Review

New Trends to Treat Muscular Atrophy: Systematic Review

Iris Jasmin Santos German , Karina Torres , Jesus Carlos Andreo , Joao Shindo , Camila Dallantonia , Luiza Haber , Cláudia Rucco Penteado Detregiachi , Adriano Araujo , Elen Landgraf Guiguer , Raul Girio , Patricia Bueno , Maricelma Souza , Marcia Rocha , Sandra Maria Barbalho * , Andre Shinohara

Posted Date: 30 June 2023

doi: 10.20944/preprints202306.2260.v1

Keywords: epicatechin; skeletal muscle; muscular atrophy; catechins; myogenic regulatory factors

Review

New Trends to Treat Muscular Atrophy: Systematic Review

Iris Jasmin Santos German [1,*], Karina Torres Pomini [2,3], Jesus Carlos Andreo [1], João Vitor Tadashi Cosin Shindo [1], Camila Tiveron Dall'Antonia [3], Luiza Santos de Argollo Haber [3], Claudia Rucco P. Detregiachi [2,3], Adriano Cressoni Araújo [2,3], Elen landgraf Guiguer [2,3], Raul José da Silva Girio [4], Patrícia Cincotto dos Santos Bueno [3,4], Maricelma da Silva Soares de Souza [3], Marcia Gabaldi Rocha [3], Sandra Maria Barbalho [2,3] and André Luis Shinohara [1]

[1] Department of Biological Sciences (Anatomy), School of Dentistry of Bauru, University of São Paulo, (FOB-USP). Alameda Doutor Octávio Pinheiro Brisolla, 9-75, 17012-901, Bauru – São Paulo, Brazil
[2] Postgraduate Program in Structural and Functional Interactions in Rehabilitation, University of Marilia (UNIMAR), 17525-902, Marília - São Paulo, Brazil
[3] Department of Biochemistry and Pharmacology, School of Medicine, University of Marília (UNIMAR), Avenida Hygino Muzzy Filho, 1001, 17525-902, Marília, São Paulo, Brazil
[4] Department of Animal Sciences, School of Veterinary Medicine, University of Marília (UNIMAR), Avenida Hygino Muzzy Filho, 1001, 17525-902, Marília, São Paulo, Brazil
* Correspondence: irisgerman@usp.br; Tel.: 5514991635033

Abstract: Epicatechin has been described as a polyphenol compound that promotes skeletal muscle restructuring, by expressing muscle regulation factors, activation of satellite cells and modulation of the main pathways associated with catabolism. However, the literature shows contrasting results of therapeutic effects and treatment protocols. Thus, the aim of this systematic review was to analyze the current literature addressing the molecular mechanism and clinical protocol of epicatechin on skeletal muscular atrophy in humans and animals. A search was conducted in PubMed/MEDLINE, Embase, Web of Science, and Cochrane Library databases. The qualitative analysis showed a prevalence of the inhibitory action of epicatechin in myostatin expression and atrogenes FOXO, MAFbx and MuRF1. Epicatechin showed positive effects on increased follistatin and on the activation of the myogenic regulatory factors (Myf5, MyoD and myogenin). In addition, the studies evidenced the impact of epicatechin on the mitochondrias' biosynthesis in muscle fibers, activation of the signaling pathway of AKT/mTOR protein synthesis, and improvement of skeletal musculature performance, particularly when associated with physical training. Epicatechin showed promising clinical applicability through beneficial results under conditions that negatively affect the skeletal musculature. However, there is no protocol standardization allowing to draw more specific conclusions on its therapeutic use.

Keywords: epicatechin; skeletal muscle; muscular atrophy; catechins; myogenic regulatory factors

1. Introduction

The study of catechins has shown growing interest in the properties reported in the scientific literature related to their antioxidant, regenerative and anti-inflammatory capacity [1-3].

There are four major subclasses of catechins: Epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin (EGC), and Epigallocatechin gallate (EGCG) [2]. Of the different catechins, EC and EGCG cover more effects on the skeletal musculature. Moreover, EC promotes mitochondrial biogenesis and angiogenesis [4].

Epicatechin (EC) is a polyphenolic compound found at high concentrations in certain fruits and vegetables, such as tea leaves, black grapes, chocolate, apples, raspberries and cherries [2,5,6]. EC is extracted mainly from the leaves of green tea (Camellia sinensis) [4,5]. The consumption of such polyphenol has been associated with a number of positive effects on diseases involving oxidative

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1

stress, such as cancer, diabetes, and cardiovascular and degenerative diseases [7,8]. Figure 1 shows the biological properties of epicatechin.

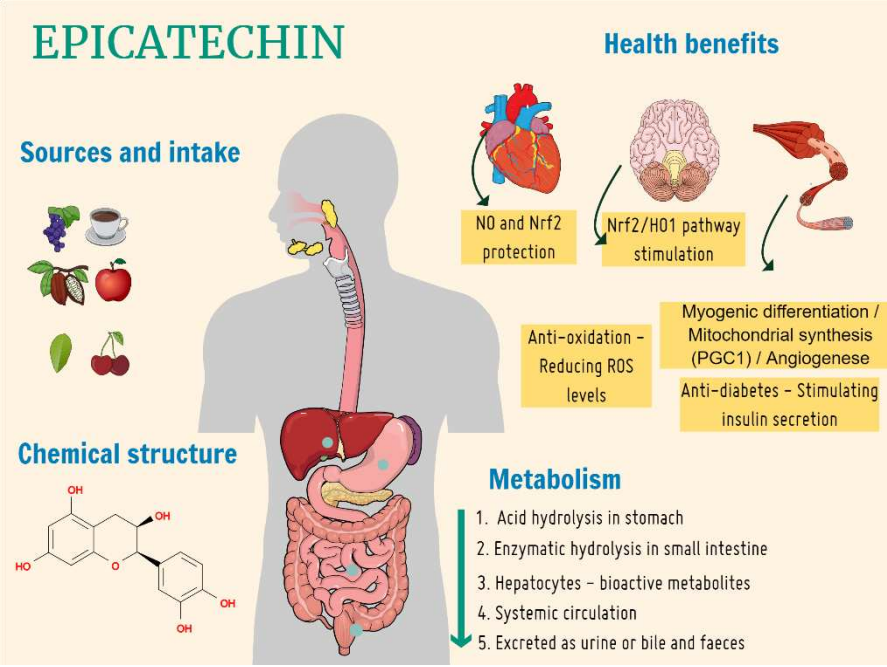


Figure 1. Main food and beverage sources, chemical structure, metabolic route and biological properties of epicatechin. NO, nitric oxide; Nrf2, nuclear factor-like 1; HO, heme oxygenase; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1-alpha; ROS, reactive oxygen species.

More recently, the beneficial effects and potential capacity to mitigate and delay the loss of muscle mass are being investigated in the prevention and treatment of diseases that affect the musculoskeletal system [4,9-11].

Clinical studies have related the catechins to the protective effects of the skeletal musculature by inducing myogenic differentiation, and improving muscle structure and function [12,13].

In the skeletal muscle, epicatechin acts directly and indirectly in the protein synthesis signaling [14], reduces the catabolic effect [15,16], by stimulating the PI3K/Akt pathway and by inactivating the autophagic genes FoxO, MAFbx, and MuRF [2]. Such mechanism of action of epicatechin in the muscle occurs by inhibiting such degradation proteins and increasing mitochondrial biogenesis [6,17].

Research in animals that received catechins presented an increase in the Muscle Regulatory Factors (MRF), including MyoD, Myf5, and Myogenin, and a decrease occurred in myostatin, a protein identified as modulatory of the main catabolic pathways, participating in the signaling which regulates the muscular atrophy [9,18].

Due to the great benefits of epicatechin supplementation and clinical relevance in the treatment of diseases that affect the musculoskeletal system, it is necessary to summarize the evidence available on the effects of this polyphenol, with the search strategy used. Despite the positive effects of epicatechin, there are conflicting results and non-standardized therapeutic protocols.

