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Hormonal regulators of muscle and metabolism in aging (HORMA): design and conduct of a complex, double masked multicenter trial

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Abstract

Background—Older persons often lose muscle mass, strength, and physical function. This report describes the challenges of conducting a complex clinical investigation assessing the effects of anabolic hormones on body composition, physical function, and metabolism during aging.

Methods—HORMA is a multicenter, randomized double masked study of 65–90-year-old community dwelling men with testosterone levels of 150–550 ng/dL and IGF-1 < 167 ng/dL. Subjects were randomized to transdermal testosterone (5 or 10 g/day) and rhGH (0, 3, or 5 µg/kg/day) for 16 weeks. Outcome measures included body composition by DEXA, MRI, and ²H₂O dilution; muscle performance (strength, power, and fatigability), VO₂peak, measures of physical function, synthesis/breakdown of myofibrillar proteins, other measures of metabolism, and quality of life.

Results—Major challenges included delay in startup caused by need for 7 institutional contracts, creating a 142-page manual of operations, orientation and training, creating a 121-page CRF; enrollment inefficiencies; scheduling 16 evaluations/subject; overnight admissions for invasive procedures and isotope infusions; large data and image management and transfer; quality control at multiples sites; staff turnover; and replacement of a clinical testing site. Impediments were largely solved by implementation of a web-based data entry and eligibility verification; electronic scheduling for multiple study visits; availability of research team members to educate and reassure subjects; more frequent site visits to validate all source documents and reliability of data entry;

and intensifying quality control in testing and imaging. The study exceeded the target goal of 108 ($n = 112$) completely evaluable cases. Two interim DSMB meetings confirmed the lack of excessive adverse events, lack of center effects, comparability of subjects, and that distribution of subjects and enrollment will not jeopardize outcomes or generalizability of results.

Conclusions—Flexibility and rapidly solving evolving problems is critical when conducting highly complex multicenter metabolic studies.

Introduction

This article describes the challenges encountered during the conduct of a complex randomized, double masked multicenter trial investigating the interaction of testosterone and growth hormone in regulating muscle mass, strength, and physical function in older men at risk for muscle wasting and frailty. The study was funded by the National Institutes of Health and designed to determine the efficacy of testosterone and growth hormone administration in older men. The study involved three clinical sites located in the Western, Midwest, and Eastern United States. We describe the strategies implemented to overcome a number of major impediments that enabled this study to be completed in a timely manner with reliable results generated by the specific aims.

Background and rationale

Age-related loss of muscle mass (sarcopenia) is an important complication of aging [1–3]. Sarcopenia contributes to the decline of skeletal muscle strength and function, risk of falls and fractures, and diminished quality of life [4]. In the Baltimore Longitudinal Aging Study of 346 men 20–93 years of age, quadriceps strength decreased by approximately 30% between 50 and 70 years of age [5]. In the Copenhagen Heart Study, leg strength in 80-year-olds was about 30% less than in 70-year-olds [6,7]. With substantial losses in strength, physical function is often impaired, resulting in difficulty rising from a chair, climbing stairs, generating gait speed, maintaining balance [8] and eventually frailty, which may result in loss of independence, social isolation, depression, and inactivity increasing the risk for pulmonary embolism, osteoporosis, and bone fractures.

Systemic levels of anabolic hormones decline with aging [9–11]. Approximately 25–30% of men over 60 years of age have testosterone levels below thresholds used to define hypogonadism [11] that may be associated with loss of muscle mass and strength [12–14]. Whereas, restoring testosterone to youthful levels increases synthesis of mixed skeletal muscle proteins [15], total body cell mass [16], and muscle strength [15,17,18], but its unclear whether this intervention will enhance functional performance.

