# Relationship of Anabolic Hormones With Motor Unit Characteristics in Quadriceps Muscle in Healthy and Frail Aging Men

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**Context:** Anabolic hormones are important factors in maintaining muscle mass for aging men, but their role in overall motor unit structure and function is unclear.

**Objective:** The objective of this work is to determine associations of anabolic and reproductive hormone levels with motor unit characteristics in quadriceps muscle in older healthy and frail men.

**Design:** This work is an observational cohort study of community-dwelling men.

**Participants:** Participants included healthy and frail men younger than 65 years.

**Intervention:** No intervention was performed.

**Outcome measure:** Quantitative assessments of electromyography-derived motor unit potential size (MUP) and compound muscle action potential size (CMAP) of the vastus lateralis muscle.

**Results:** We studied 98 men (mean ± SD: age 73 ± 6 years; body mass index [BMI] 25.7 ± 4.0 kg/m$^2$; diabetes 11%) of whom 45% were prefrail and 18% frail. After adjusting for age, BMI, and prevalent diabetes, higher total and free testosterone levels were significantly related to larger CMAP (total testosterone: β [95% CI]: 0.3 [0.08-0.53]; free testosterone: 0.34 [0.13-0.56]). Exploratory analysis showed the relationship between free testosterone and CMAP was stronger in frail rather than robust men. In univariate analyses, estradiol was associated with CMAP size (0.37 [0.16-0.57]); and vitamin D was associated with MUP size (0.22 [0.01-0.43]) but these relationships were no longer significant after adjusting for potential confounders.

**Conclusion:** Our data highlight the associations between androgen levels and the electrophysiological characteristics of older men, particularly in the frail. Clinical trials involving

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Abbreviations: BMI, body mass index; CMAP, compound muscle action potential; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; EMG, electromyography; FI, frailty index; FP, frailty phenotype; MU, motor unit; MUP, motor unit potential; MVC, maximum voluntary contraction; VL, vastus lateralis.

administration of androgens will help to elucidate the potential benefits of intervention on neuromuscular function and/or frailty status. (***J Clin Endocrinol Metab*** **105: e2358–e2368, 2020)**

**Key Words:** anabolic hormones, electromyography, frailty, motor unit, muscle, testosterone

Adverse outcomes associated with frailty, including reduced mobility and falls, might be linked to underlying sarcopenia, characterized by low muscle mass and related physical dysfunction. Lower cross-sectional area and total number of muscle fibers, both features of sarcopenia, have been linked with impaired anabolic signaling, increased levels of proinflammatory cytokines, and declining numbers of motor units (MU) (1–4). Although anabolic hormones are considered key factors in maintaining muscle fiber cross-sectional area through the effects on muscle protein turnover, their role in the broader neuromuscular system, including MU structure and function, during healthy aging and frailty, is less clear.

A MU includes a single α motor neuron and all the skeletal muscle fibers it innervates. Activation of individual motor units during movements ensures the precise matching of muscle forces to meet the task requirements. Various methods of electromyography (EMG) have been used to study human MU characteristics. Intramuscular EMG employing needle electrodes is able to provide detailed information on the structure and function of MUs via the measurement of consecutive action potentials during voluntary contractions in a "localized" fashion (5–7). Similarly, involuntary electrically stimulated contractions provide a more "global" view of the electrophysiological characteristics of muscle via skin-surface measures (8).

The declining numbers of MUs with advancing age (9, 10) may cause denervation of muscle fibers and constrain the ability of the central nervous system to control voluntary movements. By way of compensation to preserve muscle function, some denervated fibers can be reinnervated by axonal branching from neighboring motor neurons (11). This remodeling process leads to an increase in the size of surviving motor units in older adults compared with young, but contributes to fiber atrophy and fiber losses when reinnervation fails (12, 13). The underlying regulation of MU remodeling in sarcopenia and frailty remains poorly understood, but may be associated with hormonal changes during aging, particularly declines in anabolic hormone levels.

The neuromuscular protective effects of androgens have been studied in animal models in which male castration led to MU dendrites' atrophy, which was reversed by testosterone (T) administration (14, 15). Similarly, when compared with controls, T therapy attenuated atrophy of motor neuron dendrites and muscle fibers in female rats with spinal cord injury (16). Exogenous T accelerated regeneration of facial (17) and sciatic nerves (18) postinjury and in humans, T treatment protected neuron cultures from cell death caused by T deprivation (19). More recent studies, however, suggest that dihydrotestosterone might be a more potent anabolic hormone in mammalian skeletal muscle, exerting effects on force in slow-twitch and fast-twitch fibers alike (20). Dehydroepiandrosterone sulfate (DHEA-S) is a weak androgen with neuroprotective and antiapoptotic properties that are independent of any anabolic effects exerted after conversion to T. In vivo and in vitro models both suggest that DHEA-S promotes neurogenesis, neuronal survival, and prevents neurotoxicity because of its antiglucocorticoid effects (21). None of these properties, however, have been studied in the context of the peripheral nervous system and motor neuron preservation in humans.