Thus, the systematic review was substantiated through the PICO strategy [14] - P: use of epicatechin in humans and animals; I: application of epicatechin in muscular atrophy; C: comparison with the control/placebo group; O: effects on the skeletal musculature. The PubMed/MEDLINE, Web of Science, Embase, and Cochraine Library databases were based on the PICO strategy, to evaluate the clinical protocols and protein turnover effects of epicatechin supplementation on skeletal musculature atrophy condition.

 doi:10.20944/preprints202306.2260.v1

2. Materials and Methods

The databases were searched including the terms registered in the Medical Subject Headings (MeSH) and by associating the following keywords: "Catechin and muscular atrophy", "Epicatechin and muscle regeneration", "Epicatechin and muscle and damage". The search was conducted with no restrictions concerning the year of publication of the articles. In addition, studies from the gray literature were analyzed to identify potentially relevant studies for this systematic review.

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses) – PRISMA [20,21], the articles were selected considering the following eligibility criteria.

2.1. Inclusion criteria:

- Clinical and experimental studies which evaluated epicatechin in the treatment of muscular atrophy;
- Publications allowing access to the full text;
- Scientific articles in the English language;
- Studies with full specifications on the dosage of epicatechin used, as well as the treatment time and administration route.

2.2. Exclusion criteria:

- Articles that used another type of catechin;
- Literature reviews;
- Duplicated articles.
- Studies involving myoblast cell lines

In order to search, first, the keywords were combined in each database. The selection of the articles was conducted by title and by reading the abstracts, thus, they were organized, and subsequently, the articles were restricted according to the eligibility criteria, following the proposed methodology and the PRISMA checklist [20,21]. Figure 2 shows the search design strategy in the databases.

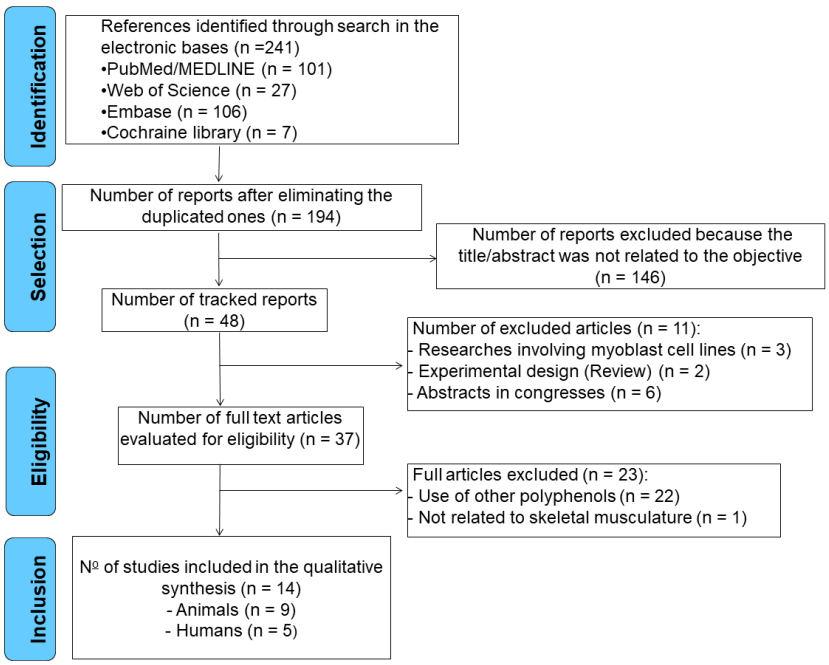


Figure 2. Search design strategy in the databases.

 doi:10.20944/preprints202306.2260.v1

3. Results

3.1. Search results

At first, 241 articles were identified in the total search for this systematic review. 101 articles were verified in the PUBMED/MEDLINE database, 27 studies in Web of Science, 106 in Embase, and 7 articles in Cochrane Library. Following the removal of the duplicated articles 194 remained, of which 146 studies were eliminated once they were not related to the subject of investigation. Of the 48 articles tracked, the following were excluded: 3 in vitro studies, 2 literature reviews, and 6 abstracts in congresses, 37 articles remaining for reading the full text, being that 23 studies were not compatible with the selection criteria. Finally, 14 articles (9 studies in animals and 5 studies in humans) matched the eligibility criteria and were included in this systematic review. Tables 1 and 2 show the main information about the 14 studies selected in humans and in animals, respectively.

3.2. Risk of bias of the studies

Among the analyzed studies, a reduced sample size was identified [22-24]; reduced evaluation time [1-3]. [25,26]; the data on the participant's diet was not collected [23,24]; absence of an evaluation of epicatechin consumption in different periods [22-25,26-32]; only one dose was studied [22-25,27-29,30,31,32-35]; variability among the responses according to the gender and/or absence of hormonal analysis [22-29,30,31-35]; difference of the period of euthanasia in the groups [34]; choices of the animal model for longevity studies - db/db BKS.Cg-Dock7m +/+ Leprdb/J mice, a mutation of the C57BLKS/J lineage [27]; lack of functional evaluation pre- and post-intervention [33]; the effect of epicatechin interruption was not evaluated [22-32,35]; the participants' gender was not reported [25]. The previous data is presented in Table 3.

 doi:10.20944/preprints202306.2260.v1

Table 1. Summary of the main epicatechin supplementation parameters – Studies in humans.

First author and year	Manufacturer	Participants	Gender/ Age	Objective	Groups	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
Taub et al., 2013 [22]	Hershey's® 60% Dark chocolate	5	Male 47-71 years old	To evaluate the skeletal muscle growth with the cocoa enriched with epicatechin in patients with heart failure and type 2 diabetes.	-Control group: Patients aged 50-53 years with no disease. -Experimental group: Patients aged 47-71 years.	100 mg a day	3 months	Oral route	The patients underwent femoral quadriceps muscle biopsies before and after consuming cocoa enriched with epicatechin.	There was a decrease in myostatin; however, it remained elevated compared to the control group. Follistatin increased above the controls with the treatment. The myogenin, MyoD, MEF2, and Myf5 levels were significantly stimulated with the epicatechin treatment.
Mafi et al., 2019 [30]	Sigma-Aldrich, St Louis, MO	62	Male / 68 ± 2.86 years old	To evaluate the plasma levels of follistatin and myostatin in men with Sarcopenia under training and epicatechin supplementation.	RT: Resistance training, EP: Epicatechin, RT+EP: Resistance training + epicatechin, PL: Double-blind placebo.	1 $mg \cdot kg^{-1} \cdot a$ day	8 weeks	Oral route (Daily capsules with 200 ml of water)	The training groups' subjects conducted the protocol at 05:00 p.m. (45 min., 3 sets, 8-12 repetitions). The placebo group received starch capsules.	Follistatin significantly increased in the RT + EP groups compared to PL group. While myostatin decreased in the RT + EP and in RT groups. The maximum supine strength significantly improved among the RT+EP and RT participants but not in EP and PL.
Corr et al., 2020 [26]	Chococru®/ Epicatechin	23	13 women and 10 men/ 24 years.	To investigate if an acute dose of flavonoid cocoa (FC) may help in muscle recovery following EIMD.	CON: Control group: Did not receive FC, n=8. CF830: High FC dose 830 mg group, n=8. CF1245: FC overdose group 1245 mg , n=7	830 mg and 1245 mg	5 days (2 adaptation days and 3 days of epicatechin)	Oral route	The EIMD protocol consisted in the hip fastening to the dynamometer at 85º of bending using straps to isolate the knee (5 series of 10 maximum concentric and eccentric contractions of the knee.	No significant differences were noted between the groups for all the measures in the bending exercises. The FC did not show benefits in muscle recovery after 24 h, 48 h, and 72 h post-EIMD.

Abbreviations: Exercise-induced muscle damage (EIMD); Flavonoid cocoa (FC); Myogenic factor 5 (Myf5); Myoblast determination protein 1 (MyoD); Myocyte Enhancer Factor 2A (MEF2a).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1

Table 1. Summary of the main epicatechin supplementation parameters – Studies in humans. *Continued*.

