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A 6-month, double-blind, placebo-controlled, randomized trial to evaluate the effect of *Eurycoma longifolia* (Tongkat Ali) and concurrent training on erectile function and testosterone levels in androgen deficiency of aging males (ADAM)

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ABSTRACT

Background: Androgen deficiency of aging males (ADAM) largely manifests as sexual symptoms. Erectile dysfunction is one of the most common symptoms of ADAM.
Aim: To ascertain the effect of concurrent training and supplementation with *Eurycoma longifolia* on erectile function and testosterone levels in men with ADAM, and the association of erectile function with levels of total testosterone.
Methods: 6-month, randomized, double-blind, placebo-controlled four-arm clinical. 45 men (47.38 ± 5.03 years) were randomized into 4 groups (G1: control + placebo; G2: control + *Eurycoma longifolia*; G3: concurrent training + placebo; G4: concurrent training + *Eurycoma longifolia*). 22 received a 200 mg supplement of *Eurycoma longifolia* and 23 underwent the intervention with concurrent training, 3 times a week for 60 min at progressive intensity.
Outcomes: International Index of Erectile Function (IIEF-5), Aging Male Scale (AMS) and total testosterone.
Results: Erectile function demonstrated improvements in both interventions; however, the most significant results were obtained by men allocated to concurrent training + *Eurycoma longifolia*.
Clinical implications: A 200 mg supplement of *Eurycoma longifolia* and the practice of concurrent training for 6 months significantly improved the erectile function of men with ADAM.
Strengths & limitations: The study's design stands out as a strength, in addition to the six-month intervention. The main limitation is the study not having groups that used only *Eurycoma longifolia* and only concurrent training.
Conclusion: The combination of *Eurycoma longifolia* and concurrent training improved erectile function and increased total testosterone levels in men with ADAM.

Clinical Trials.gov number NCT03150225.

1. Introduction

Testosterone is the main natural androgenic anabolic male hormone, and its biological effects include regulating libido, bone mass, body composition distribution and muscle strength [1]. During the male aging process, testosterone rates are expected to decrease from 1 to 3% per year [2]. ADAM (Androgen Deficiency in the Aging Male) occurs when there is exacerbated decrease associated to somatic, psychological and sexual signs and symptoms. The sexual symptoms of ADAM are the most recurrent and affect about 60 % of men [3,4]. They include decreased sexual frequency and capacity, sexual desire, beard growth and erectile dysfunction [5].

This dysfunction involves changes to any of the components of erectile response [6], and there is a consensus on its association to increased risk of cardiovascular diseases [7]. In a longitudinal study, researchers confirmed that low penile blood flow was associated with an increased risk of cardiovascular disease [8]. Studies of the systematic increase of blood flow have demonstrated that the use of herbal

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supplements [9] and physical exercise is an efficient alternative for improving ADAM and its symptoms, in addition to increasing levels of testosterone and improving erectile dysfunction [4,10,11].

Concurrent training has been studied as a potent agent for improving these aspects [12]. This training method involves aerobic and resistance exercises in the same session, optimizing the benefits [13]. Randomized clinical trials show that *Eurycoma longifolia* [14–17] is a herbal alternative for the condition. The mechanism of action of this root improves serum testosterone [18].

It is of the utmost importance to seek clinical alternatives to improve the sexual symptoms associated with ADAM and to increase testosterone of the affected men, as these are predictors for cardiovascular diseases and other pathologies and negatively influence quality of life. Moreover, non-invasive and non-pharmacological measures are better accepted by the patients and they work systemically in other health domains. It is necessary to verify whether there is an association of these sexual domains with the total testosterone indexes. The objective of this study is to ascertain the influence of concurrent training and supplementation with *Eurycoma longifolia* on erectile function and testosterone in men with ADAM and associate erectile function with total testosterone.

2. Methods

2.1. Study design

The study is a randomized, double-blind, placebo-controlled four-arm clinical trial that follows the CONSORT checklist [19]. The study duration was six months to allow for significant changes in the analyzed variables, both by supplementation and by concurrent training. A placebo arm was included to allow comparisons with all three active intervention arms.

The study was conducted at the Health and Sport Sciences Center of the Santa Catarina State University. It complied with institutional regulations and was approved by the ethics committee (number: 2.274.655), with registration in the international clinical trial registry platform "*Clinical Trials.gov*" (n. NCT03150225) and written consent for voluntary participation from all men.

The study was advertised for six months on social media, radio, printed newspapers, and educational and legal institutions. To be eligible, men must be aged 40–59 years, have clinical symptoms of ADAM verified by the *AMS Scale* [5] and total testosterone serum levels equal to or less than 346 ng/dL. Men under hormone therapy with testosterone, with history of cardiovascular, musculoskeletal, neurological or prostate cancer diseases, and who underwent concurrent training in the three months prior to the first data collection (investigated using a questionnaire) were excluded.

