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ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY
[ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE]

Volume 76 **2025** **Number 1**

OD REDAKTORA NACZELNEGO

Szanowni Państwo,

Bieżący numer otwiera artykuł J. Falandysza i wsp. wskazujący na ryzyko dla zdrowia człowieka związane z nowym zanieczyszczeniem środowiska, jakim są pierwiastki ziem rzadkich (REE) i ich obecnością w dziko rosnących grzybach wraz z przeglądem metod analitycznych wykorzystywanych do oznaczania REE. Dwie inne publikacje dotyczą również bezpieczeństwa środowiska i bezpieczeństwa żywności. Kwestia narażenia środowiskowego na pestycydy wśród rolników w Tajlandii została przedstawiona w publikacji N. Phitsadang i in., a D. Bencherit i in. w swojej publikacji ocenili spożycie barwników z żywności w grupie dzieci w Algierii. Pozostałe publikacje odnoszą się do sposobu żywienia i jego wpływu na stan zdrowia różnych grup populacyjnych. B. Mekkaoui i wsp. zaprezentowali problem niedoboru żelaza w grupie kobiet w wieku rozrodczym w Maroku, a M.K. Gacek i wsp. ocenili związek pomiędzy jakością diety a poziomem sprawności funkcjonalnej i jakością życia w grupie kobiet w wieku 60-85 lat uczestniczących w programie pn. „Aktywny Zdrowy Senior", który był realizowany przez Akademię Kultury Fizycznej im. Bronisława Czecha w Krakowie. Z kolei M. Gažarowa i N. Tobola przedstawiły wyniki własnych badań wskazujące na wpływ zarówno emocji negatywnych, jak i pozytywnych na wzrost pobrania żywności wraz z oceną składu ciała uczestników badania metodą bioimpedancji. W ostatniej publikacji tego numeru P. Pouyfung i wsp. ocenili, w grupie około 390 studentów w Tajlandii, wpływ spożycia napojów słodzonych cukrem na ryzyko gromadzenia się tkanki trzewnej.

W części *In memoriam* przedstawiamy sylwetkę Prof. dr hab. med. Wiktora B. Szostaka, wybitnego lekarza i naukowca, długoletniego dyrektora Instytutu Żywności i Żywienia, który odszedł w dniu 1 marca 2025 r.

Jednocześnie informujemy, że pod koniec 2024 r. zostały zaktualizowane i opublikowane „Normy żywienia dla populacji Polski". W bieżącym numerze zamieszczamy krótką informację o zaktualizowanych normach, zachęcającą do zapoznania się z tą publikacją.

Zapraszam do czytania i publikowania w Rocznikach PZH. Jednocześnie uprzejmie informuję, że rok 2025 jest ostatnim, w którym Roczniki PZH będą się ukazywały w formie papierowej.

Z poważaniem,

Prof. dr hab. Hanna Mojska
Redaktor naczelna
Roczników Państwowego Zakładu Higieny

EDITORIAL INTRODUCTION

Ladies and Gentlemen,

The current issue opens with an article by J. Falandysz et al. indicating the risk to human health associated with a new environmental pollutant, rare earth elements (REE) and their presence in wild mushrooms, together with a review of analytical methods used to determine REE. Two other publications also address environmental and food safety. The issue of environmental exposure to pesticides among farmers in Thailand was presented in the publication by N. Phitsadang et al., while D. Bencherit et al. in their publication assessed the intake of food dyes in a group of children in Algeria. Other publications refer to nutrition and its impact on the health of various population groups. B. Mekkaoui et al. presented the problem of iron deficiency in a group of women of reproductive age in Morocco, and M.K. Gacek et al. assessed the relationship between the quality of diet and the level of functional fitness and quality of life in a group of women aged 60-85 participating in the program called 'Active Healthy Senior', which was implemented by the University of Physical Education in Krakow. In turn, M. Gažarova and N. Tobola presented the results of their own research indicating the influence of both negative and positive emotions on the increase in food intake along with the assessment of the body composition of the study participants using the bioimpedance method. In the last publication in this issue, P. Pouyfung et al. assessed the effect of sugar-sweetened beverage consumption on the risk of visceral fat accumulation in a group of approximately 390 students in Thailand.

In the *In memoriam* section, we present the profile of Professor Wiktor B. Szostak, an outstanding physician and scientist, long-time director of the Institute of Food and Nutrition, who passed away on March 1, 2025.

We would also like to inform you that the end of 2024, the 'Dietary Reference Values for the Polish population' were updated and published. In the current issue, we provide a brief information about the updated DRVs, encouraging you to read this publication.

I invite you to read and publish in the journal Roczniki Państwowego Zakładu Higieny. At the same time, I would like to kindly inform you that 2025 is the last year in which our journal will be published in paper form.

Kind regards,

Prof. Hanna Mojska, PhD
Editor-in-Chief
Roczniki Państwowego Zakładu Higieny

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REWIEV ARTICLE

RARE EARTH ELEMENTS (REE) IN WILD MACROFUNGI: A REVIEW HIGHLIGHTING THE IMPORTANCE OF REQUISITE ANALYTICAL METHODOLOGY ON DATA QUALITY

Jerzy Falandysz[1], *Alwyn R. Fernandes*[2], *Anna Kilanowicz*[1], *Heesoo Eun*[3]

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ABSTRACT

The accelerating global use of lanthanides in modern consumer goods has introduced a new source of environmental pollution and potential health hazards. Evaluating risk for human exposure to these rare earth elements (REE) is hampered by limited occurrence data in foods, partly because reliable, sensitive and accurate determination is challenging. An objective of this work was to critically review lanthanide occurrence in fruiting bodies (mushrooms) of edible wild terrestrial (epigeic) and subterranean (hypogeic) macrofungi and their soil substrates, while also assessing the reported data for analytical quality. Given the paucity of information, all available literature on lanthanides in wild mushrooms was considered. Key requirements for credible REE determination in fungal biomass include avoiding cross contamination from substrates, exclusion of spectral/non-spectral interferences through robust purification and selective, sensitive measurement procedures, inclusion of the full range of lanthanides and strict quality control. In general, both high and lower resolution ICP-MS techniques were evidentially able to provide more reliable outcomes if these requirements were followed. A second objective was to propose a rational approach to assess data reliability by combining the above methodological attributes with the characteristics of lanthanide occurrence in mushrooms: (i) adherence to Oddo-Harkins order, visualised as a descending sawtooth pattern – a result of unfractionated uptake and accumulation of lanthanides from soils and other substrates (ii) typical individual concentration ratios (e.g., La/Sm, Ce/Nd, Ce/Sm) that indicate reliable determination, (iii) bio-exclusion of lanthanides by wild fungi (bioconcentration factors < 1). Data from studies that met these requirements confirmed typically low concentrations (0.07 μg kg^{-1} of Lu in *Suillus luteus* to 940 μg kg^{-1} of Ce in *Cantharellus minor*) with patterns corresponding to Oddo-Harkins order across reported fungal types, maintaining the unfractionated REE substrate patterns. However, given the upward trend in REE usage, the continued monitoring of macrofungi is prudent.

Keywords: *fungi, forest soils, macrofungi, emerging metal pollutants, Oddo-Harkins order, rare earth elements distribution patterns, REE*

INTRODUCTION

The rare earth elements (REE) include the fifteen lanthanides with atomic numbers 57-71 (Lanthanum, La 57; Cerium, Ce 58; Praseodymium, Pr 59; Neodymium, Nd 60; Promethium, Pm 61; Samarium, Sm 62; Europium, Eu 63; Gadolinium, Gd 64; Terbium, Tb 65; Dysprosium, Dy 66; Holmium, Ho 67; Erbium, Er 68; Thulium, Tm 69; Ytterbium, Yb 70; Lutetium, Lu 71), although some studies and organisations such as the IUPAC also include yttrium (Y 21) and scandium (Sc 39) because they exhibit similar chemical characteristics and often occur in the same mineral deposits [1-3]. In some sub-classifications, individual REE may be included in more than one group, e.g. lighter atomic weight elements (LREE) may include elements from La to Pr or Nd, with or without Sc; medium atomic weight REE (MREE) may include elements from Nd to Dy or Ho) and heavier REE (HREE) could include elements from Dy or Ho to Lu, with or without Y [4, 5]. They are considered as strategic metals and are essential components in a range of military equipment and consumer products such as sonar, radar, guidance systems, cellular telephones, computers and electric vehicles [6]. Unlike typical ores which contain significant deposits of some metals, REE are more widely distributed throughout the Earth's crust and soils (Figure 1) [4]. Individual environmental abundances of REE and their applications vary strongly depending on the

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element, e.g., Ce and Nd together with La, Pr and Sm which are LREE, are dominant in the Earth's crust, REE-bearing deposits and soils including forest soils. By mass, the LREE dominate the total REE volume used in applications [6-8]. Each REE has a practical but sometimes very minor specific application, including those elements that have a low occurrence in deposits and command a high price [6]. The currently known geological deposits of REE show a wide global distribution, e.g. major deposits are known at Mountain Pass in the US, Bayan Obo and other ion-adsorption deposits occur in Southern China, Myanmar and Mount Weld in Australia [9].

The rising demand for REE has encouraged more exploration for new deposits and has increased industrial activity involving extraction and processing. These activities that include primary and secondary (urban) mining, application and disposal of REE, Y and Sc products, have in recent time, raised concerns regarding the pollution of the natural environment and food webs by these "emerging contaminants" of concern [10-15]. Human activities such as oil refining that use zeolitic fluid catalytic converters, coal combustion or metal ore smelting and refining, result in emissions of particulate matter that contain a whole range of REE or an individual compound [16-21], which can potentially be emitted and deposited in the surrounding environment including forested land. Although the depositions from these thermal releases may be detectable in media that are in close vicinity to the source [21], they are not discernible when dispersed to forest soils on a wider or global scale (Figure 1) [22, 23]. Similarly, Gadolinium (Gd) is known locally or regionally as a contaminant of aquatic freshwater environments as well as drinking water and beverages but not of urban dust/soils or forest soils [1, 23-26]. This arises from its application in gadolinium-based contrast agents in medical magnetic resonance imaging, which helps to improve image clarity of internal structures, but are subsequently excreted through urine to waste waters.

Foods that are foraged from the wild either for economic reasons or as a leisure activity include numerous species of macrofungi. Some, such as chanterelles, *Cantharellus cibarius* (northern Hemisphere), boletes such as *Boletus aestivalis*, *B. bainiugan*, *B. edulis, B. pinophilus*, *Butyriboletus roseoflavus* (Europe/Asia), matsutake *Tricholoma matsutake* (Japan) are particularly prized foods while alba or white truffle *Tuber magnum* (Italy) are commercially very valuable. However most species of fungi, whether edible or not, can be impacted by anthropogenic or geogenic contamination such as As, Cd, Hg, Pb and other organic contaminants [27-29], the extent of which is a function of physiology, the biogeochemistry of the growing substrate and local pollution. Common examples of the resulting bioaccumulation are selenium which is beneficial or conversely, radioactive caesium which is toxic [30, 31].

Mushrooms, the edible fruiting bodies of many wild macromycetes (and in some cases, sclerotia) are popular foods or delicacies as in the case of species such as truffles, but over recent decades global demand has seen an increasing trend in the production of cultured species [32]. The global supply of edible mushrooms in 2021 was 44.2 million tons, of which China was a major producer, supplying 41.1 million tonnes [32], particularly from the Yunnan province which is the largest producer, consumer and exporter of wild mushrooms. In 2011, production reached 70,000 tonnes [33]. In contrast, the estimated annual quantity of wild mushrooms foraged from Polish forests amounted to 99.0 tonnes (mean value for 2006-2008). Of this total volume, 29.7 tonnes were purchased for industrial processing and export, 9.9 tonnes for roadside and market sales, leaving 59.4 tonnes for personal consumption [34]. Globally, a number of countries are home to discrete population groups that have a tradition of foraging wild mushrooms, either to supplement their food supplies or as hobbyists and gourmets who prize these foods, maintaining and fostering a tradition of identifying and collecting wild edible species [35, 36].

In recent decades, following the increasing commercialisation of REE, some studies have begun reporting the concentrations of these elements in the environment and in vegetation. This information is important to the consideration of REE as potential emerging contaminants which could pose risks for consumers, but good data quality is essential for an evidential and objective assessment. A few dozen review articles including books on various aspects of environmental occurrences of REE and potential risks associated with their production have been published since 2000, but none of these have included data on wild mushrooms and their habitats – forest topsoils and plant substrates [4, 5, 9, 11, 22, 36-46].

This is the first critical review of REE occurrence in wild macrofungi and their mycelial substrates which considers the quality and reliability of the applied determination methodologies. These attributes are crucial to the understanding of the trends in the occurrence of REE in fungi and in the environment because as seen in recent literature [47, 48] some reports of elevated levels may not be substantiated. The review used an open literature search using ISI Web of Science, Mendeley and Google Scholar (keywords: lanthanides, REE, rare earth elements, fungi and mushrooms) and additionally, targeted literature with documented analytical methodologies. It discusses studies published from 2001, the year when the first reports on all 14 REE (lanthanides) in wild mushrooms

using analytical methodologies for fungal biomass became available [2, 13, 23, 49-71]. Such data is scarce and a collation would be helpful for comparison of concentrations between species, assessing risk for the dietary intake of edible fungal parts and for examining any trends in occurrence. In recent years, some studies on Lanthanide contamination in fungi appeared to show elevated levels of some of these elements but critical reviews [47, 48, 64-67] suggested that stricter analytical control and pre-assessment of the data could lead to different outcomes. Thus, in addition to reviewing the quality of the analytics this review also assesses the reported data for natural distribution patterns of occurrence, concentrations and bioconcentration potential, and discusses the main requirements for providing reliable and credible data on REE in mushrooms.

REE IN MUSHROOMS, FOREST SOILS AND PLANT SUBSTRATES

REE display a relatively narrow range of atomic weights and also of ionic and atomic radii, and a unique electronic configuration (all exist as stable +3 ions) and Ce can easily oscillate between +3 and +4 oxidation states (the only lanthanide stable in the +4 oxidation state at physiological conditions) [4, 15]. Consequently, as a group they are characterised by similar physical and chemical features, which also influence their similar fate in the geobiosphere [22]. Apart from this common stable ionic form, Sm, Eu, Tm and Yb can also occur in the +2 oxidation state but only Eu^{2+} ions are sufficiently stable in natural aqueous solutions. In addition to Ce, Pr, Nd, Tb and Dy have also been reported to occur in the +4 oxidation state [4, 5, 25]. The natural occurrence of the lanthanides in biotic as well as abiotic environments including substrates for wild mushrooms (forest soils and various plant derived organic substrates) is characterised by a typical pattern when concentrations are plotted (Figure 1). This well-defined pattern results from the Oddo-Harkins (O-H) order of elemental abundances [77]. The O-H order states that elements, e.g. lanthanides, having an even atomic numbers as the atomic number increases are always an order of magnitude more abundant (higher Clarke concentrations) than the adjacent odd-numbered lanthanides, and display a larger number of isotopes [1]. The resulting order of lanthanide occurrence in most matrices appears as a descending sawtooth or zigzag pattern dominated by Ce, which endures despite the biodiversity of macrofungi or varying geochemical (forest soil or bedrock type) conditions (Figures 1-3, Tables 1-2). This pattern results from the undiscriminated mycelial uptake of individual lanthanides and is observed in both, wild and cultivated species, despite varying elemental

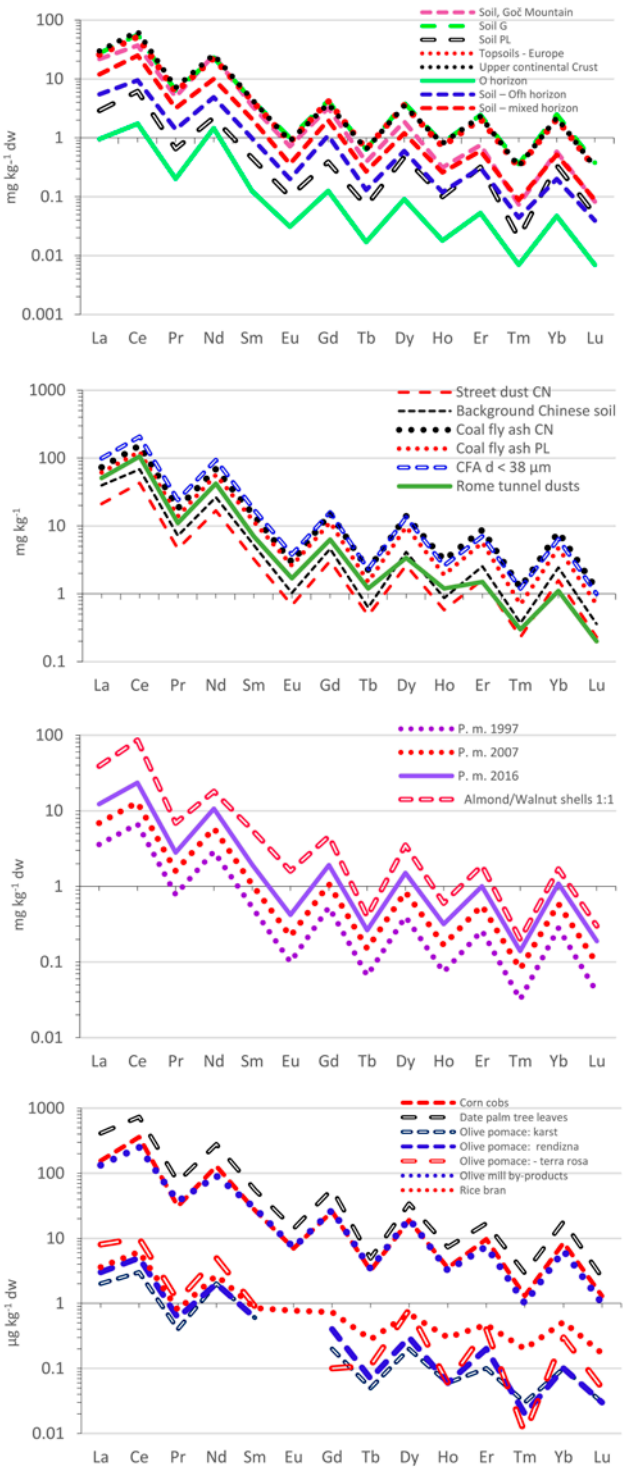

Figure 1. Distribution pattern of lanthanides concentrations in various environmental materials (forest topsoils – Serbia, Germany and Poland [8, 23, 61], topsoils in Europe) [7], upper continental crust [72], forest soil O horizon – Sweden [54], forest soil and mixed horizons – Poland [73], street dust and background soils and coal fly ash – China [86], coal fly ash – Poland and coal fly ash (< 38 µm fraction) – United Kingdom [19], tunnel dust in Roma – Italy [74], annual rings of *Pinus massoniana* – China [75], mixt of almond and walnut shells – Greece, corn combs and data palm tree leaves – Greece [78], olive pomace from olive trees grown on karst, rendzina and terra rosa soils – Adriatic Sea coastal region, olive mill by-product – Greece [78] and rice bran – Japan [76], adapted.

concentrations that arise from differences in species, the location of the sampling site, the soil or other media in which the mycelium develops, including prepared fungal compost [23, 78, 101]. Therefore, according to the O-H order, REE elements Er, Tm, Yb and Lu show the lowest concentration levels in natural environmental matrices including mushrooms, and may be several orders of magnitude lower than the most abundant REE. Practically, this can be observed in the currently reviewed data listed in Tables 1-2. The lowest concentrations are seen for the lanthanides with high atomic masses (the lowest reported concentrations is 0.07 µg kg^{-1} dw of Lu in whole fruiting bodies of *S. luteus* while the highest value of 940 µg kg^{-1} dw was reported for Ce in *C. minor*. An exception is the peridium – a thin external skin covering the flesh (gleba) of a truffle – with Ce concentrations ranging from 2100 to 4000 ± 5200 µg kg^{-1} (Table 2). Additionally, if the occurrence of individual lanthanides are logarithmically plotted, the descending sawtooth pattern persists (Figure 3) despite the wide range of species and locations (including biogeochemical and pollution profile differences) from which these fruiting bodies were collected [49, 54, 62, 71, 101].