First author and year	Manufacturer	Participants	Gender/Age	Objective	Group	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
McDermott et al., 2020 [23]	Hershey's Co.	44 participants	Male and female/ ≥ 60 years old	To evaluate if cocoa with epicatechin improves the walk performance in aged people with peripheral artery disease.	Cocoa drink/Epi (n=23) versus placebo drink (n=21) (did not contain cocoa or epicatechin)	75 mg	6 months	Oral route	The physical activity was conducted over 7 days with Accelerometer ActiGraph placed on the right hip.	There was a significant difference between the Cocoa/Epi group versus the placebo group in the 6-minute walk test 2.5 h after consuming the drink. These results suggest a therapeutic effect of cocoa/Epi in the walk performance. However, cocoa/Epi did not show significant effects on myostatin, follistatin and Pax7.
McDonald et al., 2021 [24]	cGMP facility (Syngene, Karnatak, India)	7 participants	Male/ 18-60 years old	To evaluate epicatechin capacity in mitochondrial biogenesis and in the muscle markers.	Non-randomized clinical trial (before and after)	50 mg twice a day	8 weeks	Oral route (gelatin capsules)	The participants received two capsules in the morning and two in the evening. The brachial biceps muscle biopsies were collected pre- and post-treatment.	Follistatin significantly increased, while myostatin decreased. There was a significant increment of tissue markers Myf5, MyoD, myogenin, and MEF2a.

Abbreviations: Paired box protein (Pax-7); Myogenic factor 5 (Myf5); Myoblast determination protein 1 (MyoD); Myocyte Enhancer Factor 2A (MEF2a).

Table 2. Summary of the main epicatechin supplementation parameters – Studies in animals.

First author and year	Manufacturer	Population	Gender/Age	Objective	Groups	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
Si et al., 2011 [27]	Sigma- Aldrich	29 C57BLKS/J and KS.Cg-m +/+Lepr db/J, db/db Mice	Male/ 5 weeks of age	To investigate the effects of epicatechin in obese diabetic mice.	Con: n=12 Control group: C57BLKS/J Mice; db: n=6: Diabetic rats without epicatechin. db+EC: n=11: 0.25%:	0.25% every other day	15 weeks	Oral route	To determine the contractile function, the EDL muscles were excised and attached by means of a suture to a servomotor (Aurora Scientific).	Epicatechin significantly decreased the inflammatory markers (C-Reactive Protein) in diabetic rats. The GSK antioxidant concentration and AMPKa phosphorylation were significantly higher than those of db group.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1

Hüttemann et al., 2013 [33]	Sigma-Aldrich	21 LCR rats (rats grown for low capacity to run)	Males/5 months of age	To determine the action of epicatechin on angiogenesis and mitochondrial proliferation in rats with congenital muscle dysfunction.	Diabetic rats + EC. Control: Water group for 30 days; Epi 30d: Epicatechin for 30 days; Post-Epi 15d: epicatechin for 30 days followed by 15 days without epicatechin.	1.0 mg/kg twice a day	Epicatechin for 30 days followed by 15 days without epicatechin.	Gavage	For all three groups, the plantar muscle was analyzed in order to determine the effects of epicatechin on a glycolytic muscle fiber.	EC produced a significant increase in capillarity and mitochondrial biogenesis in the 15-day treatment period, being significantly higher than in the control group, including in the 15-day period of treatment interruption. EC increased VEGF and reduced CD47 and the receptor TSP1 and activated the P38 MAPK pathways.

Abbreviations: Extensor digitorum longus muscle (EDL); Glutathione (GSH); AMP-activated protein kinase (AMPKa); Vascular endothelial growth factor (VEGF); Differentiation Cluster 47 (CD47); Thrombospondin-1 (TSP-1); Mitogen-activated protein kinase (MAPK); Myogenic factor 5 (Myf5); Myoblast determination protein (MyoD); Myocyte Enhancer Factor 2A (MEF2a).

Table 2. Summary of the main epicatechin supplementation parameters – Studies in animals. *Continued.*

First author and year	Manufacturer	Population	Gender/Age	Objective	Groups	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
Gutierrez-Salmean et al., 2014 [25]	Sigma-Aldrich	20 C57BL/6 Mice (n=20 5/group)	Young males/ 6 months and senile males/ 26 months	To examine the changes to the protein levels in the skeletal muscle of young vs senile humans and mice.	Ctrl (Young), Epi (Senile), Ctrl (Senile), Epi (Young)	1 mg/kg	2 weeks	Gavage	The control groups received water through gavage. Quadriceps muscle samples were obtained from the mice.	Epicatechin significantly decreased the myostatin levels 15% (young) and 21% (aged), while follistatin increased 56% in the senile muscle. Myogenin significantly increased in young and senile animals (16%, 21%, respectively), while MyoD increased 19% in senile rats. Myf5 incremented 12% (young) and 15% (senile) and MEF2 10%, 19%, respectively.
		12 participants	Gender not reported/ Young adults: 28 years old, n=6 Aged: 62 years old, n=6	To evaluate the effects of the treatment with epicatechin on muscle strength and on the plasma levels of myostatin and follistatin.	Young adults group (n=6) Senile group (n=6)	25 mg/day	1 week	Oral route (capsule)	The muscle strength was evaluated by hand grip dynamometry (three times with each hand, alternating the hand and resting for 10 seconds in order to prevent fatigue).	The treatment with epicatechin increases the hand's muscle strength by 7%. With age, there was a significant increase in myostatin (28%, 48%). The treatment with EC significantly increased the plasma levels of follistatin (49%).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1

Lee et al., 2015 [28]	Sigma-Aldrich, St. Louis, MO, USA	34 C57BL/6N Mice	Males/14 months of age	To determine the effect of epicatechin on angiogenesis and mitochondrial biogenesis protein markers.	C: Control group CE: Control with resistance training Epi: Epicatechin Epi-Ex: Control + training + epicatechin.	1.0 mg/kg twice a day	8 weeks	Gavage	The training groups' mice were submitted to training on the treadmill for 8 weeks (5 times/week for 60 min./session).	The Epi-Ex showed better resistance performance and a significantly higher VEGF-R2 expression and increased PGC-1b and TFAM.. FoxO1 expression was significantly reduced in the experimental groups compared to the control.

Abbreviations: Ubiquitin proteasome system (UPS); Dark chocolate drink (DC); Forkhead transcription factors family (FoxO); F-box muscular atrophy (MAFbx); Muscle RING-finger protein (MuRF1); Myocyte enhancer factor 2A (MEF2A); Gulf War Illness (GWI); Pyridostigmine bromide (PB); N,N-dimethyl-meta-toluamide (DEET); Protein kinase B (AKT); Mammalian target protein of rapamycin (mTOR).

Table 2. Summary of the main epicatechin supplementation parameters – Studies in animals. *Continued.*

First author and year	Manufacturer	Population	Gender/Age	Objective	Groups	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
Lee et al., 2016 [29]	Sigma-Aldrich, St. Louis, MO, USA	25 C57BL/6N Mice	Males/ 6 months of age	To determine if the treatment with epicatechin may mitigate the muscle mass loss in the skeletal muscle.	C: Control (water); HS-V: Suspension of the hind limbs + water; HS-Epi: Suspension of the hind limbs + epicatechin.	1.0 mg/kg twice a day (Morning and evening).	14 consecutive days	Gavage	For the hind limbs suspension protocol, the animals were placed in a cage with a steel bar. The soleus, medial, and gastrocnemius muscles were removed from both hind limbs.	HS-Epi showed significantly higher FCSA and fiber capillarity compared to HS-V. In HS-Epi there was a slight decrease in FP compared with the control group. The antiangiogenic factor TPS-1 did not change in HS-Epi and showed a significant increase in mTOR, Akt, and TFAM. PGC-1β was only induced in HS-Epi, and CcO was similar to the control. FoxO and GSK-3β were induced in HS-V.
Si et al., 2019 [34]	Millipore Sigma, Burlington MA, USA	33 C57BL/6 Mice	Males/ 9 months and 20 months of age	To investigate the effects of epicatechin on the survival rate and on the physical performance in aged mice.	OC: Control (aged mice). YC: Young control: Mice of 9 months of age. EC: 0.25% epicatechin.	0.25%	37 weeks and 44 weeks	Oral route	The samples were collected following 37 weeks, and the rest was treated for one additional week (on week 44).	Epicatechin mitigated the aging-related deterioration of the skeletal muscle; in addition, it improved physical activity, and delayed the degeneration of the quadriceps. E in senile mice presented a survival rate (69%) compared with the control group (39%).