2.2. Instruments

2.2.1. Sociodemographic and clinical profile

Self-administered questionnaire (paper and pen format), containing questions related to age, marital status, education, economic level, presence of clinically diagnosed diseases, use of medication and tobacco consumption - according to the question template from the questionnaire on smoking Multinational Monitoring Project for Trends and Determinants in Cardiovascular Diseases, from the World Health Organization.

2.2.2. AMS scale

The clinical symptoms of ADAM were analyzed using the Male Aging Symptoms Scale (*AMS Scale*) Portuguese version [5] composed of 17 questions divided into three blocks of symptoms: psychological, somatic and sexual. In this study the clinical symptomatology of ADAM was analyzed only according to sexual symptoms that are object of the study. The questions in the block of sexual symptoms are: "decreased beard growth", "decreased frequency/capacity of sexual performance" and "decreased sexual desire/libido", with responses ranging from 1 (none) to 5 (very serious).

2.3. International index of erectile function (IIEF-5)

The erectile function questionnaire developed and validated by Rosen et al. (1997) has 15 questions, grouped into five domains: erectile function, orgasmic function, sexual desire, sexual satisfaction and general satisfaction. Each question has a value ranging from 1 to 5, and the sum of the answers results in a final score for each domain, with low values indicating poor quality of sex life.

2.4. Total testosterone

A professional biochemist performed the blood collect between 7 a.m. and 11 a.m., following the recommendations of the Latin American Consensus of ADAM [20], however, in order to avoid any kind of bias, the participants went to carry out the subsequent blood collections always respecting the time of the first collection and after an 8 -h fast. The analysis was performed in duplicate, with approximately 0.2 mL of serum. The analysis procedures were as follows: 1) 4−5 ml of blood collected into a properly labeled test tube; 2) blood centrifugation and serum extraction. Chemiluminescence was the method used for analysis, in an automated Siemens device. The analyses were performed at the University Hospital of the Federal University of Santa Catarina. Needles with a special adapter for this type of collection and procedure gloves were used. All materials were correctly disposed after use.

2.5. Procedures

Initially, all baseline (BL) collections were performed (questionnaires and blood collection) with no group distinction. This was followed by a simple 1:1:1:1 randomization (www.randomization.com) into the four study groups: G1) Control + Placebo; G2) Control + *Eurycoma longifolia*; G3) Concurrent training + Placebo; 4) Concurrent training + *Eurycoma longifolia*.

The G*Power 3.1.9.2 software was used for sample calculation, considering $\alpha = 0.05$, test power of 0.8 and effect size of 0.35, applied to the four groups. The Two-way ANOVA test results with repeated measures were used for comparative analysis between factors. It resulted in 20 participants in total. However, given that a sample loss of 30 % was predicted because of the intervention duration, a total of 26 men should be distributed in the four study groups to identify significant differences. It is important to note that this study was able to reach more subjects than the sample calculation estimated (n = 38).

A total of 233 men were recruited based on the eligibility criteria, but only 45 became eligible for the study. To monitor the analyzed variables, four data collections were performed, at baseline (BL), after one month (1), four months (4) and six months (6) of intervention. All collections were performed at the Health and Sports Sciences Center, in the following order: sociodemographic questionnaire, AMS Scale, IIEF-5 and blood collection. Fig. 1 shows the study procedures and randomization.

2.6. Interventions

2.6.1. Concurrent training

Groups 3 and 4 were randomly assigned to perform concurrent training did so for six months, 60-minute session three times weekly. The classes were given by Physical Education professionals in the Santa Catarina State University gyms, in Florianopolis, and followed the protocol validated for this population by Vieira et al. [21]. The protocol was initially divided into 30 min of aerobic exercises on the treadmill, from "moderate" to "somewhat strong" according to the Borg Scale [22], followed by 30 min of resistance training at the gym, at an intensity of 40–55 % of 1RM. Intensity was increased and the resistance exercises