Similarly, cumulative evidence indicates that estradiol (E2) has a neuroprotective role in the central nervous system (22). However, animal and human research also suggests neuroprotective effects of E2 on spinal motoneurons, where the Akt antiapoptotic signaling pathway is regulated by E2 (23, 24).

The anabolic role of vitamin D in the muscle has previously been studied in health, sarcopenia, and frailty predominantly in the context of muscle protein turnover (25, 26). Despite the fact that low levels of vitamin D have been linked to impaired balance and frequent falls, no studies to date have investigated whether vitamin D levels are related to neuronal control of muscle function and MU health.

Given the possible roles of these anabolic factors for human neuromuscular function and the lack of data at the whole MU level in healthy aging and frailty, we aimed to determine the association between anabolic hormone levels and MU characteristics in quadriceps muscles in older men from the general population.

## Participants and Methods

### Participants

A total of 114 men age 65 to 90 years were recruited from the Greater Manchester area between 2014 and 2017. Participants were recruited from local universities' databases, National Health Service general practices,

and secondary care, including outpatient departments, day hospitals, and community physiotherapy centers. All participants provided written informed consent. The study was also open to the general public through poster and newspaper advertisements. A full list of the selection criteria is included in the supplemental material (27).

Ethical approval for the study was obtained from the National Research Ethics Service Committee Northwest (15/NW/0426).

## Assessments

### Questionnaires

Each participant provided details of lifestyle, medical history, and medications taken. The men also completed the Geriatric Depression Scale questionnaire (28) and the Physical Activity Scale for the Elderly questionnaire (29).

**Anthropometry measures.** Body mass (kg) and height (m) were measured and total body composition assessed by dual-energy X-ray absorptiometry (Lunar Prodigy Advance, version EnCore 10.50.086). Appendicular lean mass with appendicular bone mineral content removed was normalized to height to establish sarcopenia status (12). Appendicular lean mass strongly correlates with total body lean mass in our unpublished data of 168 older males ($r = 0.88$, $P < .001$).

**Assessment of physical function, activity, and frailty.** Physical function was assessed objectively by Short Physical Performance Battery testing, which included assessment of 4-meter (13 feet) walking speed, standing balance, and 5 chair stands. The "Timed up and go" (TUG) was also performed, in which participants were invited to stand from a seated position and walk 3 meters (10 feet) forward around a cone as quickly as possible, returning to their original seating position. Time from the command "Go" until the participants returned to their original seated position was recorded.

Grip strength was measured using a handgrip dynamometer (Jamar). Participants were invited to squeeze the handle as hard as possible for around 3 seconds and the maximum contraction force (in kilograms) was recorded. This was repeated 2 times for each hand, alternating between the right and left with 30 seconds' rest between trials. Maximal voluntary isometric contraction of the knee extensors was assessed with the participant's leg fastened to a force transducer 30 cm below the center of the knee joint, with hips and knees flexed at 90°. Participants performed a standardized warm-up of several contractions, after which they were asked to perform a maximal effort that lasted approximately 2 to 3 seconds. This was repeated a further 2 times separated by short rest intervals, and the highest of the values was accepted as the maximum voluntary contraction (MVC).

Frailty was characterized by the 2 commonly used approaches: the frailty phenotype (FP) and frailty index (FI). Frailty phenotype was adapted from the Cardiovascular Health Study based on 5 criteria: sarcopenia, exhaustion, slowness, weakness, and low activity (30). The variables used to construct FP and the population-specific cutoff points are presented in Supplemental Table 1, alongside the original Cardiovascular Health Study criteria (27). Individuals with one or more of these criteria were classed as frail and those with none were classed as robust.

The FI is composed of 37 health deficits (symptoms and signs, functional impairments) that are known to accumulate with age and are associated with adverse health outcomes. The FI was created using a standardized procedure (31). Continuous variables were dichotomized based on the distribution of participants' scores; cutoff points were set at the worst-performing tenth centile. Individuals with more than 20% of missing data on relevant deficits were excluded from the analysis. The details of the variables used to create an FI and specific cutoff points are described in Supplemental Table 2 (27).