Some plants, e.g. ferns, *Pteropsida*, species like *Athyrium yokoscence*, *Dicranopteris dichotoma* or *Dryopteris erythrosora* will accumulate REE from soil [14, 79], and further participate in humus genesis in the highly organic layer of the forest soil horizon which is essential for mycelial development and the nutrition of many fungal species [80]. Thus, the composition of REE in ecosystems is strongly influenced by their genesis in soils, and their compositional pattern in biota is considered to directly reflect this origin [81, 82]. This typical sawtooth distribution is also seen in soil substrates (that are the habitat of many fungal species) from European forest topsoils collected in the last decade at mushroom collection sites and show the lack of site specific or regional specific discrimination between individual REE [8, 23, 61, 71, 101].

This collective behaviour that preserves the O-H order underlines the lack of fractionation by individual REE which is reported by a number of studies examining elemental contents of mushrooms (Figures 3-6, Tables 1-2). For example, several fungal species (*Caloboletus calopus*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Imleria badia*, *Laccaria amethystina*, *Lactifluus piperatus*, *Leccinum scabrum*, *Suillus grevillei* and *Sutorius brunneissimus*) collected in Belarus, China and Poland did not show any signs of the fractionation of the patterns of lanthanides or Y [23], nor *Pleurotus ostreatus* and *Cyclocybe cylindracea* cultivated on a range of substrates [78] or many other edible wild species collected in Poland and Croatia [49, 63]. However, in *Suillus luteus*, examined using the same methodology (and laboratory) as in article

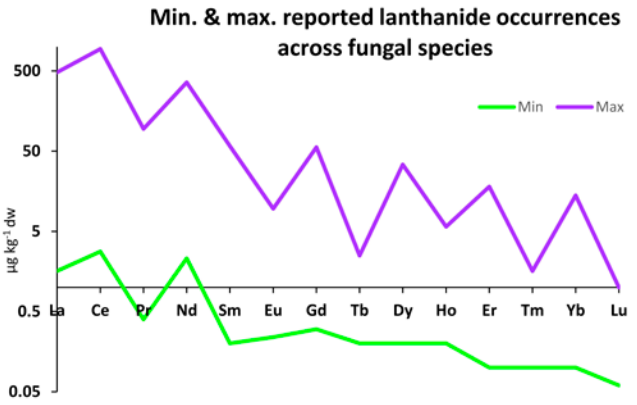

Figure 2. A plot of the range of lanthanide occurrences in mushrooms shows that the Oddo-Harkins order persists despite the different species, different biogeochemistry, varying pollution profiles and different data providers (Plot data taken from Table 1).

[23], a positive Y anomaly was detected [8], which was not detected in other *Suillus* species in independent studies [23]. Also several species of ectomycorrhizal and saprobic fungi in some other studies did not show the Y anomaly [59, 61, 63, 71, 101]. The Eu anomaly (negative and positive) has been noted in some studies, i.e. *Macrolepiota procera* and a range of saprotrophic and ectomycorrhizal species [49, 56, 59], but equally, this species did not show the Eu anomaly [61], nor did the mushrooms in some other studies. It has been reported that the Eu anomaly could be an artefact of spectrometric analysis [83, 84].

Notwithstanding their dispersion in terrestrial and marine environments and their trace or ultra-trace presence in foods of plant and animal origin, REE are not considered as essential in biology and or to human nutrition, as far as is presently known, e.g. Ce, the most abundant REE has no known biological role [85]. Nevertheless, methylotrophic bacteria from harsh environments, e.g. thermoacidophilic *Methylacidiphilum fumariolicum*, and *Methylorubrum extorquens* and *Methylobacterium radiotolerans* have been reported to utilise lanthanides (La, Ce, Pr, Nd) in methanol dehydrogenases in a similar manner to calcium [86-88].

EFFECT OF SOIL, DUST AND SAND ON DRIED FUNGAL MATERIALS FROM HERBARIA

Although REE are commonly defined as “rare”, Ce, which is the most abundant of the lanthanides, is arguably as abundant as the bio-metal zinc (Zn), and is more abundant than tin (Sn) or lead (Pb) [85]. Soils and sands at locations in China are generally richer in REE than elsewhere in the world [43, 89]. Stijve et al. [53] were the first to document that soil dust (soil and

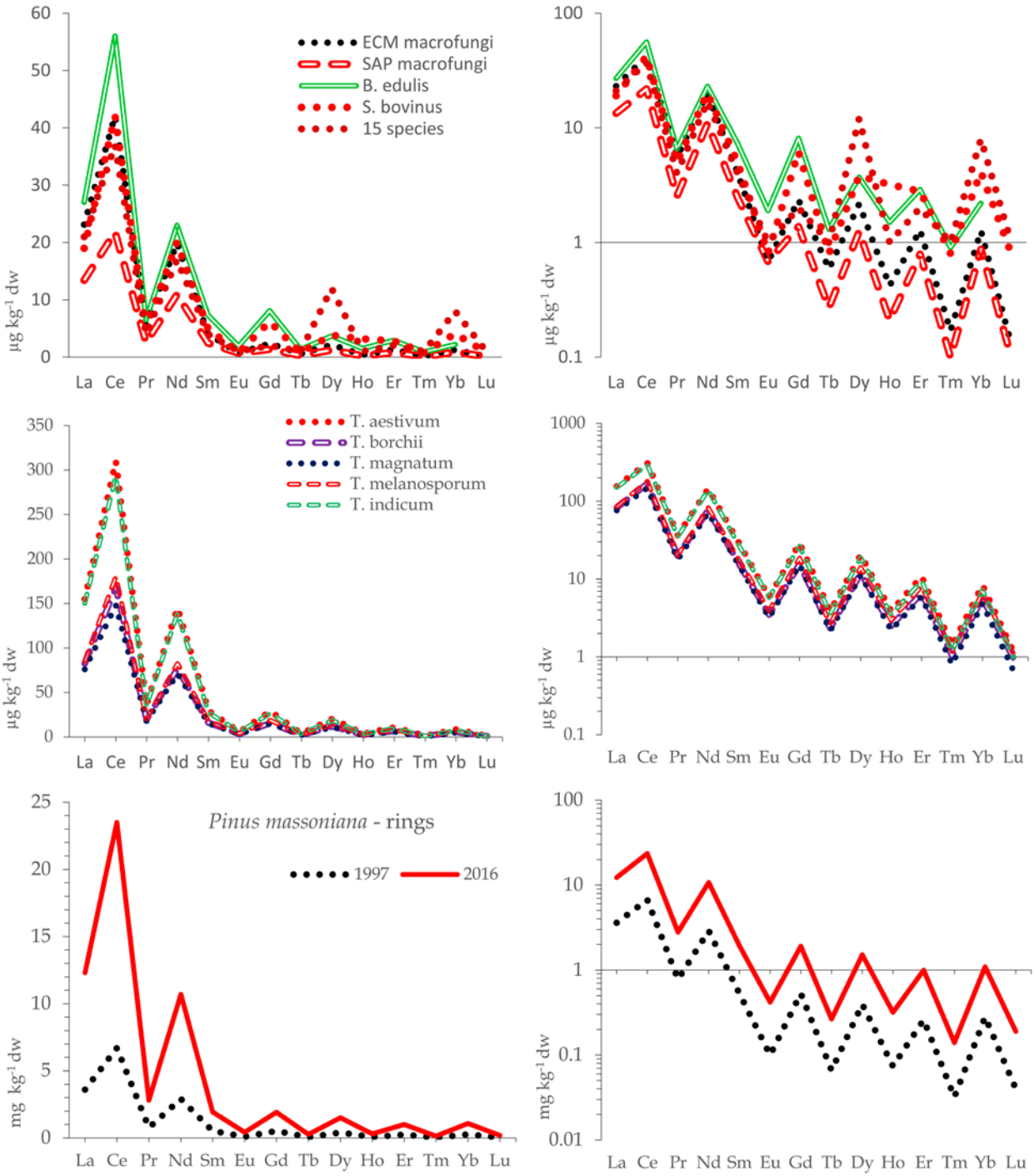

Figure 3. Distribution of REE – natural sawtooth concentrations pattern in ectomycorrhizal and saprobic mushrooms and the species *Boletus edulis* – King Bolete and *Suillus bovinus* – Cow bolete (upper plots), black (*Tuber melanosporum*, *T. aestivum* and *T. indicum*) and white truffles (*T. magnatum* and *T. borchii*) (middle plots) – as determined by direct aspiration of an acid oxidized digest into a sector field mass spectrometer and in annual tree rings of *Pinus massoniana* (bottom plots) – by quadrupole mass analyzer, after [49, 56, 62, 75].

sand particles) adhered to fruiting bodies can cause a spurious increase in actual REE concentrations. In their first study, Stijve et al. determined La, Ce, Nd, Gd, Sm, Er and Dy (also Ag, Al, Ca, Co, Fe, Ga, Mo, P, Pb, Th, V and Y) in fruiting bodies of *Albatrellus pes-caprae* (current name, *Scutiger pes-caprae* (Pers.) Bondartsev & Singer) collected in Switzerland, Germany and the USA. They also reported on the sum of Ce, La and Nd (also Al, Ca, Fe and Th) in various wild and cultivated mushrooms: "*Agaricus bisporus*, *A. arvensis*, *Agaricus bitorquis*, *Agaricus gaestrani*, *Agaricus silvicola*, *Boletopsis grisea*, *Boletopsis*

Table 1. Concentration (µg kg^{-1} dw) of lanthanides, yttrium and scandium in terrestrial fungi biomass (means and uncertainty) and selected analytical method parameters – adapted from the references cited – all data rounded for two significant figures if different from zero

Species / element	King Bolete			Slippery Jack		Cow Bolete	Larch Bolete	Hedgehog Fungus	Yellow or Golden Chanterelle		Small Chanterelle	Bitter Beech Bolete	Various mushrooms
	B. edulis			*S. luteus*		*S. bovinus*	*S. grevillei*	*H. repandum*	*C. cibarius*		*C. minor*	*C. calopus*	*15 species*
	C* *n* = 1 (3)	W *n* = 1 (1)	W *n* = 14 (261)	C *n* = 1 (3)	W *n* = 1 (3)	C *n* = 1 (3)	C *n* = 1 (4)	W *n* = (4-5)	W *n* = 3 (146)	W *n* = 22 (2235)	W *n* = 1 (153)	C *n* = 1 (11)	*n* = 19
La	27 ± 14	14	51	5.3 ± 2.5	6.4	19 ± 23	15	1.6-2.2	55 (22-91)	27	480	41	21 (3-93)
Ce	56 ± 25	24	95	9.3 ± 4.7	11	42 ± 56	28	2.8-3.6	120 (51-210)	35	940	105	37 (6-140)
Pr	6.5 ± 3.9	2.8	9.7	1.7 ± 0.6	1.2	5.0 ± 6.1	0.5	0.4-0.5	16 (12-22)	2.3	95	9.4	4 (1-17)
Nd	23 ± 12	10	32	6.7 ± 2.3	4.7	20 ± 22	15	2.3-3.5	46 (20-79)	9.2	360	38	17 (2-63)
Sm	7.3 ± 4.2	2.3	5.5	2.0 ± 1.0	1.1	5.0 ± 5.2	2.8	0.2-0.3	8.3 (3.9-14)	3.1	58	8.0	4 (1-11)
Eu	1.9 ± 0.6	0.52	1.1	0.67 ± 0.0	0.24	0.80	0.5	≤ 0.1	1.4 (0.7-2.2)	0.5	9.6	2.1	1 (< MQL-2)
Gd	8.1 ± 3.5	2.7	5.1	1.4 ± 0.8	1.2	6.2 ± 6.5	2.4	0.3	5.6 (3.2-11)	2.2	56	8.2	2 (< MQL-2)
Tb	1.3 ± 0.6	0.38	0.9	WD	0.17	0.80	0.3	< 0.1	0.9 (0.5-1.3)	0.5	2.5	1.2	1 (< MQL-2)
Dy	3.7 ± 2.5	2.3	5.0	1.1 ± 0.5	1.0	3.8 ± 3.7	1.8	0.2-0.3	4.6 (2.6-7.0)	2.0	34	6.9	12 (8-20)
Ho	1.5 ± 1.2	0.44	1.0	0.70	0.19	3.2	0.3	< 0.1	0.8 (0.5-1.1)	0.5	5.7	1.3	1 (< MQL-3)
Er	2.9 ± 1.5	1.2	3.4	0.65 ± 0.07	0.58	2.8 ± 2.8	0.8	0.1-0.2	2.0 (1.3-2.8)	2.5	18	3.4	2 (< MQL-6)
Tm	0.90	WD	0.6	0.70	WD	0.80	WD	< 0.1	WD	0.5	1.6	WD	1 (< MQL-1)
Yb	2.2 ± 1.2	0.90	3.2	0.70	0.42	4.1 ± 3.5	0.7	0.1-0.2	1.4 (1.0-1.8)	0.5	11	2.7	8 (6-14)
Lu	WD	0.12	0.5	0.70	0.066	0.80	0.1	< 0.1	0.2 (0.1-0.3)	0.5	< 1.0	0.3	1 (< MQL-1)
∑REE	140	62	310	32	36	110	43	WD	260 (110-440)	77	2070	190	120 (31-450)
Y	WD	18	42	WD	8.7	WD	4.1	1.3-1.6	23 (14-30)	22	200	44	16 (2-82)
Sc	WD	WD	35	WD	WD	WD	WD	10-20	WD	< 1.0	66	WD	34 (4-99)
Sample (mg)	WD	50	200	WD	150	WD	1000	750	1000	1000	1000	1000	50
Digestion	HNO_3	HNO_3/HCl	HNO_3/HF	HNO_3	HNO_3	HNO_3	HNO_3/HCl	HNO_3	HNO_3/HCl	HNO_3/HCl	HNO_3/HCl	HNO_3/HCl	HNO_3/HF
Acid volume	5 mL	3 + 1 mL	3 + 1 mL	5 mL	5 mL	5 mL	15 + 2.5 mL	10 mL	15 + 2.5 mL	15 + 2.5 mL	15 + 2.5 mL	15 + 2.5 mL	7 + 0.1 mL
S & P	No	Yes	No	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No
Measurement	ICP-SFMS	ICP-QMS	ICP-MS	ICP-SFMS	ICP-QMS	ICP-SFMS	ICP-QMS	ICP-MS	ICP-QMS	ICP-QMS	ICP-QMS	ICP-QMS	ICP-SFMS
Instrument	PlasmaTrace, VG Elemental	NexION 300 ICP-MS	ELAN DRC-e	PlasmaTrace, VG Elemental	NexION 300 ICP-MS	PlasmaTrace, VG Elemental	NexION 300 ICP-MS	ELAN-6000	NexION 300 ICP-MS	NexION 300 ICP-MS	NexION 300 ICP-MS	NexION 300 ICP-MS	Element 2, Thermo Scientific
Reference	[49]	[2]	[100]	[49]	[8]	[49]	[23]	[54]	[23]	[68]	[68]	[23]	[63]

Notes: *C (caps)/W (whole) and number of pools/samples (and total number of fruiting bodies); WD (without data); S & P (separation and pre-concentration).

Table 2. Concentration (μg kg^{-1} dw) of lanthanides, yttrium and scandium in subterranean (truffles) and terrestrial fungi biomass (means and uncertainty) and selected analytical method parameters – adapted from the references cited – all data rounded for two significant figures if different from zero

Species/ element	Summer truffle*			Whitish truffle*	White truffle (n=13)		Black truffle*	Asian black truffle*			Saprobic fungi	Mycorrhizal fungi	Field parasol*
	T. aestivum			*T. borchii*	*T. magnatum*		*T. melanosporum*	*T. indicum*					
	Peridium	Gleba (flesh)	Peridium	Peridium	Peridium	Blend	Peridium	Peridium	Peridium	Gleba (flesh)	WD	WD	C**
	n = 25	WD	*n* = 26	*n* = 4	*n* =13	*n* = 9	*n* = 8	*n* = 8	*n* = 1	*n* = 1	*n* = 25	*n* = 25	*n* = 19
La	520 ± 400 // WD	79	160 ± 170	82 ± 69	76 ± 46	10 0 ± 130	86 ± 59	150 ± 140	910	34	13	23	66 ± 60
Ce	4000 ± 5200 // 4300 ± 5700	140 // 350 ± 210	310 ± 380	170 ± 14	150 ± 87	190 ± 150	180 ± 120	290 ± 320	2100	73	22	42	190 ± 200
Pr	660 ± 620 // WD	WD	37 ± 42	20 ± 17	18 ± 11	24 ± 150	20 ± 14	35 ± 34	250	10	2.5	5.6	14 ± 14
Nd	1700 ± 2100 // WD	42 ± 40	150 ± 170	77 ± 62	71 ± 43	97 ± 160	82 ± 52	140 ± 130	1000	38	11	20	67 ± 58
Sm	390 ± 400 // WD	WD	30 ± 34	16 ± 12	15 ± 10	21 ± 160	18 ± 12	27 ± 25	240	10	2.5	4.1	21 ± 20
Eu	WD // 6.4 ± 77 [INAA]	WD // 6 [INAA]	5.8 ± 6.1	3.5 ± 2.3	3.2 ± 2.0	5.4 ± 180	3.9 ± 2.6	5.8 ± 4.3	68	3	0.68	0.68	3 ± 3
Gd	350 ± 430 // WD	WD	29 ± 32	16±11	15 ± 9.7	21 ± 140	19 ± 11	27 ± 23	230	10	1.4	2.3	11 ± 11
Tb	62 ± 54 // 68 ± 58	WD	3.9 ± 4.2	2.3 ± 1.7	2.2 ± 1.4	3.1 ± 210	2.7 ± 1.9	3.6 ± 3.0	46	2	0.27	0.59	1.5 ± 1.3
Dy	260 ± 31 // WD	WD	21 ± 22	12 ± 8.0	11 ± 6.7	18 ± 180	14 ± 9.7	19 ± 16	200	8	1.2	2.2	6.6 ± 6.2
Ho	68 ± 59 // WD	WD	3.9 ± 4.0	2.5 ± 1.7	2.2 ± 1.4	3.3 ± 260	3.0 ± 2.2	3.5 ± 2.8	48	2	0.21	0.42	1.2 ± 1.1
Er	150 ± 180 // WD	WD	11 ± 11	6.6 ±**4.4**	6.0 ± 3.4	9.5 ± 200	8.1 ± 5.5	9.5 ± 7.7	120	5	0.79	1.3	2.8 ± 2.5
Tm	25 ± 20 // WD	WD	1.4 ± 1.4	1.0 ± 0.8	0.80 ± 0.47	1.3 ± 260	1.2 ± 1.1	1.2 ± 1.0	24	1	< MQL	0.17	1.1 ± 0.7
Yb	210 ± 190 // WD	WD	8.4 ± 8.5	5.7 ± 3.8	4.8 ± 2.7	7.9 ± 210	6.7 ± 4.5	7.2 ± 5.8	97	4	0.87	1.3	2.4 ± 2.1
Lu	28 ± 21 // WD	WD	1.2 ± 1.2	0.99 ± 0.75	0.68 ± 0.40	1.2 ± 250	1.1 ± 1.0	1.0 ± 0.8	23	1	0.10	0.13	0.4 ± 0.3
∑REE	8423 // WD	WD	770	416	376	503	446	720	5356	201	56	104	388
Y	1500 ± 1600// WD	190 ± 61	110 ± 110	70 ± 46	65 ± 43	WD	99 ± 54	110 ± 86	1307	46	WD	WD	30 ± 27
Sc	WD // 390 ± 480 [INAA]	22 ± 16 [INAA]	57 ± 67	29 ± 31	33 ± 20	WD	24 ± 25	47 ± 37	1022	17	WD	WD	113 ± 73
Sample (mg)	250	250	100	100	100	50	100	100	100	100	250-350	250-350	500
Digestion	HNO_3/HF/H_3BO_3	HNO_3/HF/H_3BO_3	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3/H_2O_2	HNO_3	HNO_3	HNO_3/H_2O_2
Acid volume	6+2 mL /+12 mL	6 + 2 mL /+ 12 mL	4 + 1 mL	4 + 1 mL	4 + 1 mL	1 + 4 mL	4 + 1 mL	4 + 1 mL	4 + 1 mL	4 + 1 mL	WD	WD	7 + 1 mL
S & P	No	No	No	No	No	No	No	No	No	No	No	No	
Measurement	ICP-MS // INAA	ICP-MS // INAA	ICP-Q-MS	ICP-Q-MS	ICP-Q-MS	ICP-MS	ICP-Q-MS	ICP-Q-MS	ICP-Q-MS	ICP-Q-MS	ICP-SFMS	ICP-SFMS	ICP-MS
Instrument	PerkinElmer Elan DRC II	PerkinElmer Elan DRC II	Agilent 7700x	Agilent 7700x	Agilent 7700x	Agilent 7500cx	Agilent 7700x	Agilent 7700x	Agilent 7700x	Agilent 7700x	Element 2, Thermo Scientific	Element 2, Thermo Scientific	iCAP Q, Thermo Scientific X series 2
Reference	[60]	[60]	[62]	[62]	[62]	[101]	[62]	[62]	[62]	[62]	[56]	[56]	[61]