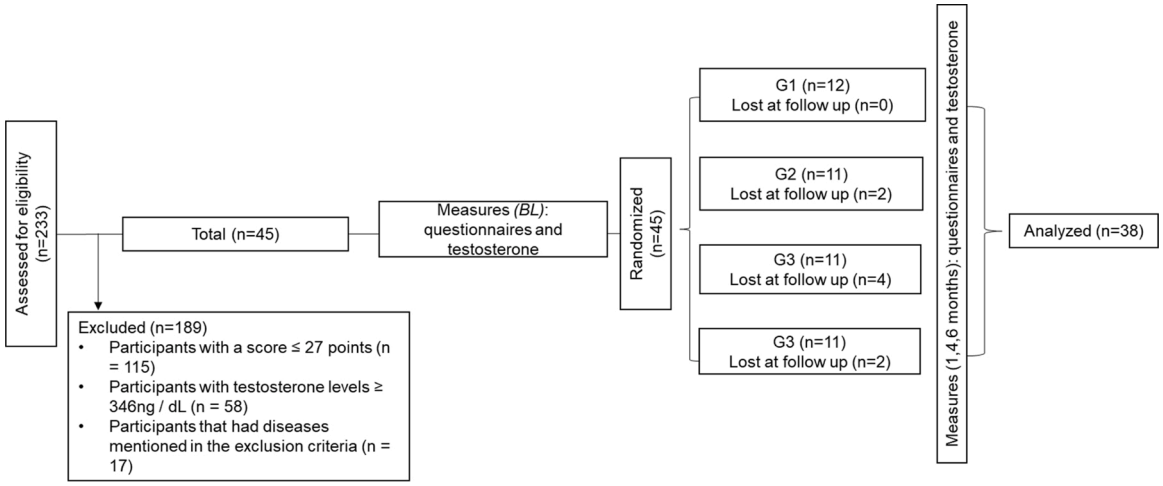

Fig. 1. flow subjects. Excluded: AMS Scale score ≤27 points (n = 115); total testosterone ≥346 ng / dL (n = 58), participants who had any disease mentioned in the exclusion criteria (n = 17). Losses on follow-up were motivated by: surgery (n = 2), hormonal changes (n = 2), dropout (n = 1), work (n = 3). Produced by the authors (2020).

were changed on a monthly basis, and in the last month included ten minutes of training on the treadmill at "very difficult" to "maximum" intensity and 50 min of resistance training with a load of 80–90 % 1RM.

2.7. Supplementation

Eurycoma longifolia capsules were produced by a local compounding pharmacy. The capsules had the same visual characteristics, so there was no distinction between the products (*Eurycoma longifolia* or placebo). The product label was not specific and included only the University's name, dosage and the participant's name. The placebo capsules contained starch while the *Eurycoma longifolia* capsules contained 200 mg of standardized dry extract (2-dihydro-18-dedihydrolongilactone: 0.32 % (w/w); 9-hydroxycanthin-6-one 0.49 % (w/w); Eurycomanone: 1.21 % (w/w); total protein: 26.3 %; total polysaccharaide: 28.8 % · glycosaponin: 44.2 %). All participants were instructed to take the supplementation in the morning, after breakfast, in order to avoid any discomfort. There were no reports of discomfort during the study.

2.8. Statistical analysis

The SPSS software version 20.0 (IBM Crop. Armonk, NY) was used for statistical analysis. A descriptive analysis was performed with mean, standard deviation and relative frequency distribution. The Two-way ANOVA model with repeated measurements was used for inferential analysis to verify the effect of interventions in sexual domains and testosterone (dependent variables). The effect of the group, time and group interaction vs. time, with inter-and intra-group comparison were analyzed. The Bonferroni test was used to adjust multiple comparisons. The data were presented in mean ($\overline{X}$) ± standard deviation, and the value of F. was shown for the significant variables.

The (1) delta value of the difference from BL to the 6-month value (2) correlation (Pearson) between independent samples and the deltas (3) and multiple linear regression analysis for the deltas were used to verify the correlation between sexual domain variables and testosterone. The model was adjusted using G4 (concurrent training + *Eurycoma longifolia)* as the reference, total testosterone delta as an independent variable and the delta for each sexual domain variable as a dependent. The test was used to verify the normality of all regression models and the residues, all of which presented normal distribution with $p \geq 0.05$.

The intention-to-treat analysis was not performed, since the men who left the study were not available for post-intervention collection (6 months). The level of significance adopted was 5% for all the analyses.

3. Results

Of the 45 men who started the study, 38 completed it. The power of the test was calculated as 99 % *a posteriori,* using the final number of participants. This difference is justified by the estimated sample loss (30 %). The actual loss in this study was 15.5 %, so the power value of the *a posteriori* was greater than estimated. Moreover, study compliance was calculated at 87.5 %. Mean participant age was 47.38 ± 5.03 years. Most men reported living with their partner (81.25 %), had no clinically diagnosed diseases (70.12 %), did not use medications (91.32 %), did not use tobacco (89.92 %), and most were classified in economic stratum "D" (46.2 %). Was realized a previous analysis for differences between groups in baseline, and the four groups showed homogeneous (erectile function, p = 0.166; sexual satisfaction, p = 0.213; sexual desire, p = 0.105; orgasmic function, p = 0.591; general satisfaction, p = 0.123 and AMS-S, p = 0.643) (Data not shown in the table).

Comparing the different treatment lines, men who participated in the study with concurrent training (G3) (Δ = 1) and with concurrent training associated with *Eurycoma longifolia* supplementation (G4) (Δ = 4.84) had improved erectile function scores (4.87 %; 15.21 %, respectively), changing erectile dysfunction classification from moderate to mild. In the intergroup analysis, there was significant difference between G1 and G4 (p = 0.047), G2 and G3 (p = 0.035) and G2 and G4 (p = 0.007) in the 6-month collection. Regarding intragroup differences, G4 showed significant increase in the erectile dysfunction score from BL to 6 months (p = 0.012). The size of effect was considered moderate for this domain ($f^2 = 0.222$) (Table 1).