### Hormone measurements

A fasting venous blood sample was used for all hormone measurements. All samples were collected between 8:30 and 9:30 AM. A validated liquid chromatography-mass spectrometry system was used to analyze total T (intra-assay, and interassay coefficients of variation [CVs]: 1.4% and 8.3%), E2 (CVs: 5.4% and 3.1%), dihydrotestosterone (DHT; CVs: 8.3%), vitamin D (CVs: 6.2% and 5.1%), and DHEA-S (CVs: 1.9% and 3.1%). Free T (fT) levels were derived from total T, sex hormone-binding globulin (analyzed using chemiluminescence), and albumin (measured by bromcresol purple) concentrations using Vermeulen's formula (32).

### Electromyography

The EMG data were collected from around the motor point of the vastus lateralis (VL). The parameters of interest were the supramaximal compound muscle action potential (CMAP) and motor unit potential (MUP).

The CMAP represents the sum of the electrophysiological signal from all motor units detectable by the

recording electrode when simultaneously activated at the same time using supramaximal stimulation of the peripheral nerve. It has been used clinically to track disease progression in spinal muscular atrophy and amyotrophic lateral sclerosis (33, 34). The CMAP was recorded at the VL motor point by surface EMG after percutaneous femoral nerve stimulation.

MUP represents the sum of electrophysiological signals as action potentials propagate along the sarcolemma of individual muscle fibers of a single MU. MUPs were recorded using a 25 mm intramuscular needle electrode inserted into the VL muscle at the motor point to a depth of approximately 1 to 2 cm. The participant then performed a sustained voluntary isometric contraction at 25% of his or her maximal effort and held it for 12 to 15 seconds. In between contractions, the needle was repositioned using combinations of 180° needle rotations and needle withdrawals of approximately 5 mm to obtain a minimum of 6 recordings from spatially distinct areas. The details of the EMG technique used, data recording, and analysis are described in the supplemental material (27).

### Statistical analysis

Descriptive statistics are presented as the mean ± SD or n (%), and statistical significance of between-group differences was assessed using ANOVA.

Linear regression models determined relationships between predictors (hormone level) and outcome (MUP or CMAP). Each predictor as well as CMAP was considered as an untransformed value standardized as a Z score [(raw score – mean)/SD] to allow comparison of results between predictors. MUP area, in view of significant skewing, was log-transformed before being standardized as a Z score to meet the linear regression assumptions.

Models were adjusted for age, body mass index (BMI), diabetes, and alcohol excess because these correlated with the predictors and therefore were potential confounders. The analyses in which E2 was a predictor were further adjusted for total T—the main precursor of E2 production in men. The results of these analyses were displayed as standardized coefficients (β) with 95% CI.

In an exploratory analysis, we introduced an interaction term (hormone × frailty phenotype or hormone × frailty index) as well as an FP or FI variable, as appropriate, to the fully adjusted models to assess whether the relationships between hormone levels and EMG parameter values varies in health in relation to the level of frailty.

All analyses were performed using STATA 13 SE software (StataCorp, 2013. Stata Statistical Software: Release 13. StataCorp LP).

## Results

Out of 114 men who participated in the study, 98 men had complete data on MUP and CMAP and were included in the analysis. The mean age of the men was 73 years and mean BMI 25.7 kg/m$^2$ (Table 1). Sixteen percent were current smokers and 39% consumed more than 14 units of alcohol per week. Cardiovascular disease was present in 19% of participants and diabetes in 11%.

We assessed relationships between hormone predictors and clinical variables (age, BMI, diabetes, smoking, and alcohol excess) that could potentially confound relationships between hormone levels and EMG parameters (Table 2).

These data indicated that age, BMI, diabetes, and alcohol excess might be potential confounders, and therefore we included these as covariates in subsequent models.

In unadjusted analysis T, fT, DHT, and E2 were positively related to CMAP size: 1-SD higher level of T, fT, DHT, and E2 was associated with larger CMAPs normalized as SD units (total T: β [95% CI]: 0.48

**Table 1. Clinical characteristics**

| Characteristics | Data |
|---|---|
| No. | 98 |
| Age, y | 73 ± 6 |
| BMI, kg/m$^2$ | 25.7 ± 4.0 |
| Current smoker | 16 (16) |
| Alcohol excess, ≥ 14 units/wk | 31 (39) |
| Respiratory disease | 14 (16) |
| Cardiovascular disease | 16 (19) |
| Diabetes | 9 (11) |
| Osteoarthritis/Rheumatoid arthritis | 24 (28) |
| Taking ≥ 3 medications | 36 (42) |
| Frailty index | 0.1 (0, 0.65) |
| Frailty phenotype | |
| Robust | 28 (36) |
| Prefrail | 35 (45) |
| Frail | 14 (18) |
| Sarcopenia | 49 (51) |
| Exhaustion | 12 (14) |
| Low activity | 16 (19) |
| Weakness | 13 (14) |
| Slowness | 15 (16) |
| Total testosterone, nmol/L | 18.5 ± 8.8 |
| Free testosterone, pmol/L | 293 ± 116 |
| Dihydrotestosterone, nmol/L | 1.7 ± 1.0 |
| Estradiol, pmol/L | 85 ± 33 |
| DHEA-S, nmol/L | 2.1 ± 1.3 |
| Vitamin D, nmol/L | 62 ± 26 |
| MUP area, μV.ms | 1400 ± 661 |
| CMAP amplitude, mV | 7290 ± 2592 |

