REVIEW

Effects of Citrulline alone or combined with exercise on muscle mass, muscle strength, and physical performance among older adults: a systematic review

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Purpose of review
To Establish the potential of Citrulline supplementation (CIT) combined or not with exercise on muscle function and physical performance via a systematic review of randomized controlled trials (RCTs) in human aged 50 years and older.

Recent findings
Based on Preferred Reporting Items for Systematic Reviews and Meta-analysis guideline, 103 references have been identified. Among this number, only six RCTs (250 participants) matched the inclusion criteria and were included in the present systematic review. Among the included studies, five of six reported beneficial effects of CIT on muscle mass. Then, four of six studies reported CIT effects on muscle strength but also that CIT when combined to exercise results in further improvements in upper muscle strength. Finally, three of six studies reported beneficial CIT effect on physical performance and suggested that CIT with exercise displayed greater improvements in walking speed than exercise alone.

Summary
CIT supplementation seems to be able to improve muscular and physical factors in frail elderly people (malnourished, hypertensive, obese, dynapenic-obese) compared to placebo. More importantly, CIT combined to exercise is more efficient than exercise or CIT alone. However, because of the small number (six) and heterogeneity (dose, duration, population) of the studies realized in older adults, further studies are needed to confirm its promising potential.

Keywords
aging, citrulline, functional capacity, muscle function, nutrition, physical activity

INTRODUCTION

Normal aging is known to derive from an accumulation of detrimental changes like the loss of muscle mass, strength, and quality, leading to a poor physical function [1]. Muscle atrophy lead to increased direct costs, such as healthcare expenditure [2]. Thus, the implementation of preventive and therapeutic interventions has become a challenge because of the growing number of older individuals. It has been suggested to prevent or manage the loss of physical function among older adults using non-pharmalogical strategy, such as nutrition. Recently, citrulline (CIT) raised some attentions because its supplementation leads to positive effects on muscle function [3] and fat metabolism [4] in animal or cell models. CIT is a nonessential alpha-amino acid, an intermediate of the urea cycle produced in the liver and from arginine during nitric oxide production [5]. In contrast with arginine or other nutrients such as omega-3 or proteins, CIT escapes splanchnic

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KEY POINTS

- Six RCTs (250 participants) were included in this systematic review assessing the effects of CIT alone or combined with exercise on muscle mass, muscle strength, and physical performance of human aged 50 years or older.
- Exercise intervention has a beneficial impact on muscle mass, muscle strength, or physical performance in individuals aged 50 years and older.
- The additional effect of CIT supplementation has only been reported on a limited number of studies, specifically in less healthy population such as dynapenic older adults.
- Studies assessing the impact of CIT alone or combined with exercise are still lacking in more frail subjects, for example in hospital or in nursing homes.
- The promising avenue of CIT on muscle mass, muscle strength, or physical performance needs more attention to be able to determine subjects in whom it could be more beneficial.

extraction [5]. Nearly 75% of CIT produced by the gut is taken up by the kidney and the intestinal synthesis rate of CIT is a key regulatory element of renal arginine synthesis [6]. An oral CIT supplementation increases muscle protein synthesis, skeletal muscle mass, muscle fiber size, lean mass, muscle strength, and mobility [7], but also decreases adipose tissue mass, particularly visceral depot [8] in old malnourished rats. However, only few investigations have been realized using CIT supplementation on muscle function and mobility in older adults [9▪,10,11▪,12▪]. In addition, nutrition in combination with exercise is considered as optimal strategy for maintaining muscle function during aging process. In this sense, some studies investigated beneficial additive effect of CIT supplementation when combined to exercise training on muscle function in older adults [10,11▪]. However, because of the heterogeneity of the randomized controlled trials (RCTs) performed, evaluating these aspects by conducting a systematic review is important to help clinicians or researchers on this topic.

METHODS

The protocol of this systematic review has been published in the PROSPERO database under registration ID 145681. The Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement has been followed throughout the whole steps of this systematic review. Our research project can be summarized by the following PICOS strategy: Population or disease: men and women aged 50 years or older; Intervention: citrulline alone or combined with exercise; Comparator: nothing or placebo; Outcome: muscle mass, muscle strength and physical performance; Study design: RCTs.

Literature search

A comprehensive literature review was performed, searching the Medline (via Ovid), Cochrane central register for controlled trials (CENTRAL via Ovid) and Scopus databases. Preregistered search strategies (on PROSPERO) adapted to the vocabulary for each database were used (see Appendix 1), combining key words and Medical Subject Heading (MeSH) terms concerning the intervention of interest, the outcomes and the type of study. Additional studies were identified through a manual search of bibliographic references from selected articles. We searched the databases from inception to 20 July 2019, limiting the searches to English and French language publications and to studies conducted in humans.

