

The effects of beetroot and nitrate supplementation on body composition: a GRADE-assessed systematic review and meta-analysis

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Abstract

This systematic review and meta-analysis aimed to investigate the effects of beetroot (BR) or nitrate supplements on body composition indices. A systematic search was conducted for randomised controlled trials (RCT) published up to August 2022 among online databases including Scopus, PubMed/Medline, Web of Science and Embase. Meta-analyses were carried out using a random-effects model. The I^2 index was used to assess the heterogeneity of RCT. A total of twelve RCT met the inclusion criteria for this meta-analysis. The pooled effect size of included studies indicated that BR or nitrate supplementation did not change body weight (weighted mean differences (WMD): -0.14 kg, 95% CI -1.22 , 1.51 ; $P=0.836$; $I^2=0\%$), BMI (WMD: -0.07 kg/m², 95% CI -0.19 , 0.03 ; $P=0.174$, $I^2=0\%$), fat mass (WMD: -0.26 kg, 95% CI -1.51 , 0.98 ; $P=0.677$, $I^2=0\%$), waist circumference (WMD: -0.28 cm, 95% CI -2.30 , 1.74 ; $P=0.786$, $I^2=0\%$), body fat percentage (WMD: 0.18% , 95% CI -0.62 , 0.99 ; $P=0.651$, $I^2=0\%$), fat-free mass (WMD: 0.31 kg, 95% CI -0.31 , 1.94 ; $P=0.703$, $I^2=0\%$) and waist-to-hip ratio (WMD: 0 , 95% CI -0.01 , 0.02 ; $P=0.676$, $I^2=0\%$). Subgroup analyses based on trial duration, BR or nitrate dose, study design, baseline BMI and athletic status (athlete *v.* non-athlete) demonstrated similar results. Certainty of evidence across outcomes ranged from low to moderate. This meta-analysis study suggests that BR or nitrate supplements cannot efficiently ameliorate body composition indices regardless of supplement dosage, trial duration and athletic status.

Key words: Beetroot: Beetroot juice: Nitrate: Body composition: Meta-analysis: Systematic review

Body composition is a determinant of general population health and athletic performance⁽¹⁾. Various methods are recommended to ameliorate body composition such as adherence to different dietary approaches, along with exercise; they have mostly focused on increasing lean body mass and reducing body fat and/or weight^(2,3). In addition to dietary and exercise interventions, dietary supplements have received increasing attention for body composition improvement among the general population and athletes^(4,5). One of these dietary supplements is beetroot juice (BRJ)⁽⁶⁾.

Red beetroot (BR) is a source of nitrates (NO₃), antioxidants, betanin, phenolic compounds, minerals (Na, K, Fe, Ca, Cu, P, Mg

and Zn), dietary fibres and vitamins (B complex, ascorbic acid and retinol)⁽⁷⁾. BR has a variety of edible roots that are used as a source of nutrients. It also has anti-inflammatory, antioxidant, anti-diabetic, anti-carcinogenic, hepatoprotective, wound healing and hypotensive properties⁽⁸⁾. BRJ is a rich source of NO₃, which is especially popular among athletes for improving athletic performance and endurance⁽⁹⁾. Therefore, BRJ is considered a useful ingredient in food supplements, especially for athletes^(10,11). On the other hand, dietary supplements containing NO₃ lead to limiting proton leakage in the mitochondrial electron transport chain, which increases energy production

Abbreviations BFP, body fat percentage; BR, beetroot; BRJ, beetroot juice; FM, fat mass; FFM, fat-free mass; RCT, randomised controlled trial; WC, waist circumference; WHR, waist-to-hip ratio; WMD, weighted mean difference.

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per oxygen unit (O₂) and improves mitochondrial respiration⁽¹²⁾. Some studies have reported that during drinking BRJ, NO₃ is converted into NO₂ by oral anaerobic bacteria and xanthine oxidase, and after swallowing NO₂, it is immediately converted into nitric oxide (NO) in the stomach⁽¹³⁾. Hypothetically, NO is a vasodilator compound that leads to increased blood flow and oxygen supply to skeletal muscles and improved contraction force in type II muscle fibres that contribute to rapid body contraction⁽⁹⁾. Moreover, NO inhibits cytochrome oxidase activity and can increase oxidative phosphorylation⁽¹⁴⁾.

Based on the International Olympic Committee, Australian Institute of Sport and International Society of Sports Nutrition guidelines, BRJ and nitrate are classified as supplements that may improve athletic performance^(15–17). According to the characteristics mentioned for BRJ as a performance-enhancing supplement, it can be effective in body composition improvement. However, evidence regarding body composition improvement following BRJ and/or nitrate is equivocal. Animal studies showed that BRJ significantly decreased the body weight and body weight gain⁽⁶⁾. A mechanistic study revealed that BJ may activate brown adipose tissue through increased UCP1 gene expression⁽¹⁸⁾. Conversely, human studies failed to support the results of animal studies. However, the general impact of BR and nitrate supplements on body composition changes is unsettled; thus, it is required to conduct a comprehensive systematic review and meta-analysis of randomised controlled trials (RCTs) on this topic. Therefore, we aimed to conduct a systematic review and meta-analysis of the pooled data from RCT to compare the efficacy of BR or nitrate supplements on body composition variables, including body weight, BMI, waist circumference (WC), fat mass (FM), body fat percentage (BFP), fat-free mass (FFM) and waist-to-hip ratio (WHR).

Methods and materials

Search strategy

This study was performed in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses statement (PROSPERO registration number: CRD42022378139). We carried out a comprehensive search with no language and time restrictions among online databases including Scopus, PubMed/Medline, Web of Science and Embase for the period up to August 2022. We used the following MeSH and non-MeSH terms in our search strategy to identify potentially relevant studies: 'Beetroot juice' AND weight, 'Beetroot juice' AND ('body mass index' OR BMI), 'Beetroot juice' AND ('waist circumference' OR WC), 'Beetroot juice' AND ('percentage of body fat' OR 'body fat percentage'), 'Beetroot juice' AND ('fat free mass' OR 'lean body mass' OR FFM OR LBM), 'Beetroot juice' AND ('fat mass' OR FM), 'Beetroot juice' AND ('waist-hip ratio' OR 'waist-to-hip ratio' OR WHR), Beetroot AND weight, Beetroot AND ('body mass index' OR BMI), Beetroot AND ('waist circumference OR WC'), Beetroot AND ('fat free mass' OR 'lean body mass' OR FFM OR LBM), Beetroot AND ('percentage of body fat' OR 'body fat percentage'), Beetroot AND ('fat mass' OR FM), Beetroot AND ('waist-hip ratio' OR 'waist-to-hip ratio' OR WHR), nitrate AND weight, nitrate AND ('body mass index' OR BMI), nitrate

AND ('waist circumference' OR WC), nitrate AND ('fat free mass' OR 'lean body mass' OR FFM OR LBM), nitrate AND ('percentage of body fat' OR 'body fat percentage'), nitrate AND ('fat mass' OR FM), nitrate AND ('waist-hip ratio' OR 'waist-to-hip ratio' OR WHR), 'nitric oxide supplement' AND weight, 'nitric oxide supplement' AND ('body mass index' OR BMI), 'nitric oxide supplement' AND ('waist circumference' OR WC), 'nitric oxide supplement' AND ('fat free mass' OR 'lean body mass' OR FFM OR LBM), 'nitric oxide supplement' AND ('percentage of body fat' OR 'body fat percentage'), 'nitric oxide supplement' AND ('fat mass' OR FM), 'nitric oxide supplement' AND ('waist-hip ratio' OR 'waist-to-hip ratio' OR WHR). Furthermore, all references of the included searches and previous review articles were searched to avoid omitting any relevant trials.