Data are mean ± SD, median (range), or n (%).
Abbreviations: BMI, body mass index; CMAP, compound muscle action potential; DHEA-S, dehydroepiandrosterone sulfate; MUP, motor unit potential.

**Table 2. Relationships between hormonal predictors and potential covariates (n = 98)**

| | | Tertiles of Hormone Level | | | |
|---|---|---|---|---|---|
| **Predictor** | **Covariate** | **Tertile 1** | **Tertile 2** | **Tertile 3** | ***P*** |
| Testosterone | Age, y | 76 ± 7 | 72 ± 5[a] | 72 ± 5[a] | .070 |
| | BMI, kg/m$^2$ | 28.0 ± 5.1 | 25.1 ± 2.9[a] | 23.7 ± 2.5[a] | .002 |
| | Diabetes | 6 (22) | 0 | 1 (4) | .017 |
| | Smoking | 6 (20) | 3 (11) | 6 (21) | .553 |
| | Alcohol excess | 8 (31) | 9 (43) | 9 (43) | .608 |
| Free testosterone | Age, y | 76 ± 6 | 73 ± 6 | 70 ± 4[a,b] | < .001 |
| | BMI, kg/m$^2$ | 27.5 ± 5.1 | 25.8 ± 3.1 | 24.6 ± 3.2 | .054 |
| | Diabetes | 6 (22) | 0 | 1 (4) | .017 |
| | Smoking | 4 (14) | 7 (25) | 4 (14) | .459 |
| | Alcohol excess | 7 (27) | 11 (58) | 8 (35) | .099 |
| Dihydrotestosterone | Age, y | 74 ± 7 | 73 ± 6 | 72 ± 5 | .750 |
| | BMI, kg/m$^2$ | 27.8 ± 5.0 | 25.1 ± 3.1[a] | 23.9 ± 2.9[a] | .008 |
| | Diabetes | 4 (15) | 2 (8) | 1 (5) | .463 |
| | Smoking | 5 (17) | 5 (17) | 5 (19) | .967 |
| | Alcohol excess | 10 (37) | 5 (24) | 11 (55) | .120 |
| Estradiol | Age, y | 74 ± 7 | 74 ± 6 | 71 ± 5 | .166 |
| | BMI, kg/m$^2$ | 25.9 ± 5.0 | 26.8 ± 3.9 | 24.3 ± 2.8 | .079 |
| | Diabetes | 4 (14) | 2 (9) | 1 (4) | .485 |
| | Smoking | 5 (17) | 5 (19) | 5 (18) | .983 |
| | Alcohol excess | 8 (30) | 10 (48) | 8 (40) | .437 |
| DHEA-S | Age, y | 75 ± 7 | 72 ± 5 | 72 ± 6 | .073 |
| | BMI, kg/m$^2$ | 25.1 ± 3.4 | 26.0 ± 4.8 | 25.9 ± 4.2 | .926 |
| | Diabetes | 3 (11) | 3 (14) | 1 (4) | .522 |
| | Smoking | 5 (16) | 3 (12) | 7 (25) | .415 |
| | Alcohol excess | 7 (30) | 8 (36) | 11(48) | .467 |
| Vitamin D | Age, y | 74 ± 6 | 74 ± 7 | 72 ± 4 | .487 |
| | BMI, kg/m$^2$ | 26.4 ± 4.5 | 25.9 ± 4.0 | 24.6 ± 3.7 | .306 |
| | Diabetes | 4 (17) | 2 (9) | 1 (4) | .257 |
| | Smoking | 6 (21) | 6 (21) | 3 (11) | .500 |
| | Alcohol excess | 12 (52) | 1 (6) | 13 (48) | .004 |

Data are mean±SD or n (%).
Abbreviations: BMI, body mass index; DHEA-S, dehydroepiandrosterone sulfate.
[a]Significantly different compared to tertile 1 value.
[b]Significantly different compared to tertile 2 value.