Notes: *Summer truffle or Burgundy truffle; Whitish truffle or bianchetto truffle; Black truffle, or Périgord truffle or French black truffle; Asian black truffle or Chinese black truffle; Field parasol or Parasol mushroom; **C (caps)/W (whole) and number of pools/samples (and total number of fruiting bodies); WD (without data); S & P (separation and pre-concentration).

leucomelaena, *Bovista plumbea*, *Clathrus crispus*, *Endoptychum agaricoides*, *Endoptychum depressum*, *Entoloma caccabus*, *Entoloma lividoalbum*, *Geastrum triplex*, *Gyrophragmium dundalli*, *Inocybe haemacta*, *Laterna pusilla*, *Lepista nuda*, *Limacella guttata*, *Longula texensis*, *Mycena pura*, *Panaeolus retirugis*, *Phaeolepiota aurea*, *Phallus impudicus* - gelatinous layer, *Podaxis pistillaris*, *Protubera maracuja*, *Psilocybe subcubensis*, *Psilocybe cubensis*, *Psilocybe semilanceolata*, *Russula amoena*, *Russula velenovskyi*, *Sepultaria sumneriana*, *Suillus placidus*, *Tricholoma imbricatum*" purchased from markets in the Lake Geneva region and also obtained from the USA, the Netherlands, Germany, France, Switzerland, Thailand and Brazil [50]. The study investigated all REE (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) in the previously mentioned species – *A. gaestrani*, *E. caccabus*, *G. triplex*, *I. haemacta*, *P. pistillaris* and *T. imbricatum*. The concentration levels for the sum of La, Ce, Nd, Gd, Sm, Er and Dy in *A. pes-caprae* ranged from 74 to 2420 μg kg^{-1} dw (rounded values; some REE were not determined quantitatively because they were below the individual method limit of quantification (MQL) of 50 μg kg^{-1} [50]. It is evident that the MQL of 50 μg kg^{-1} dw for individual REE elements in biological materials such as mushrooms or in staple foods is much too high to allow for reliable measurement given the generally low concentrations. Where measured, the sum of Ce, La and Nd was around 50 μg kg^{-1} dw in the samples of *A. bisporus* and *P. subcubensis*, around 100 μg kg^{-1} dw in *E. agaricoides, P. maracuja* and *R. velenovskyi*, and from 160 μg kg^{-1} dw in *A. silvicola,* up to 62,000 μg kg^{-1} dw in *P. pistillaris*. The sum of 14 REE in six selected species of mushrooms was reported to range from 3800 to 74,600 μg kg^{-1} dw [50, 51].

These concentrations appear to be uncharacteristically high and in a follow-up to the above study, Stijve et al. [53] explained that contamination of fungal material with soil particles had been a major source of error (and erroneously high values) in the determination of the lanthanides (and also for Al, Ca, Fe and other elements) in the earlier study. Both studies used a mass analyzer with a quadrupole mass filter for the measurement. The method used a sample aliquot of 0.75 g which was decomposed with 10 mL of HNO_3. Indium (^{115}In) was used as an internal standard in all samples, blanks, and standards [50, 53, 90].

Soil incorporated at 0.1 % in the dried fungal biomass was reported to contribute 70 mg kg^{-1} of Al, 40 mg kg^{-1} of Ca and 60 mg kg^{-1} of Fe [53]. Karkocha and Młodecki (1965) [91] determined from their study that the amount of sand in commercial consignments of dried mushrooms (*A. bisporus*, *Boletus edulis*, *Cantharellus cibarius* and *Gyromitra esculenta*), ranged from 0.55 to 1.8%. Contamination of fungal materials with particles of sand or debris from the soil substrate will substantially influence the reported results, not only of REE but also of Al, Ca, Co, Cr, Fe, Li, Ni, Sr, Th, Ti or V, but not of Hg, Cd or Se elements [53, 92]. In other words, unusual concentrations of REE, Al, Ca, Co, Cr, Fe, Li, Ni, Sr, Th, Ti or V in mushrooms could arise from secondary contamination of a sample, if there is no other valid reason. Thus, data from dried fungal samples from herbaria, which are very difficult to clean, may not be entirely reliable [56]. Assuming that the methodology used is reliable and well validated, secondary contamination from soil dust will result in elevated concentrations of REE in fungal materials but will not affect the natural distribution reflected by the typical sawtooth pattern when concentrations are plotted.

OVERVIEW OF ANALYTICAL METHODS OF REE DETERMINATION IN WILD MUSHROOMS

In general, the methods that are discussed here on trace REE determination in fungal matrices are restricted to results from laboratory-based studies and in particular, from studies that have not been the subject of subsequent comments regarding inconsistencies in presented data [64-67]. As evidenced (sections 1 and 2, Figures 1-3), the uptake of REE in wild mushrooms reflects the growing substrate. Some indications in a few publications on possible fractionation of some REE by fungi appear controversial and are not supported by other reports with well-validated data, suggesting that the analytics may contribute to the anomalies [69]. Concentration data obtained for many species of fungi from diverse environments does not support fractionation, for example, no anomalies regarding individual REE and Y composition against the European Shale Composite were observed [23]. However, a lower concentration of Dy, Ho, Er, Yb and Lu in the topsoil seemed to favour their slightly better bioconcentration than of the La, Ce, Pr, Nd, Pm, Sm, Eu, Gd and Tb in the fruiting bodies [23], but the relevance of this needs to be investigated. A study on biological behaviour of REE through omics approaches using a unicellular fungus *Saccharomyces cerevisiae* showed a higher toxicological risk of a group from Dy to Lu than from La to Tb (Ce not studied) [15]. It is known that Tb, Dy, Ho, Er, Tm, Yb and Lu, as heavier lanthanides (with increasing atomic number), have smaller atomic and ionic radii than the lighter La, Ce, Pr, Nd, Pm, Sm, Eu and Gd (lanthanides contraction effect), while none of the REE are considered nutritional for fungi, at least in typical Ca-rich soil environments. Many forest fungi are mutualistic feeders and their mycelial networks readily uptake inorganic compounds

from the soil solution while also actively searching for nutrients originating from rock and mineral bio-weathering, by excreting chelating agents [93]. The literature data reviewed in this work, showed that LREE (from La to Sm) comprise 87% (74 to 96%) of Σ13-14 REE (Tables 1-2).

The moisture normalized concentrations of REE in mushrooms are far lower than in the surrounding soil or substrate when compared on a dry weight basis or in other words, REE and Y are bio-excluded, i.e. the bioconcentration factor (BCF), is less than one. In the present context, BCF is the quotient of an element's concentration in the mushroom and in the substrate (on a dry weight basis) [8, 23, 61]. BCF data are considered as environmental characteristics that are helpful in assessing the reliability of analytical results for the full range of REE determination in biological matrices. Additionally, plotting of the data in log scale enables rapid visualisation of wide concentration ranges, from several hundred ppm down to about 0.1 ppb (Figs 1-6), and also allows the credibility of the data to be verified along with the identification of REE anomalies, e.g. by normalisation against a shale, chondrite, etc. [2, 8, 23].

Various spectrometry techniques with varying outcomes have been used in studies of REE in wild mushrooms and their growing substrates and bioconcentration potential. The so called "non-destructive" (of the sample matrix) techniques used in determinations were X-ray fluorescence analysis (XFR) and neutron activation analysis (instrumental – INAA and prompt gamma-ray – PGAA, PGNAA). Other techniques require acid digestion (decomposition – oxidization) to dissolve the solid biological matrix prior to analysis to obtain solubilised REE (dissolved minerals in the sample of < 0.2%) suitable for determination by inductively coupled plasma (ICP) mass spectrometry (MS). The literature describes a range of techniques and applications (with and without sample pre-treatment) used for REE determination in organic and inorganic matrices other than mushroom and includes laser ablation-ICP coupled with mass spectrometry (LA-ICP-MS) [2, 40, 82]. Similarly, isotope dilution-thermal ionization mass spectrometry (ID-TIMS) is also a recent technique for sensitive REE analysis, but it is not used for determining mono-isotopic REE (Pr, Tb, Ho, and Tm).

XRF

XRF (secondary emission) spectrometry is generally applied to the determination of the elemental composition of materials and is "applicable to the concentration range of REEs from 100% down to absolute 0.01%" [5]. As mentioned, XRF is non-destructive of the sample matrix, but the disadvantages of using this technique in REE analysis are the possibility of high error and inadequate MQLs which range from 1 to 10 mg kg^{-1} [94]. When XRF was applied to the determination of Ce and Nd, elevated and atypical concentrations were reported in mushrooms [55, 57], e.g. Nd ranged at 2800 ± 650 to 7100 ± 490 μg kg^{-1} dw in mature fruiting bodies of nine species of wild mushrooms [55]. In the later study by Campos et al. [57], Ce ranged from 6000 to 14,000 μg kg^{-1} dw, and Nd, ranged from 1000 to 9000 μg kg^{-1} dw in another set of eighteen (ectomycorrhizal, saprotrophic and epiphytic) species of mushrooms. However, as reported by Borovička et al. [56], the result of using XRF for this application was not satisfactory.

INAA and PGAA

Řanda and Kučera [52] used long-term INAA and provided concentration data on La and Sc (Ce, Sm and Eu were not detected above MQL) in a series of wild mushrooms collected in Bohemia. Lanthanum concentrations were in the range from 12 ± 3 μg kg^{-1} dw in *Lycoperdon perlatum* to 320 ± 22 μg kg^{-1} dw in *Agaricus xanthodermus* (total range < 7 to 840 μg kg^{-1} dw for 115 samples). Scandium in these mushrooms was detected in the range from 2.5 ± 0.3 in *L. perlatum* to 76 ± 2 in *C. cibarius* (total range from 2 to 240 μg kg^{-1} dw). Yttrium was determined in 3 samples but was only detected in *A. xanthodermus* at 0.0051 μg kg^{-1} dw. More recently, Rossbach et al. (2019) [60] determined Eu by INAA, and Gd and Sm by prompt-gamma neutron activation analysis (PGAA) in a set of truffles (*T. aestivum*). These activation techniques have several drawbacks, the major ones being access to a nuclear reactor, high cost, length of time required for analysis and safety issues when working with radioactivity. The rapid developments in the use and application of ICP-MS for elemental analysis saw a decline in the use of INAA in REE determination [95].

ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) with appropriate mass resolution (double focusing sector field mass spectrometry, which uses a static electric or magnetic sector, or a combination of the two as a mass analyzer) is a proven, established technique enabling the determination of REE in parallel with other metallic elements occurring in trace and ultratrace concentrations in biological materials [5, 90, 96, 97]. REE and Y determination can be strongly affected by polyatomic molecular interferences and careful corrections need to be applied "based on matrix-matched determinations of the yields of molecular ions" [98].

Reliable determination using this technique requires acid treatment of the solid biological matrix to provide

a digest which is internally standardised in order to control interferences. Typically, the sample digestion and purification process used for ICP-MS analysis involves oxidation of the dried fungal material using concentrated nitric acid (65%) either on its own or in combination with hydrogen peroxide (H_2O_2, 30%) or ultrapure or pro-analysis grade hydrofluoric acid (HF, 40% to 48%) in a pressurised polytetrafluoroethylene vessel with the aid of microwave energy. At low or ultralow REE concentrations, the digested solution obtained from this process can be further treated to exclude other metals "to remove the effects of potential isobaric interferences from molecular ions of non-REE and Y analytes", e.g. double charged ions (Ca, Ba, Sr) and particularly of Ba in Ba-rich matrices [2, 82, 98], and preconcentrated – if a direct spectrometric analysis for all REE and Y is not feasible [2, 8, 23, 99]. In order to control the recovery of REE after the matrix separation step, a Tm spike can be used [2] (Tm typically occurs at ultra-low concentration in biological samples) but this results in the loss of the original Tm concentration (see Fig. 5 A). REE were often determined in mushrooms with other metallic elements (in multi-element methods) and other internal standards were used to monitor for changes in MS operating conditions and sample-specific matrix effects, e.g. ^{115}In [49, 63], ^{6}Li, ^{45}Sc, ^{115}In and ^{159}Tb [61] or ^{102}Ru, ^{185}Re and ^{209}Bi [2, 8]. These extracts may be directly aspirated into the instrument plasma for ICP-MS measurement.

Other ICP-MS techniques with varying mass resolutions that have been successfully used for the analysis of some or all REE in cap mushrooms and truffles (genus *Tuber*), include double focusing sector field mass spectrometry (ICP-SFMS with higher mass resolution) [49, 56, 63] and quadrupole mass spectrometry (ICP-QMS; mass resolution can vary depending on the age and type of instrument) [2, 8, 23, 53, 54, 59, 62, 68, 100]. ICP-MS methodologies used in the studies of REE in wild edible mushrooms and associated outcomes are discussed below in more detail. Other studies that commonly use ICP-OES (ICP coupled with optical emission spectroscopy) for the determination of multiple elements including REE in forest mushrooms have been discussed, but the occurrence patterns and elemental ratios obtained for REE do not follow the normal distribution as predicted by the O-H order [after 48, 64-67, 69].

One of the early studies of 14 REE in mushrooms used SFMS [49] (Figure 3, Table 1). The determinations were carried out by direct elementary measurements of acid oxidized solutions without any pre-treatment. The high mass resolution achieved by SFMS analysis helps to achieve "high sensitivity for ultratrace levels of elements, the simultaneous measurement capabilities of multiple isotopes for precise isotope ratio measurements and the high-resolution capabilities to resolve spectral interferences" [102].

Apart from elimination/reduction of spectral interferences, the higher resolution of up to 10,000 res. [116, 102] provides low instrumental detection and quantification limits which results in more sensitive measurement ("the limits of detection are one to two orders of magnitude lower") compared to QMS without preconcentration [5, 96, 102]. SFMS was also used in later studies on trace and ultra-trace multi-elemental analysis including REE in the fruiting bodies of terrestrial (epigeic) and subterranean (hypogeic) fungi, i.e. truffles (Figures 3 and 4; Tables 1 and 2) [56, 62, 63].

The early studies of REE in mushrooms using SFMS showed low levels, i.e. Σ13/14 REE occurred at a concentration of 32 μg kg^{-1} dw in *S. luteus* (caps), 82 μg kg^{-1} dw in *Tricholoma equestre* (previous name *T. flavovirens*; caps), 114 μg kg^{-1} dw in *Suillus bovinus* (caps), 140 μg kg^{-1} dw in *B. edulis* (caps), 160 μg kg^{-1} dw in *L. amethistina* (whole fruiting bodies) and 363 μg kg^{-1} dw *Armillariella mellea* (caps) [49]. Borovička et al. [56] determined Σ14 REE at median concentrations of 103 μg kg^{-1} dw of ectomycorrhizal, and 57 μg kg^{-1} dw of saprobic, mushrooms, which agreed well with results from the earlier study of Σ13/14 REE which ranged from 32 μg kg^{-1} dw to 363 μg kg^{-1} dw [49]. The SFMS determination of heavy REE such as Tb, Dy, Ho, Er, Tm, Yb and Lu which can occur in mushrooms at concentrations below 1 μg kg^{-1} dw (< 0.1 μg kg^{-1} fresh weight) can be challenging (Table 1).

ICP-QMS

Low resolution mass spectrometers (e.g., ICP-QMS with collision and reaction cells, CRCs) are commonly used in the spectroscopic determination of REE in mushrooms. As far as currently reported, a triple quadrupole ICP-MS (ICP-QQQ) has not been used to study REE in fungi. The mass resolution of the ICP-QMS systems that have been used in mushrooms research is limited in comparison to SFMS and is related to the number of serial quadrupoles used (usually two quadrupoles – the main analyser and a dynamic collision cell in tandem, ICP-MS/MS) to reach the required resolution. Although considered as a lower resolution MS, QMS can be a powerful and reliable tool for the determination of REE in biological materials provided that the additional stages of analyte separation from interfering background and pre-concentration are carried out before instrumental analysis.

Thus, direct aspiration of acid oxidized (thermally digested) solid sample digests (without further treatment) into the plasma of the ICP-QMS in low-resolution mode is not suitable for determination of

REE in mushrooms. On the other hand, analytical procedures in which acid digests were further purified to exclude interfering macroelements and pre-concentrated before ICP-QMS measurement provided reliable data on REE in mushrooms and their soil substrates [2, 8, 23]. Separation from the interfering background in low resolution mode is also required for the precise determination of ultra-low concentrations of REE in inorganic materials, e.g. iron-rich monominerals, e.g. Fe-olivine and meteorites, using high resolution ICP-MS, was achieved after effective iron removal (by around 99%) following chemical purification by polyurethane foam [99]. There are a number of other independent studies utilizing various ICP-QMS instruments for the determination of REE. These have used direct aspiration of acid digested fungal matrices into the argon plasma with appropriate AC/AQ protocols [51, 53, 59, 61, 62, 68, 100], and some results from these studies are plotted and presented in Figure 4.