Study selection and eligibility criteria

This meta-analysis included RCT with a parallel or cross-over design that investigated the effects of BR or nitrate supplements on body composition measures (e.g. body weight, BMI, FM, BFP, FFM and WHR); their findings were presented as standard deviation and mean in the control and intervention groups. Articles that did not meet the eligibility criteria were omitted by reviewing the title, abstract and full text. Exclusion criteria were (1) all studies that investigated another combination with BRJ or nitrate, (2) experimental, animal and review studies, (3) studies conducted on children and pregnant women and (4) studies without a control group.

Data extraction

Data were extracted from eligible studies by three investigators (VF, RA), and a head investigator (DA) performed the final evaluation. The obtained data are (1) surname of the first author, (2) place of study, (3) year of publication, (4) duration of the study, (5) type and dose of BR or nitrate supplements, (6) sex, (7) age, (8) BMI and (9) health status of the participants.

Quality assessment

The risk of bias was assessed in eligible and included studies by using the Cochrane scoring system. In each study, several specific items were evaluated: randomisation process, allocation concealment, participant and staff blindness, outcome assessor blindness, inadequate outcome data, selective reporting and other biases. Three groups were created as a result: high risk of bias (general risk of bias > 2 high risks), unclear risk of bias (general risk of bias = 2 high risks) and low risk of bias (general risk of bias < 2 high risks). Table 2 provides a summary of the analyses' findings.

Statistical analysis

Risk of bias assessment in eligible and included studies was performed using the Cochrane scoring system. In each study, several specific items were evaluated: the mean and standard deviation of the outcome measures (body weight, BMI, FM, BFP, FFM and WHR) examined for the control and intervention groups were used to obtain general results. If the standard deviation of the mean difference was not reported in the studies,



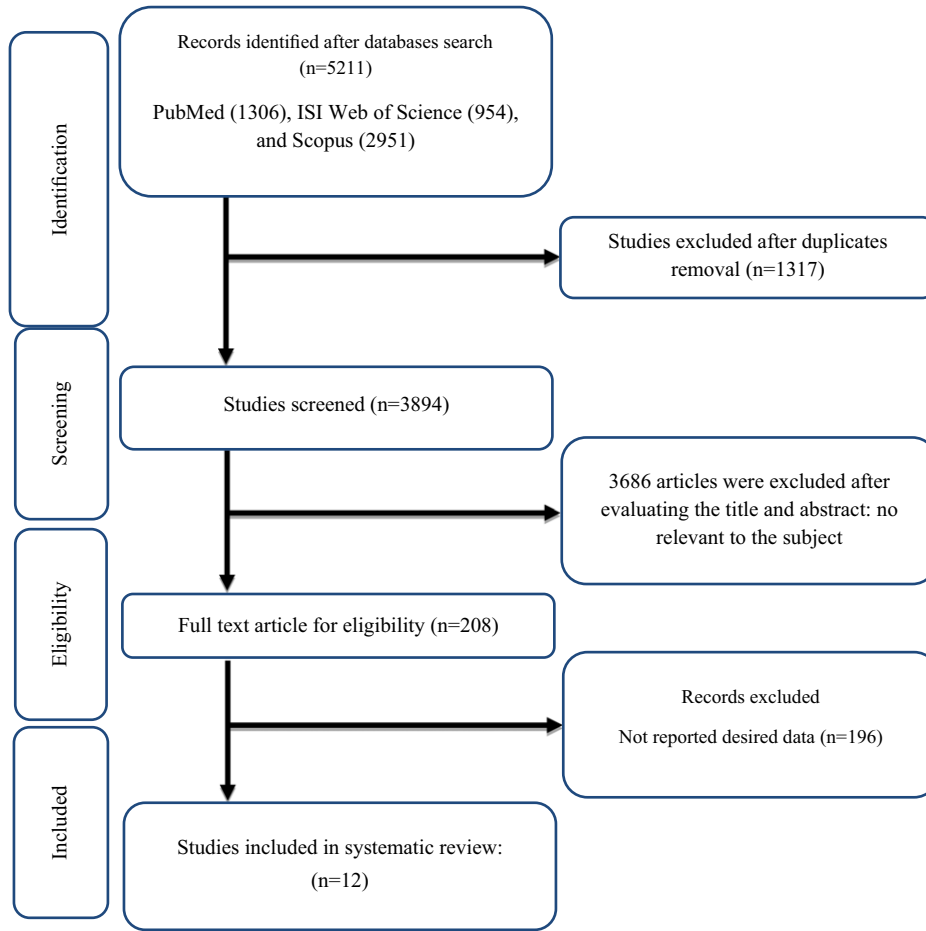


Fig. 1. Flow chart of study selection for inclusion trials in the systematic review.

we calculated it using the following formula: $SD\ change = \sqrt{(SD\ baseline)^2 + (SD\ final)^2 - (2R \times SD\ baseline \times SD\ final)}$ ($R = 0.8$)⁽¹⁹⁾. The combined effect size was expressed as the weighted mean difference and 95% CI. Heterogeneity between study estimates was investigated using the Q-test and the Cochrane I² index. Sensitivity analysis was performed to determine the impact of each study on the overall effect size. Publication bias was evaluated using Egger's test and funnel plot analysis. To examine sources of heterogeneity, we accomplished subgroup analyses based on trial duration, intervention dose, baseline BMI, study design and athletic status.

Assessment of certainty

The entire level of evidence certainty across the research was assessed and summarised using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system⁽²⁰⁾.

Results

Study selection

The initial multi-database search identified 5211 publications. After removing duplicates, 3894 records were screened and

3686 reports were excluded. The eligibility of remaining 208 articles was assessed and twelve articles that met the inclusion criteria were selected for this meta-analysis. The selection and screening process of eligible studies is summarised in Fig. 1.

Study characteristics

A total of twelve RCTs involving 500 participants (301 cases and 299 controls) were included in this meta-analysis^(14,21–31). Six of the studies had two trial arms^(14,25,26,28,29,31). Table 1 provides the characteristics of the twelve included trials. The studies were published between 2016 and 2022. Three were cross-over trials^(14,25,31) and nine studies used a parallel design^(21–24,26–30). The duration of interventions ranged from 4 to 12 weeks and sample sizes in the trials were between 10 and 80 participants. Their mean age and BMI varied between 22 to 70 years and 22.5 to 36 kg/m², respectively. Study settings were Spain^(28,29), Poland^(14,25), Australia⁽³¹⁾, Egypt⁽²²⁾, Iran⁽²³⁾, the Netherlands⁽²⁶⁾, the UK⁽²¹⁾, the USA⁽²⁷⁾, India⁽²⁴⁾ and Tanzania⁽³⁰⁾. Eight arms were conducted among both sexes^(21,23,24,26,30,31), while six and four arms included only men^(14,25,27–29) or women^(14,22,25,28), respectively. The trials were carried out among triathletes⁽²⁹⁾, baseball players⁽²⁷⁾, elite fencers^(14,25), older healthy participants⁽²¹⁾, elderly men and women⁽²⁸⁾. In addition, the studies enrolled patients with pre-hypertension^(26,31),