[0.29-0.68]; fT: 0.48 [0.29-0.67]; DHT: 0.37 [0.16-0.57]; E2: 0.37 [0.16-0.57]). After adjusting for age, BMI, alcohol excess, and prevalent diabetes, only total and fT remained significantly related to CMAP (Table 3).

In unadjusted analysis, 1-SD higher level of fT and vitamin D was associated with larger mean MUP. However, these associations were no longer statistically significant after BMI adjustment (Table 3).

Exploratory analysis suggested the relationship between fT and CMAP was much stronger in frail men compared to the robust (β [95% CI]: 0.82 [0.05-1.60], *P* for interaction of .038), as assessed by frailty phenotype (Fig. 1).

The relationship between fT and CMAP was greater with increasing frailty levels as assessed by the frailty index (β [95% CI]: 1.54 [0.02-3.06], *P* for interaction of .047; Fig. 2).

This secondary analysis also suggested a positive relationship between DHEA-S and CMAP in prefrail (β [95% CI]: 0.58 [0.15-1.00], *P* = .009 for interaction) and frail men (β [95% CI]: 0.54 [0.03-1.05], *P* = .039 for interaction) (Fig. 3).

When we explored associations between physical function and EMG parameters, TUG was negatively related to MUP size, and the association was partially attenuated after adjusting for lean muscle mass in keeping with a partial mediation model (Supplemental Table 3) (27). TUG was negatively linked with CMAP, and the associations appeared largely independent of lean muscle mass (Supplemental Table 3) (27). Knee extensor maximum voluntary contraction was associated with MUP size, and this association appeared to be explained by lean muscle mass (Supplemental Table 3) (27). We did not observe a significant relationship between knee extensor MVC and CMAP.

Finally, we performed an analysis assessing the potential influence of selection bias. Compared to the 16 men (14%) who were excluded because of incomplete data, the 98 men in the study cohort were less likely to be frail

**Table 3. Unadjusted and multivariable-adjusted cross-sectional relationships between hormone level and electromyography parameters**

| | | Log MUP | | | CMAP | | |
|---|---|---|---|---|---|---|---|
| **Endocrine Parameter** | **Models and Covariates** | **β** | **95% CI** | ***P*** | **β** | **95% CI** | ***P*** |
| Total testosterone | 1. Unadjusted | 0.16 | –0.05 to 0.37 | .130 | **0.48** | **0.29** to **0.68** | **< .001** |
| | 2. BMI | 0.07 | –0.17 to 0.31 | .573 | **0.40** | **0.18** to **0.61** | **< .001** |
| | 3. BMI + age | 0.03 | –0.21 to 0.28 | .781 | **0.33** | **0.12** to **0.54** | **.002** |
| | 4. BMI + age + DM | 0.01 | –0.26 to 0.27 | .965 | **0.30** | **0.08** to **0.53** | **.009** |
| | 5. BMI + age + DM + alcohol excess | 0.03 | –0.11 to 0.17 | .655 | **0.38** | **0.13** to **0.63** | **.003** |
| Free testosterone | 1. Unadjusted | 0.22 | 0.01 to 0.43 | .036 | **0.48** | **0.29** to **0.67** | **< .001** |
| | 2. BMI | 0.17 | –0.04 to 0.39 | .119 | **0.40** | **0.20** to **0.60** | **< .001** |
| | 3. BMI + age | 0.13 | –0.10 to 0.36 | .270 | **0.31** | **0.11** to **0.51** | **.003** |
| | 4. BMI + age + DM | 0.05 | –0.20 to 0.32 | .671 | **0.34** | **0.13** to **0.56** | **.002** |
| | 5. BMI + age + DM + alcohol excess | 0.08 | 0.18 to 0.35 | .528 | **0.34** | **0.12** to **0.56** | **.003** |
| Dihydrotestosterone | 1. Unadjusted | 0.04 | –0.18 to 0.25 | .731 | **0.37** | **0.16** to **0.57** | **.001** |
| | 2. BMI | –0.07 | –0.30 to 0.16 | .533 | **0.27** | **0.05** to **0.48** | **.018** |
| | 3. BMI + age | –0.09 | –0.32 to 0.14 | .444 | **0.23** | **0.03** to **0.44** | **.025** |
| | 4. BMI + age + DM | –0.04 | –0.30 to 0.22 | .772 | 0.21 | –0.01 to 0.44 | .058 |
| | 5. BMI + age + DM + alcohol excess | –0.01 | –0.28 to 0.26 | .914 | 0.20 | –0.04 to 0.43 | .097 |
| Estradiol | 1. Unadjusted | 0.11 | –0.10 to 0.32 | .303 | **0.37** | **0.16** to **0.57** | **.001** |
| | 2. BMI + total testosterone | 0.08 | –0.21 to 0.37 | .587 | 0.15 | –0.11 to 0.42 | .248 |
| | 3. BMI + age + total testosterone | 0.06 | –0.23 to 0.35 | .674 | 0.12 | –0.13 to 0.37 | .345 |
| | 4. BMI + age + DM + total testosterone | 0.01 | –0.37 to 0.37 | .988 | 0.13 | –0.18 to 0.44 | .405 |
| | 5. BMI + age + DM + alcohol excess + total testosterone | –0.08 | –0.51 to 0.35 | .711 | 0.50 | –0.30 to 0.40 | .777 |
| DHEA-S | 1. Unadjusted | 0.01 | –0.20 to 0.23 | .907 | 0.12 | –0.10 to 0.34 | .270 |
| | 2. BMI | 0.02 | –0.19 to 0.23 | .859 | 0.12 | –0.08 to 0.33 | .241 |
| | 3. BMI + age | –0.04 | –0.26 to 0.18 | .740 | 0.01 | 0.19 to 0.21 | .932 |
| | 4. BMI + age + DM | –0.04 | –0.29 to 0.20 | .716 | 0.04 | –0.18 to 0.25 | .739 |
| | 5. BMI + age + DM + alcohol excess | –0.02 | –0.28 to 0.24 | .881 | –0.01 | –0.23 to 0.22 | .950 |
| Vitamin D | 1. Unadjusted | 0.22 | 0.01 to 0.43 | .037 | 0.19 | –0.03 to 0.41 | .083 |
| | 2. BMI | 0.19 | –0.02 to 0.40 | .082 | 0.13 | –0.08 to 0.34 | .213 |
| | 3. BMI + age | 0.16 | 0.05 to 0.37 | .133 | 0.07 | –0.13 to 0.27 | .480 |
| | 4. BMI + age + DM | 0.18 | –0.05 to 0.41 | .118 | 0.10 | –0.11 to 0.31 | .345 |
| | 5. BMI + age + DM + alcohol excess | 0.21 | –0.02 to 0.45 | .077 | 0.07 | –0.14 to 0.29 | .485 |