Because sarcopenia and weakness may occur [19] despite normal testosterone levels, other anabolic mediators (e.g., growth hormone [GH] and IGF-1) may contribute to age-related declines in muscle mass and function. After puberty, GH levels decrease progressively [20, 21]. A total of 35% of men 60 years of age or older were GH-deficient [22], and GH secretion for adults in their 70's was <50% of that in 20-year-olds [23]. Similarly, circulating levels of IGF-1, a mediator of several but not all anabolic effects of GH, decline

through the eighth and ninth decades of life [24]. These declines in GH and IGF-1 are associated with loss of lean tissue from the second to seventh decades [24,25].

Understanding the relative contributions of low testosterone, GH, and IGF-1 to sarcopenia and impaired muscle performance could have therapeutic implications [26]. We hypothesized that endogenous testosterone and GH are important regulators of myofibrillar protein metabolism, skeletal muscle strength, power, and physical function throughout life into advanced age and that these anabolic hormones regulate muscle contractile proteins by different but complementary mechanisms. To test this hypothesis, we initiated a study (HORMA) in older, community dwelling men with low testosterone and IGF-1 to: (1) determine the independent effects of gonadal status and GH-IGF-1 levels on myofibrillar protein synthesis and breakdown, (2) determine if there are independent beneficial effects at different levels of testosterone and IGF-1 on muscle mass and performance, (3) determine the change in local molecular regulators of skeletal muscle protein anabolism (e.g., IGF-1, Akt, mTOR) and muscle catabolism (ubiquitin-proteasome and ligase activity), and (4) whether there are positive interactions achieved at different levels of these anabolic hormones. We herein report the challenges encountered in initiating and conducting this complex multicenter trial to test these specific aims.

Study organization

Design development

During late 1998 and early 1999, a group of academic physician scientists (endocrinologist [SB], rheumatologist [RR], physiologist [KEY], and infectious diseases specialist studying muscle wasting in HIV [FS]) met at several scientific meetings to discuss potential collaboration to study sarcopenia. These meetings were followed by teleconferences, which included a senior statistician (SA) and the first grant submission to the National Institute of Aging later in 1999. Others joined the team prior to resubmission of the grant application (exercise physiologist [ETS], physical performance expert (TS) and nutritionist [CCS] to assist with meal planning to control energy and protein intake prior to metabolic measurements). Prior to study initiation, two master level statisticians (YW, MK) and experienced study coordinator-nutritionist (YS) were recruited to assist with development of the case report form (CRF) and electronic database.

Manual of operations and CRFs

During the initial 3 months of the study, members of the research team worked to develop a 142-page manual of operations (MOP) to standardize protocol interventions, measurements and data management at the three study sites. In brief, the MOP included methods for collection and processing of blood and tissue specimens and their transport to off site research laboratories; dosage and administration procedures for study medications and stable isotopes; MRI and DEXA acquisition protocols and analysis procedures, transfer procedures for images to the Central Reading Center; adverse event reporting; etc. For a complete outline of the MOP, see the Clinical Trials supplemental data website. Based on the MOP, a 121-page hard copy case report form (CRF) was created.

Contracts

One particular challenge was the need to work with the legal staff from the four participating institutions and three pharmaceutical sponsors who provided study hormones. The problems were complicated by the requirement from the parent institution that all contracts be identical in structure and content. This delayed startup by nearly 6 months.

Data coordinating center

The Data Coordinating Center (DCC) was based at USC and managed by three study statisticians. The DCC developed an electronic on-line data entry system based on the 121-page CRF for acquisition, storage, and collation of data, and rapid distribution of information. Quality control features included: (a) range checks based on allowable values, (b) pop-up flags for required data fields, (c) 'use-case' scenarios to permit skipping data that are not needed/appropriate for a specific participant. The data was exported to a Statistical Analysis System 9.1 (SAS, Institute Inc, Cary, NC; ODBC-compliant) file, merged, and quality control-checked using a SAS program. Summary reports were generated on a weekly basis for the principal investigator and biostatisticians.

