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Systematic Review

Micronutrient status in children and adolescents with Down syndrome: systematic review and meta-analysis

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Abstract

Background Down syndrome (DS) is the most common genetic disorder. To date, the scientific literature regarding micronutrient status in children and adolescents with DS has not been systematically reviewed. Therefore, our aim was to provide a systematic review and meta-analysis on this topic.
Methods We identified all relevant case–control studies published by 1 January 2022, by searching the PubMed and Scopus databases for original English-language articles analysing the micronutrient status of individuals with DS. Forty studies were included in the systematic review and 31 in the meta-analysis.
Results Statistically significant differences between individuals with DS (cases) and non-DS (controls) ($P \leq 0.05$) were obtained for zinc, selenium, copper, vitamin B12, sodium and calcium. Serum, plasma and whole blood analyses showed lower zinc levels in cases than controls {standardised mean difference [SMD] serum [95% confidence interval (CI)] = −2.32 [−3.22, −1.41], $P < 0.00001$; SMD plasma [95% CI] = −1.29 [−2.26, −0.31], $P < 0.01$; SMD blood [95% CI] = −1.59 [−2.29, −0.89], $P < 0.00001$}. Similarly, plasma and blood selenium concentrations were significantly lower in cases than controls (SMD plasma [95% CI] = −1.39 [−2.26, −0.51], $P = 0.002$; SMD blood [95% CI] = −1.86 [−2.59, −1.13], $P < 0.00001$). Intraerythrocytic copper and serum B12 were higher in cases than controls (SMD Cu [95% CI] = 3.33 [2.19, 4.46], $P < 0.00001$; SMD B12 [95% CI] = 0.89 [0.01, 1.77], $P = 0.048$). Blood calcium was lower in cases than controls (SMD Ca [95% CI] = −0.77 [−1.34, −0.21], $P = 0.007$).
Conclusions This study provides the first systematic overview of micronutrient status in children and adolescents with DS and has shown that relatively little consistent research has been executed in this field. There is a clear need for more well-designed, clinical trials to study the micronutrient status and effects of dietary supplements in children and adolescents with DS.

Keywords adolescents, children, Down syndrome, meta-analysis, micronutrients, nutrition, supplements

Introduction

Down syndrome (DS), an expression of complete or partial trisomy of chromosome 21, is the most

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common genetic disorder known to date, observed in 1 in 400–1500 new-borns worldwide (Kazemi *et al.* 2016). Children/adolescents with DS suffer varying degrees of cognitive disabilities, morphogenetic abnormalities and a number of specific comorbidities. The development of children with DS is characterised by a decrease in the developmental quotient with age and frequent infections (Ram & Chinen 2011; Chan *et al.* 2017). They are also at increased risk for cardiac defects, autoimmune diseases, leukaemia, impaired thyroid function and development of Alzheimer's disease early in adulthood (Antonarakis *et al.* 2020). Muscular hypotonia is one of the most prevalent characteristics in children with DS. Accordingly, they may have trouble with feeding and swallowing, which can be additionally disturbed by periodontal disease and tooth loss (Pisacane *et al.* 2003; Anders & Davis 2010; Shukla *et al.* 2014). Furthermore, hypotonia of the abdominal muscles can cause chronic constipation and disturbances of intestinal motility (Field *et al.* 2003). Also, many individuals with DS suffer from celiac disease and leaky gut, which can lead to malabsorption of vitamins or minerals (Cartlidge & Curnock 1986) and, finally, to disturbances in nutrient metabolism (Alagiakrishnan *et al.* 2013). Due to these clinical conditions, children/adolescents with DS are biochemically different from children/adolescents without DS. Quantitative differences in blood calcium, selenium and zinc levels have been reported, as well as other disturbances in trace element and mineral metabolism. It is thought that these disturbances may contribute to problems in organ development and neurological dysfunction of DS (Gombart *et al.* 2020; Huggard *et al.* 2020). For example, Zn deficiency may contribute to growth retardation and immune deficiency (Saghazadeh *et al.* 2017; Pecze *et al.* 2020).

There is now a great deal of clinical interest in the question of whether children with DS benefit from therapeutic nutritional supplementation to improve their development, cognitive decline and overall health, especially if started early in childhood. Although advances in metabolic disorder research and targeted pharmacological treatment have improved certain comorbidities and life expectancy in children/adolescents with DS, no therapeutic options have significantly improved their intellectual abilities (de Graaf *et al.* 2017; Dierssen *et al.* 2020; Hendrix *et al.* 2021). Nonetheless, the use of various products that supposedly improve health and intellectual functions has continued to increase (Ani *et al.* 2000; Ergović & Obradović 2021; van der Haar & Zeinstra 2021).

In terms of human body, vitamins and minerals are micronutrients required to carry out a range of normal functions. They are not produced in our bodies and must be derived from the food or supplements we intake. Vitamins are organic substances classified as fat soluble (vitamins A, D, E and K) or water soluble (vitamin C and the B-complex vitamins). Minerals are inorganic elements classified into two groups: major minerals and trace minerals. The major minerals are used and stored in large quantities in the body (calcium, chloride, magnesium, phosphorus, sodium, potassium and sulphur), while the trace minerals are required in smaller amounts (1–100 mg/day). Minerals in this category include chromium, copper, iodine, iron, selenium, zinc, fluoride, manganese and molybdenum (Savarino *et al.* 2021; WHO 2022). Considering that oxidative stress is part of the basic biology of DS, it is also worth noting that some of the substances mentioned earlier (vitamins C and E, selenium and manganese) can act as antioxidants (Ferrari & Stagi 2021; NCCIH 2022).

To date, the relevant scientific literature regarding micronutrient status in children and adolescents with DS has not been systematically reviewed and organised. Therefore, this study was designed to provide a systematic review and meta-analysis of the micronutrient status in children and adolescents with DS.

Materials and methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page *et al.* 2021).

Literature search and identification of studies

We identified all relevant case–control studies published up to January 2022, by searching the PubMed and Scopus databases for original English-language articles that analysed the

micronutrient status of individuals with DS. Journal articles with the terms 'Down syndrome' + 'antioxidants' + 'micronutrients' + 'nutrition' + 'minerals' + 'diet' + 'supplements' + 'vitamins' or 'trisomy 21' + 'antioxidants' + 'micronutrients' + 'nutrition' + 'minerals' + 'diet' + 'supplements' + 'vitamins' were identified. The search was performed independently by two authors. All retrieved articles were compared to avoid duplications and potential disagreements were discussed and resolved with consensus.

