



Nutrition

Brazilian preschool children attending day care centers show an inadequate micronutrient intake through 24-h duplicate diet



Isabelle Nogueira Leroux^a, Ana Paula Sacone da Silva Ferreira^a, Fernanda Pollo Paniz^a, Fábio Ferreira da Silva^{b,c}, Maciel Santos Luz^d, Bruno Lemos Batista^b, Dirce Maria Marchioni^e, Kelly Polido Kaneshiro Olympio^{a,*}

^a Laboratório de Análises da Exposição Humana a Contaminantes Ambientais, Departamento de Saúde Ambiental, Faculdade de Saúde Pública, Universidade de São Paulo, Av. Dr. Arnaldo, 715, Cerqueira César, CEP 01246-904, São Paulo, SP, Brazil

^b EnvironMetals, Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Avenida dos Estados, 5001, Bairro Santa Terezinha, Santo André, SP, CEP 09210-580, Brazil

^c Agilent Technologies, Alameda Araguaia, 1142, Alphaville Industrial, CEP: 6455000, Barueri, São Paulo, SP, Brazil

^d Laboratório de Processos Metalúrgicos, Centro de Tecnologia em Metalurgia e Materiais, Instituto de Pesquisas Tecnológicas do Estado de São Paulo (IPT), Av. Prof. Almeida Prado, 532, Cidade Universitária, Butantã, CEP 05508-901, São Paulo, SP, Brazil

^e Departamento de Nutrição, Faculdade de Saúde Pública, Universidade de São Paulo, Av. Dr. Arnaldo, 715, Cerqueira César, CEP 01246-904, São Paulo, SP, Brazil

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ABSTRACT

Inadequate micronutrient intake in childhood harms growth and development, and it is related to increased rates of morbidity and mortality. The aim of this study is to evaluate the dietary intake and prevalence of inadequate micronutrient intake in preschool children (1–4 years old) attending two-day care centers. To assess children's dietary micronutrient intake, 24-h duplicate diets (n = 64) were collected for one week-day, including everything the children ate and drank both at home and in kindergarten. Anthropometric measurements were carried out to evaluate the children's nutritional status. The micronutrients copper, iron, calcium, magnesium, selenium, zinc, potassium, sodium, and manganese were determined by inductively coupled plasma mass spectrometry or graphite furnace atomic absorption spectrometry. Calcium and selenium were found with high inadequate intake rates: 50% and 42%, respectively, for children aged 1–3 years old, and 93% and 90% for children aged 4 years. Potassium was consumed in very low amounts, 13% and 5% of children aged 1–3 and 4 years old, respectively, achieved the adequate intake for the nutrient. Sodium intakes were excessive: 23% of the 1–3-year old and 42% of the 4-year-old children, respectively, had an intake higher than the tolerable upper levels. Regarding the nutritional status, overweight and obesity prevalence was 17%. Therefore, considering the damaging health effects for children of micronutrient deficiency and overweight and obesity status, it is necessary that government authorities be aware and update public policies and educational programs in order to promote healthy eating habits in early childhood.

1. Introduction

Essential trace elements play a role in several human body basic functions such as hormones, enzymes, receptors, and genetics; as a result, disorders of trace element metabolism are related to an extensive range of diseases. Half of the world's population is affected by trace

element deficiency [1]. In childhood, inadequate micronutrient intake is of major concern because once it harms growth and development, infant morbidity and mortality rates increase [2]. In addition, children are a vulnerable group for micronutrient deficiencies since they have higher nutrient requirements by body weight due to their developmental needs and their immune and physiologic immaturities [3].

Abbreviations: AI, adequate intake; BMI, body mass index; Ca, calcium; CRM, certified reference material; Cu, copper; DCC, day care centers; EAR, estimated average requirement; F AAS, flame atomic absorption spectrometry; Fe, iron; ICP-MS, inductively coupled plasma mass spectrometry; IRB, Institutional Review Board; K, potassium; Mg, magnesium; Mn, manganese; Na, sodium; PAHO, Pan American Health Organization; Se, selenium; Zn, zinc

* Corresponding author.

E-mail addresses: isabelle.leroux@usp.br (I.N. Leroux), saconeap@usp.br (A.P.S.d.S. Ferreira), fernandapollo@hotmail.com (F.P. Paniz), fabiofersil@gmail.com, fabio_silva@agilent.com (F.F.d. Silva), macielluz@ipt.br (M.S. Luz), bruno.lemos@ufabc.edu.br (B.L. Batista), marchioni@usp.br (D.M. Marchioni), kellypko@usp.br (K.P.K. Olympio).

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The school meals play an important role in improving children's nutrient intake [4,5], what impact their nutritional status, growth and development. In Brazil, the National School Feeding Program is a government program that is responsible for managing the school meals for all public schools in national territory. This program, coordinated by the Ministry of Education, declares that school meals must provide 70% of the nutritional requirements for full-period students. The program represents one of the most important national programs and does not allow schools to offer ultra-processed foods, candies, or sugary drinks [6].

It is recognized that food consumption assessed through recall methods, the most frequently used method, is subject to mistakes, especially at this stage of life. Therefore, the aim of this study was to evaluate the daily dietary intake of the micronutrients copper, iron, calcium, magnesium, selenium, zinc, potassium, sodium, and manganese by children attending public kindergartens and to assess the prevalence of inadequate intake or intake above tolerable upper levels through the 24-hour duplicate diet, a method more accurate than the 24-h recall. We hypothesized that children attending day care centers, where they spend about 10 h/day, receive an adequate nutritional intake through the school feeding.

2. Material and methods

2.1. Subjects

This cross-sectional study was conducted with 64 children from two public day care centers (DCC) in the city of São Paulo. The first was located in the East Zone, and the second in the South Zone of the municipality, where they spend approximately 10 h/day. As we aimed to assess the dietary micronutrient intake by children attending DCC, the children's age range was equivalent to the age range of those attending kindergarten (1–4 years old). The details about the population of this study were already well described previously [7,8]. The children's parents/guardians were informed about the study in a meeting with the researchers and written informed consent was obtained from all subjects. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Board (IRB) of the School of Public Health of the University of Sao Paulo, Brazil (Protocol #1.127.698).