Web-based data entry allowed randomization authorization to the local research pharmacists only after all eligibility criteria were electronically validated. The Web-based system also facilitated monthly enrollment reports, to centrally monitor progress of all subjects from the first preentry visit to the week 28 evaluation, and generate DSMB reports. As data were entered, limits for outliers automatically detected suspicious results as well as providing real time assessment of adverse events. This volume of data could not have been managed manually without a greater work force and greater cost. Finally, because clinical studies often take a number of years to complete before results are available, our electronic database will facilitate analysis of outcomes and more expeditious emanation of manuscripts describing the results of this important study.

Quality control

Data quality audits were conducted at regular sequential intervals by DCC staff traveling to each clinical center during the course of the trial. All data entered in the hard CRF and the electronic data base were verified with the source documents including physician's notes, nurse flowcharts, and laboratory test reports by the DCC.

Data safety and monitoring board

It was anticipated that substantial adverse events unrelated to study would occur since more than 100 subjects at least 65 years with multiple medical and musculoskeletal problems will be evaluated over nearly 25 cumulative years of subject follow-up. Subjects were evaluated by study physicians at weeks 4, 8, 12, and 16/17, 28 and additionally at weeks 2, 6, 10, and 14 by study coordinators when subjects picked up their rhGH syringes.

The Data Safety Monitoring Board (DSMB) was composed of six experts in aging, endocrinology-metabolism, biometry and statistics. The DSMB met prior to initiation of the study to assess the appropriateness and ethics of the study design and has held two interim

analyses as prescribed by the MOP after the first 30 and 70 subjects had completed study week 17 to monitor the conduct of the study and occurrence of adverse events.

Participating centers

The study was initiated at three clinical sites (Tufts University, Charles Drew University, and University of Southern California [USC]), with the Analytical Laboratory at Washington University, and Central Image Reading Center at USC. Because of slow accrual at one site and the PI moving to Boston University, a new clinical site was opened at Washington University in the third study year by a new PI conducting aging research of anabolic hormones and bone metabolism (EB).

Orientation and training

Prior to initiation of the trial, investigators and study coordinators met at USC for a day-long orientation and training session whereby all procedures were reviewed by respective experts responsible for writing sections of the MOP. Training also occurred in the USC exercise physiology laboratory to demonstrate proper techniques in muscle function testing and separate practical sessions on how to use web-based log-in, data entry, editing, etc.

Recruitment

Table 1 shows that 1036 subjects were screened by telephone as a result of extensive media advertising (local newspapers, magazines, and radio stations) to assess eligibility. From these subjects, 242 (23%) were consented for formal screening and 122 (50%) were randomized to receive study therapy. At USC, extensive group orientation sessions with up to 10–12 potential candidates per session were scheduled following the telephone screening. Sessions were led by members of the research team with power point presentations describing the potential importance of testosterone and GH in aging, results of the investigators' prior studies, a detailed description of the protocol design, and commitments needed for the intense nature of the study including what was involved at each visit. This was followed by a tour of the exercise physiology laboratory and GCRC where subjects were introduced to the staff and shown where they would stay for their overnight evaluations. Similar but less comprehensive group screening was done at the other two clinical sites.

At the second interim analysis, the DSMB recommended that 36 evaluable subjects at Tufts and 25–30 evaluable subjects at Washington University should provide sufficient numbers of subjects for generalizability of the results. The study finished in May 2007 with a total of 112 evaluable subjects including 53 from USC, 35 from Tufts University, and 24 from Washington University (Table 1).

Staff and resource standardization

Our study was funded in 2002 and completed enrollment in 2007, thereby providing a challenge when staff turnover occurred. Fortunately, there was no change in investigators, study coordinators, GCRC metabolic and exercise staff, exercise specialists, and DCC at USC. However, limited staff turnover at the other clinical sites resulted in the need for extra orientation and training sessions, extra site visits for source documentation and verification

of data entry and weekly central monitoring to ensure that the MOP was followed correctly. This resulted in some delays and initial errors in the collection of data. Data not collected according to the standard protocol listed in the MOP (e.g., incorrect subject positioning or acquisition parameters during imaging) was highlighted and some data may have to be censored from secondary analyses.