Study eligibility

RCTs assessing the effects of CIT alone, or combined with exercise, on muscle mass and muscle strength, and physical performance among older adults were eligible for inclusion in this systematic review. There were no particular considerations concerning ethnicity, gender, or country of origin of the trial participants. The detailed eligibility criteria are presented in Table 1.

Study selection

After the duplicates have been removed, the list of articles provided by the search strategy was first reviewed by two investigators (F.B. and M.A.L.) independently by reading their titles and abstracts, to exclude only obvious irrelevant studies according to the predefined eligibility criteria described above.

Table 1. Eligibility criteria AQ11

Inclusion criteria
Topic: the effects of Citrulline alone or combined with exercise on muscle mass and muscle function among older adult
Participants: older adults (human >50 years)
Design: Randomized controlled trials
Outcome: Muscle mass and/or muscle function
Date: No limit
Exclusion criteria
Langage: Non-English or French language publications

Any discrepancies between both investigators were resolved through discussion and consensus. Once selected by both investigators based on title and abstract review, the references were then screened for final eligibility by the two same investigators by reading the full-text paper. Once again, any discrepancies were resolved by discussion and consensus. The articles that did not meet the selection criteria were excluded, and the reason for the exclusion was reported by each reviewer.

Data extraction

A pilot-tested standard data extraction form was used for data extraction by two independent reviewers (F.B. and M.A.L.). For each study, the following data were extracted:

(1) Characteristics of the manuscript: authors, title, journal, year of publication, funding, conflicts of interest
(2) Objective of the study
(3) Characteristics/design of the study: study design, country, length of the intervention
(4) Characteristics of the population (intervention group): sample size, gender distribution, ethnicity, age range, description of population
(5) Characteristics of the population (control group): sample size, gender distribution, ethnicity, age range, description of population
(6) Efficacy results (outcome 1 = muscle mass): measurement, tool, results INT group, results CONT group
(7) Efficacy results (outcome 2 = muscle strength): measurement, tool, results INT group, results CONT group
(8) Efficacy results (outcome 3 = physical performance): measurement, tool, results INT group, results CONT group
(9) Main conclusion of the study

Disagreements about data extraction have been solved by discussion and consensus between reviewers.

Quality assessment

Methodological quality of each included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. The tool consists of four key domains namely: patient selection, index test, reference standard, and flow and timing. For each domain, the risk of bias can be graded as 'low risk' of bias, 'unclear risk' of bias or 'high risk' of bias based on the appraisal of the reviewer. Evaluations were done in duplicate (F.B. and M.A.L.). The two investigators resolved disagreement by consensus.

Data synthesis

After a description of the included studies, results were summed descriptively, by outcomes, based on the information provided by each of the studies. Because of the huge heterogeneity observed in the protocols of exercise and citrulline supplementation, but also in the outcomes, no meta-analysis was undertaken.

RESULTS

Studies characteristics

After removing duplicates ($n = 46$), 103 references were included in the database for screening. After excluding unappropriated references based on title/abstract screening ($n = 97$), six references were estimated as potentially relevant by both reviewers and fall in the second phase of screening based on full-text review. Finally, the six studies [9▪,10,11▪,12▪,13,14▪] matched the inclusion criteria and were included in the present systematic review (Fig. 1).

The six studies included assessed, through an RCT design, the effects of CIT alone [9▪,12▪] or combined with exercises [10,11▪,13,14▪] on muscle mass, strength, or physical performance among older adults.

First the protocol and the population varied throughout the six selected studies (Table 2). About the supplementation, the amount of citrulline administered depends on the studies: 5 g/day [13], 6 g/day [9▪,10], or 10 g /day [11▪,12▪,14▪]. In addition, CIT supplementation is combined to exercise protocols in four studies but diversified: high intensity interval training (HIIT) [11▪,14▪], whole body vibration training (WBVT) [10], or step reduction [13]. The duration of the intervention also varied from 1 week [9▪] to 12 weeks [11▪,15]. When regarding the population, the number of participants ranged from 24 (11 in the intervention group and 13 in the control group) [13] to 73 (40 in the intervention group and 33 in the control group) [14▪] with a total of 250 participants once all studies combined (108 men and 142 women). Then, the population was only women in the study of Figueroa *et al.* [10] and only men in the study of Devries *et al.* [13]. The other four studies included both men and women. Finally, two studies included healthy elderly [9▪,13] whereas two studies focused on obese [10,14▪] or dynapenic obese elderly [11▪] and one study focused on malnourished elderly [12▪].