A recent study on REE in edible and inedible mushrooms and their topsoil and plant (tree) substrates also used aspiration of sample digests directly into the ICP-MS – PlasmaQuant MS Q with integrated Collision Reaction Cell (iCRC) [103]. The soil mushrooms that were investigated were: *Agaricus arvensis*, *Calvatia gigantea*, *Chlorophyllum rhacodes* (inedible), *Lyophyllum fumosum*, *Paxillus involutus* and *T. equestre*, and the wood growing mushrooms were: *Auricularia auricula-judae*, *Cerioporus squamosus*, *Flammulina velutipes*, *Fomitopsis betulina*, *Ganoderma applanatum*, *Ganoderma resinaceum*, *Laetiporus sulphureus*, *Pholiota aurivella*, *Pleurotus ostreatus* and *Sparassis crispa*. The study, reported that "detection limits" were "at the level of 1 to 10 µg kg^{-1} dw for all elements determined (3 times standard deviation of blank analysis (n=10))" [103]. The reported mushroom concentrations of Ce, Nd, Pr, Er and Tm (no data was reported for La, Sm, Eu, Gd, Tb, Dy, Ho, Er, Yb and Lu) did not follow the natural distribution pattern of REE (the sawtooth or zigzag pattern predicted by the O-H order). Additionally, the absolute concentrations of particularly, Er and Tm but also of Ce, Nd and

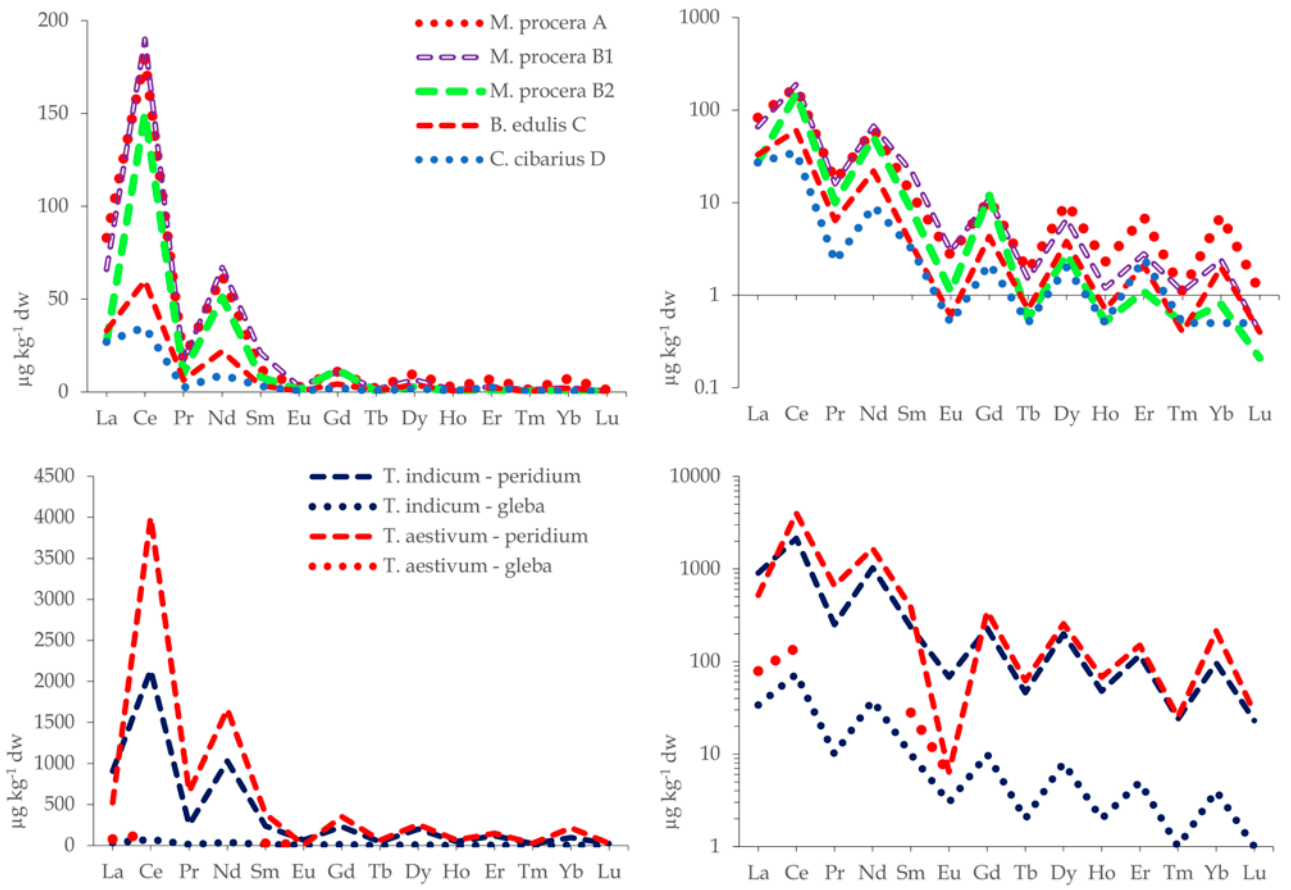

Figure 4. Normal and logarithmic scale distribution patterns of REE in several species of mushrooms (caps of *Macrolepiota procera*, *B. edulis* and a whole *C. cibarius*) as determined by aspiration of sample digests directly into the argon plasma of a quadrupole mass analyzer (upper plots) and in the peridium and gleba of the truffles, *T. indicum* (by sector field mass spectrometer) and *T. aestivum* (by quadrupole mass analyzer; Eu by neutron activation) (bottom plots), after [59, 60-62, 67, 100].

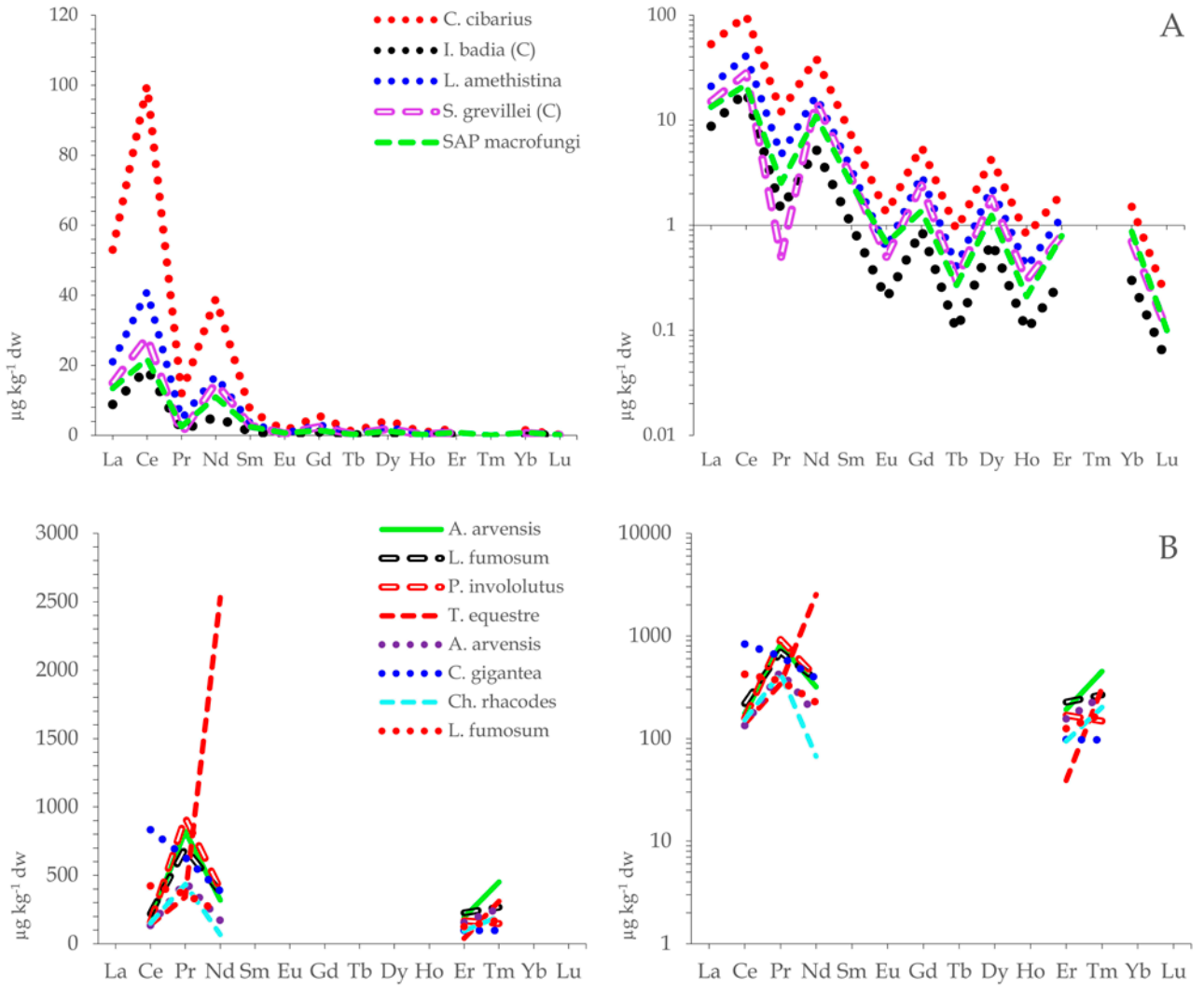

Figure 5. Normal and logarithmic scale distribution patterns of REE – natural concentrations (µg kg^{-1} dw) in mushrooms of *C. cibarius*, *Imleria badia*, *Laccaria amethistina* and *Suillus grevillei*, determined by quadrupole mass analyzer after separation and pre-concentration on ion exchange resin [23], in a collection of saprotrophic species by sector field mass spectrometer [56] (upper plots A), and in some species of mushrooms (lower plots B), adapted – cited from [64].

Pr appeared to be highly elevated relative to other reported data (data for Ce, Nd, Pr, Er and Tm are plotted in the lower half of Figure 5) [65].

ICP-OES

ICP-OES has some basic drawbacks (potential spectral interference and relatively poorer sensitivity) which results in insufficient instrumental and method detection and quantification limits when used for the determination of metallic elements in biological matrices [104]. This can be seen at macro-, micro- and ultra-trace concentration levels, particularly when sample introduction is by direct aspiration of a mineralized sample solution into the plasma [104]. It also applies to the determination of REE which typically occur in wild mushrooms at concentrations of up to a few tens of µg per kg dw as in the case of Ce and down to sub-µg per kg dw in the case of the Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu (Tables 1 and 2). Apart from the spectral interference of ions from the biological sample matrix [5], the resulting REE emission spectra can also be very complex [105]. Ce, Pr, Nd which have relatively higher occurrence in mushrooms, have the most complex emission spectra while the heaviest REE (Y, La, Eu, Gd, Yb, Lu) have relatively simple spectra and also the lowest (best) detection limits [5]. However, the heavy REE occur at ultra-low concentrations which are beyond the range of ICP-OES for direct measurement. Nevertheless, there have been several reported studies of REE (a few or all 14 elements) in wild mushrooms, that have used direct aspiration of nitric acid digests into the plasma by ICP-OES [58] as discussed elsewhere [48, 64-69].

As is evident from this section on analysis techniques, a number of measurement techniques have been used for the analysis of REE in fungal matrices. The accuracy and sensitivity of the measurement process will continue to improve with more information and experience and particularly with the introduction

Table 3. Summary of analytical techniques that have been used to determine REE in mushrooms

REE analysis technique (Abbr.)	Advantages	Disadvantages	Possible application (REE)
X-ray fluorescence (XRF)	Non-destructive of sample	Poor sensitivity, possible errors from inadequate selectivity	Screening mineral content or highly contaminated soil
Instrumental neutron activation analysis (INAA) prompt-gamma neutron activation analysis (PGAA)	Non-destructive of sample	Requires access to nuclear reactor, expensive, safety issues, long duration of analysis	Screening environmental matrices or for analysis at high concentrations
Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES)	Relatively inexpensive and laboratory bench-top sized instrument	Inadequate sensitivity and selectivity, particularly when used without additional digest purification	Could be used for initial screening, but confirmation is advised. Direct sample aspiration is not advised for fungal material analysis
Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	Relatively inexpensive and laboratory bench-top sized instrument	Sensitivity may not be enough for low concentrations, stringent sample purification required to avoid interferences	Can be used for biota/fungi if levels are not too low
Inductively Coupled Plasma – Sector Field Mass Spectrometry (ICP-SFMS)	Good sensitivity and selectivity	Expensive, requires adequate space and cooling	Variety of REE analyses possible, including measurement of low concentrations in fungi
Inductively Coupled Plasma – Quadrupole Mass Spectrometry (ICP-QMS)	Adequate sensitivity and selectivity (depending on quadrupole type)	Depending on the achievable selectivity, may require stringent clean-up for biota analysis	Variety of REE analyses possible, measurement of low concentrations may require good digest purification and preconcentration

of newer instrumentation. The techniques used thus far have been summarised in Table 3 above.

RELIABILITY OF ANALYTICAL DATA ON REE IN WILD MUSHROOMS

The recent interest in the potential for increasing environmental pollution by REE has led to a number of studies on the occurrence of these elements in environmental and food matrices. As mentioned, REE in foodstuffs are relatively difficult to analyse compared to other elements, particularly because of the low concentrations and also because of the contribution of the matrix to interference during determination. The analytical methodology used for determination should give careful consideration to sample pre-treatment (a key factor that affects the determination of REE in biological materials), processing/purification techniques and the use of appropriate instrumentation, in order to avoid unreliable data. Other considerations that are specific to mushrooms are the potential for cross-contamination from substrate particles that can adhere strongly to the sample. The use of certified reference materials (CRMs) and independent validation by participation in interlaboratory studies (or performance testing) will also help to avoid unreliable data. Where occurrence data is reported, in addition to samples processing and instrumentation, detailed metrological parameters such as linearity, measuring range, instrumental limit of detection (LOD) and quantification (LOQ), method limit of detection (MDL) and quantification (MQL), repeatability or intermediate precision, and results of control materials/certified reference materials, should also be included.

Some authors reported multi-element data measured by ICP-OES, including the concentrations of 13 REE (La, Ce, Pr, Nd, Sm, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) in mushrooms that were referred to as "above-ground species" and "wood-growing species". The reported summed concentrations (Σ13 REE, Eu was not determined) in the "above-ground species" were: 1070 μg kg^{-1} dry weight (dw) in *C. cibarius* (yellow chanterelle), 870 μg kg^{-1} dw in *L. amethistina*, 730 μg kg^{-1} dw in *Leccinum scabrum*, 980 μg kg^{-1} dw in *Lepista gilva*, 860 μg kg^{-1} dw in *L. fumosum*, 600 μg kg^{-1} dw in *M. procera*, 860 μg kg^{-1} dw *P. involutus*, 750 μg kg^{-1} dw in *S. bovinus*, 5030 μg kg^{-1} dw in

S. luteus and 2180 μg kg^{-1} dw in *T. equestre*. Σ13 REE in the "wood-growing species" were: 1440 μg kg^{-1} dw in *Armillaria mellea*, 1930 μg kg^{-1} dw in *A. auricula-judae*, 960 μg kg^{-1} dw in *F. velutipes*, 4190 μg kg^{-1} dw in *G. applanatum*, 940 μg kg^{-1} dw in *Grifola frondosa*, 1660 μg kg^{-1} dw in *L. sulphureus*, 590 μg kg^{-1} dw in *Piptoporus betulinus*, 700 μg kg^{-1} dw in *P. ostreatus*, 2430 μg kg^{-1} dw in *Pleurotus* spp. and 1610 μg kg^{-1} dw in *Polyporus squamosus* [58]. These Σ13 REE values, which ranged from 590 μg kg^{-1} dw to 5030 μg kg^{-1} dw, were more than an order of magnitude above normal occurrence values in comparison to previously reported data (Tables 1 and 2) from studies using SFMS [49, 56] and additionally, individual REE data differed from those seen in normal distribution patterns. The authors reported that the mushrooms were collected in 2014 from a site located "up to 40 m from a heavily trafficked road" in a mixed forest of acacia, acer, pine and oak. The composites used for analysis were made using three to eleven samples per species, which were cleaned "from the rest of underlying substrate to prevent contamination with REE…" using distilled water [58]. Thus, primary contamination of the mushrooms samples (including the "wood-grown species") with soil debris or sand may have been avoided. The stated aim of the study: "was to compare the ability of 20 wild mushroom species growing near a busy trunk road to accumulate particular elements of PGE (platinum elements) and REE (including Y) groups, and that this is so far the broadest study on the occurrence of these elements in mushrooms" [58].

A valid hypothesis to explain the higher reported REE levels could consider whether "a busy trunk road" was potentially a source of the highly elevated fungal concentrations of Er and also of Σ13 REE. However, the study did not consider this hypothesis [58]. Ce, La, Nd and Gd are used in oil refineries, and some REE are used in electric automobiles but these vehicles were rare or absent in Poland in the year that the samples were collected. An earlier study [106], reported that the enrichment of REE in surface soil samples in public parks of São Paulo city could not be clearly attributed to automobile traffic, instead the high background concentrations were associated with the natural composition of the soils.

The occurrence and distribution of REE in soils is mainly determined by the mineral composition of parent rocks, with primary and secondary sources being minerals of acid, siliceous and sedimentary rocks [4]. REE have very similar chemical and physical properties and behave collectively as a group in the biotic environment, including in their uptake and distribution characteristics in vegetation and animals, food web relations and metabolism [1, 4, 37, 81].

A graphical representation of the REE distribution patterns in mushrooms plotted in normal or logarithmic scale (both, normalized or not, to any reference sample such as shale, chondrite or soil) is useful for a visual identification of any possible natural anomaly and can also reveal if the data is biased (not credible). The REE distribution pattern drawn for a randomly selected species of mushrooms from the study by [58] is presented in Figure 6. This distribution pattern is very different from that seen for mushrooms and other environmental materials from other studies (as referenced above) and does not follow the expected pattern arising from the O-H order (Figures 3 and 4). Instead, it shows an unprecedented anomaly of erbium (Er) concentrations but also of other REE (Figures 5 and 6).

Another approach to evaluating elevated REE concentrations would be through examination of the bioconcentration factors for REE in wild mushrooms. These data are scarce but recently Zocher et al. [8] provided data on REE in the *S. luteus* fungus and in forest top-soils. BCF values of 0.0001 for Ce and 0.0002 for Er were calculated from the concentration data on *S. luteus* and the underlying soil substrate. These very low values suggest almost total bio-exclusion of Ce and Er (and most likely of the other REE as well because they show similar behaviour) by *S. luteus*. If these BCF values are applied to the mean concentrations of Ce and Er (600 μg kg^{-1} dw, and 3500 μg kg^{-1} dw respectively) in *S. luteus* as reported by [58], it is possible to estimate the concentrations in the soils in which these mushrooms were collected. So, the above mean concentrations of Ce and Er divided by their respective BCF values [8] would yield dried soil concentrations of 6.0 g kg^{-1} and 17.5 g kg^{-1}, respectively. These concentrations are unlikely in roadside topsoil or other soils as they are far higher than REE concentrations in the most abundant ore deposits, e.g. ~66 mg kg^{-1} [107]. Similarly, the maximum ΣREE value of 5030 μg kg^{-1} dw in *G. applanatum* reported by [58], would require extremely high and therefore unlikely concentrations in the tree wood substrate in which this fungus grows.