Regarding the domain of sexual satisfaction, there was a significant difference between times (p = 0.004; $F_{(3,102)}$: 4.719); in Bonferroni's Post Hoc analysis, this difference was observed from BL to 1 month (p = 0.017), with a decrease of 2.128 points in the sexual satisfaction score and from BL to 4 months (p = 0.038), with a 2.142 point decrease. In the intra-group differences, there was improvement from BL to 1 month (p = 0.032) and from BL to 4 months (p = 0.002) in G2. There were group vs. time interaction (p = 0.004; $F_{(9,102)}$: 2.285) differences between G1 and G4 at time 6 months (p = 0.003) (Table 1). Proportionally, the size of effect for this domain ($f^2 = 0.201$), is considered moderate.

With regard for sexual desire, one can observe difference in time vs. group interaction (p = 0.001; $F_{(9,102)} = 3.442$), and intergroup difference between G2 and G4 at time 6 months (p = 0.043), with superiority in the sexual desire score for G4, which received both types of treatment. In Bonferroni's Post Hoc analysis, G3 showed a significant difference, with an increase in the score from Baseline to 6 months (p = 0.005) and between 1 and 6 months (p = 0.002) (Table 1). Regarding correlation

Table 1
2-way RM-ANOVA for comparison of the sexual function domains of the four groups in the four moments of the study. (n = 38). BL - baseline; 1 month - after one month of intervention; 4 months - after four months of intervention; 6 months - after six months of intervention. G1: control + placebo; G2: control + *Eurycoma longifolia*; G3: concurrent training + placebo; G4: concurrent training + *Eurycoma longifolia*. AMS-S: sexual aspects of the AMS Scale. f^2: effect size (Cohen). p value□: for difference between the group; p value* for difference between times; and p value# for group versus time interaction. Uppercase letters on the lines represent difference between groups, while lowercase letters on the columns represent difference between time. Different letters in lines represent significant statistically difference between groups and different letter in columns represent significant statistically difference between times. Statistically significant values ($p \leq 0.05$) are highlighted. Results presented as mean ± standard deviation.

	G1	G2	G3	G4				
	$\overline{X}$±sd	$\overline{X}$±sd	$\overline{X}$±sd	$\overline{X}$±sd	f^2	p value□	p value*	p value#
Erectile function					0.222	0.075	0.185	0.052
BL	19.5 ± 2.1	13.22 ± 2.43	20.5 ± 2.57	18.66 ± 2.43[a]				
1 month	19.83 ± 2.13	15 ± 2.46	19.5 ± 2.6	19.77 ± 2.46[a,b]				
4 months	18.75 ± 1.79	16.22 ± 2.07	20.75 ± 2.19	20.75 ± 2.07[a,b]				
6 months	18.41 ± 1.71[A]	13 ± 1.98[B]	21.5 ± 2.1[A]	21.5 ± 1.98[b,A]				
Sexual satisfaction					0.201	0.457	**0.022**	**0.004**
BL	9.66 ± 1.19	6.11 ± 1.38[a]	9.75 ± 1.46	9 ± 1.38				
1 month	10.16 ± 0.93	10.11 ± 1.07[b]	11.87 ± 1.14	10.88 ± 1.07				
4 months	8.83 ± 0.97	12 ± 1.12[b]	10.37 ± 1.18	11.88 ± 1.12				
6 months	9.58 ± 1.23	7.88 ± 1.42[a,b]	10.37 ± 1.51	12.11 ± 1.42				
Sexual desire					0.303	0.632	**0.001**	0.118
BL	7.5 ± 0.56	6.33 ± 0.65	6.75 ± 0.69	6.88 ± 0.65[a]				
1 month	7.83 ± 0.43	7.44 ± 0.49	6.75 ± 0.52	7 ± 0.49[a]				
4 months	6.91 ± 0.66	8.22 ± 0.77	6.62 ± 0.81	8.11 ± 0.77[a,b]				
6 months	7.25 ± 0.61[A,B]	6.44 ± 0.71[A]	7.25 ± 0.75[A,B]	9.33 ± 0.71[b,B]				
Orgasmic function					0.153	0.436	0.091	**0.027**
BL	8.58 ± 0.84	5.44 ± 0.97[a]	8.25 ± 1.03	7.55 ± 0.97				
1 month	8.33 ± 0.54	8.88 ± 0.63[b]	9.37 ± 0.66	8.55 ± 0.63				
4 months	7.33 ± 0.69	8.11 ± 0.80[a,b]	8 ± 0.85	8.88 ± 0.80				
6 months	8.16 ± 0.85	6.66 ± 0.98[a,b]	9.12 ± 1.04	8.66 ± 0.98				
General satisfaction					0.100	0.347	0.420	**0.050**
BL	56.16 ± 5.33	37.88 ± 6.15	56.12 ± 6.53	53 ± 6.15				
1 month	52.91 ± 6.14	46.88 ± 7.09	53.25 ± 7.52	55.55 ± 7.09				
4 months	48.58 ± 5.60	47.33 ± 6.47	56.75 ± 6.86	58.88 ± 6.47				
6 months	56.58 ± 5.12	5.11 ± 5.91	61 ± 6.27	60.44 ± 5.91				
AMS-S					0.183	0.508	0.608	**0.001**
BL	10.16 ± 1.29	11.77 ± 1.49	12 ± 1.58	12.55 ± 1.49[a]				
1 month	9.16 ± 1.03	10.55 ± 1.19	10.12 ± 1.27	10 ± 1.19[a,b]				
4 months	9 ± 1.01	10.88 ± 1.17	8.37 ± 1.24	9 ± 1.18[b]				
6 months	8.41 ± 0.89	11.44 ± 1.02	9 ± 1.09	8.66 ± 1.05[b]				