2.2. Diet sampling

The 24-hour duplicate diet [9] was the method chosen for the assessment of children's mineral intake. To apply this method in the DCCs, we counted on the parents/guardians to collect everything the children ate at home. They were instructed to duplicate, as precisely as possible, the amounts that their children ate and drank in decontaminated containers provided by the researchers, maintaining on this day the child's eating habits. To approximate the quantities of food ingested, we asked them to use household measures. For cooked meals, the instruction for parents and guardians was to make a similar plate as the one served for the children, wait until they finish the meal, and then remove or add to the duplicate plate the amounts of food comparable to what the children actually ate. The parents and guardians were also asked to remove the parts of foods that are normally not eaten and to store the food and drinks collected in the refrigerator until delivering them to researchers (Fig. 1).

The same protocol was followed by the investigators in the DCCs, and the food and drinks collected were registered by both parents/guardians and researchers in 24-hour recalls [10]. In the DCCs children spend about 10 h a day and are served five meals: breakfast, morning snack, lunch, afternoon snack, and dinner. Usually, it is offered bread or crackers and milk with coffee or chocolate at breakfast; natural fruit juice at collation; rice, beans, animal protein, vegetables, and fruit for

lunch; milk in the afternoon; and soup and fruit at dinner time. All meals were prepared in a school kitchen by cooks, who also did the plating. The main meals were served at the dining hall, and the morning and afternoon snacks were served in the classroom. When children were at the dining hall, they were free to go to the kitchen and ask for more food, although a considerable number of children did not finish their meals. Regarding the foods served at home, there was a huge difference among the children. Most parents offered dinner again at home and a portion of milk, but the kind of foods offered varied greatly. For example, some caregivers served something before the child went to school in the morning. In addition, a few children did not attend school full time. In these cases, they had the 24-hour diet collected anyway, but it was composed mostly by the meals served at home.

2.3. Sample preparation, digestion, and mineral determination

The collected samples were transported to the laboratory at the University of Sao Paulo where they were weighted (Shimadzu, Barueri, SP, Brazil), completely homogenized with a mixer (Arno model 600 W, Sao Paulo, SP, Brazil), aliquoted, and kept at -22°C until the analysis. All the polypropylene containers used were previously decontaminated, first by cleaning with a detergent solution, rinsing in HNO_3 10% overnight, and then rinsing with deionized water $18.2\text{M}\Omega\text{cm}$ at 25°C [11]. The water used throughout was high-purity water produced by a Milli-Q water purification system (Millipore, Bedford, MA), as well as high-purity nitric acid, produced in a sub-boiling system (Distilled, Berghof, Germany).

For evaluation of the reliability and accuracy of digestion methods, a certified reference material (CRM) TORT-3 lobster hepatopancreas from the National Research Council (Canada) was analyzed.

The samples were lyophilized (Lyophilizer Liotop, L101) for 48 h at a pressure of $200\mu\text{mHg}$ and -50°C and stored in a freezer at -22°C until digestion. For sample preparation, 1 mL of subdistilled HNO_3 was added to 100 mg of lyophilized sample (triplicate) and left overnight. The predigestion was followed by a water bath (Solab SL1522L, Brazil) at 90°C at 4 h, and then the samples had their volumes completed to 14 mL with deionized water $18.2\text{M}\Omega\text{cm}$ at 25°C . CRM material was prepared by the same procedure.

The minerals copper (Cu), iron (Fe), magnesium (Mg), selenium (Se), zinc (Zn), potassium (K), sodium (Na), and manganese (Mn) were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, Hachioji, Japan) at the Federal University of ABC. Multielement stock solutions containing 10 mg/L of each element were obtained from Perkin-Elmer (PerkinElmer, USA). Standards of analytical calibration were prepared daily at concentrations of 0.1; 1; 5; 10; 50; 100; 200; 500, and 1000 $\mu\text{g/L}$ for all elements by serial dilutions of multielement stock solution in 5% (v/v) HNO_3 . Germanium, yttrium, and rhodium were used as internal standards at the concentration of 20 $\mu\text{g/L}$. All the internal standard solutions were prepared by serial dilutions of single-stock solution. The stock solutions containing 1000 mg/L were obtained by Perkin-Elmer (Perkin Elmer, USA). The ICP-MS conditions are presented in Table 1.

Analyses for calcium (Ca) followed a different digestion procedure and determination method. In the acid digestion, 2 mL of hydrogen peroxide, 1 mL of Triton X-100™ (30%), and 5 mL of HNO_3 were added to 500 mg of lyophilized sample, which remained in a water bath first at 70°C for 30 min, then at 120°C for 4 h. The Ca determination was conducted by flame atomic absorption spectrometry (F AAS), model Varian AA 240FS, at the Institute of Technological Research.

2.4. Anthropometric measurements and nutritional status

The children's weight and height were assessed by a trained nutrition student during the school day in the DCC. The height was measured once with a stadiometer fixed on the wall and without the child using shoes. To assess children's weight, a portable digital scale, model Tec-

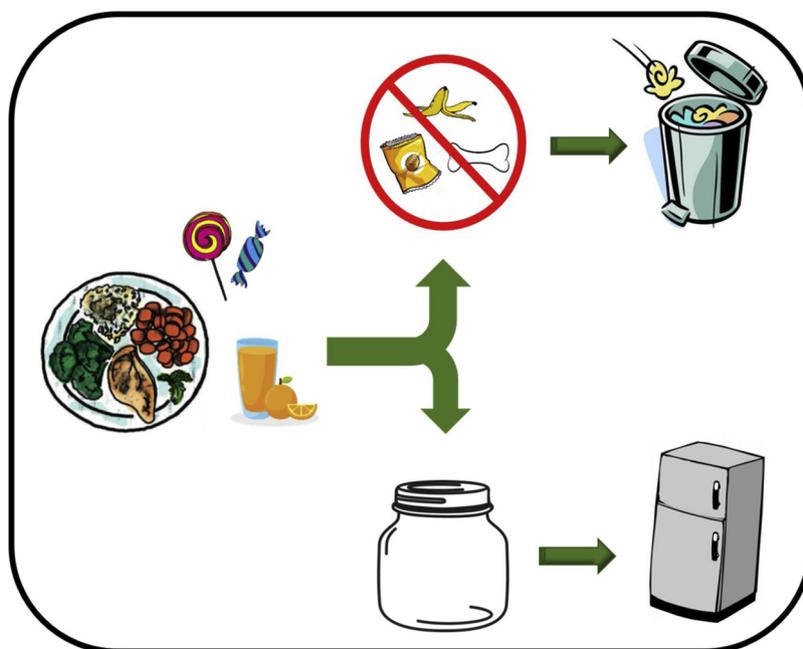


Fig. 1. Instructions given to parents and guardians about food collection for the 24-hour duplicate diet method.