Abbreviations: Vascular endothelial growth factor receptor 2 (VEGF-R2); Fiber cross-sectional area (FCSA); fiber perimeter (FP); Forkhead transcription factors family (FoxO); Thrombosponding antiangiogenic factor (TPS-1); Mitochondrial transcription factor A (TFAM); Protein kinase B (AKT); Mammalian target protein of rapamycin (mTOR); Peroxisome proliferator-activated receptor coactivator-1 (PGC-1); Cytochrome c oxidase (CcO); Enzyme Glycogen Synthase Kinase 3 Beta (GSK-3b).

Table 2. Summary of the main epicatechin supplementation parameters – Studies in animals. *Continued* .

First author and year	Manufacturer	Population	Gender/Age	Objective	Groups	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
Gonzalez-Ruiz et al., 2020 [35]	Sigma-Aldrich	36 Long-Evans Rats	Females/ 11 weeks	To analyze the effects of epicatechin on the regulation of UPS proteins in the hind limbs.	SCI+water 7 days: n=6. SCI+Epi 7 days: n=6. SCI+water 30 days: n=9. SCI+Epi 30 days: n=9. Sham: Only laminectomy n=6.	1 mg/kg/ day	Evaluation periods: 1 week and at 30 days	Gavage	The spinal cord was sectioned (Region of the T8 to T10 vertebrae). The left side gastrocnemius and soleus muscles were dissected.	At 30 days, the spinal cord injury group lost 49.52 ± 2.023% of the cross-sectional area of the muscle, and the epicatechin groups lost 24.28±15.45%. In the period of 7 days, the SCI+Epi had only one significant difference in MuRF decrease compared with SCI+water. The treatment with epicatechin induced a significant decrease in atrophy markers FOXO, MAFbx, and MuRF1 compared to the control group (VEH) after 7 and 30 days from the lesion.

Abbreviations: Ubiquitin proteasome system (UPS); Dark chocolate drink (DC); Forkhead transcription factors family (FoxO); F-box muscular atrophy (MAFbx); Muscle RING-finger protein (MuRF1); Myocyte enhancer factor 2A (MEF2A); Gulf War Illness (GWI); Pyridostigmine bromide (PB); N,N-dimethyl-meta-toluamide (DEET); Protein kinase B (AKT); Mammalian target protein of rapamycin (mTOR).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1

Table 2. Summary of the main epicatechin supplementation parameters – Studies in animals. *Continued* .

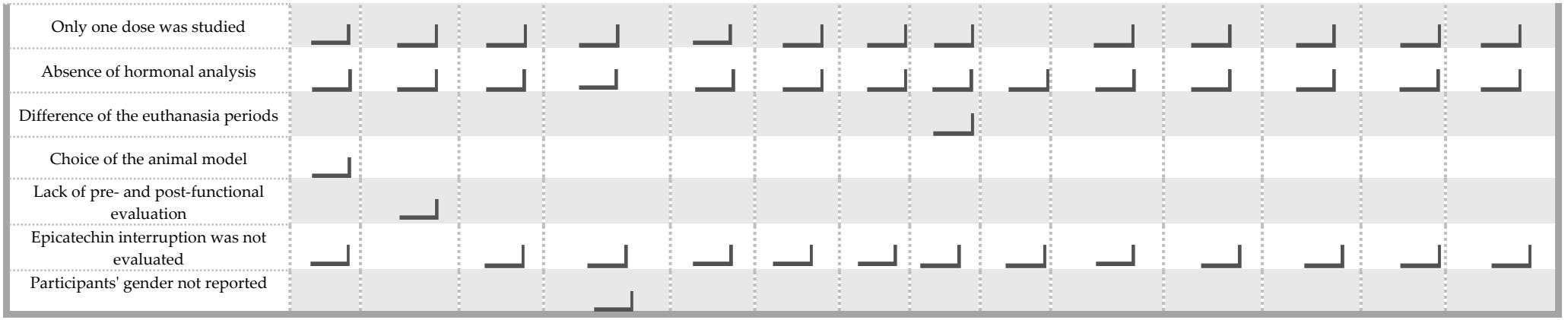
First author and year	Manufacturer	Population	Gender/Age	Objective	Groups	Dosage	Experimental time	Route of administration	Procedure	Effects of epicatechin (Main results)
Munguia et al., 2020 [31]	Sigma-Aldrich Co. (St. Louis, MO, USA)	15 C57BL/6 Mice induced to a high-fat diet.	Males/ 10 weeks	To evaluate the benefits of the flavonoids in the improvement of the physical activity decreased by age/ high-fat diet.	Three interventions: Control: Water; High-flavonoid dark chocolate (DC) drink (2 mg EC + 12.8 mg procyanidins/kg) EC: Epicatechin (2 mg EC/kg).	2 mg EC/kg	5 weeks of treatment with EC. Week 49 – 15 weeks of obesity induction. Week 64 – Change from normal diet + 5 weeks of treatment. Total: 69 weeks.	Gavage	Muscle samples from the gastrocnemius were collected. The inverted screen and front limbs functional test consisted in the longest time hanging, establishing a fixed time of 120 seconds and 130 seconds, respectively.	Epicatechin increased follistatin and myocyte enhancer factor 2A (MEF2A) expression. DC and EC decreased the FoxO and MURF; however, the MAFbx decrease was not significant. DC and EC induced a significant decrease in the fat content and increased physical performance compared with the control.
Ramirez-Sanchez et al., 2021 [32]	Sigma-Aldrich, Inc./ Hershey, PA, USA	30 Wistar Rats	Males/ 3 months of age	To examine the potential restorative effects of epicatechin in muscular atrophy-induced rats.	Control group (n=15): Without physical restriction (water): The experimental group (n=15): Physical restriction (2 weeks). Rats were divided into two groups: Epi GWI-Epi group (n=8) and Water GWI group (n=7).	1 mg/kg/day	2 weeks of EC. Atrophy induction (3 weeks) + 1 maintenance week + 2 weeks of EC. On week 6 – Functional test and euthanasia.	Gavage	The atrophy induction consisted of the administration of pyridostigmine bromide (PB) 1.3 mg/kg/day through the oral route, permethrin (PM) 0.13 mg/kg/day (skin), DEET 40 mg/kg/day (skin). The animals were physically contained for 5 min./day for 3 weeks.	The treatment with epicatechin in animals with muscular atrophy induced a partial recovery of muscle strength and run distance on treadmill. MURF, Fbox40, and atrogin-1 were partially recovered by EC. Epicatechin significantly increases AKT and mTORC1 activation.

Abbreviations: Ubiquitin proteasome system (UPS); Dark chocolate drink (DC); Forkhead transcription factors family (FoxO); F-box muscular atrophy (MAFbx); Muscle RING-finger protein (MuRF1); Myocyte enhancer factor 2A (MEF2A); Gulf War Illness (GWI); Pyridostigmine bromide (PB); N,N-dimethyl-meta-toluamide (DEET); Protein kinase B (AKT); Mammalian target protein of rapamycin (mTOR).

Table 3. Risk of bias of the studies.

Risk of bias	Si et al., 2011 [27]	Hüttemann et al., 2013 [33]	Taub et al., 2013 [22]	Gutierrez-Salmean et al., 2014 [25]	Lee et al., 2015 [28]	Lee et al., 2016 [29]	Mafi et al 2019 [30]	Si et al., 2019 [34]	Corr et al., 2020 [26]	Gonzalez-Ruiz et al., 2020 [35]	McDermott et al., 2020 [23]	Munguia et al., 2020 [31]	McDonald et al., 2021 [24]	Ramirez-Sanchez et al., 2021 [32]
Reduced sample size			⌞								⌞		⌞	
Reduced evaluation time				⌞					⌞					
Failure to collect the participants' diet											⌞		⌞	
Only 1 period was evaluated	⌞		⌞		⌞	⌞	⌞		⌞		⌞	⌞	⌞	⌞

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 June 2023 doi:10.20944/preprints202306.2260.v1

4. Discussion

The coadjuvant approaches in diseases involving the musculoskeletal system have provided beneficial results to the health and quality of life of the affected individuals [36-38]. The epicatechin supplementation has presented promising clinical applicability in the regeneration of muscle tissue. Thus, this systematic review aimed to summarize the existing literature addressing the molecular effects and clinical protocol design of phytocompound epicatechin on skeletal muscle atrophy in humans and animals.

The signaling pathways involved in muscular atrophy have been a study target once they exercise a critical role in several clinical conditions associated with the skeletal muscle degeneration [39-42].