analysis, there was a high and significant correlation between sex drive and testosterone ($r = 0.963$; $p = 0.008$), and in the multiple linear regression analysis, adjusted by treatment type (adjusted R^2: 0.243; β: 0.559; $p = 0.002$). It was found that testosterone influenced the change in the sex desire score, and there is an improvement in the sex desire score of 0.559 for each 1 ng/dL increase in testosterone. Moreover, the model proved to be significant ($p = 0.047$). The increase in the sex desire score depends on the type of treatment offered, and participants randomly assigned to G4 (concurrent training + *Eurycoma longifolia*) tend to have a lower sex desire score value when compared to other types of treatment. Furthermore, the effect of size for this domain was considered moderate ($f^2 = 0.303$).

For the domain related to orgasmic function, there was a significant difference between the times ($p = 0.027$; $F_{(3,102)}$: 2.611) independent of the treatment group. In the intragroup analysis, a significant improvement was observed for G2 from BL and 1 month ($p = 0.012$). The effect of size of this domain was classified as low ($f^2 = 0.153$).

The last area of male sexual health is general satisfaction. There was a time difference ($p = 0.050$; $F_{(3,102)}$: 1.273) for this variable, independent of group, with an improvement of 4.99 points from BL to 6 months ($p = 0.05$) in the overall satisfaction score. A high correlation was also observed between overall satisfaction and testosterone ($r = 0.856$; $p = 0.031$). When the group-adjusted multiple linear regression analysis (adjusted R^2: 0.182; β: 0.440 $p = 0.006$) was performed, the model proved significant and showed a behavior similar to the domain "sexual desire". The type of treatment offered had greater influence on the improvement of overall sexual satisfaction, and G4 received the most benefit.

Regarding the AMS sexual block (AMS-S), there was a difference in time ($p = 0.001$; $F_{(3,102)}$: 6.260) independent of group, from BL to 4 months ($p = 0.030$) and from BL to 6 months ($p = 0.006$), with score reduction of 2.109 and 2.243, respectively. In addition to the significance between times, there was an intragroup difference in G4 from Baseline to 6 months ($p = 0.024$), with a decrease of 3.89 points in this block, i.e., a lower representation of sexual symptoms of ADAM. There was high correlation for this variable ($r = 0.786$; $p = 0.05$) and in multiple linear regression (adjusted R^2: 0.121; β: 0.212; $p = 0.048$). However, as the model was not significant, it can be said that although testosterone partially influences the improvement in this variable, it is not related to the type of treatment. Therefore, with regard to the domains of sexual health, the following are explained by testosterone variation: sexual desire, general sexual satisfaction and Sexual AMS (Table 2).

For comparison analysis of testosterone behavior, there was significant difference between times ($p < 0.001$), observed from BL to 6 months ($p < 0.001$; -76.198 ng/dL), 1 and 6 months ($p < 0.001$; -68.440 ng/dL) and 4 and 6 months ($p = 0.001$; -51.417 ng/dL) and in the interaction group vs. time ($p = 0.004$). Intergroup difference was observed between G1 and G2 at 6 months ($p = 0.05$), where G2 (*Eurycoma longifolia*) had 55 % higher testosterone rates than the final G1 value. Regarding intragroup differences, G2 showed an improvement in testosterone from BL to 6 months ($p = 0.005$) and between 1 and 6 months ($p = 0.003$). For the group that performed the intervention with concurrent training and ingested placebo (G3), there was a considerable increase in testosterone between 1 and 6 months ($p = 0.021$) and 4 and 6 months ($p = 0.012$). The men assigned to G4 underwent the two types of intervention (concurrent training + *Eurycoma longifolia)*, showed substantial and significant improvement from BL to 6 months ($p = 0.005$)

Table 2
Multiple linear regression analysis correlating total testosterone with the domains of sexual health in a model adjusted by the groups. (n = 38). C: correlation / LR: linear regression.