An example of a genuine and explained anomaly of REE occurrence is that of Gadolinium (Gd) which can pollute surface waters (possibly also sediments), through its application in magnetic resonance imaging [24] as described in section 1. After use, Gd is excreted from the body via urine, survives waste water treatment, and contaminates riverine waters downstream of the source. This was reported as a high positive anomaly of Gd with slightly negative Er (compared to typical occurrence) in a study of riverine waters downstream from a municipal sewage treatment plant [24]. In another recent example, *Boletus edulis* sampled from an abandoned military area overgrown with forest showed higher REE levels and a more perturbed occurrence pattern than mushrooms

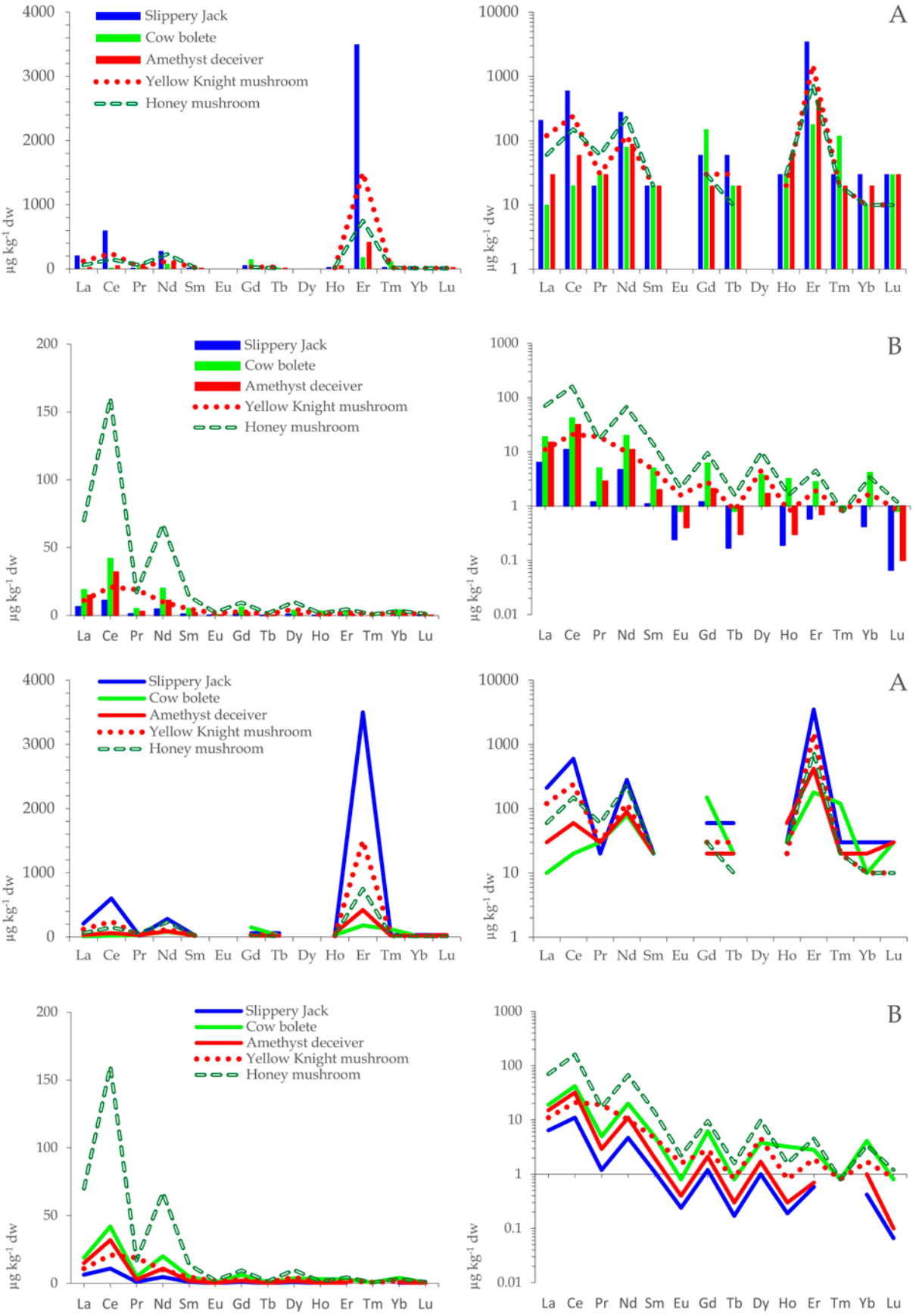

Figure 6. Normal and logarithmic scale distribution patterns of REE – natural concentrations (µg kg^{-1} dw) in the mushrooms *Suillus luteus* – Slippery Jack, *Suillus bovinus* – Cow bolete, *Laccaria amethistina* – Amethyst deceiver, *T. equestre* – Yellow Knight mushroom and *Armillariella mellea* – Honey mushroom – plots labelled A (after [58]) and in the same species by other authors – plots labelled B (after [8, 23, 49], respectively).

from other forested sites, probably as a result of earlier military activities, but this was not studied [100]. So, there is no wider evidence so far that "anthropogenic activities" disrupt the balances and relationships of lanthanides in natural forest soil and their uptake by macromycetes.

Thus, the disposition of lanthanides in most natural matrices including soils, mushroom, other biota, etc. follows a pre-defined pattern – one that was predicted by the Oddo-Harkins rule and which is reflected in the pre-historic bedrock. This does not preclude the possibility of anomalies in some of these patterns that can arise from diverse anthropogenic sources but the appearance of these should be investigated and rationalised if the sources are identified or reasonably hypothesised when the supporting information is not available.

DISCUSSION

Analytical chemistry considerations

The determination of REE in biological matrices and particularly mushrooms has developed with the concurrent exploitation and application of these elements in high-technology applications. Many of the studies on REE in fungal materials were initiated by the potential of REE to become more prominent environmental and food contaminants in line with their increasing production and applications. It is evident from Tables 1 and 2 that reliable REE occurrence data on mushrooms is scarce. Part of the reason for this is related to the higher threshold of analytical accessibility, particularly in achieving the relatively low method detection limits that are essential for reliable determination in biological matrices. It is clear from the earlier sections that the key requirements for providing reliable and credible data on REE in mushrooms are:

- Avoiding cross-contamination from the substrate - which requires stringent cleaning of the freshly picked mushrooms to remove substrate particles. Lack of care at this stage would result in erroneously higher concentrations originating from the residual substrate.
- Using effective sample digestion and digest purification methodology – which should allow thorough removal of the sample matrix that could lead to spectral and non-spectral interferences and provide preconcentration of the digest to allow adequate MQLs to be achieved during measurement (e.g. at least 0.1 μg kg^{-1} dw or better). The inclusion of matrix matching, and standard addition techniques could also be considered to correct for matrix effects and improve reliability.
- Using instrumental techniques that are sensitive enough to achieve the required MQLs while being simultaneously capable of sufficient resolution to exclude interferences (e.g. the use of ICP-SFMS at high resolution (≥ 10,000) or the newer triple-quadrupole mass spectrometers – the enhanced ion filtering achieved by the additional quadrupole can effectively exclude both plasma-based ions and prevent unwanted reactions with residual matrix ions.
- Incorporating effective QA/QC tools – such as the use of procedural blanks, internal standards (e.g. ^{115}In, ^{45}Sc, ^{115}In, ^{159}Tb etc.), recovery spike(s) and the use of reference materials such as BCR-668 (mussel tissue; REE, Th and U), NCS ZC73018 (citrus leaves; multielement), NCS ZC73022 (scallop; multielement) or REE-1 (an ore; REE, Zr and Nb). These measures would allow validation of the reported data.

At the end of the 20th century some laboratories participated in an interlaboratory study on REE determination which aimed to certify reference materials such as tuna muscle, mussel tissue, aquatic plants and estuarine sediment samples [108]. Since then, other CRMs have been developed which can be helpful to maintain AQ/AC standard in REE determination [2, 8].

The use of lower resolution ICP-MS instrumentation is feasible for the analysis of REE providing the purification procedure used prior to measurement are effective in removing non-spectral interferences (due to matrix effects and instrument drift) – through discussed internal standardisation, standard additions or isotope dilution, and in removing spectral interferences [98]. Direct measurement of biological sample digests is ill-advised with these systems, particularly for some fungal digests which are rich in mono- and divalent metallic ions (e.g. median concentrations of K and Rb in *B. edulis* and *C. cibarius* can range from 20,000-38,000 and 39,000-60,000 mg kg^{-1} dw, respectively with rubidium occurring at 190 and 590-1600 mg kg^{-1} dw respectively [109, 110]. Although SFMS and the newer triple-quadrupole mass spectrometers (TQMS; with two quadrupole mass analysers in series and with a non-mass-resolving quadrupole – collision cell in between) allow exclusion of isobaric and polyatomic spectral interferences that arise from these high concentration co-extractives, and overcome spectral interferences caused by REE themselves (REE oxides and hydroxides interfere with other REE) and chloride species, reliable measurement with lower resolution instruments would require additional purification and concentration stages to minimise or remove these interferences [97, 105]. However, despite the better sensitivity afforded by SFMS/TQMS, care should be taken when analysing low concentration fungal materials as some spectral interferences (e.g., from the formation of oxides,

hydroxides and doubly charged ions) may still persist. Resolving some of these at higher resolution can incur the cost of a consequential loss in sensitivity and additionally, requires frequent recalibration of the mass axis.

Peer review as a control on reliability of data

Good peer-review of REE data is critical in order to evaluate whether the reported concentrations in mushrooms and other biological materials are credible or have been compromised during the determination process. Review of fresh data is considerably aided by the adherence of REE occurrence in most biotic and abiotic matrices to the very characteristic pattern predicted by the O-H order as visualised in Figures 2-5 in earlier sections. This distinctive pattern derives from the predicted elemental occurrence and the fact that individual REE show a collective behavioural similarity in biotic and abiotic environments arising from their similar physical and chemical characteristics. The occurrence pattern is thus maintained from the parent bedrock and mineral sources through environmental processes like soil formation [81], reflected in topsoil concentrations and through the process of uptake by plants and mushrooms [8, 23]. The normal (natural) or logarithmic plot of these occurrences provides a simple but effective evaluation of data, particularly when concentrations for all or most of the REE are reported. The persistence of this pattern (Figures 1, 3 and 4) across the data reported listed in Tables 1 and 2, notwithstanding the differences in species, location, biogeochemical substrate influences and pollution profiles, demonstrates the validity of this assessment for the full set of lanthanides. When data is partial, i.e. only some of the REE are reported, then evaluation of the individual concentration ratios (e.g. La/Sm, Ce/Nd, Ce/Sm and La/Tm) also provides a good indication of the reliability of the determination [47, 65]. Anomalous concentrations are possible as seen in the case of Gd in waste waters and beverages [24, 25] and the increasing use of individual REE in specific applications could result in data that does not follow the predicted patterns, but these anomalies should be identified and explained, at least through hypothesis. As reviewed by Migaszewski and Gałuszka, an example of such an anomaly is seen in the data on La, Ce and Sm in < 1.1 μm particulate matter during studies on local pollution of ambient air [1].

The potential of REE to become more prominent food contaminants and pose a health risk is inherent in the rapidly increasing production and utilisation of these chemicals. It is therefore important that occurrences in environmental and food matrices are monitored for any increasing trends, but this requires a very reliable baseline for evaluation. It is clear from published data [8, 23, 56, 76, 111, 112, 113] and from Tables 1 and 2, that such a resource is currently very sparsely populated, and more credible data is essential. Most reported concentrations of individual REE in wild mushrooms and other terrestrial vegetation are low, typically ranging from sub-ppm to low or sub-ppb levels (Tables 1 and 2 [114]), although seaweed species may show higher values [115]. These lower concentration levels are expected not only because of the relatively lower amounts of REE that are naturally available, but crucially, also because plants and particularly mushrooms are known to bio-exclude REE [23, 61, 76]. Highly elevated REE concentrations in mushrooms are therefore unlikely even if the data demonstrate the predicted sawtooth pattern, because in this case, cross-contamination by the substrate is a possibility [53]. Higher individual concentrations are seen for the light REE such as La, Ce, Pr and Nd, and also Sc and Y, which geologically are more naturally abundant.

The credibility of data is essential, particularly in reports on elevated individual REE concentrations in wild mushrooms which may suggest a rapid rise in environmental pollution. This is of course a plausible scenario given the increase in production and use (and also disposal of REE containing products), but data that suggests such elevations above the background should be backed by good quality protocols and critical peer review. It is also very clear that considerably more data on REE occurrence in wild (and cultivated) mushrooms is required to indicate any trend to higher levels in this important food and indeed, the environment.

CONCLUSIONS

REE have no currently known biological or nutritional role but they have the potential to become contaminants of emerging concern because of their rapidly increasing applications in consumer goods and other products. An evaluation of this status, particularly any upward trend in environmental and food levels requires reliable occurrence data. Mushrooms, a popular food, absorb and accumulate these elements from their growing substrates and could provide an early indication of any such trend.

In the absence of any external sources, REE occur in a characteristic pattern in wild mushrooms which reflects the composition of their substrates without any fractionation of individual elements. Any anomalies to this pattern that cannot be attributed (even hypothetically) to proximate sources are like to arise from inadequacies in analytical methodologies or analytical instrumentation. This is due to the very low concentration level (< 1 μg kg^{-1} dry weight) of certain REE (Eu, Tb, Ho, Er, Tm, Yb, Lu) in fungi

and the additional challenge of overcoming matrix and instrumental interferences.

Reliable data can be obtained by avoiding cross-contamination, using effective digest purification methodology and sensitive measurement techniques that are capable of excluding spectral and other interferences. Confidence in the data can be enhanced through the use of rigorous QA/QC protocols and review of the REE occurrence patterns for any unexplained deviations from the natural distribution. Data from studies that met these requirements confirmed typically low concentrations of REE in mushrooms (0.07 μg kg^{-1} of Lu in *Suillus luteus* to 940 μg kg^{-1} of Ce in *Cantharellus minor*), confirming the bio-exclusion of REE and preserving the elemental Oddo-Harkins patterns of their growing substrates. However, the database is currently very small so further monitoring is essential in order to confirm the current findings and additionally, the widespread and increasing global use of REE does not preclude an increase in occurrence in the future.

Disclosure conflict of interest

The authors declare that have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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ORIGINAL ARTICLE

OCCUPATIONAL PESTICIDE EXPOSURE AND COGNITIVE IMPAIRMENT AMONG ADULT FARMERS IN NORTHERN THAILAND

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ABSTRACT

Background. Thai farmers are directly exposed to pesticides, which may result in adverse effects including cognitive impairment.

Objectives. The aim of this study was to examine the association between occupational pesticide exposure and cognitive decline among adult farmers in northern Thailand.

Material and Methods. This cross-sectional study included 303 pesticide-using farmers over the age of 50 from Doi Tao District in Chiang Mai Province. Pesticide exposure score was calculated using an algorithm that considered personal protective equipment (PPE) scores and exposure intensity scores, as well as lifetime application days. The scores were classified as high or low exposure based on their median. The Thai version of the Montreal Cognitive Assessment (MoCA) test was used to assess cognitive function.

Results. The mean age of adult farmers was 58.74 years. The prevalence of cognitive impairment was 93.7%, with an average score of 19.6. Spearman's rank correlation coefficient showed that the MoCA score was adversely correlated with lifetime application days (r_s = -0.145), PPE score (r_s = -0.163), exposure intensity score (r_s = -0.184), and pesticide exposure score (r_s = -0.225). Linear regression revealed that high exposed farmers had significantly lower MoCA scores than low exposed farmers, as measured by PPE score (B = -0.75; 95% CI: -1.46, -0.05), exposure intensity score (B = -0.97; 95% CI: -1.66, -0.27), and pesticide exposure score (B = -0.77; 95% CI: -1.47, -0.06), after controlling for sex, age, education, income sufficiency, and body mass index.

Conclusions. Thai farmers are at risk of cognitive impairment linked to occupational pesticide exposure, depending on their PPE use and exposure intensity. There is still a critical need for action to reduce the risk of negative health effects from pesticide exposure among Thai farmers.

Keywords: *pesticide, cognitive function, MoCA, agriculture, farmer*

INTRODUCTION

Pesticides are widely used to prevent and control pests, and their usage continues to trend upward. In 2022, total pesticide use in agriculture had doubled since 1990, with usage per arable area increasing by 94 percent [1]. In Thailand, pesticides are commonly used, including herbicides (e.g., glyphosate and paraquat), insecticides (e.g., abamectin, chlorpyrifos, and cypermethrin), and fungicides (e.g., carbendazim and propineb) [2, 3]. These chemicals can directly affect the health of farmers exposed to them through multiple routes, including dermal contact, ingestion, and inhalation. In particular, exposure to organophosphates and carbamates is known to inhibit acetylcholinesterase activity, increasing the risk of pesticide poisoning. A previous study revealed that 17.3% of Thai farmers experienced pesticide poisoning after applying pesticides [3]. Chronic health effects may include an increased risk of cancers such as leukemia, non-Hodgkin lymphoma, brain, prostate, bladder, colorectal, lung, kidney, and pancreatic cancers [4-6]. Pesticide exposure may also impact the mental health of farmers [3, 7, 8] and contribute to neurodegenerative diseases, including Alzheimer's disease and dementia [9-11].

The global number of people living with Alzheimer's disease and other dementias more than doubled from 1990 to 2016, with the number of deaths increasing by 148%, making it the second-largest cause of death in individuals aged 70 and older, following ischemic heart disease, in 2016 [12]. In Thailand, deaths from Alzheimer's and other dementias among individuals aged 75 and older rose to 8.1% in 2019, doubling from

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3.9% in 2015 [13]. Alzheimer's disease can develop in individuals with mild cognitive impairment, highlighting the importance of early identification for effective intervention [14]. Evidence also indicates that pesticides adversely affect farmers' cognitive function [15], potentially contributing to dementia and Alzheimer's disease [16].

The association between pesticide exposure and cognitive function has been investigated in many study populations, such as those in the U.S. [17, 18], France [19, 20], Chile [21, 22], Korea [23], Costa Rica [24], and Indonesia [25]. In Thailand, it was found that farmers had a higher risk of cognitive impairment compared to nonfarmers [26]. However, the effects of long-term and high-level pesticide exposure on cognitive performance warrant further investigation [27]. The objective of this study was to investigate the association between occupational exposure to pesticides and cognitive function among adult Thai farmers. The findings can provide essential insights to guide health surveillance and inform actions to protect farmers from pesticide-related risks.

MATERIAL AND METHODS

Design, setting, and subjects

This is a cross-sectional study of adult farmers in Doi Tao District, Chiang Mai Province, in northern Thailand. Doi Tao District covers an agricultural area of approximately 70 square kilometers, with about 63% of all households registered as farmer households. Longan is a popular fruit in the area, often grown alongside other crops such as rice, corn, and shallots. Farmers commonly use insecticides, herbicides, and fungicides in the cultivation of these crops.

Participants were selected using convenience sampling, with public relations efforts by local organizations. Leaflets and community loudspeakers were used to invite farmers who met the inclusion criteria. Thai farmers aged 50 and older, representing farming households, were eligible if they had at least five years of experience in agriculture, had a history of pesticide use, had no history of mental illness or neurological disorders requiring treatment, and were literate in Thai.

The sample size for the study was estimated using the G*Power program for two independent mean comparisons, with a power of 80% and a confidence level of 95% (2-tailed). A mean difference in cognitive performance scores between the low and high pesticide exposure groups was set at 1 point, with a standard deviation (SD) of 3 points [23]. The required sample size was 143 participants per group, with an additional 10% added to account for incomplete data, resulting in a total of 326 adult farmers. Ultimately, 303 farmers provided complete data for the study. The Committee of Research Ethics, Faculty of Public Health, Chiang Mai University, approved the study (No. ET021/2023). Prior to data collection, all participants provided written informed consent.

Data collection

Data were collected between October and December 2023. The interviewer-administered questionnaire was conducted by three researchers who had received training prior to data collection. The questionnaire consisted of three sections: (a) demographic characteristics, such as sex, age, education, marital status, household income, body mass index (BMI), underlying diseases, smoking, alcohol use, entry into agriculture, distance from home to the nearest farm, and household insecticide use; (b) work characteristics, including agricultural work experience, farm size, and history of pesticide use (insecticides, herbicides, and fungicides); and (c) pesticide exposure estimation, including the duration (in years) of pesticide use, frequency of pesticide application per year, pesticide use characteristics (mixing/spraying), frequency of personal protective equipment (PPE) use (dust mask/mask with carbon filter, goggles, gloves, long-sleeve shirt, long pants, and boots), and the practice of showering and changing clothes after pesticide application.

To estimate cumulative lifetime pesticide exposure, the study adapted a semi-quantitative algorithm developed for low- and middle-income contexts in previous studies [3, 28, 29]. The pesticide exposure score was calculated based on lifetime days of pesticide application and the intensity level of exposure (Equation (1)).

Lifetime application days were calculated by multiplying the number of days per year by the number of years of pesticide use. The exposure intensity score was estimated based on five exposure-modifying factors: mixing pesticides, spraying pesticides, showering, changing clothes, and using PPE (Equation (2)).

Two factors – mixing active ingredients and spraying pesticides – were assigned scores of 5 and 8, respectively. Showering and changing clothes

$$\textit{Pesticide exposure score} = \textit{lifetime application days} \times \textit{exposure intensity score} \quad (1)$$

$$\textit{Exposure intensity score} = (\textit{mix} + \textit{spray}) \times \textit{shower} \times \textit{clothes} \times \textit{PPE score} \quad (2)$$

$$\textit{PPE score} = 0.1 \times \textit{mask} + 0.1 \times \textit{goggles} + 0.4 \times \textit{gloves} + 0.2 \times \textit{shirt} + 0.1 \times \textit{pants} + 0.1 \times \textit{boots} \quad (3)$$

immediately after pesticide application were scored as follows: 0.7 for *always*, 0.8 for *sometimes*, 0.9 for *rarely*, and 1 for *never* [28]. The PPE score was calculated as shown in Equation (3).

The frequency of PPE use was scored as follows: *always* (dust mask, shirt, and pants = 0.3; gloves = 0.2; mask with carbon filter, goggles, and boots = 0.1), *often* (dust mask, shirt, and pants = 0.48; gloves = 0.4; mask with carbon filter, goggles, and boots = 0.33), *sometimes* (dust mask, shirt, and pants = 0.65; gloves = 0.6; mask with carbon filter, goggles, and boots = 0.55), *rarely* (dust mask, shirt, and pants = 0.83; gloves = 0.8; mask with carbon filter, goggles, and boots = 0.78), and *never* (all PPE = 1) [28]. The possible PPE score ranged from 0.14 to 1, with lower scores indicating reduced exposure.