Two proteolytic systems are involved in the pathophysiology of muscle atrophy, which regulate protein turnover and muscle homeostasis: the ubiquitin proteasome system (UPS) and the autophagy-lysosome system. The main genes of the UPS system are MuRF1 and MAFbx (atrogin-1) [42]. An imbalance of these systems can lead to an excessive activity of protein degradation, and consequently contribute to the loss of muscle mass and compromise the contraction of myofibers [39].

Another signaling pathway that participates as a negative regulator of muscle growth is the myostatin-Smad2/3 pathway [4]. Myostatin inhibits IGF1-AKT-mTOR signaling and acts as a synergist in the FoxO signaling pathway, leading to muscle atrophy [39]. Figure 3 shows the general effects of epicatechin on protein synthesis and degradation.

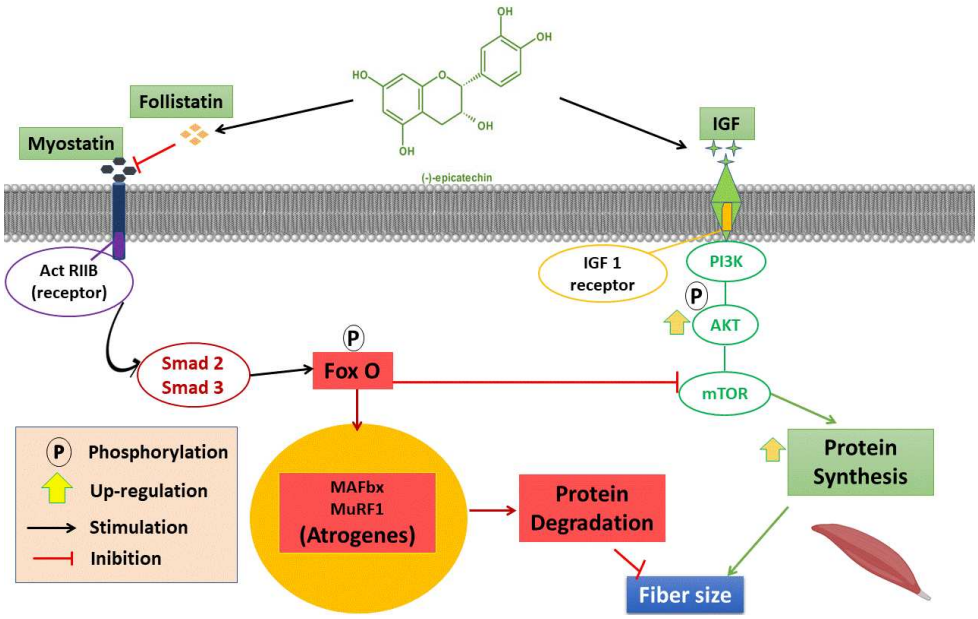


Figure 3. An overview of epicatechin effects on skeletal muscle. Modified diagram from Li et al. (2020) [4]. Act RIIB, myostatin receptor; Smad 2/3, mothers against decapentaplegic homolog 2; FoxO, forkhead transcription factor family; MAFbx, muscle atrophy F-box; MuRF1, muscle RING-finger protein-1; IGF, insulin-like growth factor-1; PI3K, phosphatidylinositol 3-kinase 9; AKT, Protein kinase B; mTOR, The mammalian target of rapamycin.

The results of the analyzed studies evidenced a decrease in myostatin expression and an increase in follistatin. The mechanism to prevent the activation of atrophic genes consists in increasing follistatin (antagonist protein of myostatin), so as to interrupt myostatin binding to the receptor [43-46].

Such an increase in the follistatin/myostatin ratio was verified in five articles in this review [22,24,25,30,31]. Taub et al., 2013 [22] and Mafi et al., 2019 [30], noted an increment in the follistatin levels; but no significant differences in myostatin decrease, comparing the epicatechin groups to the

control group. Such a result can be associated with the epicatechin stimulus in the high plasma levels of testosterone in the skeletal muscle, causing myostatin suppression [47,48].

In this review, Lee et al. (2015) [28] and Gonzalez-Ruiz et al. (2020) [35], reported the effect of EC on the ubiquitin-proteasome system (UPS) through inactivation of the autophagic genes FoxO, MAFbx, and MuRF1, resulting in the blockage of the catabolic pathways in the skeletal muscle. However, some studies did not note significant effects on the reduction of protein MAFbx MAFbx [31,32] on the increase of follistatin and Pax7 and on the decrease of atrophy markers myostatin [23], MURF, and Fbox40 [32].

Epicatechin showed positive effects on the myogenic differentiation processes of tissue markers Myf5, MyoD, and myogenin [22,24,25], in addition to increasing the activation of AKT/mTORC1 signaling [29,32] and stimulating the myocyte enhancer factor 2A (MEF2A) expression [22,24,25,31].

Concerning cell proliferation and differentiation, epicatechin was related to mitochondrial biosynthesis in the muscle fibers at the dosage of 1.0 mg/kg twice a day [28,29,33]. According to the existing studies, of the different catechins, epicatechin shows an action on the mitochondrial biogenesis of the skeletal muscle [4,6,12,49]. Such mitochondrial induction mechanism, stimulated by EC, has been proposed in the literature by Moreno-Ulloa et al. (2018) [50], and seems to be related to epicatechin bonding to receptor GPER (G protein-coupled estrogen receptor 1), expressed in several tissues of the human body, including in the metabolic homeostasis of the skeletal muscle [51].

The limitations of this systematic review can be related to the search methodology used and to the restriction of the eligibility criteria. As a comprehensive view, this qualitative analysis presented a convergence of the positive effects of epicatechin on muscle growth and differentiation modulators. As future perspectives, it would be important to clarify if epicatechin modifies the metabolic phenotype in the different types of muscle fibers, in addition, subsequent research based on the development of technologies that potentialize epicatechin bioavailability could contribute for the establishment of the therapeutic protocol to be adopted.

Another factor involved in skeletal muscle metabolism is associated with the density of the blood vessels, and has the function of supplying oxygen and metabolites through the capillaries [52]. Of the articles analyzed in this review, Hüttemann et al. (2013) [33], Lee et al. (2015) [28] and Lee et al. (2016) [29], noted a significantly higher increase of the capillaries compared to the control group, potentialized by physical exercise (LEE et al., 2015) [28]. However, Lee et al. (2016) [29] verified a significant decrease of angiogenic stimulator VEGF in the epicatechin group, followed by a slight decrease, although not significant, in the perimeter of the fiber compared with the control group, ; however, the antiangiogenic factor TPS-1 did not increase in the EC group.

Among the studies in humans which evaluated muscle function, positive effects was evidenced on the walk performance at a dosage of 75 mg of EC over six months [23] and on the increase of muscle strength at a dosage of 25 mg of EC for one week [25]. Mafi et al. (2019) [30] also obtained statistically significant results when epicatechin supplementation was combined with physical training.

However, the acute ingestion of EC, in humans, at 830 mg and 1245 mg dosages, did not show any benefit to muscle recovery 24, 48, and 72 hours after the exercise session [26]. Several studies have noted that the acute administration of cocoa polyphenols does not improve performance or post-exercise recovery [52,55].

The research in animals showed a difference in epicatechin dosages and administration time. In the functional analysis, there was a significant improvement in the walk performance at the dosage of 1 mg/kg of EC for 8 weeks [28] and in the physical activity using a dose of 0.25% for 37 weeks [34]. However, Ramirez-Sanchez et al. (2021) [32] noted a partial recovery of muscle strength when 1 mg/kg/day of EC was used for 30 days. In addition, Si et al. (2011) [27] obtained higher levels of AMPka phosphorylation, suggesting that 0.25% of EC every other day for 15 weeks improves skeletal muscle function. These results are similar to those of the study by Si et al. (2019) [34], at the dosage of 0.25% for 37 weeks, epicatechin was able to delay muscle degeneration and improved physical activity.

 doi:10.20944/preprints202306.2260.v1

Furthermore, Munguia et al. (2020) [31] used a higher dosage (2 mg of EC/kg) and obtained better results than the control, in the functional test conducted in mice, as well as epicatechin capacity was already noted in increasing the resistance to fatigue [17]. It has been reported in the literature that higher dosages of epicatechin (4 mg kg/day for 24 days) inhibit the skeletal muscle adaptations at rest or during the exercise, as a result of the blood flow impairment [56].