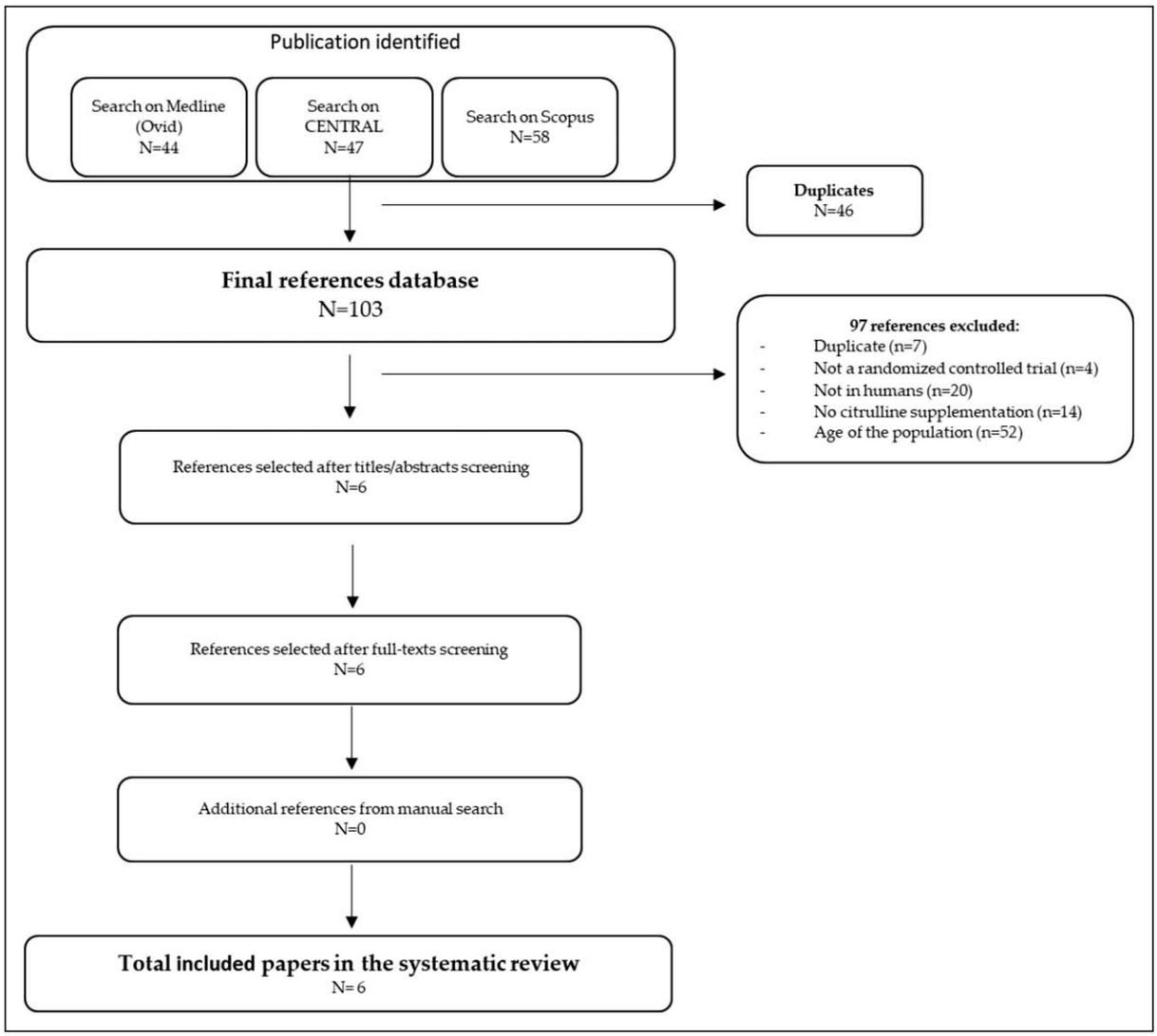

FIGURE 1. Flow chart of the search strategy.