Data screening

After removing duplicate, ineligible and irrelevant publications, 231 manuscripts were identified for review (Fig. 1). Original articles were included in which micronutrient levels were measured and expressed with mean and standard deviation (SD) both in children/adolescents with DS (cases) and in the non-DS (control group). For children and adolescent age range, we followed new recommendations (Sawyer *et al.* 2018). For studies

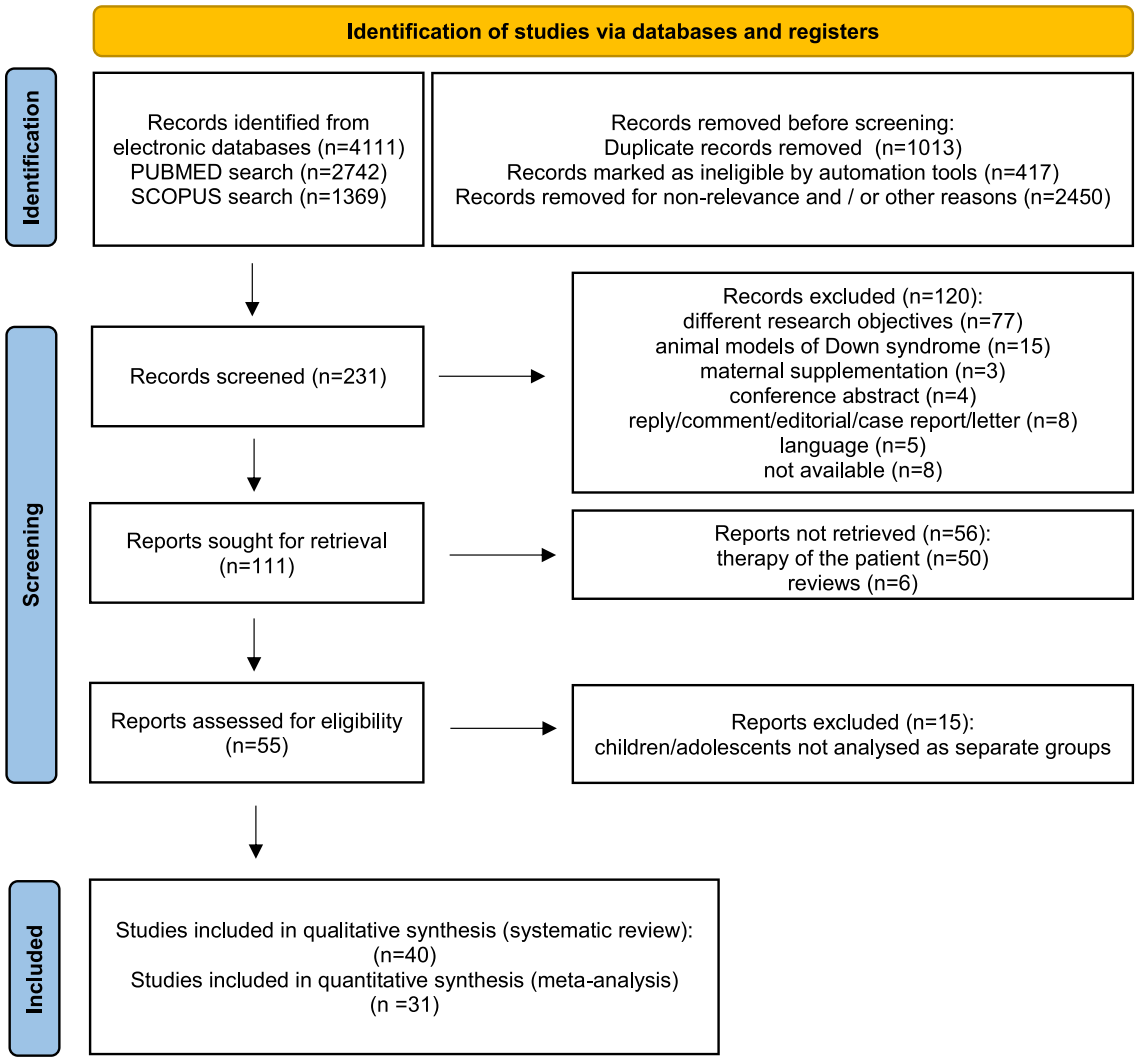

Figure 1. PRISMA 2020 flow diagram for new systematic reviews that included searches of databases and registers only.

Table 1 Characteristics of included studies

First author Year Country	Micronutrient	Patients			Controls		
		Specimen	*N* (F/M)	Age	Results Mean (SD)	*N* (F/M)	Age	Results Mean (SD)
Anneren 1985 Sweden	Selenium	Plasma	45 (24/21)	0–17 years	64.7 (17.2) ng/g	64 (28/36)	0–17 years	65.4 (12.4) ng/g
		Erythrocyte			99.7 (18.2) ng/g			82.3 (17.4) ng/g
Anneren 1985 Sweden	Copper	Erythrocyte	11 (3/8)	4–10 years	3.4 (0.8) µg/g	13 (7/6)	5–14 years	1.1 (0.4) µg/g
	Zinc				8.5 (10.0) µg/g			17.0 (6.0) µg/g
	Iron				320.0 (170.0) µg/g			560.0 (400.0) µg/g
	Manganese				1.0 (0.2) µg/g			1.4 (0.6) µg/g
	Calcium				6.2 (1.6) µg/g			4.0 (1.2) µg/g
	Magnesium				43.0 (9.0) µg/g			55.0 (19.0) µg/g
	Copper	Thrombocyte			3.0 (2.5) µg/g			1.3 (0.8) µg/g
	Zinc				4.4 (0.9) µg/g			3.6 (1.6) µg/g
	Iron				4.3 (1.4) µg/g			5.0 (1.0) µg/g
	Manganese				–			–
	Calcium				15.0 (4.0) µg/g			18.0 (7.8) µg/g
	Magnesium				23.0 (4.0) µg/g			36.0 (5.0) µg/g
	Copper	Neutrophil			3.5 (2.5) µg/g			–
	Zinc				4.8 (3.5) µg/g			5.8 (1.6) µg/g
	Iron				5.7 (1.5) µg/g			3.8 (1.6) µg/g
	Manganese				0.0 (2.0) µg/g			1.3 (0.5) µg/g
	Calcium				6.9 (9.5) µg/g			5.2 (0.9) µg/g
	Magnesium				7.2 (5.2) µg/g			6.0 (1.6) µg/g
Barden 1977 USA	Vitamin A	Serum	44 (22/22)	3–34 years (15.5)*	39.3 (9.8) µg/100 mL	40 (15/25)	1–25 (14.1)*	38.9 (10.6) µg/100 mL
	Carotene				172.8 (75.6) µg/100 mL			99.4 (29.8) µg/100 mL
Chapman 1967 UK	Sodium	Parotid saliva	33	1–12 years	26.1 (21.05) mEq/L	17	18–43 years	5.9 (3.0) mEq/L
Cutress 1972 New Zealand	Calcium	Mixed saliva	36 (17/19)	6–22 years	47.4 (11.4) µg/mL	28 (12/16)	8–23 years	38.3 (11.7) µg/mL
	Phosphorus				148.0 (50.0) µg/mL			118.0 (33.0) µg/mL
	Sodium				178.0 (79.0) µg/mL			208.0 (122.0) µg/mL
	Potassium				815.0 (154.0) µg/mL			698.0 (90.0) µg/mL
	Magnesium				4.7 (1.9) µg/mL			3.6 (2.6) µg/mL