Table 1
Operational conditions for ICP-MS in micronutrients analyses.

Operational conditions for ICP-MS	
Radio Frequency Power	1600 W
Argon Flow Rate	15 L min ⁻¹
He Flow	5.0 mL min ⁻¹
HeHE	10 mL min ⁻¹
Nebulizer Gas Flow Rate	0.68 L min ⁻¹
Collision Cell	Helium (purity > 99.99%)
Nebulizer Chamber	Scott (double pass)
Interface	Nickel cones
Sampling Cone	1 mm
Skimmer	0.45 mm

Black (Techline, China), was used. Children were weighed without shoes and wearing only lightweight clothing. The World Health Organization program Anthro 3.2.2 [12] was used to calculate the Body Mass Index (BMI) and the Z-scores. WHO patterns were used to classify the children’s nutritional status as follows: Z-scores lower than -2 for wasted, Z-scores between -2 and +2 for normal weight, Z-score higher than +2 for overweight, and above +3 for obesity [13,14].

2.5. Assessment of inadequate nutritional intake

The nutrients intake mean, and estimated average requirement (EAR) or adequate intake (AI) from dietary reference intake (DRI) were used to determinate the prevalence of inadequate intake for each nutrient and age group (1–3 years old and 4 years old). The EAR values are considered the best approach to the group’s intake assessment, and for nutrients which not have an EAR value, the adequate intake (AI) was used [15]. To estimate the prevalence of inadequate intake, the intake outlier values were disregarded once very high values increase the group’s intake means, which could underestimate the real prevalence of inadequacy. Those outliers were defined with a box plot graph, and the prevalence of inadequate intake was calculated with the formula [16]:

$$Z = \frac{EAR - \text{nutrient mean intake}}{\text{nutrient intake standard deviation}}$$

Results were reported using descriptive statistics as means and

standard deviation for the nutrients intake, and absolute and relative frequencies of inadequate nutritional intake. Stata 14.2 was used to perform the Chi-Square test in order to compare the frequencies.

3. Results and discussion

In this study, we evaluated the children’s intake from school and home with the 24-h duplicate method [9]. One of the most used methods in studies that evaluate nutrient intake is the 24-hour recall, but this method has some limitations, such as its dependence on the subjects’ memory of specific food items and quantities consumed and the accuracy of the food composition table, in addition to variations between the portion size related and consumed and different ways of preparing the food [10,17]. With the 24-hour duplicate diet, it is possible to assess the food exactly as it was consumed by the participants, that is, food prepared in the same way as intake by the individuals. Besides, it does not depend on the subject’s memory, as the food is collected at the moment of the meal, which decreases the possible difference between quantities actually intake and those evaluated by investigators.

This study assessed the mineral intake in children aged 1–4 years old with the 24-h duplicate diet method. To the best of our knowledge, there are no Brazilian studies that have evaluated children’s nutrient intake by this method. Internationally, studies were found with the duplicate diet method and they are presented below; however, those studies determined fewer minerals than this study. The children’s nutritional status by age is presented in Table 2. Overweight and obesity

Table 2
Distribution of 64 children according to nutritional status and age groups, São Paulo, Brazil, 2015.

Nutritional Status	1 year		2 years		3 years		4 years		Total	
	n	%	n	%	n	%	n	%	n	%
Low weight	1	20	–	–	–	–	–	–	1	2
Normal weight	4	80	17	81	15	79	16	84	52	81
Overweight	–	–	4	19	3	16	2	11	9	14
Obesity	–	–	–	–	1	5	1	5	2	3
Total	5	100	21	100	19	100	19	100	64	100

prevalence was 17%, while children with normal weight corresponded to 81%. Alves et al. [18] found a similar overweight and obesity prevalence (16.7%) in Brazilian children aged 4–6 years old. Higher prevalence was observed in other studies: Bueno et al. [19] found 20% and 7.7% of Brazilian children (2–6 years old) with overweight and obesity, respectively. Aly et al. [20] reported overweight prevalence of 11.2% and 15.2% for boys and girls, respectively, and obesity rates of 8.8% and 8.4% in boys and girls, respectively, from the Republic of Seychelles.

Childhood obesity is a major public health problem globally [21], with consequences for children's health and an increased risk for mortality and cardiometabolic morbidity in adulthood [22]. Besides the adverse consequences for children's health related to being overweight and obese, including poor quality of life, worsening school performance, alteration in prepubertal hormones, and attention-deficit hyperactivity disorder, children also experience stigma and discrimination about their weight, which is related to depression, anxiety, bulimia, and low self-esteem [23]. Beyond the risks during childhood lie the risks of becoming obese adults [24]. These conditions in childhood are also associated with significantly increased risk for type 2 diabetes, stroke, coronary heart disease, and hypertension in adulthood, in addition to being correlated with premature mortality [22]. In Latin America, concern about the obesity epidemic and increasing overweight and obesity rates in the past decades led to the development of the plan of action for the prevention of obesity in children and adolescents for 2014–2019, approved by the Pan American Health Organization (PAHO), which focuses on transforming obesogenic environments into healthy ones, where children have the opportunity to take in nutritive foods and perform physical activity [21,25].

Table 3 shows the estimated average requirements for each age group and nutrient [26–29], the intake values, and the prevalence of inadequate intake. Calcium and selenium showed the highest prevalence of inadequacy, and for all nutrients assessed, the inadequate intake rates are higher in the 4-year-old group. This probably occurred because 4-year-old children consumed food in similar amounts as the younger age group ($p < 0.05$), even though their intake requirements are increased [15]. The mean nutrient intake was also similar between the two DCCs, except for Cu, K, Mg, Se intakes, in which children from the PS DCC (South Zone) had higher intake values ($p < 0.05$).

Several Brazilian studies also analyzed mineral intake in children, although the most used method has been direct weighting associated with 24-hour recall or food diary [5,18,19,30]. The direct weighing was used mainly in the DCCs, while the recall was used to assess the foods consumed at home, and consists of observing the quantity of food offered to children, weighing them, as well as the quantities which remain on the plate. Hence, these quantities obtained are used to calculate the nutrient intake through composition food tables or nutrition software.

In this way, one of the possible reasons for the divergence of intake values found in the present study and in the literature is the difference between methods used to assess food intake, in addition to the fact that some studies evaluated only the food intake in the kindergartens or

with different age groups.