Muscle mass

Among the six selected RCTs, five studies evaluated the effects of citrulline supplementation on muscle mass [10,11▪,12▪,13,14▪]. In all these five studies, body composition and more specifically muscle mass was evaluated by Dual-energy X-ray absorptiometry (DXA) but the reported parameters vary according to the studies. Bouillane *et al.* [12▪] shows a significant increase in LM ($P = 0.016$) and ASMM ($P = 0.018$) in the CIT group compared with the controlled group in older malnourished women. In the study of Buckinx *et al.* [11▪], HIIT displayed an increase in total lean mass ($P \leq 0.05$) but no supplementation effect (CIT vs. PLA) was observed for body composition in dynapenic-obese older adults. Nevertheless, Buckinx *et al.* [14▪] show that, in older obese adults ingested a low amount of protein, CIT supplementation increased significantly more leg lean mass compared to PLA supplementation ($+3.1 \pm 5.2\%$ vs. $-0.1 \pm 3.6\%$ $P = 0.02$). Figueroa *et al.* [10] shows in postmenopausal women that CIT supplementation combined to vibration training (WBVT) increased more leg LM ($6.0 \pm 2.2\%$, $P = 0.05$) and ALMI ($4.8 \pm 1.8\%$, $P = 0.05$) than WBCT or CIT alone which did not affect leg LM and ALMI. Finally, Devries *et al.* [13] highlights that, in healthy older men, muscle protein synthesis was similar after ingestion of either whey isolate, micellar-whey, or micellar-whey+CIT. In addition, even if the data are not reported, Devries *et al.* [13] concluded also low-load RT, but not supplementation with CIT, can attenuate the deleterious effects of SR in total or leg FFM. Overall, studies suggested a beneficial effect of CIT alone or combined to exercise on muscle mass compared to exercise alone or combined to placebo supplementation in women or in individuals presenting low quality of diet. AQ6

Table 2. Studies' characteristics

First author, year, country	Population				Intervention	Control
	General description	Sample size	Gender distribution	Age range (years)		
Devries, 2015, Canada	Elderly men	TOT:30	Men: 30 (100%)	70 ± 1	Step reduction (<1500 steps: day) + 5 g/day of CIT	Step reduction + 5 g glycine/ day
		INT: 10	Men: 10 (100%)			
		CONT: 20	Men: 20 (100%)			
Buckinx 2018, Canada	Dynapenic-obese older adults	TOT: 56	Men: 28 (50%) / women: 28 (50%)		HIIT (3∗30 min/week) + CIT (10 g/day)	HIIT (3∗30 min /week) + PLA (maltodextrin)
		INT: 26	Men: 13 (50%) /women: 13 (50%)	65.2 ± 4.2		
		CONT: 30	Men: 15 (50%) / women: 15 (50%)	68.1 ± 4.1		
Ashley, 2018, US	Older adults	TOT: 26 (15 young adults and 11 older adults)	Young:	Young:	CIT (6 g/day) for 7 days, followed by a 2-week washout period	CIT (6 g/day)
			Men: 7 (46.7%) / women: 8 (53.3%)	22 ± 2		
			Older:			
			Men: 4 (36.4%) / women: 7 (63.6%)	74 ± 7		
Bouillanne, 2019, France	Malnourished older patients in inpatient rehabilitation units	TOT: 24	Men: 6 (25%) /women: 18 (75%)		CIT (10 g/day)	Equimolar mixture of 6 NEAAs (alanine 1,91 g, aspartic acid 2.85 g, glycine 1.61 g, histidine 3.32 g, proline 2.46 g, serrine 2.25 g)
		INT: 11	Men: 3 (27.3%) / women: 8 (72.7%)	89 (74–97)		every day
		CONT: 13	Men: 3 (23.1%) / women: 10 (76.9%)	88 (77–92)		
Buckinx, 2019, Canada	Obese older adults	TOT: 73			HIIT (3∗30 min/week) +CIT (10 g/day)	HIIT (3∗30 min/week) + PLA (maltodextrin)
		INT 1: 21	Men: 8 (38.1%) / women: 13 (61.9%)	66.5 ± 5.2		
		INT 2: 19	Men: 7 (36.8%) / women: 12 (63.2%)	67.5 ± 4.5		
		CONT 1: 19	Men: 9 (47.3%) / women: 10 (52.6%)	68.2 ± 3.5		
		CONT 2: 14	Men: 9 (64.3%) / women: 5 (35.7%)	67.8 ± 3.9		
Figueroa, 2019, US	Obese postmenopausal women	TOT: 41	Women: 41 (100%)	58 ± 1	INT 1: CIT (6 g/day)	WBVT (3 times/week) + PLA (maltodextrin)
		INT 1: 14	Women: 14 (100%)		INT 2: WBVT (3 times/week) + CIT (6 g/day)	
		INT 2: 13	Women: 13 (100%)			
		CONT: 14	Women: 14 (100%)			

CIT, citrulline; CONT, control group; HIIT, high intensity interval training; INT, intervention group; TOT, total population; WBVT, whole body vibration training.