Lastly, lifetime application days, PPE score, exposure intensity score, and pesticide exposure score were divided by the median to create high and low exposure groups.

The Thai version of the Montreal Cognitive Assessment (MoCA), a brief cognitive screening tool for mild cognitive impairment (MCI), was used to assess multiple cognitive function domains, including attention, concentration, executive function, memory, language, visuoconstruction skills, conceptual thinking, calculation, and orientation [30, 31]. The test takes approximately 10 to 15 minutes to complete, with a maximum score of 30 points. MCI is determined when the MoCA score is less than 25 points. The validity and reliability testing for the Thai MoCA for MCI screening reported a sensitivity of 80% and specificity of 80%, with a Cronbach's alpha coefficient of 0.91 [32].

Data analysis

All statistical analyses were performed using SPSS Version 28 (IBM Corp., Armonk, NY, USA). The study variables were analyzed using descriptive statistics, including frequency, percentage, percentile, mean, median, standard deviation (SD), and interquartile range (IQR). Spearman's rank correlation coefficient was used to assess the relationship between pesticide exposure data (which were not normally distributed) and MoCA scores. An independent t-test was conducted to compare MoCA scores between the high and low exposure groups. To account for potential confounding factors, linear regression was employed to investigate the association between pesticide exposure and cognitive decline. Statistical significance was set at a p-value of less than 0.05.

RESULTS

Table 1 presents the demographic characteristics of adult farmers, as well as their exposure to residential pesticides. The participants had a mean of 30.3 years (SD = 9.7) of agricultural work experience and a mean farm size of 16,200 m² (SD = 11,400). The mean duration of pesticide use was 24.8 years (SD = 9.5), with 80.2% reporting both mixing and spraying pesticides, and 19.8% spraying only. The prevalence of MCI among the farmers was 93.7% [95% confidence interval (CI) = 91.0% – 96.5%], with a mean MoCA score of 19.6 (SD = 3.3). A significant difference in MoCA scores was found for six variables: sex, age, education level, income sufficiency, BMI, and smoking. These variables were controlled for in the adjusted model for association analysis, with the exception of smoking, which was excluded due to its strong association with sex.

Table 2 summarizes the history of pesticide use reported by farmers, along with the active ingredient classification recommended by the WHO. Farmers reported using insecticides and fungicides in equal proportions (99.7%), while 97.4% reported using herbicides. The most commonly used insecticide class was avermectin (99.0%), followed by organophosphates (17.2%), carbamates (10.6%), and pyrethroids (7.6%). Some farmers used multiple active ingredients from the organophosphate and carbamate classes.

Table 3 presents the pesticide exposure assessments. The median lifetime application days was 490 (IQR = 340), and the median pesticide exposure score was 1327 (IQR = 1597). Sperman's rank correlation coefficient revealed that the MoCA score was negatively correlated with lifetime application days (r_s = -0.145), PPE score (r_s = -0.163), exposure intensity score (r_s = -0.184), and pesticide exposure score (r_s = -0.225). When the pesticide exposure score was divided into high and low exposure groups based on the median, the prevalence of MCI was 96.7% in the high-exposure group and 90.8% in the low-exposure group.

Table 4 compares MoCA scores between the high and low exposure groups using an independent t-test. Significant differences were found in MoCA scores for the PPE score (mean difference = -0.97, p = 0.011), exposure intensity score (mean difference = -1.28, p = 0.001), and pesticide exposure score (mean difference = -1.35, p < 0.001). No significant difference was observed for lifetime application days.

Table 5 shows the association between pesticide exposure and cognitive impairment among farmers after adjusting for sex, age, education level, income sufficiency, and BMI. In the adjusted model, farmers with high exposure had significantly lower MoCA scores than those with low exposure: -0.75 (95% CI: -1.46, -0.05) for the PPE score, -0.97 (95% CI: -1.66, -0.27) for the exposure intensity score, and -0.77 (95% CI: -1.47, -0.06) for the pesticide exposure score.

Table 1. Farmers' general information, residential pesticide exposure, and MoCA scores (n=303)

Variables	n (%)	MoCA score	p-value
		Mean (SD)	
Sex			0.034*
Male	158 (52.1)	19.26 (3.12)	
Female	145 (47.9)	20.07 (3.48)	
Age (years) [Mean (SD)=58.74 (5.99)]			<0.001**
50-59	179 (59.0)	20.49 (3.09)	
60-69	109 (36.0)	18.69 (3.24)	
≥70	15 (5.0)	16.60 (3.00)	
Education level			<0.001**
No	60 (19.8)	17.02 (3.05)	
Primary school	190 (62.7)	20.03 (3.00)	
Secondary school or higher	53 (17.5)	21.26 (3.08)	
Marital status			0.197*
Married	240 (79.2)	19.52 (3.33)	
Single/Divorced/Widowed/Separated	63 (20.8)	20.13 (3.27)	
Perceived income sufficiency			0.013*
Insufficient	164 (54.1)	19.21 (3.04)	
Sufficient	139 (45.9)	20.16 (3.56)	
BMI (kg/m^2) [Mean (SD)=23.15 (3.47)]			0.027**
Underweight (<18.5)	22 (7.2)	17.86 (3.27)	
Normal (18.5-22.9)	131 (43.2)	19.53 (3.42)	
Overweight (23.0-24.9)	75 (24.8)	20.25 (3.14)	
Obesity (≥25.0)	75 (24.8)	19.76 (3.18)	
Underlying disease			0.491*
No	165 (54.5)	19.77 (2.93)	
Yes	138 (45.5)	19.50 (3.73)	
Smoking			0.032*
No	221 (72.9)	19.90 (3.30)	
Yes	82 (27.1)	18.98 (3.29)	
Alcohol drinking			0.215*
No	132 (43.6)	19.37 (3.65)	
Yes	171 (56.4)	19.86 (3.03)	
Entering the farm			0.312**
<1 time per month or monthly	24 (7.9)	20.63 (3.59)	
Weekly	147 (48.5)	19.61 (3.35)	
Every day	132 (43.6)	19.51 (3.22)	
Home proximity to nearest farm			0.439**
<300 m	34 (11.2)	19.62 (3.17)	
300 m – 1 km	92 (30.4)	20.01 (3.31)	
>1 km	177 (58.4)	19.46 (3.35)	
Use of household insecticides			0.215*
No	119 (39.3)	19.35 (3.05)	
Yes	184 (60.7)	19.84 (3.47)	

* Independent t-test
** One-way ANOVA

Table 2. Farmers' pesticide use history and active ingredient classification (n=303)

Pesticide	n (%)	WHO Class	Pesticide	n (%)	WHO Class
Insecticide					
Abamectin	300 (99.0)	Ib	Methamidophos	10 (3.3)	Ib
Malathion	25 (8.3)	III	Carbosulfan	9 (3.0)	II
Cypermethrin	23 (7.6)	II	Methomyl	9 (3.0)	Ib
Chlorpyrifos	20 (6.6)	II	Monocrotophos	8 (2.6)	Ib
Carbaryl	16 (5.3)	II	Carbofuran	7 (2.3)	Ib
Herbicide					
Glyphosate	291 (96.0)	III	Diuron	6 (2.0)	III
Paraquat	153 (50.5)	II	Ametryn	3 (1.0)	II
Fungicide					
Carbendazim	300 (99.0)	U	Benomyl	12 (4.0)	U
Thiophanate	19 (6.30)	U	Copper sulfate	3 (1.0)	II
Mancozeb	17 (5.6)	U	Propineb/Maneb	3 (1.0)	U

Ib – highly hazardous; II – moderately hazardous; III – slightly hazardous; U – unlikely to present acute hazard in normal use

Table 3. Percentile of pesticide exposure and Spearman's rank correlation coefficient between pesticide exposure and MoCA score among farmers (n=303)

Pesticide exposure	P10	P25	P50	P75	P90	Spearman coefficient (r_s)	p-value
Lifetime application days	188	340	490	680	1384	-0.145	0.011
PPE score	0.22	0.27	0.46	0.63	0.63	-0.163	0.004
Exposure intensity score	1.27	1.65	2.47	4.01	4.01	-0.184	0.001
Pesticide exposure score	337	650	1327	2247	3144	-0.225	<0.001

Table 4. Mean MoCA scores of farmers classified by pesticide exposure group (n=303)

Pesticide exposure	MoCA (score)	
	Mean (SD)	p-value*
Lifetime application days		0.159
Low exposure group (≤490)	19.91 (3.40)	
High exposure group (>490)	19.38 (3.22)	
PPE score		0.011
Low exposure group (≤0.46)	20.12 (3.35)	
High exposure group (>0.46)	19.15 (3.22)	
Exposure intensity score		0.001
Low exposure group (≤2.47)	20.31 (3.26)	
High exposure group (>2.47)	19.03 (3.27)	
Pesticide exposure score		<0.001
Low exposure group (≤1327)	20.32 (3.33)	
High exposure group (>1327)	18.97 (3.17)	

* Independent t-test

Table 5. Association between pesticide exposure and MoCA score among farmers by linear regression

Pesticide exposure	B	Beta	p-value	95% CI
Lifetime application days (High vs Low)				
Unadjusted model	-0.54	-0.08	0.159	-1.29, 0.21
Adjusted model*	-0.04	-0.01	0.901	-0.73, 0.64
PPE score (High vs Low)				
Unadjusted model	-0.97	-0.15	0.011	-1.71, -0.23
Adjusted model*	-0.75	-0.11	0.037	-1.46, -0.05
Exposure intensity score (High vs Low)				
Unadjusted model	-1.28	-0.19	0.001	-2.01, -0.54
Adjusted model*	-0.97	-0.15	0.007	-1.66, -0.27
Pesticide exposure score (High vs Low)				
Unadjusted model	-1.35	-0.21	<0.001	-2.09, -0.62
Adjusted model*	-0.77	-0.12	0.033	-1.47, -0.06

B – unstandardized coefficients; Beta – standardized coefficients; 95% CI – 95% confidence interval for B
*Adjusted for sex, age in years, educational level, perceived income sufficiency, and BMI

DISCUSSION

The assessment of cognitive function in adult Thai farmers revealed a prevalence of MCI as high as 93.7%, with a mean MoCA score of 19.65, suggesting an increased risk of Alzheimer's disease and dementia. This finding may be partly due to demographic factors such as age and education, which significantly influence cognitive impairment [19, 21, 23, 26], and should be considered when interpreting MoCA scores using cut-off points [33]. Although the farmers had an average age of approximately 59 years, over 80% had completed only primary school or less, which may impact MoCA scores and contribute to the higher prevalence of cognitive impairment. Additionally, with a mean duration of pesticide use of 25 years and 80.2% of participants mixing and spraying pesticides, chronic and intensive pesticide exposure may play a role in cognitive decline. This prevalence is notably higher than in a Korean study, which reported 29.5% MCI among pesticide users, with a mean MoCA score of 24.1 [23]. However, in a Thai study, pesticide applicators had MoCA scores of 21.25 before pesticide application and 17.51 afterward [27]. Another study reported that Thai farmers had more than five times higher odds of cognitive impairment, as measured by the Mini-Mental State Examination (MMSE), compared to non-farmers [26].

Our findings show that MoCA scores were negatively associated with occupational pesticide exposure, including cumulative exposure score, PPE use score, and exposure intensity score, even after controlling for sex, age, education, perceived income sufficiency, and BMI. The lower MoCA scores in the high exposure group may reflect the health effects of pesticides on cognitive function, potentially through mechanisms such as oxidative stress, mitochondrial dysfunction, neuroinflammation, neurotransmitter abnormalities, and intestinal dysfunction [34]. Prolonged pesticide exposure may increase the risk of cognitive impairment, while proper use of PPE, bathing, and changing clothes after pesticide application may help reduce exposure and lower the risk of cognitive decline. Some farmers reported not using PPE, particularly gloves and chemical masks, during pesticide mixing and spraying – key factors in reducing exposure [27, 35]. Farmers rarely use advanced PPE due to tropical conditions, discomfort, poverty, unavailability, and high costs [36]. As a result, farmers may be exposed to significant amounts of chemicals through inhalation and skin absorption, particularly if they do not shower or change clothes immediately after pesticide use. Inadequate self-protection behaviors may, therefore, increase the risk of cognitive impairment and other health effects among farmers. However, pesticide exposure also depends on factors such as mixing and spraying practices, chemical storage, and disposal [35, 37, 38], which were not included in this study.

These findings align with those from several studies. In Korea, the exposed group had a higher prevalence of MCI and lower MoCA scores than the non-exposed group, though no significant difference was found between high and low pesticide exposure groups [23]. Our study, however, detected a significant difference in MoCA scores between high- and low-exposed farmers, which may reflect variations in study areas, population characteristics, and pesticide-related factors. A Thai study found that MoCA

scores for pesticide applicators were significantly lower after pesticide application [27]. In Chile, agricultural workers directly exposed to pesticides had significantly lower MMSE scores compared to residents living in agricultural areas with indirect exposure [21]. A 4-year follow-up in France showed a significantly greater decline in MMSE scores among exposed vineyard workers [19], while a study in Costa Rica found that exposed individuals performed worse on the MMSE than non-exposed individuals [24].

Farmers in our study reported using various pesticides, including insecticides like organophosphates (malathion), carbamates (carbaryl), and pyrethroids (cypermethrin); herbicides like glyphosate and paraquat; and fungicides such as carbendazim and maneb. Although some of the pesticides identified in this study have been banned in Thailand, their illegal use persists. The neurotoxicity of these pesticides is supported by evidence linking long-term, low-dose exposure to neurodegenerative diseases, particularly from paraquat, maneb, pyrethroids, and organophosphates [39]. A study among Indonesian farmers found that long-term exposure to organophosphates resulted in significantly lower MMSE scores compared to other pesticides [25]. Similarly, exposure to organophosphates has been linked to cognitive performance in older Mexican Americans [18] and French vineyard workers [20]. In Chile, pesticide exposure, as measured by cholinesterase inhibition (a biomarker for organophosphate and carbamate exposure), was associated with cognitive performance [22]. A Thai study also found that individuals with cognitive impairment had significantly lower blood acetylcholinesterase levels compared to those without cognitive impairment [26]. Furthermore, low-level pyrethroid exposure was linked to cognitive dysfunction in older adults in the US [17], and a positive association was found between glyphosate exposure and impaired visual memory among smallholder farmers in Uganda [29].

Regarding the study's limitations, its cross-sectional design limits the ability to infer causal relationships. Additionally, the non-probability sampling method may result in a sample that is not fully representative of the broader population. The use of a questionnaire to assess cumulative pesticide exposure through farmers' self-reports may introduce recall bias and does not specify pesticide types or account for environmental exposure. Future studies should incorporate biological indicators, such as urine and blood tests, to assess internal pesticide exposure, and consider including farmers with no history of pesticide use to better understand the impact of pesticide exposure on cognitive impairment. For low-educated older adults, a cognitive screening tool such as the MoCA-Basic (MoCA-B) may be more appropriate to address this limitation [40]. Despite these limitations, the study's findings underscore the significant differences in MoCA scores between high- and low-exposure groups among Thai farmers, even when exposure is categorized using an algorithm.

CONCLUSIONS

This study provides evidence that occupational pesticide exposure is associated with cognitive impairment in adult Thai farmers, depending on the level of exposure, including PPE use, personal hygiene practices, and other exposure characteristics. The high prevalence of MCI raises significant concerns about potential health risks. Therefore, cognitive performance should be regularly monitored among farmers for early screening and surveillance of Alzheimer's disease or dementia. Preventive measures should be implemented to reduce the risk of negative health effects from pesticide exposure among farmers.

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Conflict of interest
All authors declare they have no potential competing interest.

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ORIGINAL ARTICLE

SURVEY ON FOOD DYES ADDITIVES IN FOOD PRODUCTS COMMONLY CONSUMED BY ALGERIAN CHILDREN

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ABSTRACT

Background. Children are generally attracted to colorful foods. However, some food dyes are suspected of exacerbating the activity of children and inducing other health problems that can reach reprotoxicity and carcinogenicity.

Objective. This study aims to explore the presence of dyes such as E102, E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171 in food products widely consumed by children in Algeria notably sweets and chocolates, beverages and ice creams, yogurts and biscuits.

Material and Methods. This work was carried out on 228 products including 57 biscuits, 47 drinks and ice creams, 20 yogurts and 104 sweets and chocolates. Information mentioned on the composition label of this products were recorded to determine the presence of studied dyes.

Results. Here, we report the abundance of the yellow dyes E102 (24.1%) and E110 (18%) in the tested products. Also, apart from E121, all the other assessed dyes were found. Sweets and chocolates are the products containing the most studied dyes. The analysis of the presence of combinations of these dyes shows that 7% of analyzed foods contain 2 dyes in their composition while 20% of the products contain at least 3 dyes at the same time. Additionally, 37.5% of sweets and chocolates contain a combination of at least 3 dyes in their ingredient list.

Conclusions. In overall, except the E121, all assessed dyes were identified on the labels of food products widely consumed by children which encourage parents to be made aware of the risks associated with the ingestion of omnipresent dyes in children's diets.

Keywords: *food additives, food dyes, children, health risks, hyperactivity disorder*

INTRODUCTION

Lured by their sweet taste and flabbergasted by their smell and shape, children are obsessed with sweets in general, especially candies. This temptation is even greater if the candy is brightly coloured. In fact, the dye, influences the decision to purchase, given its psychological influence on the perception of taste [1, 2]. As a result, confectionery manufacturers "use and abuse" the use of food dyes.

Behind their cheerful and colorful appearance, food dyes hide unpleasant surprises. Their consumption is probably linked to the increase of hyperactivity disorders in children. This concerns yellow dyes E102, E104 and E110 as well as red dyes E122, E124 and E129 [3]. Apart from this study, the suspicion of a potential effect of synthetic dyes on the exacerbation of activity and disturbance of attention in children has been mentioned in 16 other scientific studies [4]. These studies had repercussions on the marketing policy for coloured sweets in Europe. Since 2010, confectionery manufacturers in this continent have had the obligation to specify the statement "may have a harmful effect on the attention of children" on the packaging of candies containing these dyes [5].

In addition to the disruption of the activity of children, food dyes would be potentially implicated in other human health concerns. In individual works it was stated that for instance, dyes E102, E110, E129 and E133 would be the cause of hypersensitivity reactions [6, 7]. On the other hand, dyes E127 and E102 would be reprotoxic. More specifically, these dyes would be

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the cause of a decrease in reproductive performance in male mice characterized by a reduction in the number of spermatozoa and an increase in abnormalities in these cells [8]. Much more serious, the dyes from petrochemical synthesis E102, E110 and E129 are contaminated with carcinogens [6, 7]. Aside from dyes E110 and E129, dyes E123, E124 and E171 are at the origin of genotoxicity characterized by the induction of DNA damage [6, 7, 9, 10]. Also, the consumption of dyes E110, E129 and E171 or dyes E121, E127, E132 and E143 was found related to the triggering of different types of tumors [6, 7, 11].

It should be emphasized that the following dyes: E121, E143 and E171 are not currently approved for use in food in the European Union (Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council, with further ammendments).

Apart from the specific name of the dye and its E code, manufacturers in the agri-food sector in Algeria are under no legal obligations to supply any additional information on the packaging of confectioneries [12]. Therefore, our work aims to assess the presence of dyes responsible for infantile hyperactivity E102, E104 and E110 as well as other dyes likely to trigger health problems, in particular E121, E123, E127, E132, E133, E143 and E171 in food products widely consumed by children in Algeria, namely sweets and chocolates, drinks and ice creams, yogurts and biscuits.