Table 1. *(Continued)*

First author Year Country	Micronutrient	Patients				Controls			
		Specimen	*N* (F/M)	Age	Results Mean (SD)	*N* (F/M)	Age	Results Mean (SD)
David 1996 Italy	Iron	Serum	17	2–5 years	72.0 (32.1) μg/dL	23	2–5 years	71.5 (26.5) μg/dL
			23	5–10 years	74.0 (25.1) μg/dL	25	5–10 years	69.2 (20.2) μg/dL
			10	10–15 years	84.2 (29.4) μg/dL	20	10–15 years	89.0 (23.8) μg/dL
	Vitamin B9	Serum	17	2–5 years	5.9 (1.5) ng/mL	23	2–5 years	6.3 (1.2) ng/mL
			23	5–10 years	6.4 (1.9) ng/mL	25	5–10 years	5.3 (1.4) ng/mL
			10	10–15 years	4.9 (1.6) ng/mL	20	10–15 years	5.3 (1.5) ng/mL
		Erythrocyte	17	2–5 years	233.3 (69.4) ng/mL	23	2–5 years	227.8 (66.3) ng/mL
			23	5–10 years	219.4 (41.3) ng/mL	25	5–10 years	238.2 (78.6) ng/mL
			10	10–15 years	184.7 (47.6) ng/mL	20	10–15 years	226.4 (47.6) ng/mL
	Vitamin B12	Serum	17	2–5 years	593.8 (219.4) pg/mL	23	2–5 years	508.9 (192.9) pg/mL
			23	5–10 years	562.2 (158.3) pg/mL	25	5–10 years	523.5 (144.7) pg/mL
			10	10–15 years	506.3 (203.7) pg/mL	20	10–15 years	386.9 (96.9) pg/mL
Fabris 1984 Italy	Zinc	Plasma	21	3–16 years	86.3 (18.3) μg/dL	11	3–25 years	105.0 (7.9) μg/dL
Farzin 2014 Iran	Selenium	Serum	56 (29/25)	6–38 years (18.0)*	87.1 (14.3) μg/L	60 (30/30)	6–40 years (20.6)*	94.1 (19.5) μg/L
	Zinc				76.5 (12.8) μg/dL			92.2 (12.3) μg/dL
	Copper				85.2 (33.9) μg/dL			90.4 (13.3) μg/dL
	Manganese				5.5 (2.4) μg/L			9.2 (2.9) μg/L
Fernández 2005 Venezuela	Zinc	Plasma	35	6 months to 6 years	1839.0 (361.0) μg/L	35	6 months to 6 years	1374.0 (867.0) μg/L
	Copper				805.0 (261.0) μg/L			767.0 (288.0) μg/L
Franceschi 1988 Italy	Zinc	Plasma	18 (7/11)	7 ± 0.83 years	85.8 (4.5) μg/dL	15 (6/9)		105.2 (2.4) μg/dL
	Copper				74.8 (3.8) μg/dL			71.4 (3.4) μg/dL
Garcez 2005 Brazil	Iron	Serum	50 (25/25)	3–24 years	63.6 (27.3) μg/dL	50	3–24 years	59.1 (18.2) μg/dL
Gericke 1977 South Africa	Vitamin B9	Serum	30 (18/12)	4–59 years (17.5)*	5.2 (0.4) μg/L	30		–
		Erythrocyte			75.2 (5.5) μg/L			196.2 (9.0) μg/L
	Vitamin B12	Serum			987.0 (70.0) ng/L			–

Table 1. *(Continued)*

		Patients			Controls		
First author Year Country	**Micronutrient**	**Specimen**	***N* (F/M)**	**Age**	**Results Mean (SD)**	***N* (F/M)**	**Age**	**Results Mean (SD)**
Grabeklis	Calcium	Hair	40	7–15 years	331.3 μg/g	40	7–15 years	535.4 μg/g
2020	Potassium				218.3 μg/g			110.3 μg/g
Russia	Magnesium				35.9 μg/g			53.7 μg/g
	Sodium				217.2 μg/g			124.5 μg/g
	Phosphorus				185.1 μg/g			153.4 μg/g
	Chrome				0.1 μg/g			0.2 μg/g
	Copper				10.2 μg/g			13.3 μg/g
	Iron				9.7 μg/g			13.2 μg/g
	Selenium				0.4 μg/g			0.4 μg/g
	Zinc				161.9 μg/g			188.2 μg/g
	Manganese				0.3 μg/g			0.3 μg/g
	Lithium				0.1 μg/g			0.1 μg/g
	Iodine				0.4 μg/g			0.4 μg/g
Gromadzinska	Selenium	Whole blood	6	6–16 years	70.2 (12.7) μg/L	77	6–16 years	101.8 (16.2) μg/L
1988		Erythrocyte			116.5 (23.8) μg/L	74		142.2 (31.5) μg/L
Poland		Plasma			47.8 (11.9) μg/L	77		76.6 (13.1) μg/L
		Whole blood	8	17–30 years (18.2)*	68.1 (14.3) μg/L	32	17–30 years (19.4)*	118.1 (16.1) μg/L
		Erythrocyte			110.3 (37.5) μg/L			167.3 (33.3) μg/L
		Plasma			46.4 (11.4) μg/L			90.2 (17.5) μg/L
Halsted	Zinc	Plasma	10	6–13 years	64.0 (8.0) μg/100 mL	26	3–13 years	89.0 (13.0) μg/100 mL
1970								
USA								
Jara	Sodium	Parotid saliva	19 (9/10)	10–25 years	22.8 (11.4) mEq/L	20 (3/17)	10–19 years	17.1 (3.1) mEq/L
1991	Potassium				21.0 (2.2) mEq/L			23.4 (2.9) mEq/L
Chile	Chloride				19.7 (8.1) mEq/L			19.3 (3.5) mEq/L
Kadrabova	Selenium	Serum	16 (8/8)	4–23 years	43.2 (1.7) μg/L	16		50.1 (1.6) μg/L
1995	Copper				1.3 (0.2) mg/L			1.1 (0.2) mg/L
Slovak Republic	Zinc				0.8 (0.1) mg/L			0.9 (0.1) mg/L
	Magnesium				21.7 (1.6) mg/L			21 (1.6) mg/L
Licastro	Zinc	Plasma	25 (6/19)	6–15 years	76.0 (3.0) μg/dL	14 (6/9)	9–13 years	97.0 (4.0) μg/dL
1992								
Italy								

Table 1. *(Continued)*

First author Year Country	Micronutrient	Patients				Controls			
		Specimen	*N* (F/M)	Age	Results Mean (SD)	*N* (F/M)	Age	Results Mean (SD)
Licastro 1994 Italy	Zinc	Plasma	21 (9/12)	7–15 years	80.9 (0.6) μg/dL 70.2 (2.3) μg/dL	18 (6/12)	10–11 years	95.2 (5.9) μg/dL 104.8 (3.6) μg/dL
Mallet 1979 France	Copper	Serum Erythrocyte	26	3–12 years	17.0 (2.6) μmol/L 8.8 (0.8) μmol/mol	55	5–12 years	19.4 (5.3) μmol/L 6.9 (0.7) μmol/mol
Marques 2007 Brazil	Zinc	Plasma Erythrocyte Urine	30 (14/16)	10–19 years	67.6 (25.6) μg/dL 49.2 (8.5) μg/gHb 244.3 (194.9) μg/24 h	32 (21/13)	13.5 years	68.9 (22.3) μg/dL 35.9 (6.1) μg/gHb 200.3 (236.4) μg/24 h
Meguid 2001 Egypt	Selenium	Whole blood	8 5 5 18	9–36 months 11–33 months 12–22 months 9–36 months	14.2 (2.2) μg/L 13.9 (0.9) μg/L 13.2 (1.2) μg/L 13.8 (1.7) μg/L	15	9–36 months	20.4 (2.7) μg/L
	Copper	Plasma	8 5 5 18	9–36 months 11–33 months 12–22 months 9–36 months	2.1 (0.8) mg/L 2.8 (0.8) mg/L 2.3 (0.3) mg/L 2.3 (0.8) mg/L			1.2 (0.2) mg/L
	Zinc		8 5 5 18	9–36 months 11–33 months 12–22 months 9–36 months	1.3 (0.6) mg/L 1.2 (0.5) mg/L 2.5 (1) mg/L 1.6 (0.9) mg/L			1.2 (0.2) mg/L
Meguid 2010 Egypt	Zinc Copper Vitamin B9 Vitamin B12 Vitamin C Vitamin E	Serum	42	5 ± 1.1 years	72.3 (9.6) μg/dL 120 (7.5) μg/dL 7.0 (3.1) ng/mL 942.0 (604.0) μg/mL 0.2 (0.0) μg/dL 1.6 (0.1) mg/dL	48	5.6 ± 2 years	98.5 (3.1) μg/dL 129 (10.6) μg/dL 11.4 (4.1) ng/mL 120 (7.5) μg/mL 0.3 (0.0) μg/dL 1.7 (0.1) mg/dL
Milunsky 1970 USA	Zinc	Plasma Red cell	10 (4/6) 6 (4/2)	3–10 years 5–14 years	64 (7.5) μg/100 mL 13.5 (2.4) μg/1 mL	16 (9/7) 15 (8/7)	5–14 years	90 (12.4) μg/100 mL 9.5 (1.4) μg/1 mL