The micronutrients Fe and Ca were widely evaluated in Brazilian studies with the methods described previously. Spinelli et al. [30] analyzed with the direct weighting method the intake of these nutrients from 106 children between 6–18 months from kindergartens in São Paulo city, Brazil. In the age group older than 12 months, they found daily intakes of 5.22 ± 1.41 mg and 369.01 ± 66.16 mg for Fe and Ca, respectively. The authors evaluated only the food ingested in the school and considered the Ca intake insufficient. At the time of Spinelli's research, the government program responsible for managing kindergartens' feeding programs was the "Feeding Program for Kindergartens," which declared that 100% of nutritional requirements were offered by the school's meals for students attending full time. However, according to the current program, the National School Feeding Program, in which the school is responsible for providing 70% of the requirements, the intake adequacy could be reasonable, considering that a portion of milk or milk derivative was served at home.

Barbosa et al. [5] evaluated 35 children between 2–3 years old from Ilha de Paquetá, Rio de Janeiro, Brazil. They found similar results to Fe intake, but adequate Ca intake. They used direct weighting to assess the diet ingested by children at DCCs and the parents' report to evaluate nutritional ingestion at home. The intake means were 7.8 ± 1.8 mg and 717.4 ± 199 mg for Fe and Ca, respectively. Furthermore, also by the direct weighting method, Alves et al. [18] found lower intakes than those found in the present study for 54 children (4–6 years old) from philanthropic kindergartens in Paraná, Brazil. The Fe and Ca intakes were 3.64 ± 0.66 mg and 119.41 ± 48.41 mg, respectively, quantities lower than intake requirements for the age group.

Despite the low prevalence of inadequate Fe intake in this study and in the Brazilian studies cited, except in Alves et al. [18], Fe deficiency in children is considered a public health problem [31]. Costa et al. [32] found anemia prevalence of 20.9% in 1–4-year-old children in public kindergartens in São Paulo. Children are considered a risk group for Fe deficiency due to some childhood feeding practices such as premature weaning, because breast milk has higher Fe bioavailability than cow milk, and late introduction of foods rich in Fe [33]. Regarding adequate Fe intake found in this study there are two points that must be considered. First, the importance of National School Feeding Program, responsible for providing 70% of nutritional requirements by school's feeding, that may be contributing to the improvement of children nutritional status [6]. At last, the mandatory supplementation of wheat and corn flour commercialized in Brazil with Fe and folic acid since 2002 [34].

Malde et al. [35] evaluated with 4 days of duplicated diet, including a weekend day, the Ca and Mg intake of 30 children aged 1–5 years in two Ethiopian villages. They found a low Ca intake of 270 ± 130 mg and 370 ± 220 mg for each village, and an adequate Mg intake of 250 ± 60 mg and 240 ± 70 mg. Low Ca intake was also found in the present study, with prevalence of inadequate intake of 50% and 93.3% in children 1–3 years old and 4 years old, respectively.

An adequate Ca intake in childhood is extremely important, once the mineral has basic cellular functions as participating in metabolic

Table 3

Requirements, intake mean (mg/day or $\mu\text{g/day}$), standard deviation (SD) and prevalence of inadequate intake by age group for nutrients with estimated average requirement (EAR) values established, São Paulo, Brazil, 2015.

Element	1–3 years (n = 45)				4 years (n = 19)			
	EAR (mg/day or $\mu\text{g/day}$)	Mean (mg/day or $\mu\text{g/day}$)	SD	Prevalence of Inadequacy (%)	EAR (mg/day or $\mu\text{g/day}$)	Mean (mg/day or $\mu\text{g/day}$)	SD	Prevalence of Inadequacy (%)
Ca (mg)	500	500.8	207.3	50.0	800	461.3	223.5	93.3
Cu (μg)	260	497.2	197.4	11.5	340	512.1	193.4	18.4
Fe (mg)	3	7.6	3.2	7.4	4	9.3	5.0	15.9
Mg (mg)	65	105.8	39.4	14.7	110	105.4	45.6	54.0
Se (μg)	17	17.7	8.5	42.1	23	15.5	5.8	90.3
Zn (mg)	2.5	6.3	2.9	9.7	4	6.3	2.4	17.1

processes of vasodilatation and vasoconstriction, muscular contractions, nervous transmissions and glandular secretion, besides compounding bones and teeth, conferring upon them hardness and endurance [29,36]. The Ca demand in childhood is maximum, because of the mineralization of the growing bones, and an adequate intake during this period also contributes for prevention of obesity, insulin resistance, kidney stones and colon cancer in other life stages [36,37]. Moreover, the adequate Ca intake is essential for children's health once it can decrease gastrointestinal absorption and its toxic effects to organism from environmental contaminants such as lead [38]. Children's exposure to lead is associated mainly with learning difficulties, attention and IQ (intelligence quotient) deficits and antisocial behavior [39,40]. In this way, inadequate Ca intake may increase lead absorption by children, once the two elements compete for the same proteinic binder in intestinal mucous membrane [38]. Inverse association between blood lead levels and Ca intake in children under 5 years old was found by Lacasaña et al. [41]. In addition to that, Turgeon O'Brien et al. [42] observed decrease of 3.8% in blood lead levels with an increase of 100 mg Ca intake from diet.

The intake values for nutrients that have not EAR and the percentage of children which reached the adequate intake are presented in Table 4 [28,43]. The K intakes of the two age groups are below the recommendations: only 13.3% and 5.3% of children aged 1–3 years and 4 years respectively reached the adequate K intake. Shibata et al. [17] found very low K intake either. They assessed the food intake by duplicated diet collected in three seasons of the year for Japanese children (n = 90) aged 3–5 years old, and the K intake was 1.19 ± 0.3 g/day. They also found similar Ca, Mg and Na intake values in comparison with the present study (Table 5).

The insufficient K intake may be related to the fact that the main K food sources are fruits, legumes, and vegetables, food that children commonly reject or are resistant to eating [36]. A way of improving the children's acceptance of these foods is the encouragement to nutritional education in schools environmental, such as vegetable gardens and cooking workshops. The qualitative analysis of the children's 24-hour recalls corroborates with the low fruits, vegetables and legumes intake by the children: 44% of them intake less than two portions of these foods per day.