Our intent was to standardize strength testing through use of Keiser pneumatic equipment at the three sites. However, by the time the study began, there was insufficient budget to repair, upgrade, and calibrate this expensive equipment at one of the sites and the replacement facility did not have this equipment. Thus, different equipment will result in different units of measurement (e.g., Newtons versus kilograms) and results in different joint mechanics for the same exercise. Thus, comparative strength results will need to be reported as relative % change to account for inherent differences in exercise equipment.

Quality control

Site visits to the three clinical centers were performed regularly to ensure that muscle function assessments and other aspects of the study were being conducted according to the MOP. Our first site visits were initiated after four-to-six subjects were enrolled. Although we established frequent communication with the sites by monthly tele-conferences and regular email, we learned at the site visits that relatively minor differences in muscle function testing and imaging procedures was occurring between sites. These were corrected but it would have been preferable to visit the sites after the first or second subjects were enrolled and images from those centers should have been evaluated immediately by the Central Reading Center.

Study methods

Eligibility

Table 2 shows the eligibility criteria for endogenous hormone levels and to assure that potential subjects did not have underlying conditions that could affect metabolism or increase their risks for treatment related adverse effects.

Study subjects and randomization

To assure diversification of participants, generalizability of outcomes, and timely completion of the study, clinical aspects of the trial were being conducted at three universities across the US. Study subjects signed local IRB-approved informed consent documents. Figure 1 shows the allocation of subjects according to treatment of the two-tiered randomization. The number of subjects randomized to each of the six intervention cells was well balanced. Two hundred and forty two (23%) of the 1036 subjects screened were consented and of 122 randomized to study therapies, 112 subjects completed the 16 weeks of study interventions and serve as the basis of this report.

The randomization efficiency for subjects consented at USC was 60% compared to the 42% and 50% at Tufts and Washington University, respectively. It is possible that the extensive orientation at USC maintained subject enthusiasm during the screening process or subjects

consented at the other sites may have had more exclusionary conditions detected at onsite evaluation. Other explanations could include local 'bad press' about GH or steroid abuse in athletes, greater concerns about subcutaneous injections, greater turn over in staff knowledgeable about the study, difficult weather in Boston and St. Louis causing some subjects to reconsider their commitment in the winter months, or other issues not immediately apparent. At USC, anxiety about parenteral injections of rhGH was largely alleviated by showing subjects an educational video, allowing them to practice injection techniques, and experienced nurses providing reassurance about administering study therapies.

Study design

HORMA was a randomized, placebo controlled, double masked investigation using a factorial design of testosterone and GH administration to achieve age-related and youthful hormone levels in community dwelling men 65–90 years of age with low or low normal testosterone and IGF-1 levels. Treatment randomization was a two-tiered process (Figure 1). Subjects were first randomized to receive one of two doses of testosterone gel, which was expected to increase testosterone levels to low to mid-normal range or mid to high-normal range utilizing a Leydig cell clamp (described below). Within these two groups, subjects were randomized (second tier) to placebo or one of two doses of rhGH. The treatment duration was 16 weeks. Subjects were evaluated at week 28 to assess the durability of effects.

Scheduling

Because of the large number of study visits required (minimum of 16), coordinating schedules of investigators, consultants (e.g., urologists and cardiologists to validate eligibility and monitor safety), nurses, nutritionists, and exercise specialists was a challenge, especially as the number of subjects on study increased. To solve this problem, the USC GCRC informatics staff developed electronic scheduling software with preset intervals for each visit and availability of members of the study teams at those time points. Once subjects were deemed eligible, baseline dates were entered manually and all follow-up dates would show automatically with holiday conflicts, dates the GCRC is closed, and dates specific members of the study teams were not available. This system allowed dates to be altered in sequence to achieve compatibility. Electronic messages were automatically sent weekly to members of the study team reminding them of subjects de-identified by codes coming for visits that week. This also provided a mechanism with prompts for coordinators to call subjects the day before to remind them of their appointments and to bring in unused syringes and sachets for adherence counting.