Abbreviations: BMI, body mass index; CMAP, compound muscle action potential; DM, diabetes mellitus, DHEA-S, dehydroepiandrosterone sulfate; MUP, motor unit potential.
Data are β coefficients (95% CIs). β represents the difference in outcome (MUP or CMAP), in SD units, associated with a 1-SD difference in baseline level of predictor (hormone). The values in bold in the tables reflect statistically significant ($P < .05$) results.

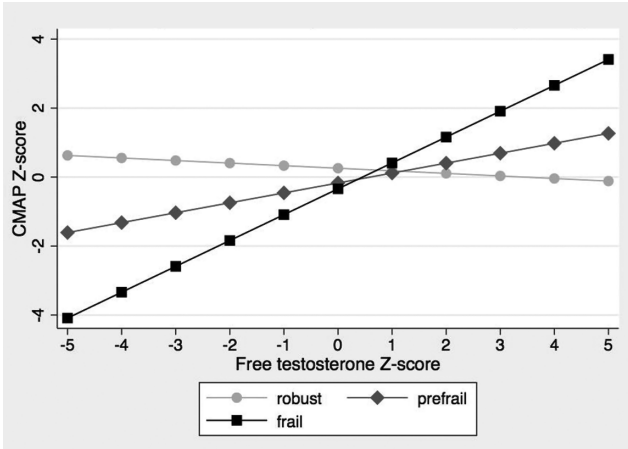


**Figure 1.** Adjusted prediction of compound muscle action potential (CMAP) by free testosterone and frailty phenotype.

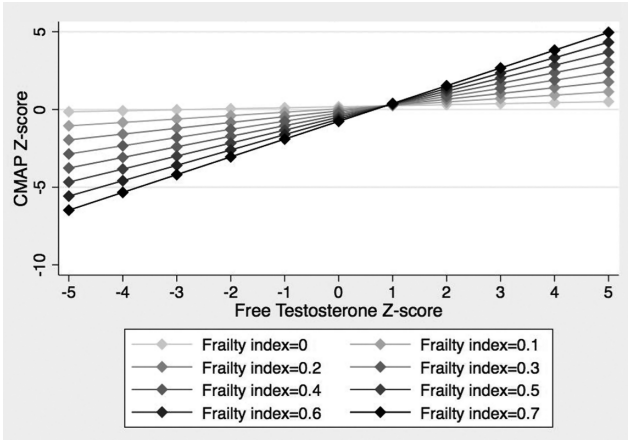


**Figure 2.** Adjusted probability of compound muscle action potential (CMAP) by free testosterone and frailty index.

and were less likely to have sarcopenia, weakness, respiratory disease, diabetes, and arthritis (Supplemental Table 4) (27). Therefore, the strength of relationships described earlier may be conservative estimates of what would have been obtained in the original cohort.