Table 1. Characteristic of included studies in meta-analysis (Mean values and standard deviations)

Studies	Country	Study design	Participant	Sample size and sex	Sample size		Trial duration (Week)	Means age				Means BMI				Intervention			
					IG	CG		IG		CG		IG		CG		Type	Dose	Control group	Adverse events
								Mean	SD	Mean	SD	Mean	SD	Mean	SD				
Blekkhorst et al., 2016 (A)	Australia	Cross-over, R, PC	Pre-hypertensive	M/F (F:10, M:20)	30	30	4	63		63		27.0	3.9	27.0	3.9	Nitrate-rich vegetables	200 g/d	Placebo	Not mentioned
Blekkhorst et al., 2016 (B)	Australia	Cross-over, R, PC	Pre-hypertensive	M/F (F:10, M:20)	30	30	4	63		63		27.0	3.9	27.0	3.9	Nitrate-poor vegetables	200 g/d	Placebo	Not mentioned
Siervo et al., 2018	Tanzania	Parallel, R, DB, PC	Hypertensive patients	M/F (F:25, M:6)	16	15	8	60.0	6.7	61.2	5.9	29.1	5.8	27.3	5.7	Nitrate-rich beetroot (BR) juice	70 ml/d	Placebo	Minor side effects
Srivastava et al., 2019	India	Parallel	NAFLD	M/F (F:43, M:37)	40	40	12	45	2.3	42.2	1.5	29.5	14	31.6	1.9	BR powder	5 gm/d	Medication and lifestyle modification	Not mentioned
Kozłowska et al., 2020 (A)	Poland	Cross-over, PC	Elite fencers	M/F (F:10, M:0)	10	10	4	22.6	4.7	22.6	4.7	NR		NR		Freeze-dried beetroot juice (BRJ)	26 g/d	Placebo	Not mentioned
Kozłowska et al., 2020 (B)	Poland	Cross-over, PC	Elite fencers	M/F (F:0, M:10)	10	10	4	27.2	5.4	27.2	5.4	NR		NR		Freeze-dried BRJ	26 g/d	Placebo	Not mentioned
Capper et al., 2020	UK	Parallel, R, PC, open label	Older healthy participants	M/F (F:22, M:14)	19	17	8	70	6	65	4	26	2	25	3	Whole BR	75 gr/d	Placebo	No adverse events reported
Kozłowska et al., 2020 (A)	Poland	Cross-over, PC	Elite fencer	M/F (F:10, M:0)	10	10	4	22.6	5.3	22.6	5.3	NR		NR		Freeze-dried BRJ	200 ml/d	Placebo	Not mentioned
Kozłowska et al., 2020 (B)	Poland	Cross-over, PC	Elite fencer	M/F (F:0, M:10)	10	10	4	27.2	5.4	27.2	5.4	NR		NR		Freeze-dried BRJ	200 ml/d	Placebo	Not mentioned
Matar et al., 2021	Egypt	Parallel, R	PCOS	M/F (F:20, M:0)	10	10	12	NR		NR		36.0	0.17	36.0	0.21	Dry BR powder	10 g/d	Diet	No adverse events
Townsend et al., 2021	USA	Parallel, R, DB, PC	Baseball players	M/F (F:0, M:16)	8	8	11	NR		NR		NR		NR		Red spinach Extraction capsule	2 g/d	Placebo	No adverse events
Cindy et al., 2021 (B)	Neatherlands	Parallel, R, controlled	Pre-Hypertensive	M/F (F:20, M:32)	26	26	12	64	10	68	9	27.0	3.8	25.9	3.2	Concentrated red BRJ	70 ml/d	No intervention	No adverse events
Cindy et al., 2021 (A)	Neatherlands	Parallel, R, controlled	Pre-hypertensive	M/F (F:16, M:35)	25	26	12	63	10	68	9	25.4	2.8	25.9	3.2	Nitrate-rich vegetable	250–300 g/d	No intervention	No adverse events
Burgos et al., 2022 (B)	Spain	Parallel, R, DB, PC	Triathletes	M/F (F:0, M:16)	8	8	9	34.35	7.95	32.75	7.01	22.54	1.63	24.52	2.53	Nitrate-rich BR extract+CIT	2.1 g/d	Placebo (citrulline)	Not mentioned
Burgos et al., 2022 (A)	Spain	Parallel, R, DB, PC	Triathletes	M/F (F:0, M:16)	8	8	9	32.67	6.54	34.01	7.03	23.25	1.86	24.01	1.89	Nitrate-rich BR extract	2.1 g/d	Placebo	Not mentioned
Karimzadeh et al., 2022	Iran	Parallel, R, DB, PC	T2DM	M/F (F:7, M:31)	19	19	12	54.08	9.23	53.89	8.78	28.28	2.77	30.93	5.4	Concentrated BRJ	24 ml/d	Placebo	No adverse events
Cordova Martinez et al., 2022	Spain	Parallel, R, DB, PC	Elderly women	M/F (F:15, M:0)	7	8	6	NR		NR		NR		NR		Dry BR Extract capsule	3–3.5 g/d	Placebo	Not mentioned
Cordova Martinez et al., 2022	Spain	Parallel, R, DB, PC	Elderly men	M/F (F:0, M:29)	15	14	6	NR		NR		NR		NR		Dry BR Extract capsule	3–3.5 g/d	Placebo	Not mentioned

IG, intervention group; CG, control group; DB, double-blinded; SB, single-blinded; PC, placebo-controlled; CO, controlled; RA, randomised; NR, not reported; F, Female; M, Male; NR, not reported; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; PCOS, polycystic ovary syndrome.

Table 2. Risk of bias assessment

Study	Random sequence generation	Allocation concealment	Selective reporting	Other sources of bias	Blinding (participants and personnel)	Blinding (outcome assessment)	Incomplete outcome data	General risk of bias
Blekkenhorst et al., 2016 (A)	L	L	L	L	H	U	L	L
Blekkenhorst et al., 2016 (B)	L	L	L	L	H	U	L	L
Siervo et al., 2018	U	L	H	H	L	U	H	H
Srivastava et al., 2019	H	H	H	H	H	U	L	H
Kozłowska et al., 2020 (A)	H	H	H	L	H	U	L	H
Kozłowska et al., 2020 (B)	H	H	H	L	H	U	L	H
Capper et al., 2020	L	U	L	L	H	U	L	L
Kozłowska et al., 2020 (A)	H	H	H	L	H	U	L	H
Kozłowska et al., 2020 (B)	H	H	H	L	H	U	L	H
Matar et al., 2021	U	L	H	H	H	U	L	H
Townsend et al., 2021	L	L	L	L	L	U	L	L
Cindy et al., 2021 (B)	L	L	L	L	H	U	L	L
Cindy et al., 2021 (A)	L	L	L	L	H	U	L	L
Burgos et al., 2022 (B)	L	L	L	L	L	U	L	L
Burgos et al., 2022 (A)	L	L	L	L	L	U	L	L
Karimzadeh et al., 2022	L	L	L	L	L	U	L	L
Cordova Martinez et al., 2022	U	U	H	U	L	U	H	U
Cordova Martinez et al., 2022	U	U	H	U	L	U	H	U

General low risk < 2 high risk.
 General unclear risk = 2 high risk.
 General high risk > 2 high risk.

hypertension⁽³⁰⁾, non-alcoholic fatty liver disease⁽²⁴⁾, type 2 diabetes mellitus⁽²³⁾ and polycystic ovary syndrome⁽²²⁾. A daily dose of BR or nitrate supplements was between 2 and 200 g. **Table 2** presents the risk of bias assessment in the twelve included studies.