Muscle strength

Out of the six RCTs included in this systematic review, four studied the effects of citrulline supplementation on muscle strength [10,11▪,13,14▪]. Two of these studies were conducted by Buckinx *et al.* [11▪,14▪]. Both the muscular strength of the upper limbs (i.e. using handheld dynamometer) and lower limbs (i.e. using a strain gauge system attached to a chair) were assessed and muscle power was also measured (i.e. using the Nottingham Leg Extensor Power rig). The intervention was identical in both studies since it was HIIT combined with citrulline (10 g/day). However, the studied population where somewhat different: dynapenic-obese older adults [11▪] on the first and obese older adults [14▪] on the other. Globally, the results were homogeneous and confirmed that HIIT is an efficient intervention to increase upper and lower limbs muscle strength in obese older adults [14▪] and in dynapenic obese older adults [11▪]. More interestingly, Buckinx *et al.* (2018) highlighted that adding CIT supplementation to HIIT results in further improvements in upper muscle strength ($+9.3 \pm 10.8\%$, $P = 0.05$) and tends to further improve lower limb muscle strength ($+12.3 \pm 21.5\%$; $P = 0.07$). In addition, only one of the two studies reported the muscular quality results (i.e. muscle strength/muscle mass) for upper and lower limb muscle [11▪]. The authors concluded that HIIT was effective in improving muscle quality but that the combination of HIIT + CIT was not more beneficial on this outcome. The third study conducted by Figueroa *et al.* in obese postmenopausal women [10] shows that leg muscle strength increased ($P < 0.001$) after WBVT alone or combined with CIT but not after L-citrulline alone. The last study conducted by Devries *et al.* [13] in healthy older men concluded (data not shown) that isometric maximal voluntary contraction of the knee extensors and leg press 1RM were not influenced by citrulline supplementation over the intervention. Altogether, the studies highlight the beneficial effects of CIT alone or combined with exercise on muscle strength in older women or in obese older adults but not in healthy men.

Physical performance

The effects of citrulline supplementation on physical performance, through different measurements, were evaluated in 3/6 studies [9▪,11▪,15]. The heterogeneity of these studies (different design and various population) makes comparisons difficult. One cross-over study concluded that seven days of L-citrulline supplementation does not improve the oxygen cost of moderate intensity walking among healthy older adults [9▪]. Nevertheless, L-citrulline supplementation improved more oxygen uptake kinetics during walking (Mean response time significantly decreased by 10%, $P = 0.04$ and the O2 deficit was reduced by 17%, $P < 0.01$) than placebo in men but not women. In the other 2 studies, the parameters studied concerned walking speed (i.e. Timed Up and Go test), mobility and aerobic capacities (i.e. 6 min walking test), balance (i.e. unipodal balance test), lower body function (i.e. chair stand test), weight shifting ability in the forward and upward directions (alternate step test) [11▪,15]. In 2018, Buckinx *et al.* observed, in dynapenic obese older adults, an improvement for all of these variables ($p \leq 0.05$) following the intervention period in HIIT+CIT group as well as in HIIT + PLA group. However, HIIT+CIT displayed greater improvements in fast-paced 'Timed Up and Go' test than HIIT + PLA (-16.1 ± 9.0 s vs. -11.8 ± 7.8 $P = 0.04$). One year later, Buckinx *et al.* confirmed that HIIT independently of the supplementation improved functional capacities ($P < 0.001$ for all parameters) in a population of obese older adults. Nevertheless, no difference between groups (i.e. HIIT+CIT vs. HIIT+PLA) was observed in obese older adults. Moreover, this study showed that the initial amount of protein intake did not influence the evolution of physical performance. All-embracing, studies show the beneficial effects of citrulline alone or combined with exercise in healthy older men, in dynapenic-obese older adults but not in obese older adults.

Quality assessment

The overall quality of studies was rather high as graphically displayed in Fig. 2.

For the domain 'patients selection', four of six studies (66.6%) were scored at high risk of biais, mainly because subjects were volunteers and recruited by social communication or advertisement in local newspapers [10,11▪,13,15]; 1 (16.6%) was scored with an unclear risk of biais, because the recruitment method was not specified in the article [9▪], and 1 (16.6%) with a low risk of biais.