Study interventions

A Leydig cell clamp was utilized to maintain serum testosterone concentrations during therapy in the low to low-normal range (250–550 ng/dL) similar to levels of aging men or mid- to high-normal range (650–950 ng/dL) typical of healthy young men. Participants received monthly injections of 7.5 mg of a long-acting GnRH agonist, leuprolide acetate depot (Lupron, Tap Pharmaceuticals) through study week 12 and were randomized to

receive blinded transdermal testosterone gel (AndroGel, Solvay Pharmaceuticals Inc) at 5 g or 10 g/day each morning for 16 weeks.

Subjects were also randomized to receive 0, 3.0, and 5.0 µg/kg of rhGH (Nutropin, Genentech Inc) subcutaneously 2–3 h after dinner each evening. The 3.0 µg/kg dose was chosen as the lowest active dose since 3.3, but not 2.0 µg/kg/day, increased whole body protein synthesis in GH deficient adults [27]. The 5 µg/kg/day dose was chosen to produce a greater anabolic stimulus at levels low enough to minimize the risks of fluid retention and adverse musculoskeletal effects as occur with higher doses [28–31]. The rhGH was prepared by study pharmacists in prefilled syringes to be kept refrigerated by study subjects.

Outcome measurements

Table 3 depicts the screening process and measurements during the study to assess outcomes and to monitor for adverse events. The primary outcome measures for this study included changes in body composition (total and regional muscle and adipose tissue), muscle function (strength, power, endurance, and physical performance); quantification of total myofibrillar protein, actin, and myosin synthesis along with breakdown of muscle proteins; assessment of local regulators of muscle function and their relationship to systemic hormone levels and safety.

Special imaging

Body composition was measured by DEXA, MRI, and D2O dilution. Scans were transferred to the Central Reading Center on 3.5 inch and optical discs, respectively. A DEXA phantom for body composition standard was used to cross-calibrate DEXA scanners at the three clinical sites. To evaluate muscle volume of the extremities by MRI, pixels associated with intramuscular fat, bone, and major arteries, veins, and nerves are subtracted from images using validated software (SliceOmatic version 4.3, TomoVision) [32].

Skeletal muscle physiology

Subjects were admitted to the local research centers for overnight infusions of 1,2-¹³C₂-leucine with pre-and postinfusion biopsies of the vastus lateralis to quantify enrichment of the tracer by mass spectroscopy and to calculate the fractional synthetic rates of total myofibrillar protein, myosin, and actin [33]. Skeletal muscle breakdown will be analyzed for ubiquitin proteasome enzyme activity.

EKG stress, aerobic capacity and endurance testing

To exclude subjects with cardiopulmonary limitations, a symptom limited graded cycle ergometry protocol with 12-lead electrocardiographic stress test was performed to achieve a heart rate (HR) of at least 85% of the predicted maximum. Resistance to pedaling is increased by 15 or 20 watts/min to exhaustion when peak oxygen uptake (VO₂) is measured. After a 45 minute rest period, subjects underwent a timed endurance test at constant work rate (80% of the maximum work rate achieved in the peak VO₂ test) to exhaustion. At one of the clinical sites, local policy would not allow subjects to achieve HRs >90% of predicted maximum, and thus data for VO₂peak and aerobic endurance were not available for initial

subjects at that site but fortunately, after numerous meetings, the policy was changed to accommodate testing.