Effect of beetroot or nitrate supplementation on body weight. Ten RCTs^(14,21,23,25–31) with 400 participants were included in a meta-analysis to investigate the effect of BR or nitrate supplementation on body weight. The pooled analysis of sixteen trial arms did not indicate any significant changes in the body weight of intervention group participants compared with those in untreated or placebo groups (weighted mean differences (WMD): -0.14 kg, 95% CI $-1.22, 1.51$; $P = 0.836$; $I^2 = 0\%$). The results of categorical subgroup analyses for the effect of BR or nitrate intake on body composition indices are summarised in **Table 3**. The sub-analyses suggested no significant differences in body weight between subgroups in terms of duration of intervention, the dose of BRJ or nitrate

supplementation, study design, baseline BMI of participants and athletic status (athlete *v.* non-athlete).

Effect of beetroot or nitrate supplementation on BMI. Six trials^(21–24,29,31) including eight arms (n 266) provided data to evaluate the effect of supplementation with BR or nitrate on BMI; the meta-analysis failed to show any significant differences in BMI between the intervention and control groups (WMD: -0.07 kg/m², 95% CI $-0.19, 0.03$; $P = 0.174$, $I^2 = 0\%$) (**Fig. 2(b)**); similar findings were observed in subgroup analyses (**Table 3**).

Effect of beetroot or nitrate supplementation on waist circumference. **Figure 2(c)** shows a forest plot of three RCTs^(23,26,31) that examined the effects of BR or nitrate supplementation on WC among individuals subjected to BRJ intervention compared with the placebo group; a pooled analysis of five trial arms with 201 participants (WMD: -0.28 cm, 95% CI $-2.30, 1.74$; $P = 0.786$, $I^2 = 0\%$) and subgroup analyses revealed no significant effects on WC (**Table 3**).

Table 3. Subgroup analyses of beetroot (BR) juice on anthropometric indices in adults (95 % confidence intervals)

	Number of study	WMD	95 %CI	P	Heterogeneity		
					P heterogeneity	I ²	P between sub-groups
The effects of BR intake on body weight (kg)							
Overall effect	16	0.14	-1.22, 1.51	0.836	1.000	0.0 %	
Trial duration (week)							
≤8	10	0.03	-1.79, 1.85	0.974	1.000	0.0 %	0.287
>8	6	0.29	-1.78, 2.37	0.782	0.999	0.0 %	
Intervention dose (g/d)							
<200	9	0.25	-1.46, 1.97	0.773	1.000	0.0 %	0.238
≥200	7	-0.04	-2.31, 2.22	0.969	1.000	0.0 %	
Baselin BMI (kg/m²)							
Normal (18.5–24.9)	2	0.47	-2.99, 3.93	0.789	0.679	0.0 %	0.250
Overweight (25–29.9)	7	-0.02	-1.92, 1.87	0.981	1.000	0.0 %	
Study design							
Cross-over	6	-0.03	-2.19, 2.13	0.978	1.000	0.0 %	0.237
Parallel	10	0.26	-1.51, 2.03	0.772	1.000	0.0 %	
Athletic status							
Athlete	7	0.28	-1.72, 2.29	0.779	1.000	0.0 %	0.273
Non-athlete	9	0.02	-1.85, 1.89	0.984	1.000	0.0 %	
The effects of BR intake on BMI (kg/m²)							
Overall effect	8	-0.07	-0.19, 0.03	0.174	0.973	0.0 %	
Trial duration (week)							
≤8	3	-0.06	-0.85, 0.72	0.869	0.980	0.0 %	0.954
>8	5	-0.08	-0.19, 0.03	0.177	0.793	0.0 %	
Intervention dose (g/d)							
<200	6	-0.07	-0.19, 0.03	0.179	0.888	0.0 %	0.836
≥200	2	-0.14	-1.33, 1.03	0.805	0.934	0.0 %	
Baselin BMI (kg/m²)							
Normal (18.5–24.9)	2	0.25	-0.61, 1.12	0.562	0.582	0.0 %	0.046
Overweight (25–29.9)	5	0.06	-0.32, 0.45	0.739	0.995	0.0 %	
Obese (>30)	1	-0.10	-0.22, 0.02	0.105	–	–	
Study design							
Cross-over	2	-0.14	-1.33, 1.03	0.805	0.934	0.0 %	0.836
Parallel	6	-0.07	-0.19, 0.03	0.179	0.888	0.0 %	
Athletic status							
Athlete	2	0.25	-0.61, 1.12	0.562	0.582	0.0 %	0.130
Non-athlete	6	-0.08	-0.20, 0.03	0.147	0.974	0.0 %	
The effects of BR intake on WC (cm)							
Overall effect	5	-0.28	-2.30, 1.74	0.786	0.614	0.0 %	
Trial duration (week)							
≤8	2	0.15	-3.12, 3.43	0.926	0.881	0.0 %	0.745
>8	3	-0.54	-3.11, 2.01	0.676	0.281	21.3 %	
Intervention dose (g/d)							
<200	2	-0.68	-3.88, 2.51	0.675	0.112	60.3 %	0.753
≥200	3	-0.01	-2.61, 2.59	0.992	0.976	0.0 %	
Study design							
Cross-over	2	0.15	-3.12, 3.43	0.926	0.881	0.0 %	0.745
Parallel	3	-0.54	-3.11, 2.01	0.676	0.281	21.3 %	
The effects of BR intake on FM (kg)							
Overall effect	5	-0.26	-1.51, 0.98	0.677	0.993	0.0 %	
Trial duration (week)							
≤8	4	-0.34	-1.64, 0.94	0.597	1.000	0.0 %	0.002
>8	1	0.90	-3.93, 5.73	0.715	–	–	
Intervention dose (g/d)							
<200	3	-0.19	-1.90, 1.52	0.825	0.893	0.0 %	0.688
≥200	2	-0.34	-2.18, 1.48	0.708	0.957	0.0 %	
The effects of BR intake on BFP (%)							
Overall effect	6	0.18	-0.62, 0.99	0.651	0.712	0.0 %	
Trial duration (week)							
≤8	3	-0.91	-4.06, 2.23	0.569	0.879	0.0 %	0.414
>8	3	0.26	-0.57, 1.09	0.536	0.339	7.5 %	
Intervention dose (g/d)							
<200	4	0.19	-0.61, 1.01	0.633	0.454	0.0 %	0.793
≥200	2	-0.61	-7.29, 6.06	0.856	0.619	0.0 %	
Baselin BMI (kg/m²)							
Normal (18.5–24.9)	2	0.23	-0.61, 1.08	0.583	0.150	51.8 %	0.726
Overweight (25–29.9)	1	-1.00	-4.57, 2.57	0.583	–	–	

Table 3. (Continued)