MATERIAL AND METHODS

This is a cross sectional study on the presence of the dyes such as E102, E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171 in food products widely consumed by children. in Algeria namely sweets and chocolates, drinks and ice creams, yogurts and biscuits. This work was carried out during the periods of March-April 2022 and June-September 2021 in convenience stores and supermarkets in the Djelfa region of Algeria. Indeed, the information on the packaging of 228 products (57 biscuits, 47 drinks and ice cream, 20 yogurts and 104 sweets and chocolates) concerning the name, brand, price, composition and origin were recorded. Of note, the repetition of products over the 2 years of the study was eliminated (so that each product was considered only once) before the start of the analyses.

RESULTS

Analysis of the frequency of labeling aberrations

We first analyzed the compliance of sweets/chocolates, beverages/ice creams, yogurt and biscuits labeling regarding Algerian's labeling requirements for the indication of food dyes on the packaging. In other words, we assessed if the E code or the specific name of the dye used is clearly specified in the composition formula of analyzed products. Results reveal that 73.7%, 91.5%, 95% and 82.7% of biscuits, beverages/ice creams, yogurts and sweets/chocolates respectively displayed compliant labels (Figure 1a). However, 26.3% and 17.3% of the packaging labels of biscuits and sweets/chocolates contain anomalies. Regarding biscuits, data indicate that almost 21% of them are visually colored without any indication of dye additives on their label. This also applies to almost 9% of sweets/chocolates. The other labeling aberration observed for biscuits (5.3%) and sweets/chocolates (1.9%) is the presence of the statement "Food color" on the ingredient label without any specification of the E code or the specific name of the food dye additive used (Figure 1b).

Frequency of assessed food dyes in sweets and chocolates

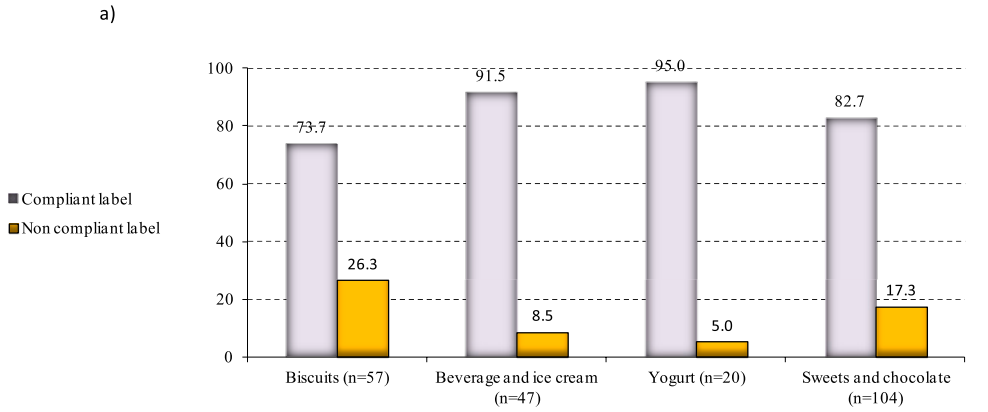
The existence of the food dyes such as E102, E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171 have been studied in children's favorite treats; sweets and chocolates. Except the E121, results show the presence of all assessed food dyes. Indeed, the E102 is the most found dye (almost 39%). In addition, dyes E110, E124, E129, E133 and E171 were found in 28.8%, 23.1%, 17.3%, 24%, and 20.2% of sweets/chocolates respectively. In contrast, reds E122, E123, and E127 were recorded in 9.6%, 4.8%, and 5.8% of sweets/chocolates labels respectively. The results also indicate the presence of the yellow E104 (4.8%), the blue E132 (1%) and the green E143 (almost 3%) (Figure 2).

Frequency of assessed food dyes in biscuits

Being regularly consumed by children, biscuits were also the subject of our investigation on the presence of food dyes, in particular E102, E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171. Our results show that red E122 (17.5%) is the most popular among the list of assessed food dyes. Also, dyes E102, E110 and E124 are each present in almost 9% of the analyzed biscuits. However, the E171 was found in 3.5% of biscuits and dyes E104, E123 and E133 were each recorded in almost 2% of biscuits (Figure 3).

Frequency of assessed food dyes in beverages and ice creams

Depending on household eating habits, beverages and ice cream can also be consumed by children or at least made accessible to them. Therefore, the labels of these foods were also searched for food dyes (E102,



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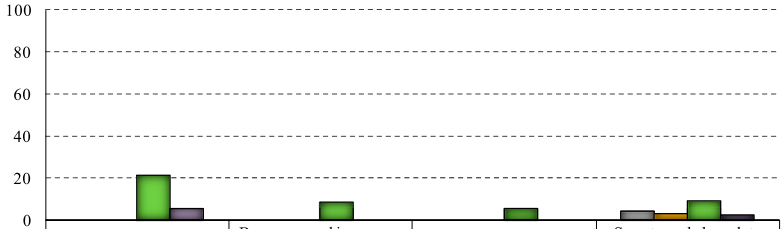


	Biscuits (n=57)	Beverage and ice cream (n=47)	Yogurt (n=20)	Sweets and chocolate (n=104)
Different combinations of dyes on the same composition label preceded by the statement "according to flavor"	0.0	0.0	0.0	3.8
Presence of an incomplete "2-digit" color code or the presence of a code of another additive among dyes	0.0	0.0	0.0	2.9
Colored product without indication of dye on composition label	21.1	8.5	5.0	8.7
The statement "food color" on the composition label without specifying the name or color code	5.3	0.0	0.0	1.9

Figure 1. Analysis of the labeling compliance. a) Frequency of labeling aberrations, b) Analysis of labeling anomalies

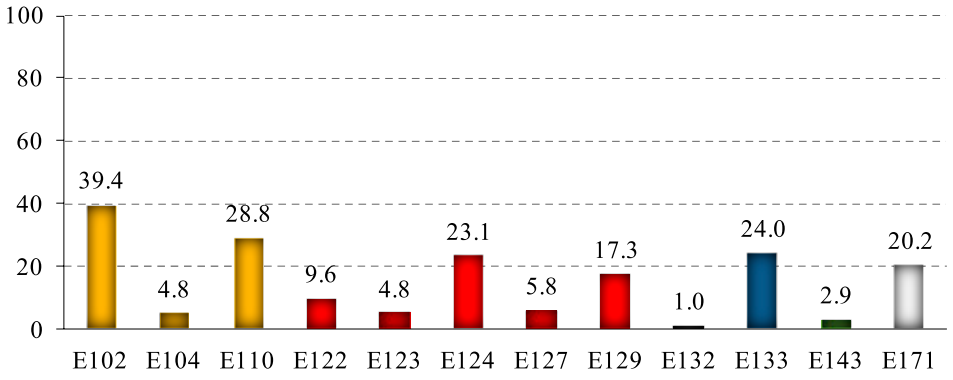


Figure 2. Frequency of assessed food dyes in sweets and chocolates

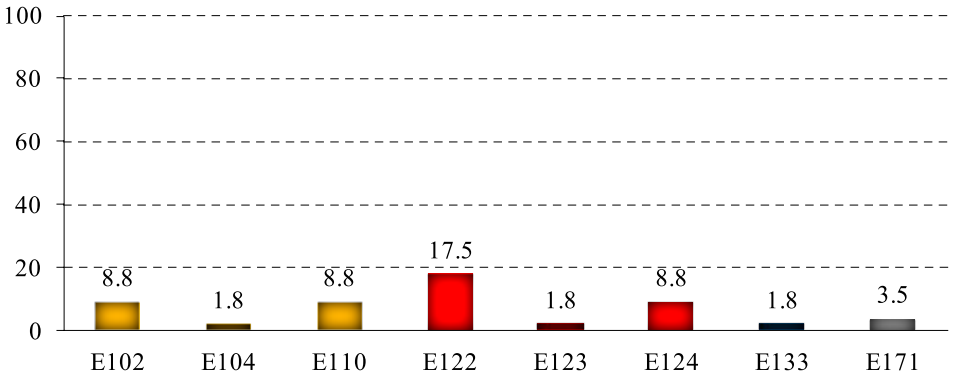


Figure 3. Frequency of assessed food dyes in biscuits

E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171). The results report the existence of dyes E102 and E122 at an equal percentage (17%). We also note the presence of dyes E104, E110, E124, E133 and E171 in 6.4%, 10.6%, 4.3%, 8.5%, 2.1% and 6.4% of beverages and ice creams respectively. However, none of dyes E121, E123, E127, E132 and E143 were found (Figure 4).

Frequency of assessed food dyes in yogurts

Considering their importance in children's diets, yogurts were also included in our survey of food colorings. The same list of food dyes (E102, E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171) assessed in the above foodstuffs was explored in yogurts. The E129 dye was the most common (15%) among the assessed food dyes. Furthermore, dyes E133 and E110 were each recorded in 10% of analyzed yogurts. However, dyes E102, E122 and E124 were found at an equal percentage of 5%. None of the dyes E104, E121, E123, E127, E132, E143 and E171 have been registered (Figure 5).

Frequency of assessed food dyes combinations

Finally, we tried to determine, among the products studied, the percentage of products containing 0, 1, 2 or 3 or more assessed food dyes. Some of the analyzed products do not contain any assessed food dyes. This corresponds to almost 54% of biscuits, almost 55% of beverages/ice creams, 60% of yogurts and almost 30% of the sweets/chocolates category. On the other hand, about 42% of biscuits, 25.5% of beverages/ice creams, 30% of yogurts and about 21% of sweets/chocolates contain studied food dyes in their manufacturing formula. More interestingly, about 11% of biscuits and 10% of yogurts contain combinations of 2 assessed food dyes. This is also true for 8% of sweets/chocolates. Surprisingly, 37.5% of sweets/chocolates contain at least 3 studied food dyes in their ingredient list. This kind of combination was also reported in 8.5% of beverages/ice creams and in 3% of biscuits (Figure 6).

DISCUSSION

The manufacture and marketing of food products containing food additives, in particular dyes, is governed, in Algeria, by executive decree no. 12-214 requiring the inclusion of the specific name and/or the E code of the used food dye on the product label [12]. The assessment of the application of these labeling guidelines in food products commonly consumed by children showed that 83.3% of the labels of analyzed products are compliant. This is representative of 73.7% of biscuits, 91.5% of beverages and ice creams, 95% of yogurts and 82.7% of sweets and chocolates. On the other hand, 16.7% of the tested product labels

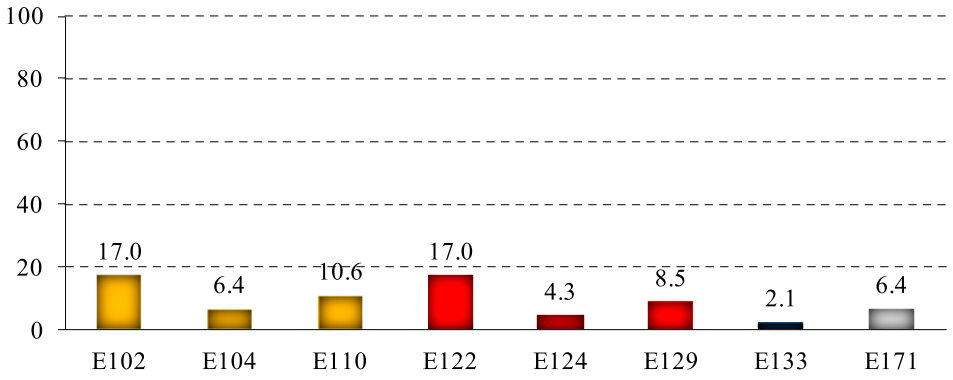


Figure 4. Frequency of assessed food dyes in beverages and ice creams

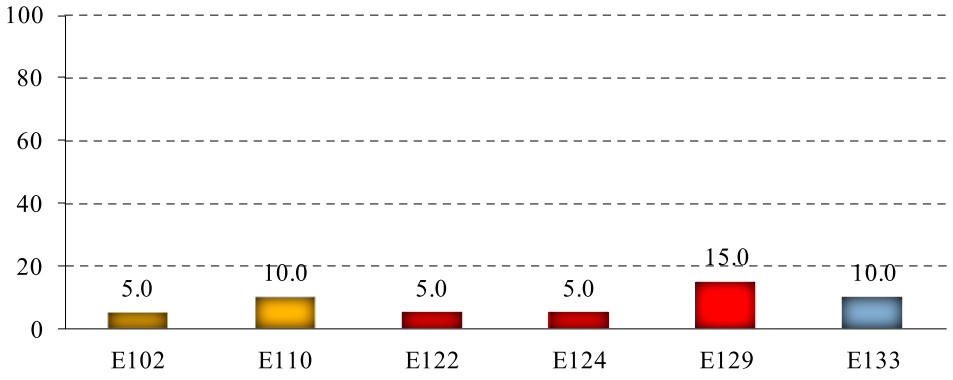
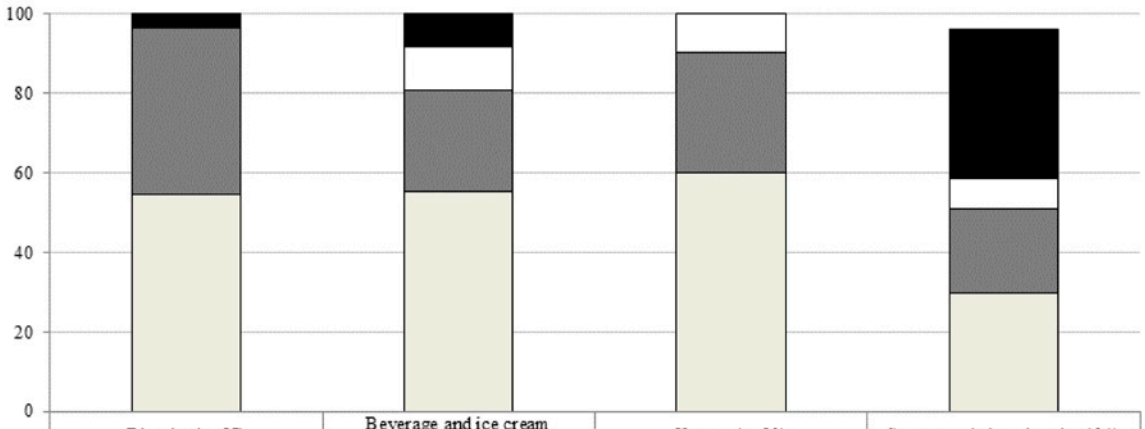


Figure 5. Frequency of assessed food dyes in yogurts



	Biscuits (n=57)	Beverage and ice cream (n=47)	Yogurt (n=20)	Sweets and chocolate (n=104)
3 or more	3.5	8.5	0.0	37.5
2	0.0	10.6	10.0	7.7
1	42.1	25.5	30.0	21.2
0	54.4	55.3	60.0	29.8

Figure 6. Frequency of assessed food dyes associations

(26.3% of biscuits, 8.3% of beverages and ice creams, 5% of yogurts, 17.3% of sweets and chocolates) proved to be non-compliant with Algerian labeling rules. These products had various labeling flaws, namely the absence of indication of the dye additive on the label despite the colorful appearance of the product, the presence of the statement "Food color" on the label without any specification of the dye additive specific name or its E code, display of an incomplete "2 digits" E code or indication of another food additive E code among food dyes as well as the inscription of several food dyes combinations on the same ingredient label preceded by the wording "according to flavor". In fact, the absence of indication of the coloring additive on the label despite the colorful appearance of the product cannot be considered as a real labeling problem given that these foods are made from fruit puree. In other situations, this aberration sometimes reflects another concern to be taken into consideration consisting of the use of the new flavoring technology called "flavoring paste" giving the product, in addition to the flavor, the taste and the dye. The problem with this concept of flavoring pastes is that these latter are made from food dyes without being able to detect them on the composition formula of the products containing these flavoring dough [13].

Also, the problem of the presence of an E code belonging to another family of food additives among the dyes could be explained by the fact that certain food additives have a coloring power despite their belonging to other categories of food additives (the case of egg yolk powder).

Apart from labeling loopholes, the closer analysis of the presence of dyes in products widely consumed by children in Algeria reveals that about 56% of the analyzed products are colored by dyes covered by our study. The yellow dyes E102 (24.1%) and E110 (18%) are the most popular among the studied food dyes. Indeed, the E102 is suspected of being responsible for the exacerbation of children's activity, contaminated by carcinogens, inducer of hypersensitivity reaction and reprotoxic [3, 6-8]. It was found in 34.9% of sweets and chocolates, in 17% of beverages and ice creams, in almost 9% of biscuits and in 5% of yogurts. Like the E102, the E110 is responsible for the increase in infantile hyperactivity disorders, the appearance of allergies, contaminated by a carcinogen in addition to its potential tumorigenic power for adrenal and testicular tissues [3, 6, 7]. This dye was also abundantly found in sweets and chocolates (almost 29%), in beverages and ice creams (almost 11%), in yogurts (10%) and in biscuits (almost 9%). These results are consistent with research carried out in Sri Lanka in 2019 and in India in 2013 [1, 14]. Specifically, Dilrukshi et al. 2019 [1] study showed that E102 (41%) and E110 (22%) were the most popular in confectionary and beverages in Sri Lanka. The predominance of E102 was also reported by dixit et al in 2013 in sweets and savories in India [14]. Even if the study carried out by Asif Ahmed et al the previous year in Saudi Arabia indicated the predominance of the dyes E133 (54.1%) and E128 (58.3%) among the food dyes of synthetic origin in the food products consumed by school going children in Saudi Arabia, this work showed that E102 and E110 existed in 42.3% and 39.1% of the products explored (chocolate, ice cream, juice and drinks, candy, jelly and gums), respectively [15].

In addition, the survey on the dyes used in the sweets sold in Muscat (Oman) showed that E133 was present in 13% of the analyzed candies [16]. In the present study, this dye was also found in 13% of studied products (sweets and chocolates 24%, yogurts

10%, beverages and ice creams 2.1%, biscuits 1.8%). Also, among the most popular food dyes in studies of dyes used in sweets sold in Oman and in children's consumed food products in Saudi Arabia are E129 (43.8% of Muscat candies and 33.9% of children's consumed food products in Sausi Arabia) and E171 (26% of Muscat candies) [15, 16]. Here, we report the presence of each of these 2 dyes in 11% of the tested products. If we focus on the results of this study, we see that sweets and chocolates are the most colorful products among the different products included in this study with a predominance of dyes E102 (39.4%), E110 (28.8%), E133 (24%), E124 (23.1%), E171 (20.2%). If it is trendy to discuss about the ability of food dyes E102, E110 and E124 to increase hyperactivity disorders in children, we must never forget that these dyes have been associated with other physiological disorders, in particular genotoxicity (E102 and E124), tumorigenicity (E110) and reprotoxicity (E102) [6-8, 10]. Like E102 and E110, E133 is involved in hypersensitivity reactions. Its ability to inhibit the development of nerve cells should also not be overlooked, especially when it comes to products consumed by growing children [6, 7]. In addition, E171 was also found to be present in almost a fifth of assessed sweets and chocolates. Prohibited, since 2020, in France and throughout the European Union, since 2022, this so-called "food color" is responsible for gastrotoxicity, hepatotoxicity, an alteration of the intestinal flora, the appearance of oxidative stress, genotoxicity and is carcinogenic [6, 7, 9, 11, 17].

On the other hand, we also note the absence of the dye E121 in all the products covered by this study which could be explained by the fact that it is forbidden to use in Algeria [18, 19]. However, all other food dyes assessed in present survey are authorized by the Algerian low [12].

Before finishing, it is important to point out that the study of the frequency of combinations of dyes shows that 7% of the studied products contain a combination of 2 assessed dyes and 20% of the products contain at least 3 dyes. This result is all the more interesting knowing that the food categories containing the most combinations of dyes are sweets and chocolates, enough to leave any parent "speechless". Under the slogan "the aims justify means" manufacturers use and abuse of coloring additives without any hindsight on their misdeeds or at least their cocktail effect with the other additives present in the food. Given their psychological influence on the purchase decision and the perception of taste, the choice of coloring additives in the food industry is often motivated by the desire to obtain the most "enticing" confectionery or more generally food without any consideration of the repercussions on health. Inevitable in the food industry, these results encourage us to increase our vigilance towards food additives particularly food dyes which are ubiquitous in our meals and in those of our children especially since 37.5% of the sweets and chocolates studied contain a combination of at least 3 assessed dyes. In overall, it is extremely important to raise parents as well as children aware of the dangers associated with dyes contained in confectionery products and to instill in them a culture of reading food labels.

