

Supplementary information:**Title:**

Compared to total serum testosterone, calculated free testosterone has a stronger association with lean mass, muscle strength, power, and physical function in older men.

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Table of contents

Supplementary table 1

Supplementary figure 1

Post-hoc statistical power analysis

Supplementary table 1. Relationship between hormone levels and the different muscle parameters by age group.

		Models							
		Unadjusted		+Age		+Age, BMI		+Age, BMI, FP	
		Std- β	p-value	Std- β	p-value	Std- β	p-value	Std- β	p-value
<i>Total Testosterone</i>									
ALM	YM	0.053	0.337	-0.106	0.081	0.096	0.030	0.040	0.330
	OM	-0.225	<.001	-0.232	<.001	0.033	0.536	-0.024	0.611
SMI	YM	-0.152	0.006	-0.178	0.002	0.066	0.068	-0.020	0.491
	OM	-0.215	<.001	-0.215	<.001	0.118	0.006	-0.042	0.183
ALM%	YM	0.317	<.001	0.257	<0.001	0.113	0.008	0.006	0.802
	OM	0.348	0.001	0.334	<.001	0.123	0.025	0.017	0.628
HGS	YM	0.046	0.423	0.004	0.944	0.069	0.226	0.021	0.701
	OM	0.086	0.178	0.079	0.207	0.163	0.011	0.120	0.055
LEP	YM	0.053	0.324	-0.013	0.803	0.084	0.082	0.067	0.186
	OM	0.031	0.666	0.018	0.784	0.171	0.009	0.140	0.032
STS	YM	0.036	0.498	-0.026	0.616*	-0.072	0.158*	-0.111	0.030*
	OM	0.198	0.005	0.173	0.005*	0.115	0.089*	0.074	0.263*
<i>Free Testosterone</i>									
ALM	YM	0.085	0.129	-0.006	0.915	0.068	0.132	0.040	0.113
	OM	0.002	0.981	-0.015	0.811	0.104	0.039	0.072	0.113
SMI	YM	0.033	0.129	-0.011	0.857	0.080	0.032	0.035	0.179
	OM	0.002	0.975	-0.006	0.926	0.144	<.001	0.100	<.001
ALM%	YM	0.286	<.001	0.155	0.003	0.093	0.035	0.036	0.203
	OM	0.287	<.001	0.260	<.001	0.158	0.003	0.099	0.003
HGS	YM	0.135	0.017	0.045	0.435	0.069	0.234*	0.039	0.491*
	OM	0.208	0.001	0.191	0.002	0.225	<.001*	0.199	<.001*
LEP	YM	0.211	<.001	0.079	0.138	0.125	0.014	0.119	0.019
	OM	0.232	<.001	0.206	0.002	0.273	<.001	0.250	<.001
STS	YM	0.147	0.005	0.012	0.816*	-0.009	0.858*	-0.020	0.702*