	Number of study	WMD	95 %CI	P	Heterogeneity		
					P heterogeneity	I ²	P between sub-groups
Athletic status							
Athlete	3	0.26	-0.57, 1.09	0.536	0.339	7.5 %	0.414
Non-athlete	3	-0.91	-4.06, 2.23	0.569	0.879	0.0 %	
The effects of BR intake on FFM (kg)							
Overall effect	7	0.31	-1.31, 1.94	0.703	0.999	0.0 %	
Trial duration (week)							
≤8	6	0.50	-1.26, 2.28	0.574	1.000	0.0 %	0.029
>8	1	-0.70	-4.78, 3.38	0.737	-	-	
Intervention dose (g/d)							
<200	3	0.16	-2.01, 2.33	0.884	0.888	0.0 %	0.482
≥200	4	0.51	-1.94, 2.97	0.681	0.985	0.0 %	
Study design							
Cross-over	4	0.50	-1.31, 2.31	0.589	1.000	0.0 %	0.091
Parallel	3	-0.44	-4.14, 3.25	0.813	0.890	0.0 %	
Athletic status							
Athlete	5	0.30	-1.35, 1.96	0.720	0.991	0.0 %	0.773
Non-athlete	2	0.70	-8.02, 9.43	0.874	0.697	0.0 %	
The effects of BR intake on WHR							
Overall effect	3	0.00	-0.01, 0.02	0.676	0.877	0.0 %	

WC, waist circumference; FM, fat mass; BFP, body fat percentage; FFM, fat-free mass; WHR, waist-to-hip ratio; WMD, weighted mean differences.

Effect of beetroot or nitrate supplementation on body fat mass. Three studies^(14,25,27) with five arms (n 84) reported the effect of BR or nitrate intake on FM of participants (BRJ or nitrate group *v.* controls) (Fig. 2(d)); the outcome analysis (WMD: -0.26 kg, 95 % CI -1.51, 0.98; P = 0.677, I^2 = 0 %) and subgroup analyses found no significant change in FM (Table 3).

Effect of beetroot or nitrate supplementation on body fat percentage. Four trials^(21,27-29) containing six arms (n 128) were included in the meta-analysis to determine the effect of BR or nitrate supplementation on BFP. The analysis did not show any significant differences in BFP between the two groups (intervention and control) (WMD: 0.18 %, 95 % CI -0.62, 0.99; P = 0.651, I^2 = 0 %) (Fig. 2(e)); no significant differences were observed between subgroups (Table 3).

Effect of beetroot or nitrate supplementation on fat-free mass. Four studies^(14,25,27,28) with seven effect sizes (n 100) were evaluated regarding the effect of BR or nitrate supplementation on FFM; the meta-analysis did not find significant differences in FFM between the intervened and placebo groups (WMD: 0.31 kg, 95 % CI -0.31, 1.94; P = 0.703, I^2 = 0 %) (Fig. 2(f)); there was no significant difference between subgroups (Table 3).

Effect of beetroot or nitrate supplementation on the waist-to-hip ratio. Two RCTs^(23,31) with three arms (n 98) reported the impact of BR or nitrate intake on the WHR of participants (intervened *v.* untreated group) (Fig. 2(g)). The pooled data analysis (WMD: 0, 95 % CI -0.01, 0.02; P = 0.676, I^2 = 0 %) and subgroup analyses did not show any significant changes in WHR (Table 3).

Publication bias

Begg's and Egger's tests did not find publication bias for the majority of evaluated outcomes (BMI, WC, BFP, FFM and WHR). However, it was detected in studies related to the effects

of BR or nitrate supplementation on body weight (Begg's test, P = 0.031; Egger's test, P = 0.028) and FM (Egger's test, P = 0.003). Funnel plots showed some level of asymmetry for body weight and BMI outcomes, while they looked symmetrical for WC, FM, BFP, FFM and WHR (Fig. 3(a)-(g)).

Sensitivity analysis

The sensitivity analysis indicated that each outcome in these meta-analyses did not change through the systematic removal of each study; none of the studies did substantially affect the overall effect size, the direction of the association and statistical significance.

Grading of Recommendations Assessment, Development and Evaluation assessment

The certainty of the evidence was assessed for all evaluated outcomes according to the GRADE framework (Table 5). The quality of evidence was graded as moderate for BMI, WC, BFP, FFM and WHR owing to a downgrade for serious imprecision. It was identified as low for body weight and FM outcomes due to the serious risk of imprecision and publication bias.

Discussion

This systematic review and meta-analysis intended to evaluate available evidence related to the effects of BR or nitrate supplementation on body composition measures. The present study failed to show any association between BR or nitrate supplementation and body composition indices (body weight, BMI, FM, WC, BFP, FFM and WHR). All included studies indicated that BR or nitrate supplementation did not significantly change the body composition indices of the intervened groups compared with controls. The subgroup analyses suggested no significant differences in body composition-related outcomes between subgroups in terms of duration of intervention, the dose of BR or