Another study using duplicate diet method was conducted by Cambrera-Vique et al. [44] with children and adolescents aged 4–14 years old in an orphanage in Guatemala. They collected 7 consecutive days of duplicated diet and found a Mn mean intake (1.96 mg/day) equivalent to Mn intake found in the present study, and a Se mean intake (62.4 µg/day), much higher than the present results. Children in the present study showed elevated inadequacy for Se intake, which is sustained by the analysis of the foods intake by them through the 24-hour recalls: the main Se food sources are Brazil nut and fish, and these foods were not intake by the children, except for one child who consumed one portion of fish at home. In a study conducted with an Italian community, the main Se food sources were fish and meat [45]. Concerning Se, an exposure comprehensive approach included the determination of the Se chemical species, once the inorganic forms of the element are toxic [46]. Filippini et al. [47] observed that Se inorganic levels in blood serum were associated with the consumption of fish,

vegetables and dry fruits, and inversely associated with dairy products intake, while organic Se species were associated with the consumption of mushroom, legume, potato and fresh fruits. Vicenti et al. [46] described serum Se chemical species in the same population as mostly organic, but with levels of inorganic forms above the expected.

In developing countries, Se deficiency is considered a public health problem [48]. Se composes the enzyme Glutathione peroxidase, which has antioxidant function, protecting cells from toxic substances produced by oxidative stress. In addition, Se participates in essential metabolic activities, such as thyroid hormone metabolism and immune system functioning, and has preventive action for hepatocyte damage and cardiovascular disease, in addition to having protective action against heavy metal toxicity due to the antioxidant activity. Se deficiency is also related to increased blood cholesterol and hepatic dysfunction. Lower Se levels are found in preterm infants, mainly the ones with low birth weight, and are associated with increased risk of respiratory diseases [48–50]. In addition, an extremely low Se intake may be related to an increased risk for developing the Keshan disease, serious cardiomyopathy. In that way, it is recommended that the mean Se intake by population be higher than the required for avoiding the disease incidence, as well as, the minimum requirement for this element has been defined as the amount needed to maximize selenoprotein synthesis [51].

Bueno et al. [19] in a multicenter study evaluated the food consumption of 3058 Brazilian children between 2–6 years by the direct individual weighting method combined with food diary to assess the home feeding. In this study all minerals investigated had a low prevalence of inadequate intake, except for Ca, with an inadequacy prevalence of 49% for the 4–6 years old age group. The intake means for this study were higher than ours and are also presented in Table 5.

Despite the high prevalence of inadequate Ca, Se, and K intake observed in the present study, the children's inadequate consumption and nutritional status could be worse if they were not attending the day care centers. Gagné et al. [4] reported that calcium and iron intakes were higher when children attended a childcare center, in addition to they better reach the recommended servings for grain products, fruits and vegetables. Barbosa et al. [5] assessed children's intake both at the admission moment and after six months of attending the DCC and observed that, after this period of attending school, there was a decrease in inadequate rates of Fe and Ca intake and an improvement in nutritional status, showing the important role of the school's meals in nutrient intake of children.

It is not enough to verify if the Na intake reached the nutritional requirements, since it's greater than the physiologic needs, which are related to health problems. The excessive consumption of Na by children impacts the blood pressure in childhood and influences arterial pressure in adulthood and even when the Na intake does not affect arterial pressure; it can bring adverse consequences to the cardiovascular system [52]. High Na consumption is also associated with weight gain and childhood obesity [53,54]. In this study, 23% and 42% of children with 1–3 years and 4 years old respectively had a Na intake higher than the tolerable upper level [43]. In Bueno et al. [19], this prevalence is 90% and 75% for the 1–3-year-old and 4-year-old age groups, respectively. O'Halloran [55] evaluated the food items that

Table 4

Requirements (mg/day; g/day), intake mean (mg/day; g/day), standard deviation (SD), and prevalence of inadequate intake (%) by age group for nutrients with adequate intake (AI) values, São Paulo, Brazil, 2015.

Element	1–3 years (n = 45)					4 years (n = 19)				
	AI [†]	Mean	SD	Above AI (%)	Prevalence of Inadequate Intake	AI [†]	Mean	SD	Above AI (%)	Prevalence of Inadequate Intake
Mn (mg)	1.2	2.0	1.3	88.2	Low	1.5	2.0	0.8	78.9	Low
K (g)	3.0	2.2	1.1	13.3	– ^a	3.8	2.0	0.9	5.3	– ^a
Na (g)	1.0	1.8	1.2	93.3	Low	1.2	1.8	0.9	78.9	Low

^a For nutrients whose reference values are adequate intake (AI) and the group mean ingestion is lower than the AI, nothing can be inferred about the prevalence of inadequate intake [15].

Table 5

Comparison of intake means ($\mu\text{g}/\text{day}$; mg/day ; g/day) in the present study, in a multicenter Brazilian study and in a Japanese study, which used the duplicate diet method.

Element (unit)	Present study				Bueno et al. [19]				Shibata et al. [17]			
	Brazil – duplicated diet				Brazil – direct individual weighing and food diary				Japan – duplicated diet			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	1–3 years n = 45		4 years n = 19		2–3 years ^a n = 1278		4–6 years ^a n = 1041		3 years n = 30		4 years n = 30	
Ca (mg)	500.8	207.3	461.3	223.5	821.6	241.7	804.1	244.5	416.0	150.0	477.0	177.0
Cu (μg)	497.2	197.4	512.1	193.4	1736.7	1622.2	1429.9	1022.6	–	–	–	–
Fe (mg)	7.6	3.2	9.25	5.0	13.5	2.9	13.3	2.6	–	–	–	–
Mg (mg)	105.8	39.4	105.4	45.6	261.2	62.1	254.9	47.9	104.0	27.7	115.0	31.1
Se (μg)	17.7	8.5	15.5	5.8	83.8	12.5	84.2	11.9	–	–	–	–
Zn (mg)	6.3	2.9	6.3	2.4	9.8	1.9	9.4	1.9	–	–	–	–
K (g)	2.2	1.1	2.0	0.9	–	–	–	–	1.13	0.3	1.22	0.4
Na (g)	1.8	1.2	1.8	0.9	2.2	0.4	2.3	0.4	1.5	0.7	1.6	0.4

^a Bueno et al. [19] evaluated the intake of children in both public and private kindergartens, but the results in this table are regarding only the public schools, once the present study was carried out in public day care centers.

most contribute to Na intake in 251 Australian children aged 3.5 ± 0.19 years. They found that ultraprocessed foods contributed with 48% of children's Na intake, followed by processed food, contributing with 35%. According to Hanevold [52], newborns have no preference for salty taste, but salt consumption can increase the children's interest for this taste, which shows the relevance of the parents and the caregivers in the development of feeding practices that will have a significant impact on adult patterns of consumption.