Table 1. *(Continued)*

First author Year Country	Micronutrient	Patients				Controls		
		Specimen	*N* (F/M)	Age	Results Mean (SD)	*N* (F/M)	Age	Results Mean (SD)
Neve 1983 France	Selenium	Plasma	28	9–36 years (16.0)*	0.9 (0.2) µmol/L	32	5–36 years (19.0)*	1.2 (0.3) µmol/L
		Erythrocyte			4.7 (1.1) nmol/gHb			4.3 (0.8) nmol/gHb
	Zinc	Plasma	29		13.5 (3.5) µmol/L	32		13.0 (2.0) µmol/L
		Erythrocyte	28		679.0 (73.0) nmol/gHb	31		382.0 (84.0) nmol/gHb
	Copper	Plasma	26		23.5 (6.5) µmol/L	25		20.8 (5.5) µmol/L
		Erythrocyte			57.1 (8.3) nmol/gHb			32.6 (6.1) nmol/gHb
Noble 1988 USA	Zinc	Plasma	11	0–5 years	1.5 (0.1) µg/mL	11	0–5 years	1.3 (0.1) µg/mL
Pueschel 1990 USA	Vitamin A	Serum	33 (18/15)	6–28 years (18.0)*	106.0 (87.9) µg/dL	14 (7/7)	12–33 years (20.9)*	136.5 (70.3) µg/dL
Sinet 1984 France	Selenium	Plasma	13	9–15 years	69.0 (12.0) ng/mL	10	5–15 years	103.0 (21.0) ng/mL
		Erythrocyte			345.0 (69.0) ng/gHb			335.0 (57.0) ng/gHb
Siqueira 2004 USA	Sodium	Whole saliva	22 (10/12)	6–10 years	9.5 (2.1) mEq/L	21 (10/11)	6–10 years	5.7 (1.5) mEq/L
	Potassium				5.3 (1.9) mEq/L			7.5 (1.3) mEq/L
	Phosphorus				1.2 (0.3) mEq/L			1.1 (0.3) mEq/L
	Calcium				0.6 (0.2) mEq/L			0.4 (0.1) mEq/L
	Zinc				0.002 (0.0009) mEq/L			0.002 (0.0012) mEq/L
	Magnesium				0.06 (0.014) mEq/L			0.06 (0.027) mEq/L
Siqueira 2007 USA	Sodium	Whole saliva	20	12–60 months	8.7 (0.9) mEq/L	18	12–60 months	5.4 (1.2) mEq/L
	Potassium				5.6 (1.0) mEq/L			7.9 (1.5) mEq/L
	Phosphorus				1.1 (0.1) mEq/L			1.2 (0.2) mEq/L
	Calcium				0.5 (0.1) mEq/L			0.5 (0.1) mEq/L
	Zinc				0.003 (0.0009) mEq/L			0.003 (0.001) mEq/L
	Magnesium				0.06 (0.03) mEq/L			0.06 (0.02) mEq/L
Sustrova 1994 Slovakia	Zinc	Serum	20	1–6 years	0.8 (0.1) µg/mL	19	1–6 years	1.0 (0.1) µg/mL
			45	6–15 years	0.8 (0.1) µg/mL	20	6–15 years	1.0 (0.2) µg/mL
			40	15–35 years (20.0)*	0.9 (0.1) µg/mL	20	15–35 years (21.0)*	1.0 (0.2) µg/mL

Table 1. *(Continued)*

		Patients			Controls		
First author Year Country	**Micronutrient**	**Specimen**	***N* (F/M)**	**Age**	**Results Mean (SD)**	***N* (F/M)**	**Age**	**Results Mean (SD)**
Stabile 1991 Italy	Zinc	Serum	38 (16/22)	2–15 years	0.67 (0.15) μg/dL	20 (9/11)	2–14 years	1.02 (0.24) μg/dL
Stagi 2015 Italy	25(OH)D	Plasma	31 (14/17)	4–19 years	14.3 (8.3) ng/mL	99 (48/51)	4–20 years	27.1 (7.5) ng/mL
	Calcium				2.4 (0.1) ng/mL			2.5 (0.1) ng/mL
	Phosphorus				1.3 (0.2) nmol/L			1.3 (0.3) nmol/L
Teksen 1998 Turkey	Selenium	Plasma	20 (6/14)	3–16 years	113.7 (33.6) ng/mL	15		124.5 (27.6) ng/mL
	Copper				0.9 (0.2) μg/mL			1.1 (0.2) μg/mL
	Zinc				1.4 (0.3) μg/mL			1.5 (0.3) μg/mL
Tiano 2008 Italy	Q10	Plasma	30	4–12 years	0.6 (0.1) μg/mL	30	4–12 years	0.7 (0.1) μg/mL
		Lymphocyte			6.6 (1.1) ng/10^6 cells			10.4 (0.5) ng/10^6 cells
		Thrombocyte			0.1 (0.0) ng/10^6 cells			0.1 (0.0) ng/10^6 cells
Winer 1972 USA	Calcium	Parotid saliva	20 (16/4)	12–45 years (23.0)*	1.7 (0.6) mEq/L	10 (5/5)		1.7 (0.4) mEq/L
	Sodium				22.6 (17.8) mEq/L			28.5 (19.9) mEq/L
	Potassium				20.0 (4.9) mEq/L			19.0 (2.6) mEq/L
	Phosphorus				6.4 (1.4) mEq/L			6.2 (1.3) mEq/L
	Calcium	Serum	18		2.4 (0.7) mEq/L			2.4 (0.9) mEq/L
	Sodium				134.8 (22.0) mEq/L			126.4 (30.2) mEq/L
	Potassium				4.4 (2.3) mEq/L			4.8 (2.1) mEq/L
	Phosphorus				2.1 (1.9) mEq/L			1.7 (0.3) mEq/L
Yamato 2009 Japan	Calcium	Neutrophil	27 (11/16)	8.6 ± 4.6 years	70.6 (28.0) nmol/L	14 (6/8)	12.0 ± 3.9 years	44.4 (16.0) nmol/L
		Serum			9.2 (0.3) mg/dL			8.2 (0.9) mg/dL
Yarom 1987 Israel	Calcium	Tongue muscle	15 (7/8)	1–16 years	245.0 (107.0) μg/g	8 (4/4)	1–65 years	98.0 (25.0) μg/g
	Iron				41.0 (11.0) μg/g			36.0 (7.0) μg/g
	Copper				4.5 (3.6) μg/g			2.1 (1.2) μg/g
	Zinc				33.0 (7.0) μg/g			30.0 (6.0) μg/g
Yenigun 2004 Turkey	Zinc	Hair	19	2–6 years	95.2 (56.1) ppm	11	2–6 years	208.9 (152.4) ppm

*Mean age.