For 'Index test' and 'Reference standard', no study was scored at high risk of bias, 1 (16.6%) was scored with an unclear risk of biais because of the study design (i.e. crossover study), and 4 (83.3%) with a low risk of bias. For 'Flow and timing', all the studies (100%) were scored with a low risk of biais. Finally, the applicability was judged with a low risk of biais in five of six studied (83.3%) for each domain.

DISCUSSION

Based on previous promising results from animal studies, we aimed to establish the potential of CIT

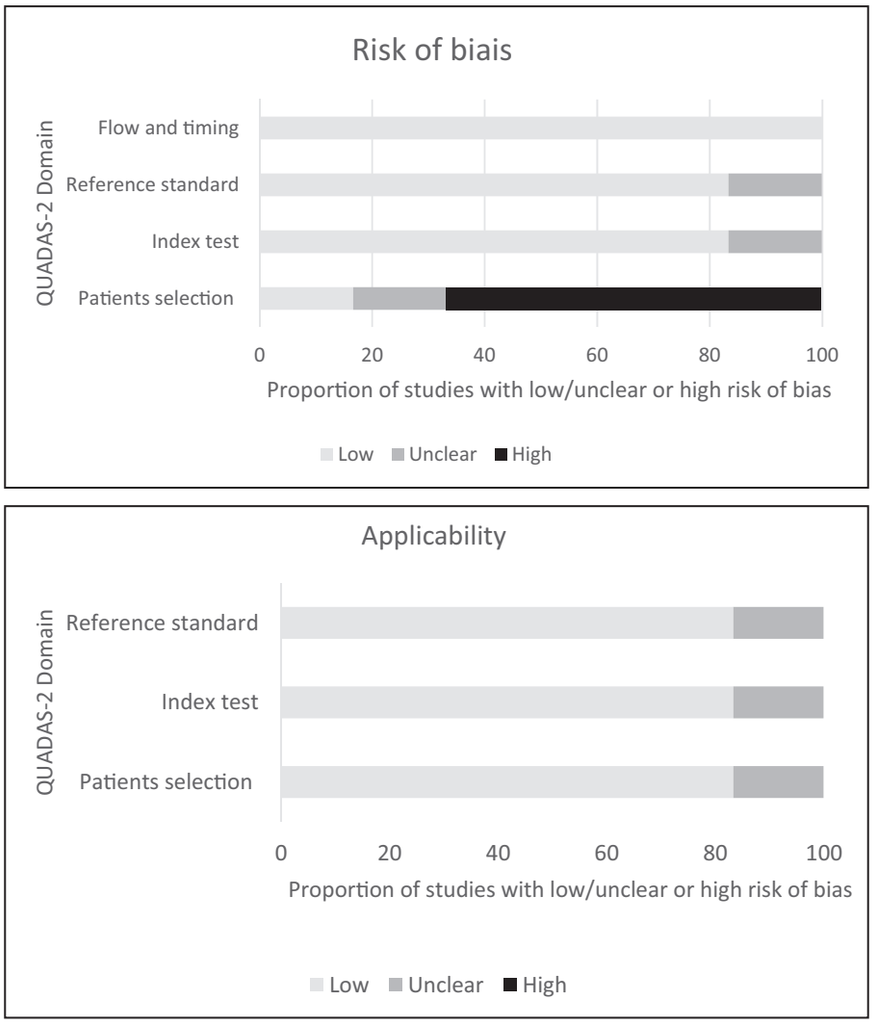

FIGURE 2. Quality assessment by QUADAS-2.

supplementation combined or not with exercise on muscle function and physical performance via a systematic review of RCTs in individuals aged 50 years and older.

Firstly, the studies conducted in women or in individuals with low quality of diet suggested a beneficial effect of CIT alone or combined to exercise on muscle mass compared to exercise or placebo supplementation [10,11▪,12▪,13,14▪]. The physiological explanation is that the upregulation of peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC-1α) in the skeletal muscle by CIT is associated with increased exercise performance and muscles mass [16]. Second, among the six RCTs included in the systematic review, four RCTs assessed the impact of intervention (i.e. CIT alone or combined with exercise) on muscle strength in older adults [10,11▪,13,14▪]. In the four studies, upper and lower limbs muscle strength increased with the exercise intervention [10,11▪,13,14▪]. Nevertheless, only one of the four studies suggested that adding CIT supplementation to exercise results in further improvements in muscle strength [11▪]. Because the intervention was exactly the same in both studies, a hypothesis that could explain this inconsistency is the health status of the population. Indeed, one study included dynapenic obese older subjects [11▪] whereas the other included obese older adults (i.e. with or without dynapenia) [14▪]. Muscle strength was probably more likely to improve in subjects who had a lower value at baseline (i.e. among dynapenic subjects), because the margin of progression is greater. Effectively, Mangine *et al.* [17] recently highlighted that a physical activity program (i.e. 8 weeks of a high-volume, moderate-intensity training program) stimulated adaptations in all participants, but significantly greater improvements in upper body strength was observed in weaker participants compared to stronger participants at baseline. Third, although the protocols were heterogeneous in terms of intervention (i.e. dosage, AQ7