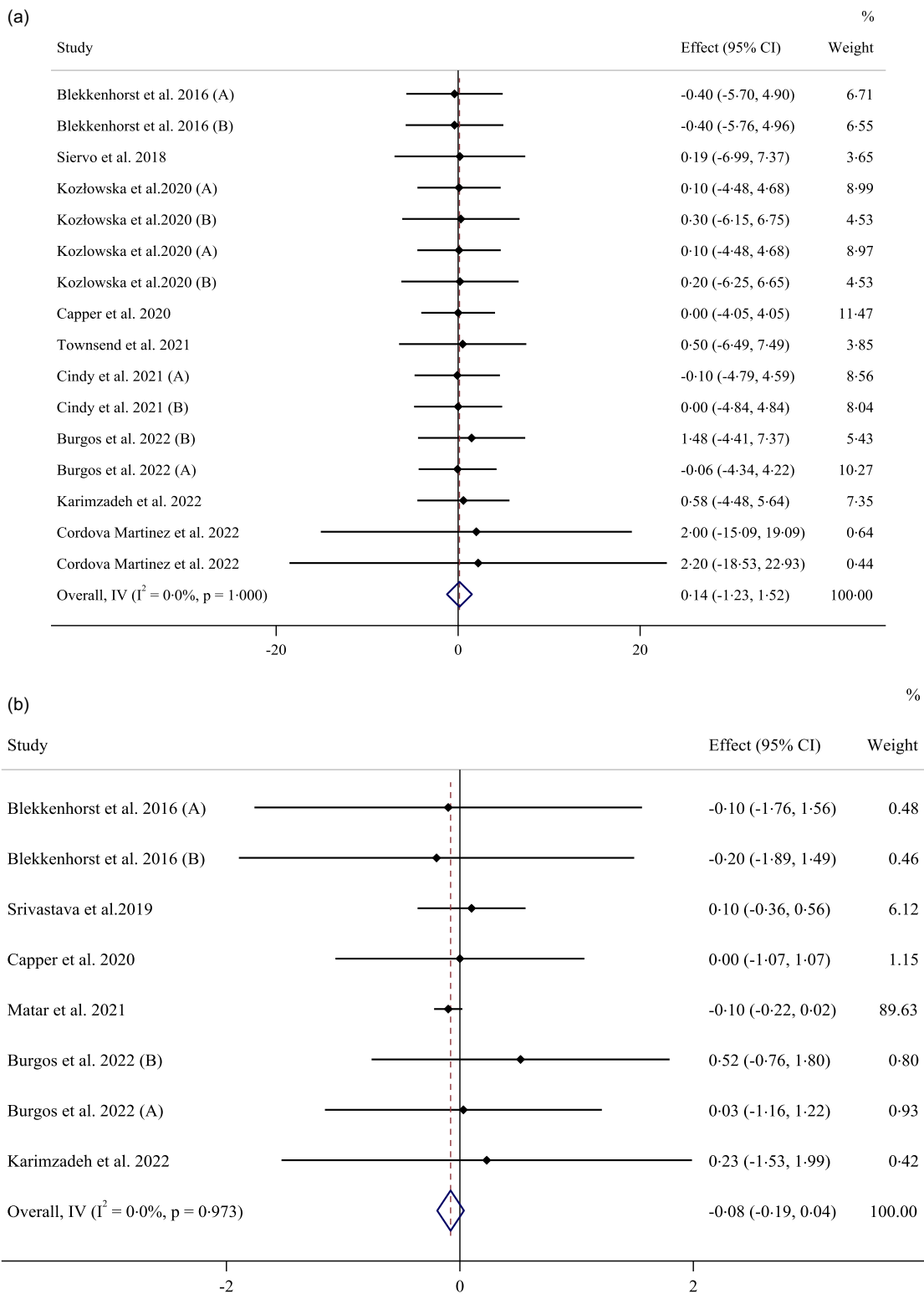


Fig. 2. Forest plot detailing weighted mean difference and 95 % CI for the effect of beetroot or nitrate intake on (a) body weight (kg); (b) BMI (kg/m²), (c) waist circumference (cm), (d) fat mass (kg), (e) body fat percentage (%), (f) fat-free mass (kg) and (g) waist-to-hip ratio.

nitrate supplement, study design, baseline BMI of participants and athletic status (athlete *v.* non-athlete). The findings indicated that BR or nitrate supplementation did not significantly change

the body composition indices of the intervened groups compared with controls. However, the present study revealed that the majority of claims about the advantages of BR consumption

The effects of beetroot on body composition

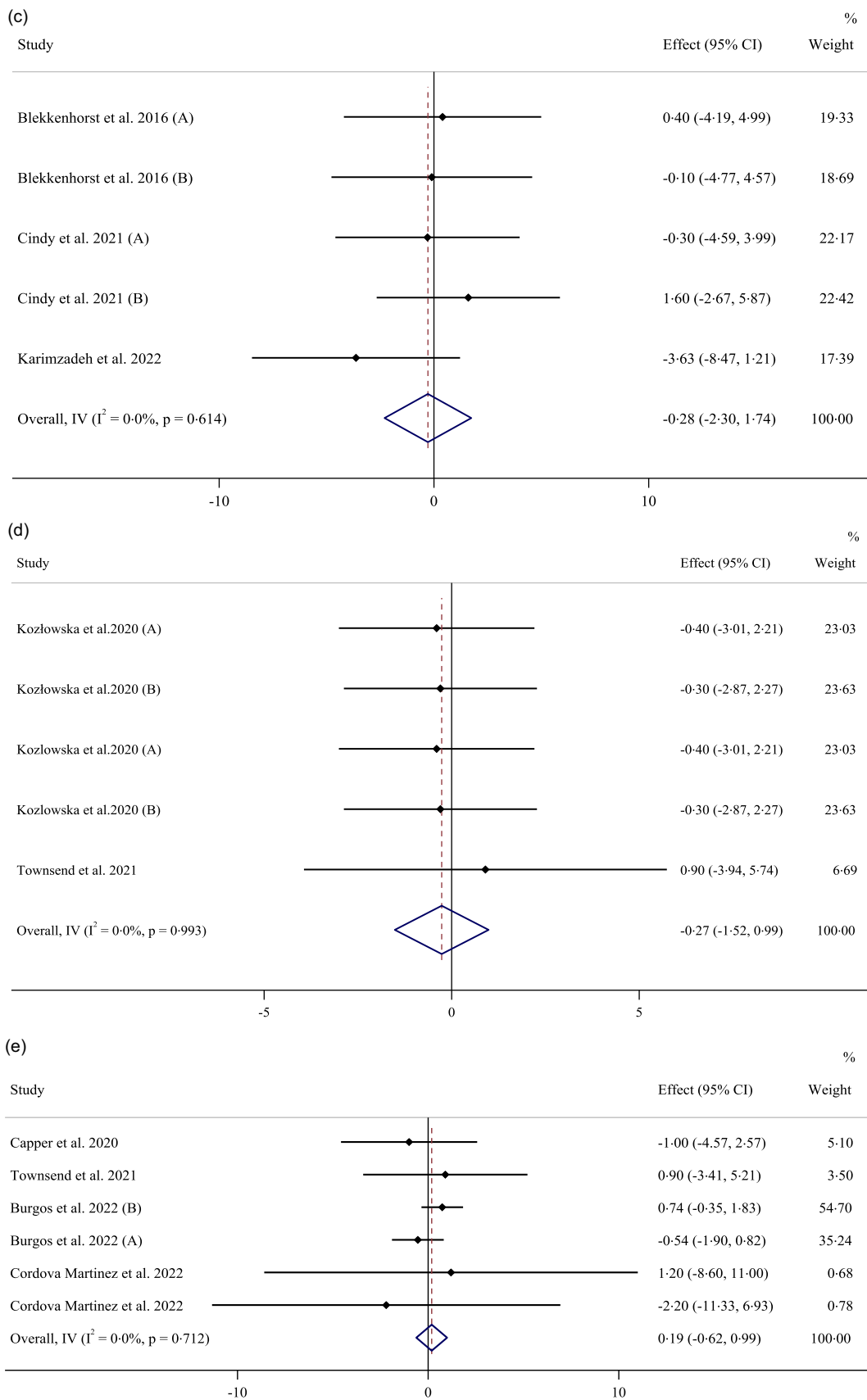


Fig. 2. (Continued)