	Erectile function		Sexual satisfaction		Sexual desire		Orgasmic function		General satisfaction		AMS-S	
	C	LR	C	LR	C	LR	C	LR	C	LR	C	LR
	r(p*)	β(p#)	r(p*)	β(p#)	r(p*)	β(p#)	r(p*)	β(p#)	r(p*)	β(p#)	r(p*)	β(p#)
Testosterone	0.070	0.036	0.282	0.210	0.963	0.559	0.091	0.189	0.856	0.440	0.786	0.212
	(0.675)	(0.128)	(0.086)	(0.221)	**(0.008)**	**(0.002)**	(0.589)	(0.055)	**(0.031)**	**(0.006)**	**(0.050)**	**(0.048)**
Adjusted R^2	0.170		0.150		0.243		0.105		0.182		0.121	
p value of model	0.176		0.239		**0.047**		0.436		**0.050**		0.053	

Model adjusted according to the treatment group (G4 being the reference). p: p value of the correlation. p#: p value of linear regression.

and between 4 and 6 months (p = 0.05). The effect of size for testosterone was considered moderate ($f^2 = 0.342$) (Table 3).

4. Discussion

ADAM is a condition that affects male somatic, psychological and sexual behavior according to the frequency of symptom onset. The study by Corrêa, Rombaldi, Silva (2011) showed that sexual symptoms are those men report the most (644%), where erectile dysfunction is the most frequent sexual symptom [4,23]. Erectile dysfunction can lead to unsatisfactory sexual life and, consequently, quality of life [6]. In this study, the influence of concurrent training and EL supplementation on erectile function and testosterone in men with ADAM was studied and associated with total testosterone.

The main evidence of this study was improvement in the five domains of sexual health, namely, erectile function, sexual satisfaction, sexual desire, orgasmic function and general satisfaction. There was progress in the sexual block of the AMS Scale for men who underwent the two interventions (G4). Men assigned to G3 (concurrent training + placebo), showed great improvement in the five domains up to the 4th month, with a decrease observed at the 6-month collection. The hypothesis is that it may be necessary to combine supplementation with physical exercise in order to maintain or even improve these parameters, as observed for G4. The group that underwent concurrent training only showed discrete and gradual improvement of the five aspects, reaching their best value in the last collection (6 months). It can thus be said that it is necessary for the study population to undergo both interventions (*Eurycoma longifolia* + concurrent training) to have more positive results for these variables.

The sexual domains specifically associated with total testosterone were sexual drive, general satisfaction and AMS-S. When multiple linear regression analysis was performed, the response was positive for the type of treatment received (*Eurycoma longifolia* or concurrent training or *Eurycoma longifolia* + concurrent training), not for testosterone values, i.e. for this population, intervention with *Eurycoma longifolia* + concurrent training was more effective for these domains than positive testosterone variation. Regarding testosterone response, the group that showed the most variation and positive results was G4, with a 48 % increase in total testosterone from BL to 6 months.

The effect of *Eurycoma longifolia* on total testosterone in men with ADAM needs to be explained since the group that only used supplementation with this root showed a 43 % increase in testosterone and reached levels within the normal range for total testosterone (>346 ng/dL). In previous studies using *Eurycoma longifolia* supplementation at doses similar to those used in this study for 3 or 6 months yielded significant increases in total testosterone for middle-aged men (38. 6% and 46.8 %, respectively) [14,24]. Therefore, *Eurycoma longifolia* seems to be an excellent natural alternative with no side effects for men with ADAM. Some mechanisms of action involved in increasing total testosterone through supplementation with *Eurycoma longifolia* are shown in Fig. 2. Even when supplementation is associated to concurrent training (G4), improvement in total testosterone was 48 %, showing that these two interventions alone are beneficial and extremely positive when combined.

The group that performed concurrent training only (G3) also showed an evident increase in total testosterone, reaching 29.3 %. The following important variables contributed to this increase: duration, intensity and volume worked during concurrent training. These should be observed when the objective is to raise total testosterone values in middle-aged men. This was also observed in the randomized clinical trial by Khoo et al. [25], where larger volumes (200 min//week, moderate-vigorous intensity) provided better results for this hormone. Regarding practice duration, the recommendation is that it should be for more than 12 weeks, as suggested in the study by Lovell et al. [26], given that changes in testosterone levels in men are maintained after 16 weeks of training. The same authors highlight the need to maintain physical exercise for free testosterone modulation, as the gains obtained in the training period can be lost after only four weeks without training.

This study also showed improved IIEF-5 score for the group that supplemented *Eurycoma longifolia* (G2) especially in the 4-month collection for the five sexual health domains, reaching a 96 % improvement in sexual satisfaction, with significant difference and 49 % in orgasmic function. In other studies using supplementation of *Eurycoma longifolia* for 3 and 6 months, there were no positive associations of supplementation with improvement in the total IIEF-5 score [24,27]. Further to these studies, a systematic review with meta-analysis aimed

Table 3
2-way RM-ANOVA for comparison of total testosterone of the four groups in the four moments of the study. (n = 38). BL - baseline; 1 month - after one month of intervention; 4 months - after four months of intervention; 6 months - after six months of intervention. G1: control + placebo; G2: control + *Eurycoma longifolia*; G3: concurrent training + placebo; G4: concurrent training + *Eurycoma longifolia*. f^2: effect size (Cohen). p value□: for difference between the group; p value* for difference between times; and p value# for group versus time interaction. Lowercase letters on the lines represent difference between time, while uppercase letters on the columns represent difference between groups. Different letters in lines represent significant statistically difference between times and different letter in columns represent significant statistically difference between groups. Statistically significant values (p ≤ 0.05) are highlighted. Results presented as mean ± standard deviation.