Several recent studies evaluated children nutritional intake and status, and found similar results, in addition to some associations between them. Choy et al. [56] assessed nutritional intake of Samoan children aged 2–4 years through food frequency questionnaire and also found out a concerning scenario: 21% of children exceed lipid intake recommendation, while 59% and 45% of them didn't reach Ca and K necessities, respectively. Furthermore, they observed a Na intake higher than the upper limit for 80% of children. Markiel-Pawlowska and Chalcarz [57] evaluated the nutrient intake of children aged 4–6 years from Poland, and as in the present study, they observed a high prevalence of Ca and K inadequate intake, besides a high Na intake. They also found a significative difference between boys' and girls' intake of Ca, Mg and phosphorus. In the present study, it was observed that boys and girls aged 4 years old presented a statistically significant difference for Mg intake (117.3 ± 44.6 and 92.2 ± 45.5 mg/day, respectively, $p = 0.037$), Cu (569.9 ± 109.2 and 416.0 ± 187.5 $\mu\text{g}/\text{day}$, respectively, $p = 0.047$), and Na (2.2 ± 1.0 and 1.4 ± 0.6 g/day, respectively, $p = 0.026$). Between children aged 1–3 years old, statistically different intake was observed according to nutritional status only for Mg intake ($p = 0.031$). The Mg intake was: 104.3 ± 40.1 mg/day for children with normal weigh, and 119.4 ± 34.8 mg/day for the ones with overweight and obesity.

That scenario characterized by insufficient essential nutrients intake added to an increasing prevalence of overweight and obesity can be easily associated with the consumption of ultra-processed foods. The intake of these products has been rising in the past years, and their nutritional profile (energy-dense, poor fat quality, low contents of fiber and micronutrients) matches with obesity rising rates and micronutrients insufficient intake. Furthermore, the type of carbohydrates present in ultra-processed foods lead to high glycemic loads and an increased insulin response, which can promote weight gain [58]. Recently published Brazilian studies found an association between the household availability of ultra-processed food and obesity, and ultra-processed foods consumption and obesity [58,59]. Longo-Silva et al. [60] evaluated the time that took for ultra-processed foods to be offered to Brazilian preschool children attending day-care centers: until the 6th month of life, 75% of the 359 children had already received that kind of food.

An alternative and promising strategy for combating micronutrient deficiency is the fortified fertilizing of the soils, which allows the improvement of nutritional content in food and also helps solve environmental problems, since when plants are absorbing trace elements from the soil, they could prefer to absorb an essential element instead of a toxic one. To implement this strategy, studies in chemical speciation, bioaccessibility, and bioavailability are still necessary in order to elucidate interactions between elements during their absorption and translocation in plants [48].

Although the present study brings important data about trace elements intake by children, there are some limitations to be considered. The diet evaluation of one-week day is a limitation, once one day of diet assessment does not represent the individual's habitual intake, and the prevalence of inadequate micronutrients intake can be overestimated or underestimated. However, the dietary assessment of a single day has been used for describing groups' average intake, once means are not affected by within-person variation [61].

4. Conclusions

Significant inadequacy prevalence for copper, iron, magnesium, manganese, and zinc and elevated prevalence of inadequate calcium, selenium, and potassium intake were found in children attending day care centers. In addition, the study found excessive sodium intake, which for 23% and 42% of children aged 1–3 and 4 years old, respectively, exceeds the tolerable upper level. Beyond that, the overweight and obesity rate of 17.2% is approaching the prevalence found for children at the same age in Latin America. Overall, our findings show the need to reinforce public policies aimed at promoting healthy feeding habits in childhood and nutritional education programs for children as well as their parents and caregivers. Those actions should be in the timetable planning of public health officials as extremely necessary for improving the adequacy of micronutrient intake and for preventing children from becoming overweight and obese.

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Conflict of interest

None.