F, female; M, male; SD, standard deviation.

that included adult cases with DS, we have chosen the ones with mean age of participants in the recommended age range (0–25). This information is included in the brackets (Table 1). Exclusion criteria were papers with other research objectives, data from animal models of DS, maternal supplementation studies, conference abstracts, responses, comments, editorials, case reports, letters, non-English studies and reports with incomplete or unavailable data.

Screening for eligibility

The remaining 111 publications were retrieved for detailed analysis, and 56 additional publications were excluded: 50 articles because the focus was on therapy for children/adolescents with DS and 6 articles because they were classified as reviews. Finally, another 15 articles were excluded because they did not analyse children/adolescents as a separate group. Ultimately, 40 studies were included in the systematic review. A meta-analysis was conducted if there were at least three studies comparing the title.

Variables included in the data analysis

Relevant data were extracted from the included studies and Table 1 was created with the following characteristics: author's first name, year and country of publication; name of selected micronutrient; type of sample collected from subjects; number and demographic characteristics of subjects and controls; mean ± SD of nutrient values; and nutrient value scale used.

Meta-analytical methods

Comprehensive Meta-Analysis software version 3.0 (Biostat, Inc., Englewood, NJ, USA) was used for the meta-analyses. Statistical heterogeneity was assessed using the Cochran's Q-statistics and the I-statistics. According to Cochrane guidelines, a random-effects model was used if heterogeneity between studies was detected ($I^2 > 40\%$). If the I^2 was less than 40%, the fixed-effects model was used as the analysis model. The standardised mean difference (SMD) was used to measure the effect, and a *P* value of less than 0.05 was considered statistically significant. In addition, Egger's linear regression test was used to assess publication bias.

Results

The PRISMA 2020 flow chart shows the details of the literature search (Fig. 1). After a comprehensive analysis, 40 studies were finally included in the systematic review. The characteristics of the included studies and their relevant findings are summarised in Table 1. The studies were published between 1967 and 2020, and all but 10 were conducted in Europe or the USA. A range of different micronutrients and samples were analysed in the studies. The number of patients included per group also varied. Some studies

Table 2 Summary of meta-analyses results associated with the significant *P* value

Micronutrient	Specimen	Comparisons (*n*)	Cases/controls (*n*)	Inconsistency I^2 (%)	Effect measure SMD [95% CI]	Overall effect *Z* (*P* value)
Zinc	Serum	4	177/110	88	−2.32 [−3.22, −1.41]	5.02 (<0.00001)
	Plasma	12	269/258	95	−1.29 [−2.26, −0.31]	2.59 (<0.01)
	Blood	17	502/428	95	−1.59 [−2.29, −0.89]	4.39 (<0.00001)
Selenium	Plasma	5	120/230	90	−1.39 [−2.26, −0.51]	3.11 (0.002)
	Blood	8	224/430	91	−1.86 [−2.59, −1.13]	5.01 (<0.00001)
Copper	Erythrocyte	3	63/93	78	3.33 [2.19, 4.46]	5.76 (<0.00001)
Vitamin B12	Serum	3	122/146	88	0.89 [0.01, 1.77]	1.98 (0.048)
Sodium	Parotid saliva	4	108/75	33	0.76 [0.45, 1.07]	4.79 (<0.00001)
	Whole saliva	6	186/142	89	1.06 [0.29, 1.82]	2.73 (0.006)
Calcium	Mixed saliva	3	78/67	11	0.49 [0.16, 0.83]	2.91 (0.004)
	Blood	3	76/123	60	−0.77 [−1.34, −0.21]	2.68 (0.007)

SMD, standardised mean difference; CI, confidence interval.

reported a sex ratio, but the number of these studies was not relevant for further sex-specific analyses. The age of the patients and controls was consistent with the aim of the study to analyse different micronutrients in children and adolescents. The results were expressed as mean ± SD in different scales but later standardised by the authors.

Thirty-one studies were included in the meta-analysis. Table 2 provides an overview of the results of the meta-analyses in terms of significant *P* value. Statistically significant differences between individuals with DS and controls ($P \leq 0.05$) were obtained for zinc, selenium, copper, vitamin B12, sodium and calcium. However, the most significant differences were obtained for zinc. Specifically, serum (Fig. 2), plasma (Fig. 3) and whole blood (Fig. 4) analyses showed lower zinc levels in children and adolescents with DS than non-DS (SMD serum [95% confidence interval (CI)] = −2.32 [−3.22, −1.41], $P < 0.00001$; SMD plasma [95% CI] = −1.29 [−2.26, −0.31], $P < 0.01$; SMD blood [95% CI] = −1.59 [−2.29, −0.89], $P < 0.00001$). Similarly, plasma (Fig. 5) and blood (Fig. 6) selenium concentrations were significantly lower in DS than non-DS (SMD plasma [95% CI] = −1.39 [−2.26, −0.51], $P = 0.002$; SMD blood [95% CI] = −1.86 [−2.59, −1.13], $P < 0.00001$). In contrast, intraerythrocytic copper (Fig. 7) and serum B12 (Fig. 8) were higher in DS than non-DS (SMD Cu [95% CI] = 3.33 [2.19, 4.46], $P < 0.00001$; SMD B12 [95% CI] = 0.89 [0.01, 1.77], $P = 0.048$). Salivary sodium (Fig. 9) was slightly elevated (SMD Na [95% CI] = 1.06 [0.29, 1.82], $P = 0.001$). Blood calcium (Fig. 10) was lower in cases with DS than

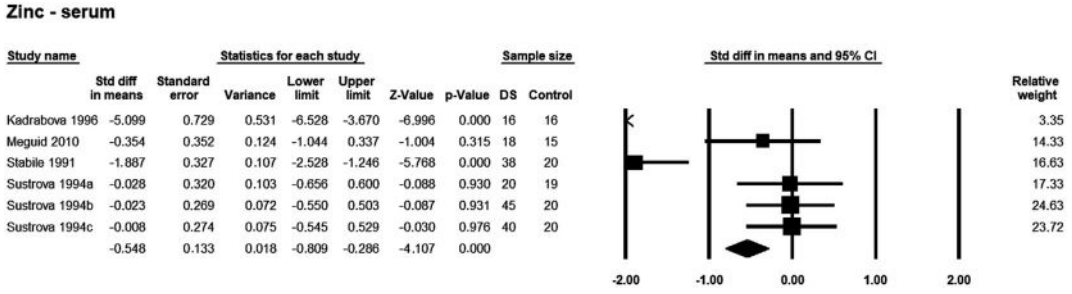

Figure 2. Serum zinc in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 1.36$; Q = 66.4, df = 5 ($P < 0.0001$); $I^2 = 93\%$.