Oral dosages of 1-2 mg/kg of epicatechin do not cause adverse effects in animals [57]. However, dosages that represent more than 5% of the daily diet and consumed for more than 3 months can produce acute cytotoxicity in liver cells, oxidative damage to pancreas DNA [58-60] and an enlargement of the thyroid [58].

Concerning the EC protocols, an important variability was identified in the studies with humans, between 25 mg and 1245 mg [22-26,30]. However, the most frequent dosage used in animals corresponded to 1.0 mg/kg, although the experimental time is very divergent among the studies [25,28,29,32,33,35].

The limitations of this systematic review can be related to the search methodology used and to the restriction of the eligibility criteria.

As a comprehensive view, this qualitative analysis presented a convergence of the positive effects of epicatechin on muscle growth and differentiation modulators. As future perspectives, it would be important to clarify if epicatechin modifies the metabolic phenotype in the different types of muscle fibers, in addition, subsequent research based on the development of technologies that potentialize epicatechin bioavailability could contribute for the establishment of the therapeutic protocol to be adopted.

5. Conclusions

This systematic review provided important evidence concerning the effects of epicatechin on the regulation of the atrogenes (FOXO, MAFbx, and MuRF1) expression and the activation of the muscle regulatory factors (Myf5, MyoD and myogenin). The results evidenced the AKT/mTOR pathway signaling and mitochondrial biosynthesis induction, stimulated by epicatechin. Despite the discrepancies in the different parameters shown, the results are of great relevance due to the potential biological activities of such polyphenol. However, the scarce existing clinical studies are a barrier to validate epicatechin's therapeutic applicability in muscular atrophy-associated diseases.

6. Future Directions

Despite the biological properties of catechins, there are certain limitations for their clinical application, such as low bioavailability and degradation according to pH and temperature [61,62]. In order to improve the stability of catechins and produce a prolonged release, nanotechnology has been applied for therapeutic use [63,64].

Nanodeliveries are biocompatible systems with physicochemical properties that increase bioavailability, pharmacokinetics and pharmacodynamics. Some of the nanosystems used to encapsulate catechins include polymer nanoparticles, liposomes, lipids, proteins/peptides, gold nanocarriers and liquid crystal nanocomposites [65,66].

One of the main challenges of nanotechnology is to develop delivery systems based on nanocarriers that target specific cells or tissues [67]. Also, it is necessary to consider the advantages and drawbacks of each nanoparticle system in order to guarantee the effectiveness of the therapeutic effects [68] and reduce the toxic effects of polyphenol overdose, allowing greater safety of its clinical application [66.]

Author Contributions: Conceptualization, I.J.S.G.; methodology, I.J.S.G. and K.T.P.; investigation, I.J.S.G., J.V.T.C.S., C.T.D., E.L.G. and L.S.A.H.; data curation, I.J.S.G., A.C.A., M.G.R., and M.S.S.S.; writing—original draft preparation, I.J.S.G.; writing—review and editing, S.M.B., R.J.S.G. and P.C.S.B.; visualization, I.J.S.G., K.T.P., J.V.T.C.S., E.L.G., C.T.D., L.S.A.H., A.C.A., M.S.S.S., S.M.B., R.J.S.G., P.C.S.B., J.C.A., C.R.P.D. and A.L.S.; supervision, J.C.A., C.R.P.D. and A.L.S.

Funding: This systematic review was supported in part by The Brazilian National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq), Brazil [Nº. 140808/2021-3].

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable.

Acknowledgments: Authors would like to acknowledge Professor Elizabeth Schroder for providing valuable information related to the topic of this review.

Conflicts of Interest: The authors declare no conflict of interest. Furthermore, the funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Nichols: M.; Zhang, J.; Polster, B.M.; Elustondo, P.A.; Thirumaran, A.; Pavlov, E.V.; Robertson, G.S. Synergistic neuroprotection by epicatechin and quercetin: activation of convergent mitochondrial signaling pathways. *Neuroscience* **2015**, 12, 75-94.
2. Bernatoniene, J.; Kopustinskiene, D.M. The role of catechins in cellular responses to oxidative stress. *Molecules* **2018**, 23, 965.
3. Araújo, C.R.R.; de Melo Silva, T.; Dos Santos, M.G.; Otton,i M.H.F.; de Souza Fagundes, E.M.; de Sousa Fontoura, H.; de Melo Geba.; de Carvalho Alcântara, A.F. Anti-inflammatory and cytotoxic activities of the extracts, fractions, and chemical constituents isolated from luehea ochrophylla mart. *BMC Complement Altern Med* **2019**, 19, 284.
4. Li, P.; Liu, A.; Xiong, W.; Lin, H.; Xiao, W.; Huang, J.; Zhang, S.; Liu, Z. Catechins enhance skeletal muscle performance. *Crit Rev Food Sci Nutr* **2020**, 60, 515-528.
5. Lan, X.; Han, X.; Li, Q.; Wang, J. Epicatechin, a natural flavonoid compound, protects astrocytes against hemoglobin toxicity via nrf2 and ap-1 signaling pathways. *Mol Neurobiol* **2017**, 54, 7898-7907.
6. Daussin, F.N.; Heyman, E.; Burelle, Y. Effects of epicatechin on mitochondria. *Nutr Rev* **2021**, 79, 25-41.
7. Shay, J.; Elbaz, H.A.; Lee, I.; Zielske, S.P.; Malek, M.H.; Hüttemann, M. Molecular mechanisms and therapeutic effects of epicatechin and other polyphenols in cancer, inflammation, diabetes, and neurodegeneration. *Oxid Med Cell Longev* **2015**, 2015, 181260.
8. Farkhondeh, T.; Yazdi, H.S.; Samarghandian, S. The protective effects of green tea catechins in the management of neurodegenerative diseases: a review. *Curr Drug Discov Technol* **2019**, 16, 57-65.
9. Lee, S.J.; Leem, Y.E.; Go, G.Y.; Choi, Y.; Song, Y.J.; Kim, I.; Kim, D.Y.; Kim, Y.K.; Seo, D.W.; Kang, J.S.; Bae, G.U. Epicatechin elicits myod-dependent myoblast differentiation and myogenic conversion of fibroblasts. *PLoS One* **2017**, 12, e0175271.
10. Zbinden-Foncea, H.; Castro-Sepulveda, M.; Fuentes, J.; Speisky, H. Effect of epicatechin on skeletal muscle. *Curr Med Chem* **2022**, 29, 1110-1123.
11. Munguia, L.; Ortiz, M.; González, C.; Portilla, A.; Meaney, E.; Villarreal, F.; Najera, N.; Ceballos, G. Beneficial effects of flavonoids on skeletal muscle health: a systematic review and meta-analysis. *J Med Food* **2022**, 25, 465-486.
12. Barnett, C.F.; Moreno-Ulloa, A.; Shiva, S.; Ramirez-Sanchez, I.; Taub, P.R.; Su, Y.; Ceballos, G.; Dugar, S.; Schreiner, G.; Villarreal, F. Pharmacokinetic, partial pharmacodynamic and initial safety analysis of epicatechin in healthy volunteers. *Food Funct* **2015**, 6, 824-33.
13. McDonald, C.M.; Henricson, E.; Oskarsson, B. Epicatechin enhances mitochondrial biogenesis, increases dystrophin and utrophin, increases follistatin while decreasing myostatin, and improves skeletal muscle exercise response in adults with becker muscular dystrophy (BMD). *Neuromuscular Disorders* **2015**, 25, S314-S315.
14. Rodríguez-Ramiro, I; Martín, M.A.; Ramos, S; Bravo, L; Goya, L. Comparative effects of dietary flavanols on antioxidant defences and their response to oxidant-induced stress on caco2 cells. *Eur J Nutr* **2011**, 50, 313-322.
15. Meador, B.M.; Mirza, K.A.; Tian, M.; Skelding, M.B.; Reaves, L.A.; Edens, N.K.; Tisdale, M.J.; Pereira, S.L. The green tea polyphenol epigallocatechin-3-gallate (EGCG) attenuates skeletal muscle atrophy in a rat model of sarcopenia. *J FrailtyAging* **2015**, 4, 209-215.
16. Kim, A.R.; Kim, K.M.; Byun, M.R.; Hwang, J.H.; Park, J.I.; Oh, H.T.; Kim, H.K.; Jeong, M.G.; Hwang, E.S.; Hong, J.H. Catechins activate muscle stem cells by myf5 induction and stimulate muscle regeneration. *Biochem Biophys Res Commun* **2017**, 489, 142-148.