## Discussion

### Main findings

Our study presents several novel findings: First, T levels were positively associated with skeletal muscle electrophysiological characteristics as assessed by CMAP in unadjusted models and also models adjusted for BMI, age, and prevalent diabetes. We have also observed a similar trend for DHT; however, adjustment for diabetes attenuated the DHT-CMAP relationship. Second, we showed that E2 was related to muscle CMAP, but this relationship was rendered nonsignificant after adjusting for total T levels, indicating that the effect is likely to be T-related. Third, we showed that vitamin D was

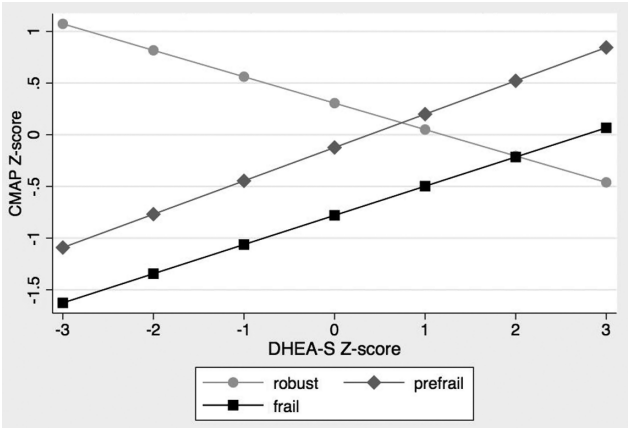


**Figure 3.** Adjusted probability of compound muscle action potential (CMAP) by dehydroepiandrosterone sulfate (DHEA-S) and frailty phenotype.

positively related to MUP size in unadjusted models, but this effect was no longer significant after adjusting for BMI. Finally, the significance of fT and DHEA-S relationships with muscle contractility (assessed by CMAP) appeared to be greater in frail men. These novel human observations may have important implications for future clinical research and clinical care.

### Prior studies and mechanistic insights

Data from our previous work, and that from other groups, indicate that muscle strength and the number of MUs declines progressively from old (~ 66 y) to very old (~ 82 y) (35) age and that CMAP and MUP size differ according to sarcopenic and frailty status (12, 36).

Although these past studies demonstrate important links between MU, muscle function, and health status in older age, they do not provide more detailed underpinning mechanisms that may identify causes of MU changes with advancing age. The pathophysiological mechanisms linking neuromuscular health with frailty in humans remain largely unknown. Although our study is the first to investigate associations between hormone levels and MU function of older adults, others have previously suggested such a link may exist based on the observed age-related decline in anabolic hormones occurring in parallel with development of physical impairments, sarcopenia, and frailty (37).

The role of T in neuromuscular function has largely been investigated in the context of its effects on muscle mass. Both in animal and human studies, T has been shown to increase skeletal muscle size by, in part, increasing the number of muscle satellite cells to support muscle fiber hypertrophy (38).

There is a paucity of research into the role of T in MU health and remodeling processes. Available evidence comes from animal models of motor neuron injury. Byers et al, in an experimental model of spinal cord

injury, found that T treatment of female rats prevented atrophy of motor neuron dendrites and muscle fibers (16). Similarly, castration of adult male rodents led to motor neuron atrophy that was almost completely reverted by T administration (14, 15). In other rodent studies, exogenous T accelerated regeneration of injured facial and sciatic nerves (17, 18, 39).

The effects of T are not uniform across the nervous system owing to reduced expression of androgen receptors in typical somatic motor neurons, such as those of the quadriceps, compared to the cranial motor neurons. Nonetheless, rodent research data suggest that T has a neuroprotective role in the L2 spinal segment (40–42).

Gonadal hormones regulate the brain-derived neurotrophic factor receptor, trkB (43). Work by Osborne suggests androgen-mediated expression of the brain-derived neurotrophic factor receptor could help maintain motor neurons (41).

In humans, an age-related decline in T levels has been linked to the loss of muscle mass and sarcopenia; interestingly, the magnitude of a concomitant decline in muscle strength and neuromuscular coordination appears to be far greater than expected from the degree of the muscle mass loss only (44).

Similarly, T-induced muscle hypertrophy in healthy older men does not necessarily translate into significant gains in muscle function and improved physical performance, raising questions about the previously unexplored role of T in neuronal control of muscle function.