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References

- [1] M. Bost, W. Sanchez, Ecotoxicology and toxicology: problems and decisions, *Environ. Sci. Pollut. Res. Int.* 25 (3) (2018) 2005–2006, <https://doi.org/10.1007/s11356-017-1044-5>.
- [2] R.L. Bailey, K.P. Jr West, R.E. Black, The epidemiology of global micronutrient deficiencies, *Ann. Nutr. Metab.* 66 (Suppl. 2) (2015) 22–33, <https://doi.org/10.1159/000371618>.
- [3] D.B. Lima, E. Fujimori, A.L. Borges, et al., Feeding in the two first years of life, *Rev. Esc. Enferm.* 45 (2011) 1705–1709.
- [4] D. Gagné, R. Blanchet, E. Vaissière, et al., Impact of a childcare centre nutrition program on nutrient intakes in Nunavik Inuit children, *Can. J. Diet Pract. Res.* Spring 74 (1) (2013) e311-7.
- [5] R.M. Barbosa, E.A. Soares, H.S. Lanzillotti, Assessment of nutrients intake of children in a charity daycare center: application of dietary reference intake, *Rev. Bras. Saude. Mater. Infant.* 7 (2007) 159–166.
- [6] Brazilian Ministry of Education, The National Programme School Feeding, (2013) (Accessed 18 January 2018), <http://www.fnde.gov.br/index.php/ae-legislacao>.
- [7] I.N. Leroux, A.P.S.D.S. Ferreira, F.P. Paniz, et al., Lead, cadmium, and arsenic bioaccessibility of 24 h duplicate diet ingested by preschool children attending day care centers in Brazil, *Int. J. Environ. Res. Public Health* 15 (8) (2018), <https://doi.org/10.3390/ijerph15081778> pii: E1778.
- [8] I.N. Leroux, A.P.S.D.S. Ferreira, J.P.D.R. Silva, et al., Lead exposure from households and school settings: influence of diet on blood lead levels, *Environ. Sci. Pollut. Res. Int.* 25 (31) (2018) 31535–3131542, <https://doi.org/10.1007/s11356-018-3114-8>.
- [9] F.V. Zohoori, M.A.R. Buzalaf, C.A.B. Cardoso, et al., Total fluoride intake and excretion in children up to 4 years of age living in fluoridated and nonfluoridated areas, *Eur. J. Oral Sci.* 121 (2013) 457–464.
- [10] G.S. Castell, L. Serra-Majem, L. Ribas-Barba, What and how much do we eat? 24-hour dietary recall method, *Nutr. Hosp.* 26 (31 Suppl. 3) (2015) 46–48, <https://doi.org/10.3305/nh.2015.31.sup3.8750>.
- [11] T. Watanabe, H. Nakatsuka, S. Shimbo, et al., High cadmium and low lead exposure of children in Japan, *Int. Arch. Occup. Environ. Health* 86 (2013) 865–873.
- [12] World health organization, WHO anthro for personal computers, Software for Assessing Growth and Development of the World's Children Version 3.2.2 (software), (2011) (accessed 15 December 2017), <http://www.who.int/childgrowth/software/en/>.
- [13] WHO Multicentre Growth Reference Study Group, WHO Child Growth Standards: Length/Height-for-Age, Weight-for-age, Weight-for-length, Weight-for-Height and Body Mass Index-for-age: Methods and Development, World Health Organization, Geneva, 2006 (Accessed 11 January 2018), http://www.who.int/childgrowth/standards/technical_report/en/.
- [14] World Health Organization, Training Course on Child Growth Assessment, World Health Organization, Geneva, 2008 (Accessed 11 January 2018), http://www.who.int/childgrowth/training/module_h_directors_guide.pdf.
- [15] Institute of Medicine, J.J. Otten, J.P. Hellwig, L.D. Meyers (Eds.), DRI - Dietary Reference Intakes: The Essential Guide to Nutrient Requirements, Institute of Medicine of the National Academies, Washington, 2006 (Accessed 10 January 2018), <https://www.nap.edu/catalog/11537/dietary-reference-intakes-the-essential-guide-to-nutrient-requirements>.
- [16] B. Slater, D.L. Marchioni, R.M. Fisberg, Estimating prevalence of inadequate nutrient intake, *Rev. Saude Pública* 38 (4) (2004) 599–605.
- [17] T. Shibata, T. Murakami, H. Nakagaki, et al., Calcium, magnesium, potassium and sodium intakes in Japanese children aged 3 to 5 years, *Asia Pac. J. Clin. Nutr.* 17 (3) (2008) 441–445.
- [18] G. Alves, E.V. Colauto, J.K. Fernandes, et al., Anthropometric and food intake assessment of preschoolers in day-care centers in Umuarama, Paraná, *Arq. Cienc Saude Unipar.* 12 (2008) 119–126.
- [19] M.B. Bueno, R.M. Fisberg, P. Maximino, et al., Nutritional risk among Brazilian children 2 to 6 years old: a multicenter study, *Nutrition* 29 (2) (2013) 405–410, <https://doi.org/10.1016/j.nut.2012.06.012>.
- [20] R. Aly, B. Viswanathan, G. Mangroo, et al., Trends in obesity, overweight, and thinness in children in the Seychelles between 1998 and 2016, *Obesity Silver Spring (Silver Spring)* 26 (3) (2018) 606–612, <https://doi.org/10.1002/oby.22112>.
- [21] M. Onis, Preventing childhood overweight and obesity, *J. Pediatr.* 91 (2) (2015) 105–107, <https://doi.org/10.1016/j.jpeds.2014.10.002>.
- [22] J.J. Reilly, J. Kelly, Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review, *Int. J. Obes. (Lond.)* 35 (7) (2011) 891–898, <https://doi.org/10.1038/ijo.2010.222>.
- [23] E.P. Williams, M. Mesidor, K. Winters, et al., Overweight and obesity: prevalence, consequences, and causes of a growing public health problem, *Curr. Obes. Rep.* 43 (3) (2015) 63–70, <https://doi.org/10.1007/s13679-015-0169-4>.
- [24] P. Gordon-Larsen, N.S. The, L.S. Adair, Longitudinal trends in obesity in the United States from adolescence to the third decade of life, *Obesity* 18 (9) (2010) 801–804.
- [25] Pan American Health Organization, 154th Session of the Executive Committee, Plan of Action for the Prevention of Obesity in Children and Adolescents, Resolution CE154.R2 PL, Pan American Health Organization, Washington (DC), 2014 (Accessed 18 January 2018), http://www.paho.org/hq/index.php?option=com_content&view=article&id=11373%3Aplan-of-action-prevention-obesity-children-adolescents&catid=8358%3Aobesity&Itemid=4256&lang=pt.
- [26] Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride, National Academies Press (US), Washington (DC), 1997.
- [27] Institute of Medicine (US) Panel on Micronutrients, Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids, National Academies Press (US), Washington (DC), 2000.
- [28] Institute of Medicine (US) Panel on Micronutrients, Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, National Academies Press (US), Washington (DC), 2001.
- [29] Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, A.C. Ross, C.L. Taylor, A.L. Yaktine, et al. (Eds.), Dietary Reference Intakes for Calcium and Vitamin D, National Academies Press (US), Washington (DC), 2011.
- [30] M.G. Spinelli, R.M. Goulart, A.L. Santos, et al., Consumo alimentar de crianças de 6 a 18 meses em creches, *Rev. Nutr.* 16 (2003) 409–414.
- [31] Centro Brasileiro de Análise e Planejamento, PNDS 2006: Dimensões Do Processo Reprodutivo E Da Saúde Da Criança, Ministério da Saúde, Brasília, DF, 2009 (Accessed 13 January 2018), <http://bvms.saude.gov.br/bvs/pnds/index.php>.
- [32] C.A. Costa, E.H. Machado, C. Colli, et al., Anemia in pre-school children attending day care centers of São Paulo: perspectives of the wheat and maize flour fortification, *J. Braz. Soc. Food Nutr.* 34 (2009) 59–74.
- [33] J.A. Braga, M.S. Vitale, Iron deficiency in infants and children, *Rev. Bras. Hematol. Hemoter.* 32 (Suppl. 2) (2010) 38–44.
- [34] Ministério Da Saúde, Programa Nacional De Suplementação De Ferro: Manual De Condutas Gerais, Ministério da Saúde, Brasília, DF, 2013 (Accessed 14 January 2018), <http://dab.saude.gov.br/portaldab/biblioteca.php?conteudo=publicacoes/manualferro2013>.
- [35] M.K. Malde, L. Zerihun, K. Julshamn, et al., Fluoride, calcium and magnesium intake in children living in a high-fluoride area in Ethiopia. Intake through food, *Int. J. Paediatr. Dent.* 14 (3) (2004) 167–174.
- [36] M.E. Shils, A.C. Ross, M. Shike, et al., Tratado De Nutrição Moderna Na Saúde E Na Doença, 2 ed., Manole, Barueri, 2009.
- [37] G.L. Silva, M.H.A. Toloni, R.C.E. Menezes, et al., Intake of protein, calcium and sodium in public child day care centers, *Rev. Paul. Pediatr.* 32 (2) (2014) 193–199.
- [38] C. Ros, L. Mwanji, Lead exposure, interactions and toxicity: food for thought, *Asia Pac. J. Clin. Nutr.* 12 (4) (2003) 388–395.
- [39] K.P.K. Olympio, C. Gonçalves, W.M. Gunther, et al., Neurotoxicity and aggressiveness triggered by low level lead in children: a review, *Rev. Panam. Salud Publica* 26 (Sep 3) (2009) 266–275.
- [40] K.P.K. Olympio, P.V. Oliveira, J. Naokusa, et al., Surface dental enamel lead levels and antisocial behavior in Brazilian adolescents, *Neurotoxicol. Teratol.* 32 (2) (2010) 273–279.
- [41] M. Lacasaña, I. Romieu, L.H. Sanin, et al., Blood lead levels and calcium intake in Mexico city children under five years of age, *Int. J. Environ. Health Res.* 10 (4) (2000) 331–340.
- [42] H. Turgeon O'Brien, D. Gagné, E. Vaissière, et al., Effect of dietary calcium intake on lead exposure in Inuit children attending childcare centres in Nunavik, *Int. J. Environ. Health Res.* 24 (5) (2014) 482–495.
- [43] Institute of Medicine (US) Panel on Micronutrients, Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate, National Academies Press (US), Washington (DC), 2005.
- [44] C. Cabrera-Vique, M. Briones, J.J. Muros, et al., A pilot duplicate diet study on manganese, selenium and chromium intakes in institutionalised children and adolescents from Guatemala, *Br. J. Nutr.* 28 (10) (2015) 1604–1611, <https://doi.org/10.1017/S0007114515003207>.
- [45] T. Filippini, B. Michalke, L.A. Wise, et al., Diet composition and serum levels of selenium species: a cross-sectional study, *Food Chem. Toxicol.* 115 (2018) 482–490, <https://doi.org/10.1016/j.fct.2018.03.048>.
- [46] M. Vinceti, P. Grill, C. Malagoli, et al., Selenium speciation in human serum and its implications for epidemiologic research: a cross-sectional study, *J. Trace Elem. Med. Biol.* 31 (2015) 1–10, <https://doi.org/10.1016/j.jtemb.2015.02.001>.
- [47] T. Filippini, S. Cilloni, M. Malavolti, et al., Dietary intake of cadmium, chromium, copper, manganese, selenium and zinc in a Northern Italy community, *J. Trace Elem. Med. Biol.* 50 (2018) 508–517, <https://doi.org/10.1016/j.jtemb.2018.03.001>.
- [48] J. Naozuka, Elemental enrichment of foods: essentiality and toxicity, *Nutri. Food Sci. Int. J.* 4 (3) (2018) 555640, <https://doi.org/10.19080/NFSIJ.2018.04.555640>.
- [49] M. Navarro-Alarcón, C. Cabrera-Vique, Selenium in food and human body. A review, *Sci. Total Environ.* 400 (1–3) (2008) 115–141.
- [50] R.G. Freitas, R.J. Nogueira, M.A. Antonio, et al., Selenium deficiency and the effects of supplementation on preterm infants, *Rev. Paul. Pediatr.* 32 (1) (2014) 126–135.
- [51] M. Vinceti, T. Filippini, S. Cilloni, et al., Health risk assessment of environmental selenium: emerging evidence and challenges (Review), *Mol. Med. Rep.* 15 (5) (2017) 3323–3335, <https://doi.org/10.3892/mmr.2017.6377>.
- [52] C.D. Hanevold, Sodium intake and blood pressure in children, *Curr. Hypertens. Rep.*