Muscle performance

Maximal voluntary strength was assessed using the one-repetition maximum (1-RM) method for the bilateral leg press, leg extension, leg flexion, latissimus pull-down, and chest press exercises on Keiser pneumatic equipment at USC and on selectorized weight stack resistance exercise machines at Tufts and Washington universities. The highest of the respective 1-RM values assessed at preentry 3 or baseline was used as the pretreatment value. Muscle endurance was evaluated with subjects performing as many repetitions as possible at a leg press resistance of 80% and the chest press resistance of 70% of their respective best pretreatment 1-RM values. Unilateral leg extension power was measured with the Bassey leg extension rig [34], since leg power has been highly correlated with physical performance measures in frail elderly subjects [35]. To further evaluate physical function, two performance-based tests with higher ceilings than commonly used tests were selected (Margaria stair climb power test [36] and 400 meter timed walk to determine maximum habitual gait speed [37]).

Hormones and cytokines

For screening, total testosterone was measured in the local clinical university laboratories and IGF-1 at Quest Diagnostics. Batched samples for total and free serum testosterone levels will be measured by liquid chromatography tandem mass spectroscopy (LC-MS/MS) [38] at Boston University. Insulin, adiponectin, and IGF-1 levels will be measured in the USC GCRC Core Laboratory by standard methods.

Dietary monitoring and meal preparation

Because protein and amino acid intake are potent stimuli for skeletal muscle protein synthesis [39], energy and protein intake was tightly controlled to minimize potential dietary confounding based on 3-day food diaries, to maintain protein intake at 0.9–1.1 g/kg/day (Nutritionist V software). Registered dietitians prepare food for subjects to be eaten at home for 3 days prior to the isotope studies. Food diaries and exercise questionnaires were monitored by study nutritionists monthly to assure that subjects do not deviate from their usual diets and activity levels.

Statistical considerations

Sample size

Sample size calculations were based on detectable effect sizes using one-way ANOVA with pairwise comparisons (adjusting for multiple comparisons) for baseline to end-of-treatment changes for the primary endpoints across the three dosing groups of rhGH for each of the testosterone groups. A sample size of 18 in each of the six groups (108 evaluable subjects) has 80% power to detect a small effect size of 0.19 at $\alpha=0.05$. Based on our prior studies with dropout rates <7%, and considering a higher dropout rate from adverse effects of rhGH, HORMA was designed to over-accrue the placebo, 3 and 5 $\mu\text{g/kg/day}$ rhGH arms by 10, 15, and 20%, respectively.

Statistical analysis

Two-way ANOVA will be used to compare baseline characteristics across the six groups and to identify potential covariates for subsequent analyses. Two-way ANOVA (ANCOVA), including pairwise multiple comparisons (two-sided), will be utilized to analyze baseline to end-of-treatment changes in endpoints for evaluable subjects for each intervention factor (testosterone, rhGH), and treatment interaction. Repeated measures ANOVA (ANCOVA) will be used for contrasting endpoints at baseline, 17 and 28 weeks. Imputation methods will be used to estimate 17-week endpoints for nonevaluable subjects and results for the full cohort will be contrasted with those obtained for evaluable subjects.

Mixed (random effects) models will be conducted for longitudinal data within treatment group and overall (with treatment as a covariate) to evaluate: (1) baseline characteristics predictive of change in muscle protein synthesis, regulators of muscle protein metabolism, body composition, muscle strength, and physical function; (2) on-trial changes in muscle protein synthesis and breakdown, levels of local muscle regulators, body composition, strength, physical function, and QOL; and (3) role of diet and lifestyle variables on the rate of change in study endpoints. Treatment effect modifiers (e.g., BMI, total LBM, testosterone) will be evaluated using similar methods. Statistical analyses will be conducted at the $\alpha=0.05$ level using SAS.

Subject compliance and retention

The 16 study visits was burdensome for some subjects to complete evaluations and measurements, pickup growth hormone syringes every 2 weeks, and suture removal 5–7 days after two overnight admissions to the clinical research units. Yet, adherence to study visits exceeded 98% and only 10 (8%) of the 122 randomized subjects dropped out of the study during study interventions. A number of strategies proved valuable in solving intervention and retention challenges. We believe compassionate and dedicated research teams who spent adequate time addressing concerns of potential subjects and showing interest in all aspects of their health and well being was an important factor in subject satisfaction, which likely accounted for the high adherence rate.