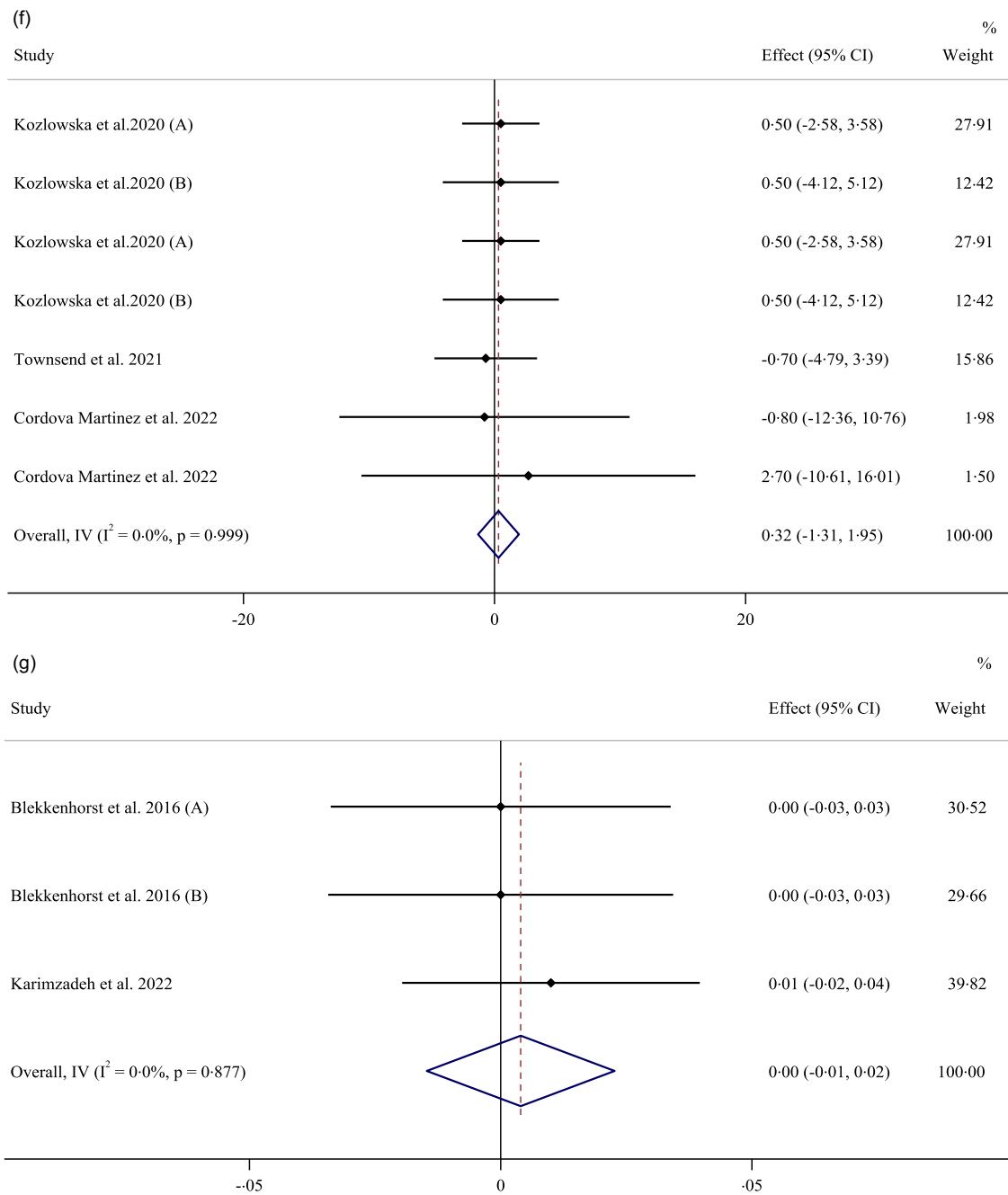


Fig. 2. (Continued)

on weight loss or body composition improvement found on the labels of several products are not supported by clear scientific data.

There is consensus that NO derived from BR can exert an effect on endothelial function by increasing cyclic guanosine monophosphate (cyclic GMP) in vascular smooth muscle, which can lead to increased blood flow, oxygen delivery and better resistance to fatigue during exercise efforts⁽³²⁻³⁴⁾. Due to the role of NO in oxidative phosphorylation efficiency⁽³⁵⁾, BR has been proposed to improve performance during intensive endurance efforts in which the main source of energy is oxidative

phosphorylation⁽³⁶⁾. However, the results of a recent systematic review and meta-analysis showed no changes in peak and mean power output, two main measures of athletic performance, during high-intensity interval training and sprint interval training following chronic or acute supplementation of BRJ⁽³⁷⁾. Therefore, the evidence regarding the effective dose of BRJ supplementation, maximum useful dose and appropriate duration of BRJ supplementation to improve performance and endurance measures is equivocal⁽¹¹⁾. It can be the first reason why the recent studies failed to show any significant changes in body composition indices after BRJ supplementation.

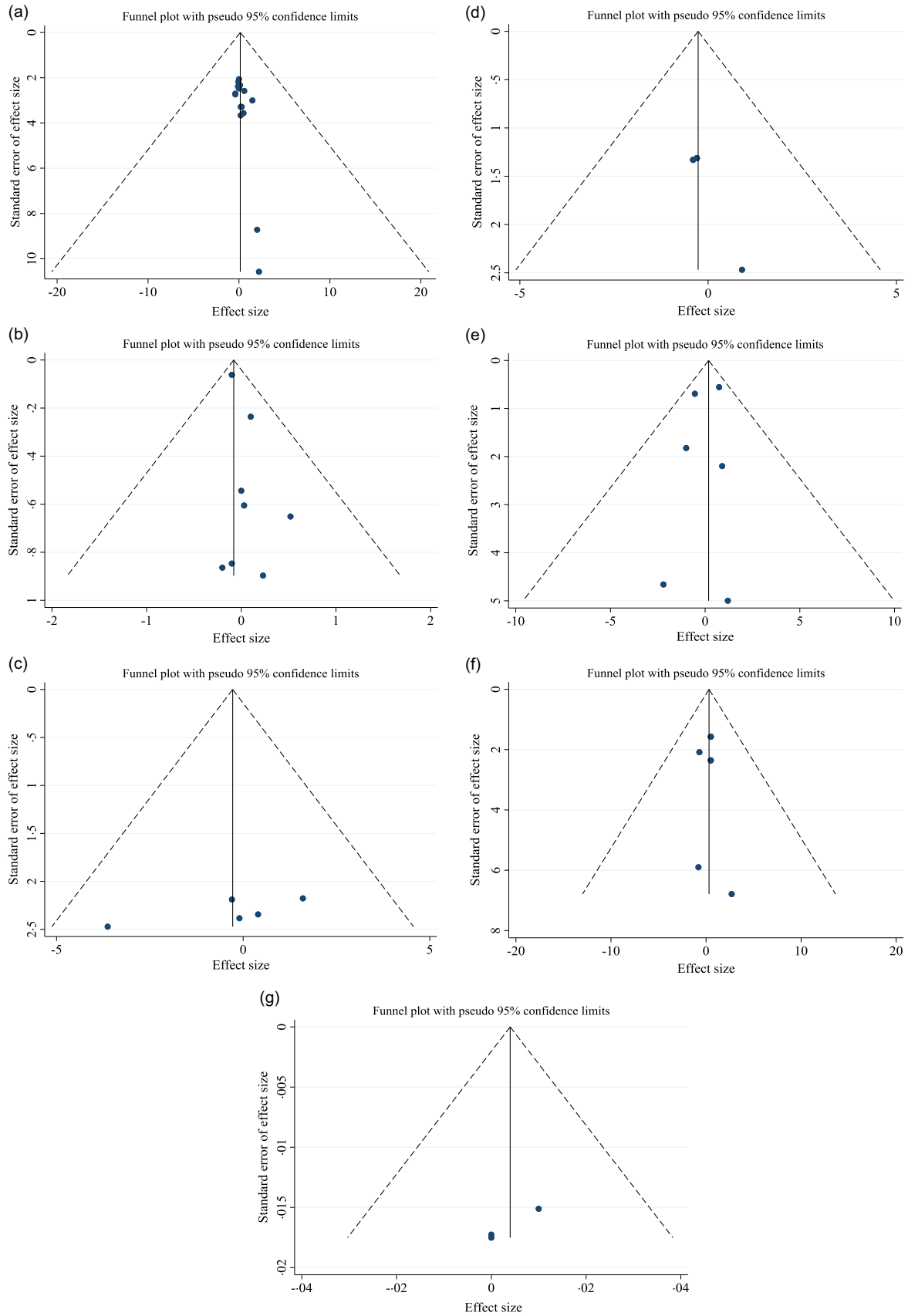


Fig. 3. Funnel plots for the effect of beetroot or nitrate intake on (a) body weight (kg); (b) BMI (kg/m²), (c) waist circumference (cm), (d) fat mass (kg), (e) body fat percentage (%), (f) fat-free mass (kg) and (g) waist-to-hip ratio.