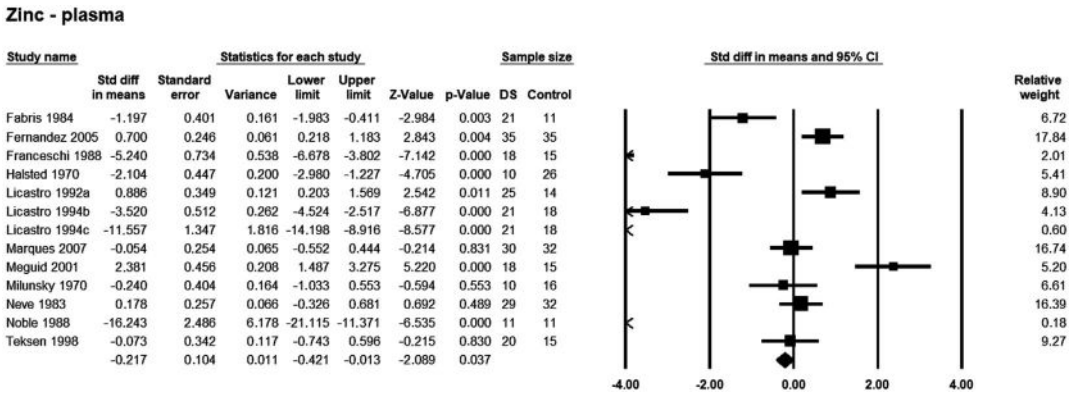

Figure 3. Plasma zinc in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 3.34$; Q = 283.9, df = 12 ($P < 0.0001$); $I^2 = 96\%$.

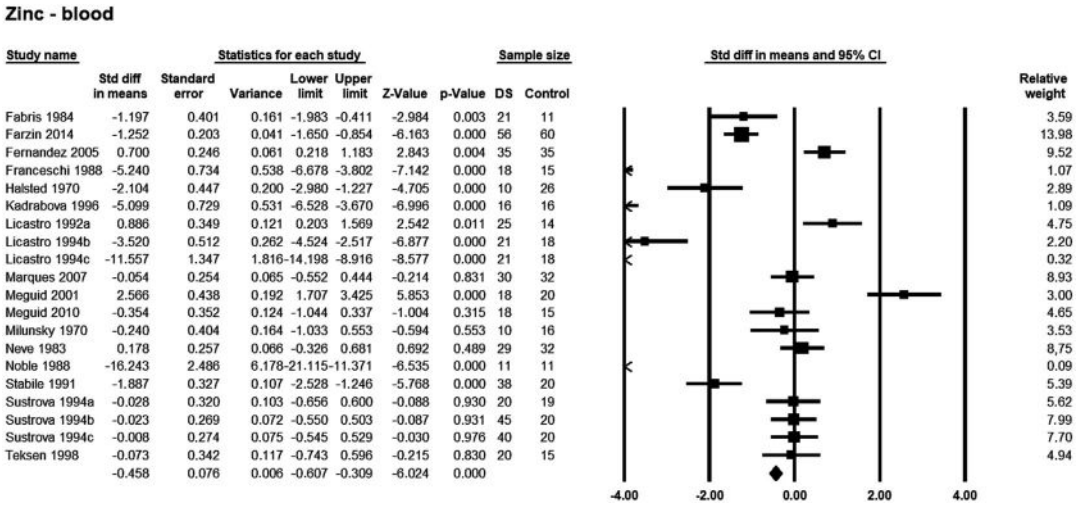

Figure 4. Whole blood zinc in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 2.25$; Q = 380.3, df = 19 ($P < 0.0001$); $I^2 = 95\%$.

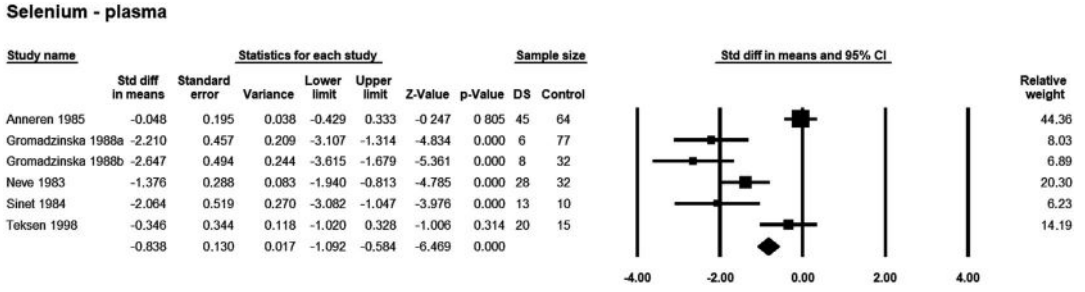

Figure 5. Plasma selenium in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 1.04$; Q = 50.1, df = 5 ($P < 0.0001$); $I^2 = 90\%$.

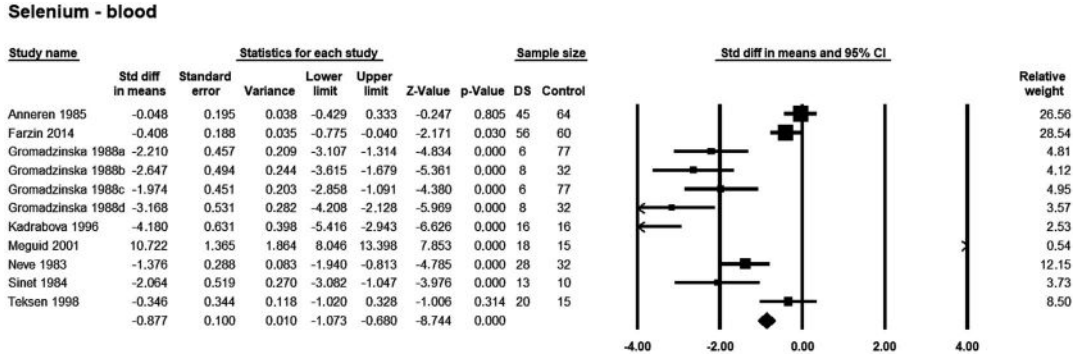

Figure 6. Blood selenium in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 2.10$; Q = 180.5, df = 10 ($P < 0.0001$); $I^2 = 94\%$.

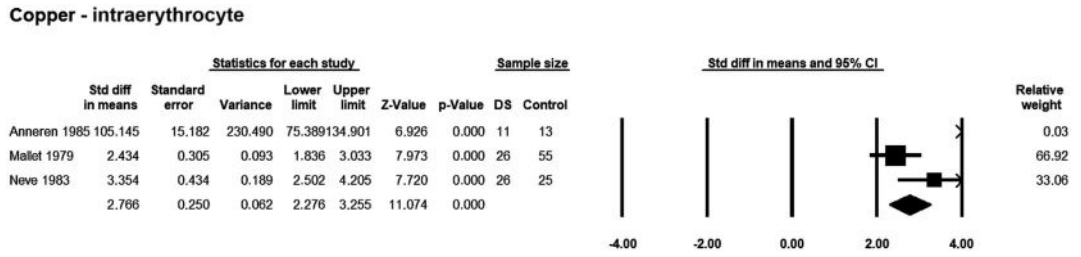

Figure 7. Intraerythrocytic copper in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 6.55$; Q = 48.5, df = 2 ($P < 0.0001$); $I^2 = 96\%$.

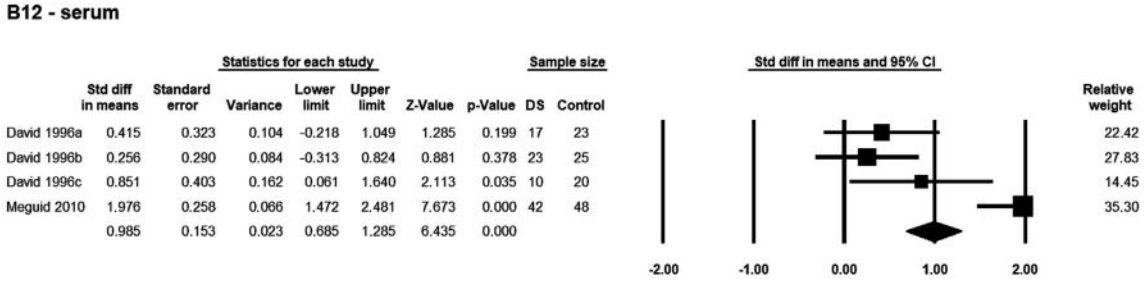

Figure 8. Serum vitamin B12 in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 0.68$; Q = 24.4, df = 3 ($P < 0.0001$); $I^2 = 88\%$.