Discussion

Despite the complexity of testing procedures, enormity of data generated, and successes in enrollment, we learned a number of important lessons from the challenges occurring while initiating and conducting this complex metabolic multi-center investigation.

Recommendations

- For multicenter trials, contracts should be executed well in advance of the targeted start dates. Principal investigators should work with their contracts and grants officers to facilitate reconciliation of differences in legal goals between participant sites.
- Web-based data entry greatly facilitates screening subjects for eligibility, monitoring adverse events and data out of range, and generating DSMB and monthly accrual reports and should be valuable for other complex studies.

- Because some procedures at the testing sites may not be performed properly despite a comprehensive manual of operations and extensive orientation session of investigators and study coordinators, study sites should be visited to validate performance of procedures and data entry after the first 1–2 subjects are entered. Similarly, special imaging scans (DEXA and MRI) should be reviewed at the reading center after 1–2 scans have been completed.
- When changes in study personnel occur, ad hoc training sessions, intensified monitoring, and immediate site visits should be conducted to assure that all specified procedures are being exactly followed.
- When accrual is slow to be initiated as identified in monthly census reports, complicating factors should be investigated promptly, strategies to improve performance instituted, but if accrual remains below target, consideration for recruitment of other study sites should be expeditiously considered after review with DSMB.
- Since enrollment, randomization, retention efficiency will inherently differ by sites, sharing successful strategies for advertising, screening, orientation, subject satisfaction by the more successful sites or limitations caused by local factors of sites enrolling at slower rate, should be shared by the group at large.
- When equipment or testing procedures do not allow standard procedures to be followed, attempts must be made to rectify local procedures or policies. Regardless, the impact on data must be assessed by the principle investigator and DCC and alternate strategies for testing or data analyses need to be considered.
- Creating software packages to plot scheduling based on availability of research team members, identification of holidays, and to provide weekly prompts for subjects to receive telephone reminders, can substantially facilitate coordination of the work force and attendance by study subjects.

Summary

Complex multicenter clinical studies such as HORMA require intensive oversight, rapid identification of problems and deviation from standardized procedures, and participation of research team members with expertise in informatics, biometry, statistics, data management as well as investigators with expertise in the respective areas of biomedicine. Rapid implementation of strategies as we did to correct problems encountered in real time allowed this important study to be completed in a timely manner and the specific aims were able to test the hypothesis underlying the study design, and so that results may be expeditiously submitted for publication.

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Appendix

A. Committee members

Data safety and monitoring board

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Figure 1.
Randomization schema.

Table 1

Enrollment and randomization efficiency

	USC	Tufts University	Washington University	Total
No. screened by telephone	270	560	206	1036
No. enrolled (consented)	94 (35%) ^a	100 (18%)	48 (23%)	242 (23%)
No. randomized	56 (60%) ^b	42 (42%)	24 (50%)	122 (50%)
No. dropped by week 17	3 (5%) ^c	7 (16%)	0 (0%)	10 (8%)
No. completing week 17	53	35	24	112 (92%)
Potentially evaluable	53	35	24	112

^aPercentage of those screened by telephone who provided formal informed consent at or after interview/orientation sessions.

^bPercentage of those consented and were randomized to study interventions.

^cPercentage of those randomized to study therapies who did not complete study interventions or end of treatment measurements.