- 15 (5) (2013) 417–425, <https://doi.org/10.1007/s11906-013-0382-z>.
- [53] L. Libuda, M. Kersting, U. Alexy, Consumption of dietary salt measured by urinary sodium excretion and its association with body weight status in healthy children and adolescents, *Public Health Nutr.* 15 (3) (2012) 433–441, <https://doi.org/10.1017/S1368980011002138>.
- [54] Y.S. Yoon, S.W. Oh, Sodium density and obesity; the Korea national health and nutrition examination survey 2007–2010, *Eur. J. Clin. Nutr.* 67 (2) (2013) 141–146, <https://doi.org/10.1038/ejcn.2012.204>.
- [55] S.A. O'Halloran, C.A. Grimes, K.E. Lacy, et al., Dietary sources and sodium intake in a sample of Australian preschool children, *BMJ Open* 6 (2) (2016) e008698.
- [56] C.C. Choy, A.A. Thompson, C. Soti-Ulberg, T. Naseri, M.S. Reupena, R.L. Duckham, et al., Nutrient intake among Samoan children aged 2–4 years in 2015, *Ann. Hum. Biol.* 45 (3) (2018) 239–243, <https://doi.org/10.1080/03014460.2018.1473491>.
- [57] S. Merkiel-Pawłowska, W. Chalcarz, Gender differences and typical nutrition concerns of the diets of preschool children - the results of the first stage of an intervention study, *BMC Pediatr.* 17 (1) (2017) 207, <https://doi.org/10.1186/s12887-017-0962-1>.
- [58] M.L. Louzada, L.G. Baraldi, E.M. Steele, A.P. Martins, D.S. Canella, J.C. Moubarac, et al., Consumption of ultra-processed foods and obesity in Brazilian adolescents and adults, *Prev. Med.* 81 (2015) 9–15, <https://doi.org/10.1016/j.ypmed.2015.07.018>.
- [59] D.S. Canella, R.B. Levy, A.P. Martins, R.M. Claro, J.C. Moubarac, L.G. Baraldi, et al., Ultra-processed food products and obesity in Brazilian households (2008–2009), *PLoS One* 25 (9–3) (2014), <https://doi.org/10.1371/journal.pone.0092752> e92752.
- [60] G. Longo-Silva, J.A.C. Silveira, R.C.E. Menezes, M.H.A. Toloni, Age at introduction of ultra-processed food among preschool children attending day-care centers, *J. Pediatr.* 93 (5) (2017) 508–5516, <https://doi.org/10.1016/j.jpmed.2016.11.015>.
- [61] F.E. Thompson, A.F. Subar, Dietary assessment methodology, in: A.M. Coulston, C.J. Boushey (Eds.), *Nutrition in the Prevention and Treatment of Disease*, 2nd ed., Elsevier Academic Press, Burlington, 2008, p. 22.