type of intervention, duration), population (i.e. older adults [9▪], dynapenic obese older adults [11▪], obese older adults [15]) and measured parameters (i.e. VO2 kinetic parameters, walking speed, mobility and aerobic capacities, balance, lower body function, weight shifting ability in the forward and upward directions), the three studies showed an improvement of the physical performance after a CIT supplementation [9▪,11▪,15]. Furthermore, CIT when combined to exercise displayed a greater improvement in walking speed, assessed using the Timed up and Go, than exercise alone, in a population of dynapenic obese older adults [11▪]. This result strengthen the view that CIT supplementation can improve functional and muscular capacities in Humans [5]. However, the additional effect of CIT when combined with exercise on walking speed was not confirmed in a population of obese older adults, independently of the dynapenia status [14▪]. This is not surprising because there are many reports that lower strength (i.e. dynapenia), is strongly related physical function, particularly to locomotion, balance ability and IADL [18,19] and, according to the literature, subjects with lower baseline functional capacity are more likely to experience meaningful improvement in physical function from rehabilitation [20]. This supports the hypothesis that patients with less reserve have more to gain from rehabilitation program compared to subjects with greater baseline functional capacity.

These studies in humans therefore seem to confirm the promising results previously observed in old rats regarding the positive effects of CIT supplementation on muscle protein synthesis, skeletal muscle mass, muscle fiber size, lean mass, muscle strength and mobility [7]. This reinforces the positive effects of citrulline observed in other populations. Indeed, the effects of CIT in athletes are well known and have been summarized as follow: Dietary supplementation with CIT alone does not improve exercise performance. Indeed, the ergogenic response of L- citrulline supplements depends on the training status of the patients. Dietary CIT supplements may increase levels of NO metabolites, although this response has not been directly related to an improvement in athletic performance. CIT may develop beneficial effects on the elimination of NH3 in the course of recovery from exhaustive muscular exercise and also as an effective precursor of L-arginine and creatine.

A strength of this review is that we used a strong and rigorous methodology, based on PRISMA. That led us to define strict inclusion criteria and resulted in exclusion of a large number of studies. The exclusion of many studies could lead to loss of information and can be considered as a potential limitation of the present review. Furthermore, this review is limited by the disparity between the included studies. The exercise interventions described in the RCTs varied in regards of the types of exercises, doses, intensity, and duration. The dosage of citrulline supplementation was also different according to the studies. Then, that lack of placebo or non-isonitrogenous placebo in some studies could induce a response bias, which can affect the internal validity of the studies. Moreover, adherence to these protocols were rarely reported, which impacts the assessment of the real effect of CIT (+ exercises) on muscle parameters. Another limitation of this review concerns the population because only volunteers were included. This point can limit the external validity of the results.

In conclusion, CIT supplementation seems to be able to improve muscular and physical factors in frail elderly people (malnourished, hypertensive, obese, dynapenic-obese) compared to placebo. More importantly, even if exercise is well recognized to impact positively on muscle function or physical performance in older adults, CIT combined to exercise is more efficient than exercise or CIT alone. Nevertheless, the additional effect of CIT supplementation has only been reported on a limited number of studies, specifically in less healthy population such as dynapenic older adults, malnourished adults or in women only. However, the studies included in this systematic review were performed on volunteer older subjects. Therefore, studies assessing the impact of CIT alone or combined with exercise are still lacking in more frail subjects, for example in hospital or in nursing homes. Further well designed and well conducted studies performed on these types of setting should be implemented. It seems likely that interventions with CIT in populations who are presenting poor physical function or strength deficiencies would be more beneficial than interventions in populations with better physical capacity or strength. Overall, the promising avenue of CIT on muscle mass, muscle strength, or physical performance needs more attention to be able to determine subjects in whom it could be more beneficial.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