	BL $\overline{X}$±dp	1 month $\overline{X}$±sd	4 months $\overline{X}$±sd	6 months $\overline{X}$±sd	f^2	p value□	p value#	p value*
TT					0.342	0.369	**<0.001**	**0.004**
G1	281.5 ± 17.7	249.0 ± 32.8	261.5 ± 31.1	258.5 ± 33.7^{A}				
G2	278.2 ± 20.5^{a}	286.4 ± 37.9^{a}	341.5 ± 35.9^{a,b}	400.3 ± 38.9^{b,B}				
G3	286.7 ± 21.7^{a,b}	273.3 ± 40.2^{a}	286.8 ± 38.1^{a}	370.8 ± 41.3^{b,A,B}				
G4	253 ± 20.5^{a}	321.6 ± 37.9^{a,b}	308.6 ± 35.9^{a}	374.5 ± 38.9^{b,A,B}				

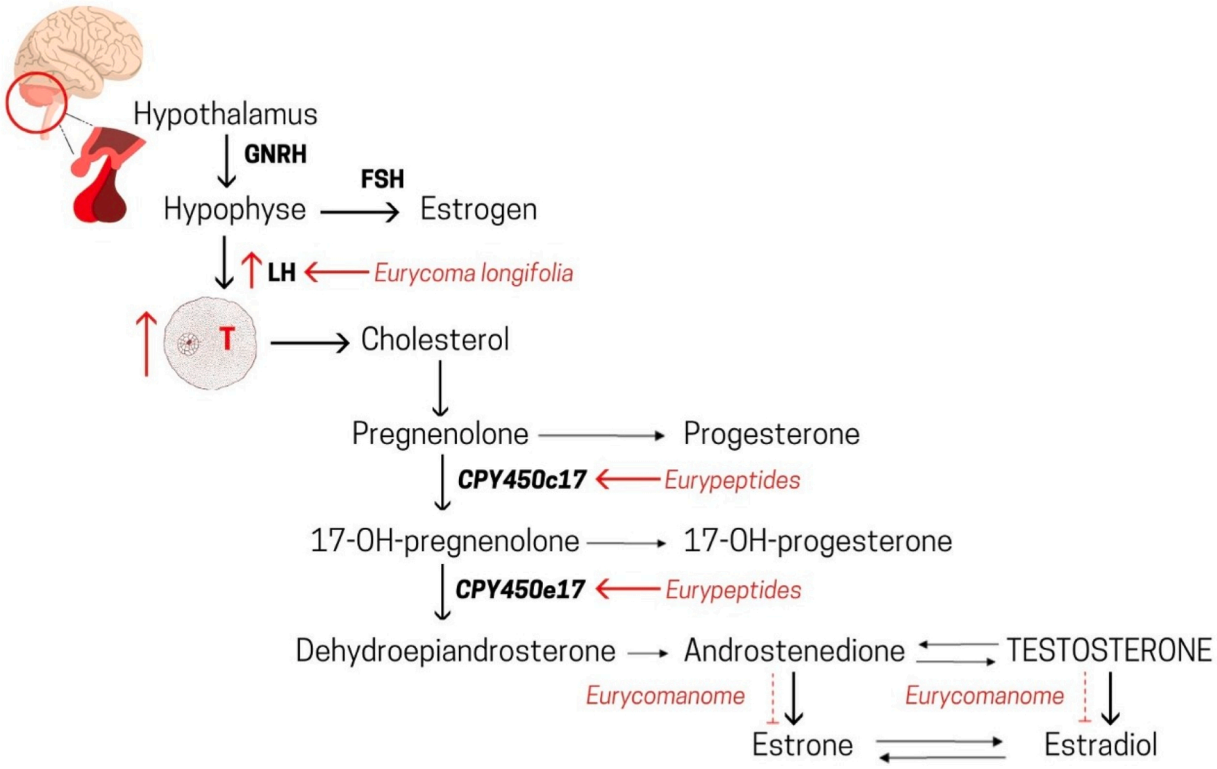

Fig. 2. Mechanism of action of *Eurycoma longifolia.* The increase in testosterone by the consumption of EL is related to several mechanisms. At the hypothalamus-pituitary axis, the EL generates a greater stimulus of LH release by the pituitary, and thereby greater production and secretion of testosterone by Leydig cells. In the testosterone synthesis process, the *eurypeptides* act enhancing the action of CYP450c17 and e17 enzymes, and by that generating a greater production of dehydroepiandrosterone and testosterone. Besides, *quassinoids* denominated *eurycomanome* act by inhibiting aromatase and diminishing the conversion of testosterone to estradiol or estrone [14,28,36,37]. GNRH: Gonadotropin-Releasing Hormone FSH: Follicle-stimulating hormone LH: Luteinizing hormone T: testosterone. Figure created by author.