Table 2**Eligibility requirements for study participation**

Inclusion criteria	
•	Men 65–90 years of age
•	Total serum IGF-1 in the lower tertile for adults (0–167 ng/dL)
•	Total morning serum testosterone of 150–550 ng/dL, which is the expected normal range for this age
Exclusion criteria	
•	BMI ≥ 35 kg/m ²
•	Weight instability (>3% change in prior 3 months)
•	Daily intake of total kilocalories 0.8 × the RDA and intake of protein 0.8 or 1.4 g/kg/day
•	Acute illness in the prior 30 days
•	Resistance training in the past 12 months
•	Vigorous aerobic sports: Competing as a Master Athlete in the prior 5 years or weekly swimming, racquet ball, cycling, tennis, in the prior 12 months
•	Use of an anabolic agent (rhGH, androgen, androgen precursor, etc) in prior 12 months
•	Use of medications that might affect amino acid metabolism (e.g., β-adrenergic blockers, β-agonists, Ca ²⁺ channel blockers, or corticosteroids)
•	Fasting blood sugar ≥ 126 mg/dL or diabetes requiring therapy
•	History of benign intracranial hypertension
•	Heart failure, active angina, or myocardial infarction in the prior 6 months or history of aortic stenosis
•	Uncontrolled hypothyroidism or hyperthyroidism
•	Rheumatoid arthritis, cirrhosis or active hepatitis
•	History of carpal tunnel syndrome
•	Prior cancer other than squamous or basal cell carcinoma of the skin
•	Sleep apnea or severe chronic lung disease
•	Anticoagulation with heparin or coumadin
•	Blood pressure that can not be controlled with medication to <180/95 mm Hg
•	Calculated creatinine clearance <50 cc/min
•	Serum prostatic antigen >4.0 or American Urological Association score ≥ 14
•	Hematocrit < 50%, or ALT >1.5 ×ULN
•	Failure to pass a modified Bruce treadmill stress test
•	Severe disability limiting strength or physical function testing
•	Dementia or cognitive impairment affecting a subject's ability to provide informed consent

Table 3

Schema of testing and study interventions

Testing	Study week									
	-1-2	0	4	8	12	16	17	28		
Pre-Screening: AM testosterone and IGF-1	X									
Screening: Complete history/physical exam, EKG, chest radiograph, CBC, chemistries, TSH, PSA, urinalysis	X									
Study treatments										
Leuprolide acetate intramuscular injections		X	X	X	X					
Daily testosterone in AM and growth hormone in PM										
Research measurements										
3-day dietary diaries/Nutritionist V analysis	X		X	X	X	X		X		
Serum total/free testosterone, SHBG, LH, IGF-1 ^a		X	X	X	X	X	X	X		
Muscle protein synthesis and breakdown										
[1,2- ¹³ C ₂]-leucine infusion		X						X		
Muscle biopsies 90 min and 14 h after infusion begun		X						X		
Body composition and muscle mass										
DEXA for total and appendicular LBM, trunk fat		X		X				X	X	
MRI of arms/legs for muscle volume; abdomen for fat		X						X		
D ₂ O isotope dilution studies for total body water		X						X		
Muscle strength and functional performance ^b										
1-repetition maximum (1-RM) ^c and endurance tests	X	X		X				X	X	
Leg extensor power (Bassey power rig)	X	X		X				X	X	
Functional performance tests ^d	X	X		X				X	X	
VO ₂ max and aerobic endurance tests						X				
Quality of life assessments		X				X			X	
Safety assessments										
History and physical examination		X	X	X	X	X		X	X	
Digital rectal exam (study urologist), PSA, AUA ^e		X				X		X	X	
Complete blood counts and comprehensive chemistries		X	X	X	X	X		X	X	

Testing	Study week						
	-1-2	0	4	8	12	16	28
Fasting lipid panel, fasting blood sugar and insulin		X		X		X	X

^a Blood was collected for sex hormone levels 24 h after the prior dose and 2 h after application at weeks 4, 8, 12, and 16 at the local GCRCs.

^b To minimize familiarization, subjects will have strength and function testing done twice within 7 days of starting hormonal interventions separated by at least 48 h.

^c Chest press, latissimus pull down, leg press, knee extension, knee flexion.

^d Margaria stair climb, 400 m walk.

^e American Urological Score for urinary obstruction.