17. Nogueira, L.; Ramirez-Sanchez, I.; Perkins, G.A.; Murphy, A.; Taub, P.R.; Ceballos, G.; Villarreal, F.J.; Hogan, M.C.; Malek, M.H. Epicatechin enhances fatigue resistance and oxidative capacity in mouse muscle. *J Physiol* **2011**, 589, 4615-31.
18. Hong, K.B.; Lee, H.S.; Kim, D.H.; Moon, J.M.; Park, Y. Tannase-converted green tea extract with high epicatechin inhibits skeletal muscle mass in aged mice. *Evid Based Complement Alternat Med* **2020**, 2020, 4319398.
19. Eriksen, M.B.; Frandsen, T.F. The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: a systematic review. *J Med Libr Assoc* **2018**, 106, 420-431.
20. Page, M.J.; Moher, D. Evaluations of the uptake and impact of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and extensions: a scoping review. *Syst Rev* **2017**, 6, 263.
21. Page, M.J.; Moher, D.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; Chou, R.; Glanville, J.; Grimshaw, J.M.; Hróbjartsson, A.; Lalu, M.M.; Li, T.; Loder, E.W.; Mayo-Wilson, E.; McDonald, S.; McGuinness, L.A.; Stewart, L.A.; Thomas, J.; Tricco, A.C.; Welch, V.A.; Whiting, P.; McKenzie, J.E. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ* **2021**, 372, n160.
22. Taub, P.R.; Ramirez-Sanchez, I.; Ciaraldi, T.P.; Gonzalez-Basurto, S.; Coral-Vazquez, R.; Perkins, G.; Hogan, M.; Maisel, A.S.; Henry, R.R.; Ceballos, G.; Villarreal, F. Perturbations in skeletal muscle sarcomere structure in patients with heart failure and type 2 diabetes: restorative effects of epicatechin-rich cocoa. *Clin Sci (Lond)* **2013**, 125, 383-9.
23. McDermott, M.M.; Criqui, M.H.; Domanchuk, K.; Ferrucci, L.; Guralnik, J.M.; Kibbe, M.R.; Kosmac, K. Kramer, C.M.; Leeuwenburgh, C.; Li, L.; Lloyd-Jones, D.; Peterson, C.A.; Polonsky, T.S.; Stein, J.H.; Sufit, R.; Van Horn, L.; Villarreal, F.; Zhang, D.; Zhao, L.; Tian, L. Cocoa to improve walking performance in older people with peripheral artery disease: the cocoa-pad pilot randomized clinical trial. *Circ Res* **2020**, 126, 589-599.
24. McDonald, C.M.; Ramirez-Sanchez, I.; Oskarsson, B.; Joyce, N.; Aguilar, C.; Nicorici, A.; Dayan, J.; Goude, E.; Abresch, R.T.; Villarreal, F.; Ceballos, G.; Perkins, G.; Dugar, S.; Schreiner, G.; Henricson, E.K. Epicatechin induces mitochondrial biogenesis and markers of muscle regeneration in adults with Becker muscular dystrophy. *Muscle Nerve* **2021**, 63, 239-249.
25. Gutierrez-Salmean, G.; Ciaraldi, T.P.; Nogueira, L.; Barboza, J.; Taub, P.R.; Hogan, M.C.; Henry, R.R.; Meaney, E.; Villarreal, F.; Ceballos, G.; Ramirez-Sanchez, I. Effects of epicatechin on molecular modulators of skeletal muscle growth and differentiation. *J Nutr Biochem* **2014**, 25, 91-94.
26. Corr, L.D.; Field, A.; Pufal, D.; Killey, J.; Clifford, T.; Harper, L.D.; Naughton, R. J. Acute consumption of varied doses of cocoa flavanols does not influence exercise-induced muscle damage. *Int J Sport Nutr Exerc Metab* **2020**, 30, 338-344.
27. Si, H.; Fu, Z.; Babu, P.V.; Zhen, W.; Leroith, T.; Meaney, M.P.; Voelker, K.A.; Jia, Z.; Grange, R.W.; Liu, D. Dietary epicatechin promotes survival of obese diabetic mice and drosophila melanogaster. *J Nutr* **2011**, 141, 1095-100.
28. Lee, I.; Hüttemann, M.; Kruger, A.; Bollig-Fischer, A.; Malek, M.H. Epicatechin combined with 8 weeks of treadmill exercise is associated with increased angiogenic and mitochondrial signaling in mice. *Front Pharmacol* **2015**, 13, 43.
29. Lee, I.; Hüttemann, M.; Malek, M.H. Epicatechin attenuates degradation of mouse oxidative muscle following hindlimb suspension. *J Strength Cond Res* **2016**, 30, 1-10.
30. Mafi, F.; Biglari, S.; Ghardashi Afousi, A.; Gaeini, A.A. Improvement in skeletal muscle strength and plasma levels of follistatin and myostatin induced by an 8-week resistance training and epicatechin supplementation in sarcopenic older adults. *J Aging Phys Act* **2019**, 27, 384-391.
31. Munguia, L.; Ramirez-Sanchez, I.; Meaney, E.; Villarreal, F.; Ceballos, G.; Najera, N. Flavonoids from dark chocolate and epicatechin ameliorate high-fat diet-induced decreases in mobility and muscle damage in aging mice. *Food Biosci* **2020**, 37, 100710.
32. Ramirez-Sanchez, I.; Navarrete-Yañez, V.; Garate-Carrillo, A.; Lara-Hernandez, M.; Espinosa-Raya, J.; Moreno-Ulloa, A.; Gomez-Diaz, B.; Cedeño-Garcidueñas, A.L.; Ceballos, G.; Villarreal, F. Restorative potential of epicatechin in a rat model of gulf war illness muscle atrophy and fatigue. *Sci Rep* **2021**, 11, 21861.
33. Hüttemann, M.; Lee, I.; Perkins, G.A.; Britton, S.L.; Koch, L.G.; Malek, M.H. Epicatechin is associated with increased angiogenic and mitochondrial signalling in the hindlimb of rats selectively bred for innate low running capacity. *Clin Sci (Lond)* **2013**, 124, 663-674.
34. Si, H.; Wang, X.; Zhang, L, Parnell, L.D.; Admed, B.; LeRoith, T.; Ansah, T.A.; Zhang, L.; Li, J.; Ordovás, J.M.; Si, H.; Liu, D.; Lai, C.Q. Dietary epicatechin improves survival and delays skeletal muscle degeneration in aged mice. *FASEB J* **2019**, 33, 965-977.
35. Gonzalez-Ruiz, C.; Cordero-Anguiano, P.; Morales-Guadarrama, A.; Mondragón-Lozano, R.; Sánchez-Torres, S.; Salgado-Ceballos, H.; Villarreal, F.; Meaney, E.; Ceballos, G.; Najera, N. Epicatechin reduces

muscle waste after complete spinal cord transection in a murine model: role of ubiquitin-proteasome system. *Mol Biol Rep* **2020**, 47, 8975-8985.