Table 4. Sensitivity analysis and publication bias

Variables	Sensitivity	Publication bias	
		Begg's test	Egger's test
Body weight	None	0.031	0.028
BMI	None	0.902	0.100
WC	None	0.221	0.149
FM	None	1.000	0.003
BFP	None	1.000	0.614
FFM	None	1.000	0.849
WHR	None	1.000	0.055

WC, waist circumference; FM, fat mass; BFP, body fat percentage; FFM, fat-free mass; WHR, waist-to-hip ratio.

Table 5. GRADE profile of beetroot (BR) intake for anthropometric indices (95 % confidence intervals)

Outcomes	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	WMD	95 % CI	Quality of evidence
Body weight	No serious limitation	No serious limitation	No serious limitation	Serious limitation	Serious limitation	0.14	-1.22, 1.51	⊕⊕○○ Low
BMI	No serious limitation	No serious limitation	No serious limitation	Serious limitation	No serious limitation	-0.07	-0.19, 0.03	⊕⊕⊕○ Moderate
WC	No serious limitation	No serious limitation	No serious limitation	Serious limitation	No serious limitation	-0.28	-2.30, 1.74	⊕⊕⊕○ Moderate
FM	No serious limitation	No serious limitation	No serious limitation	Serious limitation	Serious limitation	-0.26	-1.51, 0.98	⊕⊕○○ Low
BFP	No serious limitation	No serious limitation	No serious limitation	Serious limitation	No serious limitation	0.18	-0.62, 0.99	⊕⊕⊕○ Moderate
FFM	No serious limitation	No serious limitation	No serious limitation	Serious limitation	No serious limitation	0.31	-1.31, 1.94	⊕⊕⊕○ Moderate
WHR	No serious limitation	No serious limitation	No serious limitation	Serious limitation	No serious limitation	0.00	-0.01, 0.02	⊕⊕⊕○ Moderate

WMD, weighted mean differences; WC, waist circumference; FM, fat mass; BFP, body fat percentage; FFM, fat-free mass; WHR, waist-to-hip ratio.

1. There is significant publication bias for body weight ($P=0.028$) and FM ($P=0.003$).
2. There is significant effects of BR intake on body weight, BMI, WC, FM, BFP, FFM and WHR.

In a well-designed study, the administration of 180 mg nitrate in the form of red spinach extract before each resistance training session could not improve adaptation to resistance training and performance among baseball players, and no changes were observed in their body composition⁽²⁷⁾. Meanwhile, the absolute dose of nitrate for increasing athletic performance without side effects has not yet been determined, although some studies showed an improvement in training performance following NO₃ supplementation in dosages greater than or equal to 400 mg^(38,39) and the lower dose of NO₃ could improve athletic performance measures⁽⁴⁰⁾. Besides controversial evidence regarding effective nitrate dose, the elevation of plasma NO₃ following inorganic nitrate or BR supplementation and its effects on exercise performance is under debate⁽⁴¹⁻⁴³⁾. It has been hypothesised that nitrate-rich supplement products could improve exercise performance by increasing plasma NO₃ levels, but the evidence is equivocal^(21,44). It should be stated that the post-intervention plasma levels of NO₃ or intramuscular NO₃ concentration contributed to the ergogenic effects of BR products, while its values were not measured in some of the included studies in the present meta-analysis^(22-25,27-29). Therefore, the results of these studies should be interpreted with caution, in which plasma levels of NO₃ following BR products and their subsequent effects on body composition were not assessed.

The majority of the included studies in the current meta-analysis were conducted to determine the effects of BRJ on inflammation, oxidative stress, lipid profile levels, blood pressure and liver enzymes in patients; thus, none of these studies was designed to assess the effect of partial substitution of carbohydrate intake with BR or nitrate on weight loss. In this regard, one of the included RCT was conducted to evaluate the effects of BRJ on oxidative stress and inflammatory markers in patients with type 2 diabetes mellitus who were asked to maintain their regular dietary habits during the study⁽²³⁾. In that study, supplementation with 24 ml BRJ for 12 weeks did not show any changes in body weight, BMI, WC and WHR compared with the control group; no changes in body composition indices may partially be explained by no differences in energy content intake between the two groups and in each group at the end of the study⁽²³⁾. In another included RCT among patients with non-alcoholic fatty liver disease, supplementation with 5 mg of BR powder daily for 3 months could reduce liver enzyme levels, lipid profiles and liver size compared with controls, but BMI remained unchanged⁽²⁴⁾. The health benefits of BRJ have been ascribed to phytochemical components like polyphenols, carotenoids, betalains and anthocyanins, which manifested high stability and antioxidant capacity^(45,46).

The remaining included studies in this meta-analysis aimed to determine the effects of BRJ supplementation on athletic performance measures among trained individuals; the dietary recommendations and the training protocol of these studies were not designed to achieve weight loss or body composition changes. An RCT with cross-over design evaluated the impact of the combination of dietary recommendation and BRJ supplementation on muscle damage, oxidative stress and vo2 max in elite fencers for 4 weeks⁽²²⁾; it failed to show any differences in body composition measures compared with the group received dietary recommendations alone⁽²⁵⁾. The maximal plasma concentration of NO₃ occurs within 2–3 h after BRJ consumption and the ergogenic effects of BRJ were observed at a supplementation dose of 6–8 mmol NO₃⁽⁴⁷⁾; thus, it is important to design study protocols to consider the timing of BRJ supplementation, duration of training and dose of BRJ, as well as optimise the ergogenic potential of BRJ and its effects on body composition improvement in further studies.

It is worth mentioning that the advantages of BR consumption on body weight management may attribute to its green leaves and stems which are rich in fibres and low in energy content⁽⁴⁸⁾. Previous studies have reported a positive association between increased consumption of low-energy foods like root vegetables and weight loss⁽⁴⁹⁾. Therefore, future studies are deemed necessary to prove the weight-lowering effect of BR supplementation; trials should utilise whole BR in the context of a healthy dietary pattern instead of BR supplementation alone or substituting portion of energy-dense foods with BR.

This meta-analysis had some limitations; there was a lack of RCT that examined the concurrent effects of BR supplementation and exercise efforts or dietary plans on body composition indices. In addition, the majority of the included RCT did not measure baseline plasma concentrations of NO₃, its changes during the study period and dietary intake of NO₃ using a validated methodology. Furthermore, there was a lack of research that used body composition measures as their primary outcome and most included studies measured body composition variables as the secondary outcome. However, this study is the first one to review the effects of nitrate-rich products on body composition indices. The low heterogeneity among the included studies is one of the strengths of the current review and meta-analysis.

Conclusion

This meta-analysis declared that supplementation with BR or nitrate could not ameliorate body composition indices regardless of supplement dosage, trial duration and athletic status. So, this study backs some claims of BR product labels regarding the effectiveness of BR supplementation on body composition changes. Further longer-term trials with larger sample sizes are warranted.

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R. A. and D. A. L. were the leader in the current study and revised the manuscript. V. F., T. T. V. and S. S. K. searched

databases and O. A. analysed the data. M. G., S. M., Y. J. and M. M. contributed to the conception and writing of the manuscript. All authors have read and approved the final manuscript.

The authors declare that they have no competing interests.

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