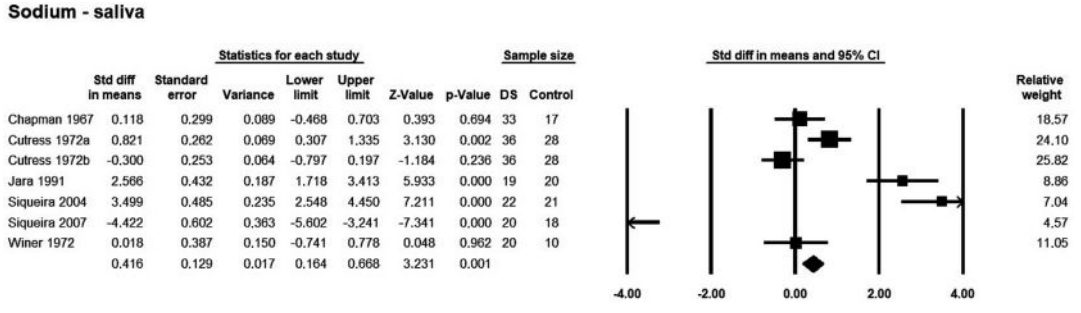

Figure 9. Saliva sodium in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 1.66$; Q = 142.1, df = 6 ($P < 0.0001$); $I^2 = 96\%$.

controls (SMD Ca [95% CI] = −0.77 [−1.34, −0.21], $P = 0.007$) (Table 2).

Discussion

The present systematic review was designed to review the current literature on micronutrient levels in children and adolescents with DS. Forty studies were included in the systematic review. Meta-analysis of data was carried out for three trace elements (Zn, Se and Cu), one vitamin (B12) and two minerals (Ca and Na).

It is important to note that some of the micronutrients included in the meta-analysis were

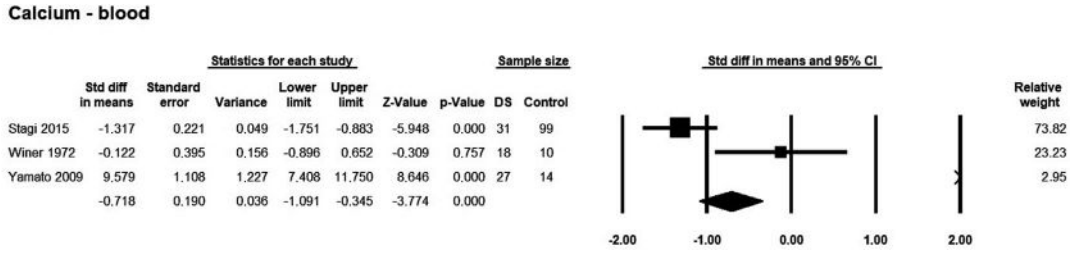

Figure 10. Blood calcium in children/adolescents with Down syndrome (DS) versus non-DS. Forest plot showing relative weights, standardised mean difference with confidence intervals (CIs). Overall average effect size is displayed by filled diamond. $Tau^2 = 8.50$; Q = 95.9, df = 2 ($P < 0.0001$); $I^2 = 98\%$.

selected based on availability in the literature and are not recommended micronutrients. Nowadays, the reference intervals and tissues for various micronutrients are determined by consensus of medical experts based on the results of clinical trials. There are also micronutrient manuals from WHO, which provide recommendations for these analyses (CDC 2020). Based on these analyses, the tissue selected for analysis correlates best with overall micronutrient status. However, the studies included in the meta-analysis measured a number of different tissues, so we have included them all in our manuscript (Table 1). Although some of them are not the first choice today, they are valuable for estimating the different concentrations of the micronutrients analysed.

Zinc, selenium and copper

According to the meta-analysis, serum, plasma and whole blood zinc and selenium concentrations were significantly lower in children and adolescents with DS than the control group. Meanwhile, intraerythrocytic copper was significantly higher in DS.

One of the reasons for the observed changes could be the oxidative stress response caused by overexpression of a gene on chromosome 21 that encodes the enzyme superoxide dismutase (SOD) (Gulesserian *et al.* 2001). Interestingly, SOD has been found to be 50% higher than normal in various cells and tissues of people with DS. It is involved in the regulation of redox homeostasis by catalysing the conversion of O_2– to H_2O_2 in the cytosol (Barone *et al.* 2018). However, it cannot be regulated by a similar increase in catalase, glutathione peroxidase (GPX), and thioredoxin peroxidase, known as cellular systems for efficient removal of H_2O_2. Accordingly, all DS tissues have elevated levels of H_2O_2, which can lead to cell degeneration (Campos & Casado 2015; Barone *et al.* 2018). The SOD can occur in the body in three isoforms: Cu/Zn SOD, an intracellular dimeric enzyme containing Cu and Zn ions in the active site (SOD1); extracellular Cu/Zn SOD, which has the same ions in the active site but different tetrameric Apo enzymes; and mitochondrial, also tetrameric, Mn SOD (SOD2), which contains the Mn ion in the active site (Muchová *et al.* 2014).

Considering that the Cu in the active site of SOD1 is directly responsible for the assembly and catalytic activity of the enzyme, it seems very likely that the elevated Cu levels in red blood cells of DS are due to the increased intraerythrocytic levels of SOD1 (Mallet *et al.* 1979; Nève *et al.* 1983). Other proposed mechanism for the elevated intraerythrocytic Cu levels could be its inadequate bioavailability, tissue distribution or interaction with other nutrients such as Zn. When copper chronically accumulates, it manifests itself in tissue degeneration, apparently due to the stimulation of oxidative damage to proteins and DNA. In normal ranges, an essential trace element, Cu, is required for growth, energy metabolism, bone mineralisation, the immune system, maturation of red and white blood cells, brain development, transport and metabolism of iron (Linus Pauling Institute 2014).

Nevertheless, the aforementioned increase in SOD activity could also lead to excessive zinc and selenium consumption: zinc for constitutional reasons (it is part of the active enzymatic centre) and selenium for functional reasons (it forms the family of selenoenzymes – e.g. GPX, which is widely consumed in the H_2O_2 equilibrium) (Campos & Casado 2015). This may be consequently reflected in their lower

plasma/blood concentrations. In addition, the aberrant amino acid metabolism of children and adolescents with DS could also explain the aforementioned impairments. For example, high cysteine levels in DS may explain Zn deficiency by its chelation and excretion of this metal–protein complex in the urine, which results in a Zn deficiency in the blood/plasma (Pecze *et al.* 2020).