36. Tsekoura, M.; Kastrinis, A.; Katsoulaki, M.; Billis, E.; Gliatis, J. Sarcopenia and its impact on quality of life. *Adv Exp Med Biol* **2017**, 987, 213-218.
37. Beaudart, C; Biver, E; Bruyère, O; Cooper, C; Al-Daghri, N; Reginster, J.Y.; Rizzoli, R. Quality of life assessment in musculo-skeletal health. *Aging Clin Exp Res* **2018**, 30, 413-418.
38. Cremonini, E.; Fraga, C.G.; Oteiza, P.I. Epicatechin in the control of glucose homeostasis: involvement of redox-regulated mechanisms. *Free Radic Biol Med* **2019**, 130, 478-488.
39. Bonaldo, P; Sandri, M. Cellular and molecular mechanisms of muscle atrophy. *Dis Model Mech* **2013**, 6, 25-39.
40. Rodriguez, J.; Vernus, B.; Chelh, I.; Cassar-Malek, I.; Gabillard, J.C.; Hadj Sassi, A.; Seiliez, I.; Picard, B.; Bonnieu, A. Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. *Cell Mol Life Sci* **2014**, 71, 4361-71.
41. Zheng, L.F.; Chen, P.J.; Xiao, W.H. Signaling pathways controlling skeletal muscle mass. *Sheng Li Xue Bao* **2019**, 71, 671-679.
42. Yoshida, T.; Delafontaine, P. Mechanisms of IGF-1-mediated regulation of skeletal muscle hypertrophy and atrophy. *Cells* **2020**, 9, 1970.
43. Sharma, M.; McFarlane, C.; Kambadur, R.; Kukreti, H.; Bonala, S.; Srinivasan, S. Myostatin: expanding horizons. *IUBMB Life* **2015**, 67, 589-600.
44. Gazdanova, A.A.; Kukes, V.G.; Parfenova, O.K.; Sidorov, N.G.; Perkov, A.V.; Solovieva, S.A.; Ryazantceva, O.V.; Lenkova, N.I. Myostatin - A modern understanding of the physiological role and significance in the development of age-associated diseases. *Adv Gerontol* **2021**, 34, 701-706.
45. Chen, M.M.; Zhao, Y.P.; Zhao, Y.; Deng, S.L.; Yu, K. Regulation of myostatin on the growth and development of skeletal muscle. *Front Cell Dev Biol* **2021**, 9, 785712.
46. Lee, S.J. Targeting the myostatin signaling pathway to treat muscle loss and metabolic dysfunction. *J Clin Invest* **2021**, 131, e148372.
47. Yu, P.L.; Pu, H.F.; Chen, S.Y.; Wang, S.W.; Wang, P.S. Effects of catechin, epicatechin and epigallocatechin gallate on testosterone production in rat leydig cells. *J Cell Biochem* **2010**, 110, 333-42.
48. Wu, Y.; Collier, L.; Pan, J.; Qin, W.; Bauman, W.A.; Cardozo, C.P. Testosterone reduced methylprednisolone-induced muscle atrophy in spinal cord-injured rats. *Spinal Cord* **2012**, 50, 57-62.
49. Chang, W.T.; Chen, C.S.; Cheng, M.C.; Wu, M.F.; Cheng, F.T.; Hsu, C.L. Effects of resveratrol, epigallocatechin gallate, and epicatechin on mitochondrial functions in c2c12 myotubes. *J. Funct. Foods* **2017**, 35, 507-512.
50. Moreno-Ulloa, A.; Miranda-Cervantes, A.; Licea-Navarro, A.; Mansour, C.; Beltrán-Partida, E.; Donis-Maturano, L.; Delgado De la Herrán, H.C.; Villarreal, F.; Álvarez-Delgado, C. Epicatechin stimulates mitochondrial biogenesis and cell growth in C2C12 myotubes via the G-protein coupled estrogen receptor. *Eur J Pharmacol* **2018**, 822, 95-107.
51. Sharma, G.; Prossnitz, E.R. G-protein-coupled estrogen receptor (GPER) and sex-specific metabolic homeostasis. *Adv Exp Med Biol* **2017**, 1043, 427-453.
52. Davison, G.; Callister, R.; Williamson, G.; Cooper, K.A.; Gleeson, M. The effect of acute pre-exercise dark chocolate consumption on plasma antioxidant status, oxidative stress and immunoendocrine responses to prolonged exercise. *Eur J Nutr* **2012**, 51, 69-79.
53. Peschek, K.; Pritchett, R.; Bergman, E.; Pritchett, K. The effects of acute post exercise consumption of two cocoa-based beverages with varying flavanol content on indices of muscle recovery following downhill treadmill running. *Nutrients* **2013**, 6, 50-62.
54. Stellingwerff, T.; Godin, J.P.; Chou, C.J.; Grathwohl, D.; Ross, A.B.; Cooper, K.A.; Williamson, G.; Actis-Goretta, L. The effect of acute dark chocolate consumption on carbohydrate metabolism and performance during rest and exercise. *Appl Physiol Nutr* Metab **2014**, 39, 173-182.
55. Decroix, L.; Tonoli, C.; Soares, D.D.; Descat, A.; Drittij-Reijnders, M.J.; Weseler, A.R.; Bast, A.; Stahl, W.; Heyman, E.; Meeusen, R. Acute cocoa flavanols intake has minimal effects on exercise-induced oxidative stress and nitric oxide production in healthy cyclists: a randomized controlled trial. *J Int Soc Sports Nutr* **2017**, 14, 28.
56. Copp, S.W.; Inagaki, T.; White, M.J.; Hirai, D.M.; Ferguson, S.K.; Holdsworth, C.T.; Sims, G.E.; Poole, D.C.; Musch, T.I. Epicatechin administration and exercising skeletal muscle vascular control and microvascular oxygenation in healthy rats. *Am J Physiol Heart Circ* Physiol **2013**, 304, H206-14.
57. Schroeter, H.; Heiss, C.; Balzer, J.; Kleinbongard, P.; Keen, C.L.; Hollenberg, N.K.; Sies, H.; Kwik-Uribe, C.; Schmitz, H.H.; Kelm, M. (-) - A epicatequina medeia os efeitos benéficos do cacau rico em flavanol na função vascular em humanos . *Proc Natl Acad Sci* **2006**, 103, 1024-1029.

58. Sakamoto, Y.; Mikuriya, H.; Tayama, K.; Takahashi, H.; Nagasawa, A.; Yano, N.; Yuzawa, K.; Ogata, A.; Aoki, N. Goitrogenic effects of green tea extract catechins by dietary administration in rats. *Arch Toxicol* **2001**, 75, 591-596.
59. Yun, S.Y.; Kim, S.P.; Song, D.K. Effects of (-)-epigallocatechin-3-gallate on pancreatic beta-cell damage in streptozotocin-induced diabetic rats. *Eur J Pharmacol* **2006**, 541, 115-121.
60. Chacko, S.M.; Thambi, P.T.; Kuttan, R.; Nishigaki, I. Beneficial effects of green tea: a literature review. *Chin Med* **2010**, 5, 13.
61. Massounga, Bora, A.F.; Ma, S.; Li, X.; Liu, L. Application of microencapsulation for the safe delivery of green tea polyphenols in food systems: review and recent advances. *Food Res Int*. **2018,** 105, 241-249.
62. Qi, C.; Liu, G.; Ping, Y.; Yang, K.; Tan, Q, Zhang, Y.; Chen, G.; Huang, X.; Xu, D.A. Comprehensive review of nano-delivery system for tea polyphenols: construction, applications, and challenges. *Food Chem X* **2023**, 17, 100571.
63. Granja, A.; Pinheiro, M.; Reis, S. Epigallocatechin gallate nanodelivery systems for cancer therapy. *Nutrients* **2016**, 8, 307.
64. Rashidinejad, A.; Birch, E.J.; Sun-Waterhouse, D.; Everett, D.W. Effect of liposomal encapsulation on the recovery and antioxidant properties of green tea catechins incorporated into a hard low-fat cheese following in vitro simulated gastrointestinal digestion. *Food and Bioproducts Processing* **2016**, 100, 238–245.
65. Granja, A.; Frias, I.; Neves, A.R.; Pinheiro, M.; Reis, S. Therapeutic potential of epigallocatechin gallate nanodelivery systems. *Biomed Res Int* **2017**, 5813793.
66. Natarajan, S.B.; Chandran, S.P.; Vinukonda, A.; Dharmalingam, S.R. Green tea catechin loaded nanodelivery systems for the treatment of pandemic diseases. *Asian J Pharm Clin Res* **2019**, 12, 1-7.
67. Lushchak, O.; Strilbytska, O.; Koliada, A.; Zayachkivska, A.; Burdyliuk, N.; Yurkevych, I.; Storey, K.B.; Vaiserman, A. Nanodelivery of phytobioactive compounds for treating aging associated disorders. *Geroscience*. **2020**, 42, 117-139.
68. Puligundla, P.; Mok, C.; Ko, S.; Liang, J.; Recharla, N. Nano-technological approaches to enhance the bioavailability and therapeutic efficacy of green tea polyphenols. *Journal of Functional Foods*, **2017**, 34, 139-151.