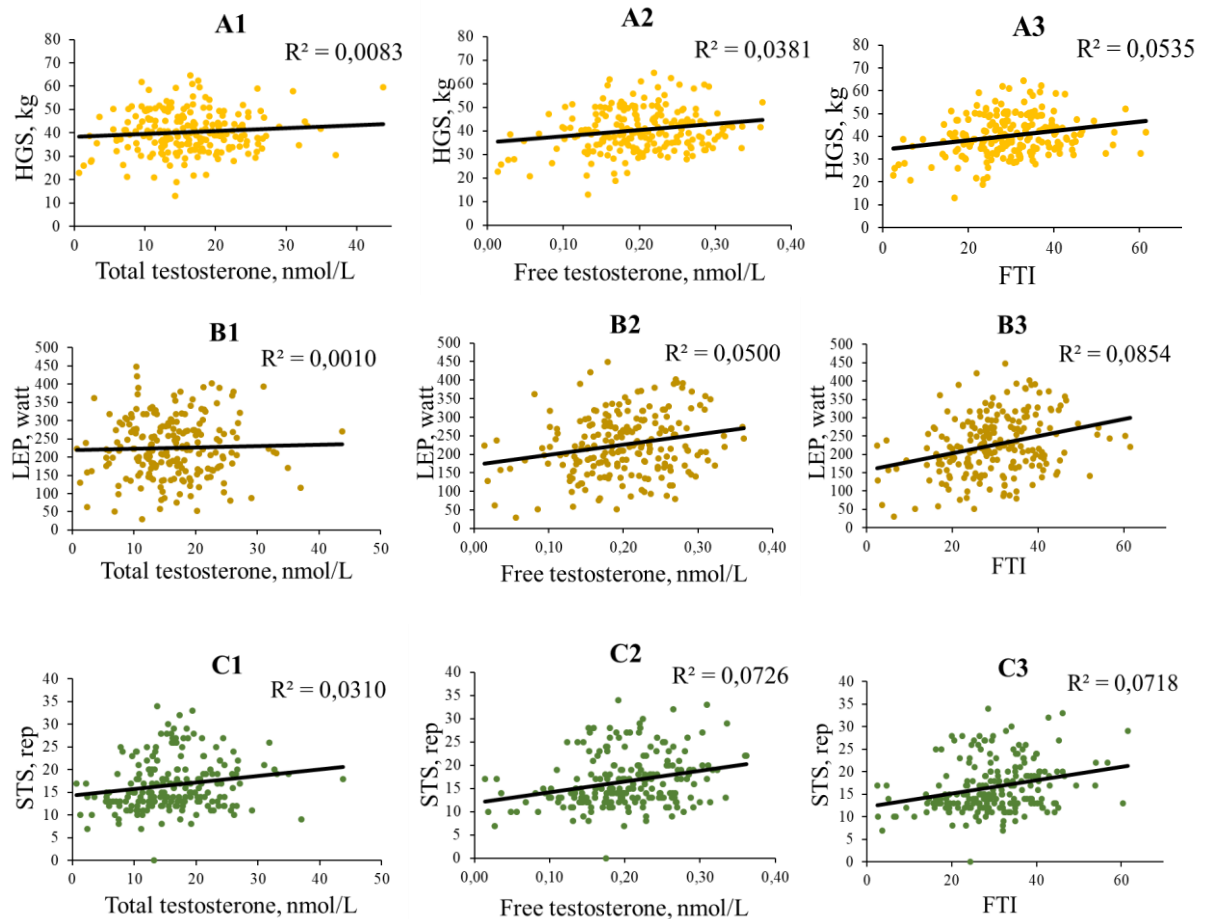
	OM	0.277	<.001	0.252	<.001*	0.219	<.001*	0.188	0.004*
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Free Testosterone Index

ALM	YM	0.171	0.002	0.068	0.250	0.023	0.631	0.019	0.640
	OM	0.118	0.069	0.099	0.126	0.107	0.03	0.088	0.054
SMI	YM	0.161	0.003	0.131	0.029	0.073	0.063	0.058	0.018
	OM	0.126	0.052	0.115	0.064	0.137	0.001	0.105	<.001
ALM%	YM	0.205	<.001	0.018	0.753	0.055	0.248	0.041	0.114*
	OM	0.201	0.003	0.162	0.011	0.155	0.004	0.115	<.001*
HGS	YM	0.190	<.001	0.084	0.168*	0.071	0.241*	0.061	0.303*
	OM	0.249	<.001	0.229	<.001*	0.230	<.001*	0.212	<.001*
LEP	YM	0.283	<.001	0.131	0.018*	0.120	0.023*	0.125	0.017*
	OM	0.303	<.001	0.271	<.001*	0.277	<.001*	0.259	<.001*
STS	YM	0.120	<.001	0.028	0.609*	0.035	0.528*	0.041	0.431*
	OM	0.270	<.001	0.236	<.001*	0.231	<.001*	0.204	0.002*

Note. YM, young men. OM, older men. Std- β , standardized β -coefficients. BMI, body mass index. FP, fat percentage. ALM, appendicular lean mass. ALM%, percentage appendicular lean mass. SMI, skeletal muscle index. HGS, hand grip strength. LEP, leg extension power. STS, 30-s sit-to-stand performance. Bold values indicate $p < 0.05$. *Significant age-by-hormone interaction, denoting significant differences between age groups ($p \leq 0.05$).

Supplementary figure 1



Note. Relationship between; handgrip strength (HGS) and total testosterone (A1), free testosterone (A2), and free testosterone index (FTI) (A3); leg extension power (LEP) and total testosterone (B1), free testosterone (B2), and FTI (B3); 30-s sit-to-stand performance (STS) and and total testosterone (C1), free testosterone (C2), and FTI (C3), in older men (age > 65 years, n=231)

Post-hoc statistical power analysis:

A post hoc statistical power analysis was performed free online software (G*power 3.1.9.7, Heinrich-Heine-Universität Düsseldorf). The analysis was performed as a F-test, ANCOVA: Fixed effects, main effects and interactions. Post hoc achieved power was computed using an alpha of 0.05, a sample size of 231 (older men sub cohort), with degrees of freedom set to 6, number of groups to 2, and number of covariates to 4 (Free testosterone, age, BMI, fat percentage). The effect size f was set to 0.55 based on the results from the general linear model (partial eta squared 0.23, older men free testosterone estimates on SMI). This resulted in a power of 0.999.

Subsequently, we lowered the sample size to 52, which resulted in a power of 0.805. In this regard, we consider this study well powered.