Many enzymes and transcription factors require zinc to function properly. It is important for brain functions because it is an integral component of most brain structures, so disruption of its homeostasis can impair cognitive functions. Zinc metabolism is associated with many brain-related ageing processes and the development of age-related neurodegenerative diseases (Mocchegiani *et al.* 2005). In addition, zinc transporters are critical for the maintenance of memory and cognitive functions. Although certain zinc transporters have been associated with the manifestations of Alzheimer's disease, their involvement in DS has yet to be fully explored (Malakooti *et al.* 2014). Zinc is involved in osteogenic activity and its deficiency in children/adolescents with DS may cause or exacerbate growth disorders (O'Connor *et al.* 2020). Moreover, it is considered as one of the most important nutrients for the immune system, as it is necessary for the production of antibodies and leukocytes (Prasad 2009). Interestingly, early thymic involution associated with low serum zinc levels is a common finding in DS, which may also lead to immunological dysfunction (Karl *et al.* 2012). When discussing immune response, a lack of Se has also been linked to numerous cases of onset and exacerbation of viral and bacterial diseases, common in children with DS. Indeed, it is believed that the lack of Se has a negative effect on the concentrations of IgG2 and IgG4 in the serum, which are thought to be part of the immune response to bacterial antigens (Annerén *et al.* 1990).

Furthermore, deficiency of Se or Zn can lead to impaired thyroid hormone metabolism, which is clinically recognised as hypothyroidism, one of the most common concomitant diagnoses in DS (Antonarakis *et al.* 2020). The T3 receptor requires zinc to maintain its physiologically active confirmation. Some of the effects of zinc deficiency may therefore be due to the loss of zinc at the T3 receptor and the impairment of T3 action (Freake *et al.* 2001). Meanwhile, the importance of Se in the thyroid gland is reflected in the fact that it contains the highest Se content per gram of tissue of all organs. As it is incorporated into iodothyronine deiodinase (types 1, 2 and 3), Se plays an important role in thyroid hormone metabolism (Huang *et al.* 2012; Ventura *et al.* 2017). The alteration in defence mechanisms associated with selenium deficiency leads to abnormal iodination of certain proteins, resulting in apoptosis of cells or exposure to unusual epitopes that can be recognised by the immune system. Apoptosis is induced by high doses of H_2O_2, while pre-incubation of human thyroid follicles *in vitro* with low doses of selenium increases GPX activity and reduces cell death. When Se supply is sufficient, intracellular GPX and the thyroid system protect the thyroid cell from peroxide and minimise oxidative damage. In essence, Se functions primarily as an activator of enzymes necessary for cellular protection from oxidative damage and maintenance of normal redox potentials (Drutel *et al.* 2013).

Vitamin B12

This meta-analysis showed elevated serum B12 levels in children and adolescents with DS, which represents an uncommon finding as the majority of studies warn of specific B12 deficiency in DS.

Interestingly, high serum B12 levels can sometimes paradoxically be accompanied by signs of deficiency associated with qualitative abnormalities that may be the result of abnormal uptake and action of vitamin B12 in the tissues (Ermens *et al.* 2003; Andrès *et al.* 2013). However, high serum B12 levels may encompass serious medical conditions such as haematological malignancies (e.g. chronic myelomonocytic leukaemia, myelodysplastic syndromes and acute leukaemia) (Ermens *et al.* 2003). Given a 10-fold to 20-fold higher risk of developing acute lymphoblastic leukaemia and acute myeloid leukaemia in children with DS than children without DS (Webb *et al.* 2007), the determination of vitamin B12 status as a possible early diagnostic marker seems to be of great clinical importance (Arendt *et al.* 2013).

It is well known that vitamin B12 is an important cofactor of enzymes required for the DNA/RNA synthesis and for remethylation of homocysteine to methionine. Indeed, adequate dietary intake and gut

absorption of B12 are essential for epi/genome stability. The key transport proteins of B12 in the blood, tissue and hepatic uptake are transcobalamins (TCBs). Specifically, the function of TCB II is important to understand the pathophysiological mechanisms that may lead to high serum B12. Some developmental, neuropsychiatric disorders are observed in children with elevated B12 and with congenital deficiencies in TCB II, which led to elevated B12, showing the crucial role of this protein for tissue and hepatic uptake of B12 (Butala *et al.* 2021).

Calcium and sodium

Our meta-analysis showed that the concentrations of Ca and Na in the saliva of subjects with DS were increased compared with the controls. It is hypothesised that the mechanism of Cl reabsorption in DS is disturbed in the context of gene overexpression, which consequently alters the mechanism of acinar ion transfer and thus saliva. Ca is actively excreted from acinar cells and is influenced by NaCl and water concentrations taken up by the ductal system. Higher Ca and Na concentrations are thought to be due to lower Cl secretion by activating the mechanism to balance osmolality maintenance in saliva (Davidovich *et al.* 2010). Salivary enzymes have been found to be altered in people with DS such that increased carbonate anhydrase activity can cause different salivary electrolyte levels (Singh *et al.* 2015). In addition, studies have shown that children with DS have significantly less tooth decay and a different salivary environment of electrolytes and pH compared with other children. It is still not known why this phenomenon occurs, taking into account all the recognised risk factors for caries present in DS, such as cariogenic diet, reduced salivary flow rate, oral respiration, imbalanced occlusal forces and poor access to oral hygiene (Shapira *et al.* 1991). Interestingly, Stabholz *et al.* (1991) found that 84% of children with DS had no caries at all.

Compared with the increased Ca concentrations in the saliva of children/adolescent with DS, the Ca concentration in the blood proved to be reduced. Human chromosome 21 contains two genes that modulate calcium: the *S100β* gene, which stimulates calcium influx, and the *DSCR1* gene, which is a regulator of calcineurin and can inhibit calcineurin-signalling pathways under certain circumstances. Calcineurin has been shown to regulate Ca^{2+} pumps and exchangers to maintain Ca^{2+} homeostasis. In the central nervous system, Ca plays an important role as a cofactor, messenger, signalling molecule and coenzyme. It is involved in the excitatory function of neuronal cells and in voltage-regulated calcium ion channels. The additional dose of genes on chromosome 21 affecting metal ion homeostasis has been linked to neurodegenerative diseases in individuals with DS (Mattson *et al.* 1993; Malakooti *et al.* 2014).

Limitations and strengths

This study has several limitations. First, the quality of the primary studies included in the meta-analyses may influence the results by obscuring or even reversing the direction of the effect due to numerous methodological problems (small samples, studying only people living in institutions and using questionable measures). Second, the heterogeneity of the studies, which includes a large number of countries from which data were collected and a wide range of publication dates, could be another source of bias. In most studies, there were also discrepancies in the age of the children, with no distinction between male and female. In addition, only a small number of studies were included in the final meta-analysis after applying a set of strict criteria, which should be taken into account when interpreting the results. No records of dietary intake are also one of the limitations in our study, given that micronutrient status is highly influenced by food choices. However, this study provides the first systematic overview of micronutrient status in children and adolescents with DS. In accordance with the PRISMA principles, we sought to conduct a high-quality study with a thorough and methodical search strategy based on a set of rigorous criteria.

Conclusions

This study provides the first systematic review of the micronutrient status of children and adolescents with DS. Serum, plasma and whole blood analyses revealed lower zinc levels in children and adolescents with DS than controls. Plasma and blood selenium concentrations were also significantly lower in

children/adolescents with DS than controls. In contrast, intraerythrocytic copper and serum B12 were higher in children/adolescents with DS than controls. Salivary sodium was slightly elevated, while blood calcium was lower in children and adolescents with DS than non-DS controls.

The results of this study have shown that relatively little consistent research has been executed on the micronutrient status of children and adolescents with DS. Consequently, doctors, parents and caretakers have little guidance on how to support their child's health in this regard. Therefore, there is a clear need for more well-designed, clinical trials to study the micronutrient status and, afterwards, effects of several dietary supplements in children and adolescents with DS.

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Conflict of interest

The authors report no conflicts of interest.

Data availability statement

Data are available on request.

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