AQ9

1. Cruz-Jentoft AJ, Bahat G, Bauer J, *et al.* Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 2018.
2. Steffl M, Sima J, Shiells K, *et al.* The increase in healthcare costs associated with muscle weakness in older people without long-term illnesses in the Czech Republic: results from the Survey of Health, Ageing and Retirement in Europe (SHARE). Clin Interv Aging 2017; 12:2003–2007.
3. Le Plenier S, Goron A, Sotiropoulos A, *et al.* Citrulline directly modulates muscle protein synthesis via the PI3K/MAPK/4E-BP1 pathway in a malnourished state: evidence from in vivo, ex vivo, and in vitro studies. Am J Physiol Endocrinol Metab 2017; 312:E27–e36.
4. Joffin N, Jaubert AM, Durant S, *et al.* Citrulline induces fatty acid release selectively in visceral adipose tissue from old rats. Mol Nutr Food Res 2014; 58:1765–1775.
5. Papadia C, Osowska S, Cynober L, *et al.* Citrulline in health and disease. Review on human studies. Clin Nutr 2017; 37:1823–1828.
6. Bahri S, Zerrouk N, Aussel C, *et al.* Citrulline: from metabolism to therapeutic use. Nutrition 2013; 29:479–484.
7. Moinard C, Le Plenier S, Noirez P, *et al.* Citrulline supplementation induces changes in body composition and limits age-related metabolic changes in healthy male rats. J Nutr 2015; 145:1429–1437.
8. Joffin N, Jaubert AM, Durant S, *et al.* Citrulline counteracts overweight- and aging-related effects on adiponectin and leptin gene expression in rat white adipose tissue. Biochim Open 2015; 1:1–5.
9. ■ Ashley J, Kim Y, Gonzales JU. Impact of l-citrulline supplementation on oxygen uptake kinetics during walking. Appl Physiol Nutr Metab 2018; 43:631–637.

This is the first RCT to test the hypothesis whether CIT would improve VO2 kinetics during walking in older and young adults

10. Figueroa A, Alvarez-Alvarado S, Ormsbee MJ, *et al.* Impact of L-citrulline supplementation and whole-body vibration training on arterial stiffness and leg muscle function in obese postmenopausal women with high blood pressure. Exp Gerontol 2015; 63:35–40.
11. ■ Buckinx F, Gouspillou G, Carvalho LP, *et al.* Effect of high-intensity interval training combined with L-citrulline supplementation on functional capacities and muscle function in dynapenic-obese older adults. J Clin Med 2018; 7:. AQ10
12. ■ Bouillanne O, Melchior JC, Faure C, *et al.* Impact of 3-week citrulline supplementation on postprandial protein metabolism in malnourished older patients: the Ciproage randomized controlled trial. Clin Nutr 2019; 38:564–574.

This is the first interventional study that research the effects of CIT supplementation on protein

13. Devries MC, Breen L, Von Allmen M, *et al.* Low-load resistance training during step-reduction attenuates declines in muscle mass and strength and enhances anabolic sensitivity in older men. Physiol Rep 2015; 3:; pii: e12493.
14. ■ Buckinx F, Gaudreau P, Marcangeli V, *et al.* Muscle adaptation in response to a high-intensity interval training in obese older adults: effect of daily protein intake distribution. Aging Clin Exp Res 2019; 31:863–874.

This is the first RCT that evaluate if the initial amount of daily protein intake could influence the combined effect of HIIT and CIT on functional capacities, physical endurance, muscle strength, power, and body composition in obese older men and women.

15. Buckinx F, Charles A, Quabron A, *et al.* Impact of frailty status on the cost of drugs and dietary supplements prescribed to nursing home residents: the SENIOR cohort. Aging Clin Exp Res 2019; 31:875–880.
16. Villareal MO, Matsukawa T, Isoda H. L-citrulline supplementation-increased skeletal muscle PGC-1alpha expression is associated with exercise performance and increased skeletal muscle weight. Mol Nutr Food Res 2018; e1701043. [Epub ahead of print]
17. Mangine GT, Gonzalez AM, Townsend JR, *et al.* Influence of baseline muscle strength and size measures on training adaptations in resistance-trained men. Int J Exerc Sci 2018; 11:198–213.
18. Bohannon RW. Association of grip and knee extension strength with walking speed of older women receiving home-care physical therapy. J Frailty Aging 2015; 4:181–183.
19. Barbat-Artigas S, Pinheiro Carvalho L, Rolland Y, *et al.* Muscle strength and body weight mediate the relationship between physical activity and usual gait speed. J Am Med Dir Assoc 2016; 17:1031–1036.
20. Minnella EM, Awasthi R, Gillis C, *et al.* Patients with poor baseline walking capacity are most likely to improve their functional status with multimodal prehabilitation. Surgery 2016; 160:1070–1079.

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