We showed that low fT, which is a biologically active fraction of circulating T, is associated with impaired muscle electrophysiology assessed by maximal CMAP. The maximal CMAP size, though not dependent on the total size of larger muscle groups (7), depends on the volume of contractile material within the recording range of the electrodes, and is proportional to the total size of the MUs activated minus any attenuation of the signal as it reaches the recording electrode (8), such as subcutaneous fat thickness, which did not differ here, as we and others have previously reported (7, 45). Smaller CMAPs in older age have been reported for a number of muscles (46). In clinical practice, the CMAP remains a useful parameter to monitor progression of neuromuscular disorders such as motor neuron disease (34). Interestingly, the relationship of fT with CMAP size was greater with increasing levels of frailty. Although this is an observational study that cannot determine causality, the differing relationships by frailty status could lead us to speculate that T supplementation might improve neuromuscular function to a greater extent in frail rather than nonfrail elderly populations. This idea is largely in keeping with the results of T trials in which T replacement in relatively healthy men resulted in very small gains in objectively assessed and self-perceived physical function (47–50).

Contrary to the relationship with CMAP, there was no observed relationship between T and MUP size, which may be explained by the nonlinear trajectory of MUP size with increasing age. Expansion of the MU occurs as a compensatory process to minimize fiber loss, via reinnervation of denervated fibers, and a failure of this process contributes to sarcopenia, evidenced by larger MUPs in healthy old individuals when compared to the young, and these are smaller again in older people with sarcopenia (12). It is therefore apparent that MUP size increases up to a certain ill-defined point when reinnervation is outpaced by denervation, the motor unit fails to expand, and proceeds to become smaller.

Our findings suggest that vitamin D may play a role in successful reinnervation occurring with aging as evidenced by the association of low vitamin D with smaller MU potentials. Adjusting for BMI attenuated the relationship between vitamin D and MUP size, and although we might have been underpowered to detect significant associations on multiple adjustments, the unadjusted model might still provide valuable insights into mechanisms linking vitamin D and sarcopenia. For example, it is possible that BMI is on the causal pathway linking vitamin D with MUP size, which could explain the effect of statistical adjustment.

In experimental models, treatment with vitamin D has been shown to induce nerve growth factor synthesis (involved in peripheral nerve recovery postinjury), reduce demyelination, and induce axonal regeneration in a spinal cord compression and peroneal nerve injury model (51–53). Certainly the evidence from randomized, placebo-controlled trials suggests that vitamin D replacement results not only in improved lower limb muscle mass and strength but also neuromuscular control and balance (54, 55).

Whereas some of the effects of vitamin D deficiency on muscle are thought to be, in part, mediated by raised proinflammatory cytokines levels (26, 56) and direct activation of the insulin-like growth factor-1 receptor (57, 58), vitamin D deficiency has previously been linked to altered muscle innervation (59), which is further supported by our findings.

## Strengths and Limitations

Our study has a number of strengths. Our cohort is representative of older community-dwelling men and although the sample size may appear small, it is relatively large for an invasive study in elderly and frail

participants. To our knowledge, it is the first study in humans to relate hormone levels to MU size and muscle electrophysiological characteristics assessed by intramuscular and surface EMG. However, we did not measure calcium or parathyroid hormone levels, which may have helped in the interpretation of the vitamin D data. We have also not performed nerve conduction studies, which could have helped in the interpretation of study findings to identify whether deficits exist in motor neuron axons. Our work was limited to men, so the generalizability to women is unknown.

### Clinical and research implications

Interventions preventing age-related MU loss are largely unknown. We have previously shown that even lifelong exercise does not attenuate this process and the muscles of master athletes show a similar loss to those of normally active older men; however, older athletes appear to be more successful at reinnervation (13, 60). The associations of fT and vitamin D with neuromuscular parameters suggest that both hormones might contribute to preservation of muscle fibers and successful reinnervation.

Whether intervention with these anabolic hormones could prevent MU loss or improves reinnervation remains unclear. We recommend additional in vivo studies and clinical trials before there is any change in clinical practice. T replacement in frail hypogonadal men resulted in improvements in physical function, but larger trials in this group of people are lacking (61). Moreover, the greater significance of relationships in frailer men suggests that hormonal manipulation aimed at improving muscle function might be of particular benefit in the frail.

In conclusion, we have shown that T was positively associated with the volume of excitable muscle tissue as assessed by CMAP. We have also shown, in univariate analysis, that vitamin D was related to MU size. These cross-sectional hypothesis-generating data suggest that it may be appropriate to design clinical trials to assess the impact of androgen therapy on neuromuscular decline in frail older men.

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## Additional Information

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***Data Availability:*** The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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