published nutrition and serum lipid studies [25–27] confirming that daily dietary consumption of eggs does not change lipid profiles, there is a general misconception that it does. In line with published literature, the results from our study demonstrated that daily use of Fortetropin for 3 months as recommended and at 3 times the recommended dose had no adverse effect on lipid profiles of study participants including serum cholesterol (high-density lipoprotein and low-density lipoprotein) and triglycerides. There were no study-related adverse events during this clinical trial.

CONCLUSIONS

The present research provided initial insight into plausible mechanisms of action for Fortetropin. Our research demonstrated that Fortetropin supplementation in a rodent model decreased catabolic signaling (ubiquitin proteasome pathway), increased anabolic (mTOR), signaling and reduced mRNA expression of the myostatin receptor ActRIIB. Consistent with these findings, our human clinical trial showed that Fortetropin supplementation resulted in positive changes in muscle thickness and lean body mass in healthy resistance-trained young males. In addition, future research should be focused on further elucidating the underlying mechanism of these outcomes and determining the effect of Fortetropin supplementation on other populations susceptible to accelerated muscle loss (i.e., cachectic or sacropenic individuals).

FUNDING

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Author Contributions

M.H.S., R.P.L., K.A.S., E.O.S., N.A., and J.M.W. were all involved in the study design, data collection, and statistical analysis of the human model. C.B.M., C.D.F., J.C.H., R.M.T., and M.D.R. were all involved in the data collection and statistical analysis of the rodent model.

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