at analyzing the effect of *EL* consumption on erectile function in men showed similar findings to previous studies. An increased erectile function score can be observed, but without significant difference [18]. The mechanism of action of *EL* for erectile dysfunction improvement has not been explored in the literature, but this improvement is possible due to the increase in total testosterone. In chemical terms, Chiou and Wu [28] isolated the effect of an *EL* compound, an alkaline β-carboline named 9-hydroxycanthin-6-one (9−HC-6-one) and identified that this compound interferes with Ca^{2+}, mobilization which, among other factors, contributes to penile erection induction. In addition, 9−HC-6-one was able to antagonize the muscle tone of certain ejaculatory tissues (such as the seminal vesicle) *in vitro* and reduce the threshold of ejaculation by inhibiting the effect of seminal vesicle pressure after *in vivo* stimulation of the epigastric nerve.

In addition to improvements observed in G2, the men assigned to G3 who underwent concurrent training showed the following IIEF-5 improvements: 5% for erectile function; 6.3 % for sexual satisfaction; 7.4 % for sexual desire; 10.5 % for orgasmic function and 8.7 % for general satisfaction. It is therefore extremely important to encourage the practice of consistent concurrent training as a positive agent in erectile dysfunction.

There is still a gap in the literature on studies that used concurrent training as an intervention to improve erectile dysfunction. Regarding the methods analyzed alone according to the systematic review by Gerbild et al. [29], the recommendation for male erectile dysfunction improvement is practicing aerobic exercises in a moderate to vigorous intensity for 40 min four times a week for at least 6 months, as in this study. The explanation for erectile dysfunction improvement through aerobic exercise is oxidative stress reduction and increased nitric oxide availability in penile tissues [30]. Nitric oxide is necessary for the muscles of the penis to relax. This relaxation allows for better blood vascularity in the cavernous body and erection [6].

In addition to the recommendations for aerobic exercises, there are recommendations for resistance exercises, to be performed at least twice weekly, at high intensity. Exercises that mobilize all muscle groups should be performed at different periods [31,32]. Resistance exercises are recommended to improve erectile dysfunction because strength training increases blood lactate, nitric oxide and cortisol production [33], which all lead to an increase in total testosterone [34].

In summary, there are limited studies that analyze the effect of these two training methods in one session (concurrent training) for the variables related to erectile dysfunction, even though practicing exercises that combine aerobic and anaerobic stimuli is encouraged to increase total testosterone (resistance exercises) and endothelial vascularization (aerobic exercises), and thus enhance the effect of exercise on male sexual health [35].

As previously mentioned, the men in this study underwent six months of intervention to have significant increases in testosterone and combined aerobic and resistance exercises in the same session (CT) for 60 min, three times weekly. Positive results for erectile dysfunction were achieved with this choice of volume and duration. When the effect of *Eurycoma longifolia* supplementation is analyzed with concurrent training intervention (G4), the percentage improvements observed were greater than for other groups: 15.2 % in erectile function, 34.5 % in sexual satisfaction, 35.6 % in sexual desire, 14.7 % in orgasmic function and 14 % in general satisfaction. These men went from moderate erectile dysfunction to mild erectile dysfunction. That is, in order to achieve the greatest benefit, it is necessary to carry out concurrent training associated with *Eurycoma longifolia* supplementation for at least 6 months.

5. Conclusion

It is important to note that one of the study's limitations was not including two more groups without placebo use to avoid the "placebo effect". Moreover, the study method should be highlighted: it was the first controlled, randomized study to evaluate the influence of *EL* and concurrent training on the erectile function of men with ADAM. Finally, this study showed the benefits of *Eurycoma longifolia* supplementation and practice of a concurrent training protocol on sexual health and total testosterone in men with ADAM. The action of *Eurycoma longifolia* was best observed in the fourth month of use, while the best result for concurrent training was observed in the sixth month. When the two interventions were associated, the improvements were superior to isolated use. These findings are important for professionals who work with this population, as this non-pharmacological treatment proved to be positive and had no side effects. It can be applied to improve total testosterone, erectile dysfunction and, consequently, quality of life of middle-aged men with ADAM.

Contributors

Alice Erwig Leitão contributed to study design, data collection, data interpretation and manuscript preparation,

Melissa Carvalho de Souza Vieira contributed to study design, and data interpretation.

Andreia Pelegrini contributed to manuscript preparation.

Edson Luiz da Silva contributed to manuscript preparation.

Adriana Coutinho de Azevedo Guimarães contributed to manuscript preparation.

All authors approved the final version of the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

This study was conducted at the Health and Sport Sciences Center of the Santa Catarina State University. It complied with institutional regulations and was approved by the ethics committee (number: 2.274.655), with registration in the international clinical trial registry platform "*Clinical Trials.gov*" (n. NCT03150225) and written consent for voluntary participation from all men.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. Data will be made available on request.

Provenance and peer review

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