From legal standpoint, it would be very interesting to update the list of authorized dyes.

CONCLUSIONS

Overall, except the E121, the dyes such as E102, E104, E110, E121, E122, E123, E124, E127, E129, E132, E133, E143 and E171 have been identified in food products widely consumed by children without any particular statement on the label. Dyes E102 (almost 24%) and E110 (18%) are the most popular among tested food products. These dyes are present by 2 (7%) or even by 3 (20%). Sweets and chocolates are the food categories containing the most combinations by 3 assessed dyes (37.5%) which could be unfavourable for children health.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

IRON STATUS IN WOMEN OF REPRODUCTIVE AGE IN MOROCCO

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ABSTRACT

Background. Women of reproductive age (WRA) are one of the vulnerable population mostly impacted by anemia and iron deficiency (ID) worldwide.

Objective. This study aimed to assess the prevalence of anemia, ID, and iron deficiency anemia (IDA) among WRA in Morocco.

Material and Methods. This study included a representative sample of 2,012 non-pregnant women aged 15-49 years covering the entire territory of Morocco. Data collection encompassed socio-demographic information, anthropometric measurements, along with blood samples. Hemoglobin (Hb) concentration, serum ferritin (SF), and C-reactive protein (CRP) levels have been analyzed.

Results. The median of SF for the entire population was 27 µg/mL (Interquartile Range (IQR): 12-50 µg/mL), and the mean of Hb was 12.2 ± 1.5 g/dL. Significant differences were observed between urban and rural areas: urban SF median was 24 µg/mL (IQR: 11-45 µg/mL) versus rural 31 µg/mL (IQR: 15-55 µg/mL, $p < 0.001$), and urban Hb mean was 12.2 ± 1.5 g/dL compared to rural 12.4 ± 1.5 g/dL ($p = 0.02$). Furthermore, the prevalence of anemia, ID and IDA are consistently high; 34.3%, 29.8%, and 16.4%, respectively, with a significant difference in favor of urban areas.

Conclusion. Our findings from this national survey reveal that despite over a decade of implementing flour fortification strategy using electrolytic iron to address iron deficiency in Morocco, anemia, ID, and IDA remain widespread among WRA. Exploring alternative strategies or adopting a different form of iron for fortification could be beneficial in reducing or even eradicating iron deficiency among Moroccan women.

Keywords: *anemia, iron deficiency, women of reproductive age, hemoglobin, serum ferritin, Morocco*

INTRODUCTION

Anemia is a condition characterized by a deficiency of healthy red blood cells, which impairs the body's ability to deliver oxygen to vital tissues such as the brain, muscles, and heart [1]. On the other hand, ID refers to low levels of stored iron, leading to reduced SF and decreased saturation of the iron transport protein transferrin [2]. The World Health Organization (WHO) defines anemia as Hb levels below 12.0 g/dL in females, while ID is indicated by SF levels below 15 µg/mL [3, 4]. These conditions are global health concerns, primarily affecting WRA and children. Anemia affects millions of women worldwide, with higher prevalence rates in low- and middle-income countries [5]. The global prevalence of anemia in WRA was 30%, with significant geographical variations [5]. Indeed, nutritional deficiencies, infectious and inflammatory diseases, as well as genetic disorders of hemoglobin are the principal causes of anemia [6]. The most common type of anemia is IDA, which accounts for 50% of all anemia cases worldwide [7]. Anemia can have adverse effects on cognitive and physical abilities, leading to reduced economic productivity [8, 9], increased morbidity, and mortality [10].

Turning our focus to the situation in Morocco, previous surveys conducted in 1994 and 2000 indicated a high prevalence of anemia among WRA, with rates of 30.8% and 32.6% respectively [11]. In response, the Moroccan Ministry of Health (MH) implemented a National Program to Fight and Control Micronutrient Deficiencies, which included measures such as food fortification, dietary supplementation,

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and nutritional education [12]. Food fortification is considered a cost-effective and sustainable approach to increase iron intake and combat micronutrient deficiencies, especially ID, in the general population [13]. ID in Morocco has been estimated to cost 2 billion dirhams per year [14]. In 2005, a national wheat flour fortification program incorporating elemental electrolytic iron (EEI) was initiated to improve iron status across the country [15]. This program was followed by two sentinel surveys conducted in 2006 and 2008, which revealed anemia prevalence rates of 31.5% and 33.3% among WRA, respectively. During the same period, IDA accounted for 63.9% and 59.1% of all anemia cases [16]. However, these results indicated that the national coverage of the fortification program was only 38.3%, highlighting the need for higher coverage rates exceeding 80% to achieve significant improvements [17].

In 2014, the World Health Organization approved a global implementation plan on maternal, infant and young child nutrition [18] specifying six global nutrition goals for 2025 [18]. The second of which is, to achieve a 50% reduction in the prevalence of anemia among WRA by 2025. In Morocco, the MH launched the National Nutrition Program in 2019 with the objective of improving the nutritional status of the Moroccan population and achieve by 2030 a one-third reduction in ID compared to the levels recorded in 2000 [14]. Therefore, the aim of this study is to provide an updated assessment of the prevalence of anemia, ID, and IDA among WRA in Morocco, following 12 years after mandatory fortification program implementation in 2008 and support from the MH.

MATERIAL AND METHODS

Study design and population

The study comes in the framework of Nutrition National Survey (2019-2020) conducted by Moroccan MH at the national level covering the 12 regions of Morocco based on the size-proportional probability sampling approach recommended by the WHO out of a total of 180 clusters [19], in each cluster, households were randomly selected based on the count sheet completed the day before the survey. A sample of 20 households was selected using the systematic approach at a point of departure with the same probability. A total of 3118 households were surveyed (60.4% in urban areas and 39.6% in rural areas). Briefly, in each selected household, a WRA between the ages of 15 and 49 years was recruited for the survey if she was present in the household, if several WRA met the criteria for inclusion and exclusion in a household, a draw based on Kish's table was conducted by the team supervisor. Each WRA younger than 15 years or older than 49 years and were taking iron supplements were excluded from the study. WRA presenting chronic or severe illness requiring hospitalization or treatment were excluded from the study. In a family meeting, the purpose of the study was explained and oral and written informed consent was obtained from women before the start of the investigation.

Ethical approval

The survey protocol was validated by a Steering Committee and a Committee Technical staff comprising representatives of all the institutions concerned (MH, Universities, CHU, HCP) and in a concerted and participatory way, were entrusted with the coordination and monitoring of all stages of the operation. The Ethics Committee for Biomedical Research in Rabat gave the favorable opinion to the realization of the National Nutrition Survey in Morocco after review of its protocol (Ethical Approval number 321; 3 April 2017).

Socio-economic assessments

A questionnaire was used to gather socioeconomic data relevant to the families of WRA. A face-to-face questionnaire included information on level of education, household size, socioeconomic variables of the participants.

Anthropometric measurement

Anthropometric measurements were taken by trained health professionals according to the WHO standard protocol and using calibrated instruments [20]. Measurements were conducted with minimal clothing and without shoes. Body weight was measured to the nearest 0.1 kg using an electronic scale (Seca GmbH and Co. KG). Height was measured to the nearest at 0.1 cm using a stadiometer (Seca GmbH and Co. KG). BMI was calculated as weight in kg divided by height in meter square (BMI; kg/m^2) to define nutritional status as follow: underweight (< 18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obese (≥ 30.0 kg/m^2) [21].

Blood sampling

Blood was withdrawn by venipuncture from fasting participants and collected in dry tubes of 5 mL for the measurement of SF and CRP a marker of the presence of infection and/or inflammation [22]. These samples were subsequently centrifuged at 5000 rpm for 10 min. The serum was then collected and transported to the laboratory in cold boxes with icepacks, and preserved at –80 °C until analysis.

Hb analysis was performed in situ using the Hemocue portable spectrophotometer (HemoCue AB, Angelholm, Sweden) on a drop of venous blood withdrawn while doing the blood sampling. Anemia

was defined as Hb levels < 12.0 g/dL and ID as SF < 15 μg/mL [23], in non-pregnant women mild, moderate and severe anemia were defined as Hb levels 11.0 to 11.9 g/dL, 8.0-10.9 g/dL and lower than 8.0 g/dL respectively [3]. The ferritin and CRP determinations were performed on an auto-analyzer COBAS c311. These tests are based on the principle of immuno-turbidimetry. Furthermore, the values of ferritin were adjusted according to the inflammation status of the WRA based on the CRP values [24].

Statistical analysis

Data were analyzed using IBM SPSS version 21.0. The Kolmogorov-Smirnov test was used to evaluate the normally distributed variables, which are presented as means ± SD, and non-normally distributed variables are presented as median (inter-quartile range (IQR)). Nominal variables are presented as a proportion and 95% confidence interval and *Chi*-square test was used to test independence between nominal variables. t-test was used to examine the difference in normally distributed variables. A p-value of < 0.05 was considered as statistically significant.

RESULTS

Characteristics of the study population

At the end of the field work, the study included a total of 2125 WRA, excluding pregnant or breastfeeding women and those who refused blood sampling. After laboratory analysis and elimination of outliers, a final sample of 2012 women was used for statistical processing. Table 1 presents the characteristics of the participants. On average, the WRA had an age of 32.8 ± 9.3 years, a weight of 69.1 ± 14.4 kg, a height of 159.7 ± 7 cm, and a BMI of 27.1 ± 5.7 kg/m^2. The majority of the women were married (73.2%), and 35.9% of them were illiterate. Regarding anthropometric characteristics, 37% of the women fell within the healthy weight range, while 32.4% were overweight and 27.5% were obese (Table 1).

When comparing between WRA in urban and rural areas, no significant differences were found in terms of age and height. However, the mean weight of WRA in urban areas was significantly higher than that of those living in rural areas (p < 0.001). Illiteracy was more prevalent in rural areas (p < 0.001), while obesity was more common in urban areas (p < 0.001) (Table 1).

Table 1. Characteristics of the participants

	Total	Urban	Rural	p-values
	N = 2012	N = 1193	N = 819	
Age (years), Mean ± SD	32.8 ± 9.3	32.6 ± 9.2	33.2 ± 9.4	0.163
Level of education				
Illiterate, % (95%CI)	35.9 (33.9-38)	24.5 (22.2-27.1)	52.6 (48.9-56.0)	< 0.001
Primary school, % (95%CI)	23.8 (21.9-25.6)	21.6 (19.2-23.8)	27.0 (23.9-30.3)	0.100
Secondary school, % (95%CI)	32.2 (30.2-34.1)	41.6 (38.5-44.3)	18.4 (15.8-21.1)	< 0.001
Superior, % (95%CI)	7.8 (6.6-8.9)	12.3 (10.4-14.3)	1.2 (0.6-2.0)	< 0.001
Other, % (95%CI)	0.4 (0.1-0.7)	0.1 (0-0.3)	0.9 (0.2-1.6)	0.034
Marital status				
Single, % (95%CI)	21.8 (20-23.5)	24.8 (22.3-27.3)	17.4 (14.7-20.2)	< 0.001
Married, % (95%CI)	73.2 (71.3-75)	69.7 (67-72.3)	78.3 (75.2-81.1)	< 0.001
Divorced, % (95%CI)	3.1 (2.3-3.8)	3.6 (2.6-4.7)	2.3 (1.3-3.6)	0.002
Widow, % (95%CI)	1.5(1-2.2)	1.7 (1-2.4)	1.3 (0.6-2.2)	0.106
Separated, % (95%CI)	0.4 (0.1-0.7)	0.3 (0-0.6)	0.6 (0.1-1.2)	0.480
Anthropometric characteristic				
Weight (kg), Mean ± SD	69.1 ± 14.4	70.5 ± 14.8	67 ± 13.4	< 0.001
Height (cm), Mean ± SD	159.7 ± 7	159.5 ± 7	160.1 ± 7.1	0.109
BMI (kg/m^2), Mean ± SD	27.1 ± 5.7	27.8 ± 6	26.2 ± 5.1	< 0.001
Underweight, % (95%CI)	3.2 (2.4-4)	2.6 (1.8-3.6)	4.0 (2.7-5.4)	0.803
Normal weight, % (95%CI)	37 (35.1-39.2)	33.5 (30.9-36.3)	42.0 (38.7-45.3)	0.040
Overweight, % (95%CI)	32.4 (30.3-34.3)	32.4 (29.7-35.1)	32.4 (29.2-35.5)	< 0.001
Obese, % (95%CI)	27.5 (25.4-29.5)	31.5 (28.8-34.2)	21.6 (18.6-24.4)	< 0.001

p-values were determined using *Chi*^{2} test or t-test

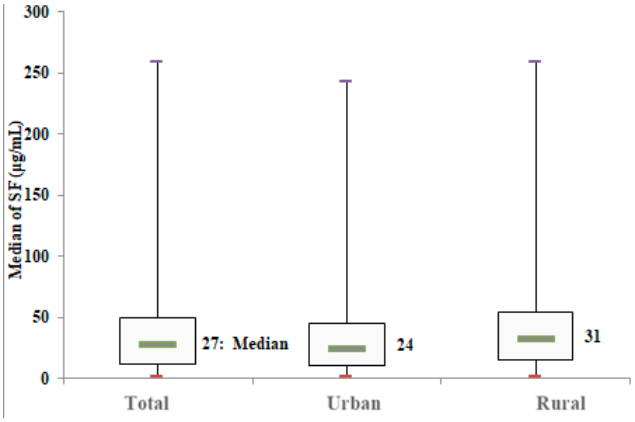
Iron status in WRA

SF status and mean hemoglobin were presented in the figure 1 (a, b). The median of SF for the entire population was 27 µg/mL (IQR: 12-50 µg/mL). Regarding the mean of hemoglobin in WRA, the overall average was 12.3 ± 1.5 g/dL. When analyzed according to living area, a significant difference was observed between urban and rural areas in terms of median serum ferritin ($p < 0.001$). In urban areas, the median serum ferritin was 24 µg/mL (IQR: 11-45 µg/mL), while in rural areas, it was 31 µg/mL (IQR: 15-55 µg/mL). There was a significant difference in the mean of hemoglobin between urban and rural areas ($p = 0.02$). In urban areas, the mean of hemoglobin was 12.2 ± 1.5 g/dL, whereas in rural areas, it was 12.4 ± 1.5 g/dL (Figure 1).

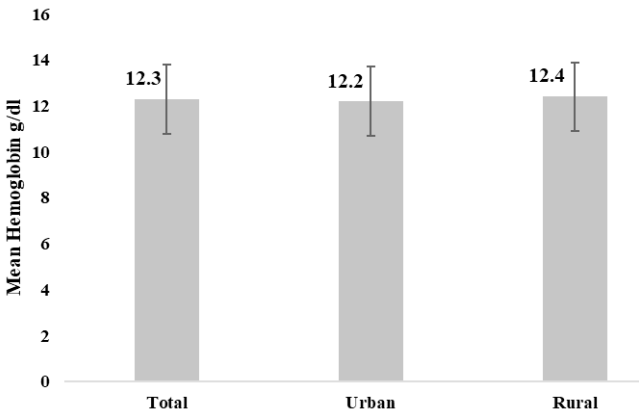
The analysis of the data revealed that out of the total enrolled WRA, 34.3% were found to be anemic, 29.8% had ID, and 16.4% were IDA which represented 47.9% of anemic women. When examining the urban vs the rural population, a highly significant difference was observed ($p < 0.001$). In urban areas 36.5% of WRA were anemic, with 33.5% having ID and 19.3% having IDA. On the other hand, in rural areas, 30.6% of WRA were found to be anemic, with 24.3% having ID and 12.1% having IDA (Figure 2).

According to anemia severity categories, the data revealed that severe anemia accounted for 1% of the total enrolled WRA. Additionally, 14.3% were classified as having moderate anemia, while 19% had mild anemia. When focusing on the urban population, the prevalence of severe anemia was 0.8%, with 16% classified as moderate anemia and 19.7% as mild anemia. In rural areas, the prevalence of severe anemia was slightly higher at 1.2%, with 11.3% classified as moderate anemia and 18.1% as mild anemia. No significant difference was observed in the prevalence of severe anemia between urban and rural areas. However, a highly significant difference was noticed in the prevalence of mild and moderate anemia, with a p-value of less than 0.001 (Figure 3).

a)

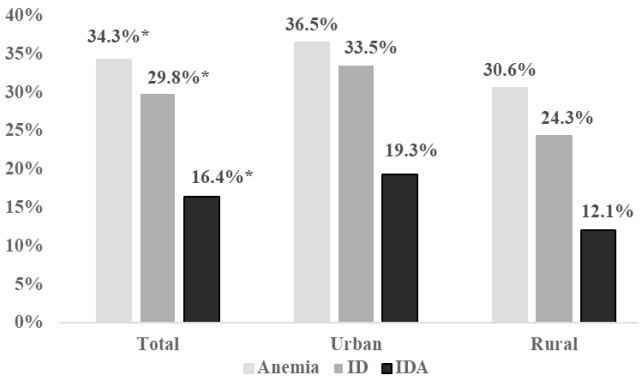


b)



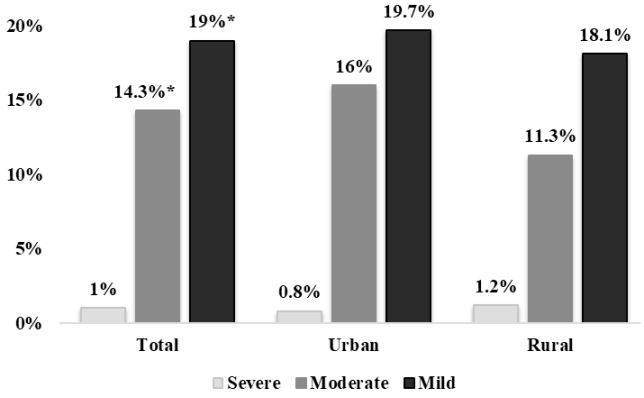
Results are presented as Median (interquartile at Q1: 25% and Q3: 75%) and Mean ± SD

Figure 1. Serum ferritin and hemoglobin status in WRA. a. Median of SF of WRA, b. Mean hemoglobin in WRA



Values are presented as percentage; p-values were determined using *Chi*^{2} test; * – a significant difference between urban and rural areas ($p < 0.05$)

Figure 2. Percentages of anemia, ID and IDA in WRA



Values are presented as percentage; p-values were determined using *Chi*^{2} test; * – a significant difference between urban and rural areas ($p < 0.05$)

Figure 3. Anemia severity categories

DISCUSSION

Anemia affecting WRA is a worldwide form of micronutrient deficiencies, especially in low- and middle-income countries. The aim of this study was to provide an updated assessment of the prevalence of anemia, ID and IDA in WRA in Morocco known that over the course of the last 12 years, the fortification program was mandated and supported by the government of Morocco.

The main finding of this study revealed that 34.3% of women were anemic, 29.8% had ID, and 16.4% had IDA. Numerous studies worldwide have emphasized that anemia caused by iron deficiency is a significant public health issue. For instance, in Turkey, 27.8% of WRA were reported to be anemic [25]. Similarly, a study conducted by Nour Abdo et al. in Jordan found that 19.3% of non-pregnant women were anemic [26]. Research in Uganda [27] and India [28] recorded anemia rates of 32% and 57.2%, respectively. Meanwhile, in Brazil, 25% of WRA were diagnosed with IDA [29]. In South Africa, the prevalence of anemia, ID, and IDA among WRA was reported at 44.0%, 19.0%, and 9.7%, respectively [30].

In Morocco, according to the survey conducted in 2000, it was found that 32.6% of WRA were diagnosed anemic. To solve this problem, in May 2002, an agreement on the fortification of flour with EEI and vitamin B group was signed jointly by the MH and the national flour milling federation. The choice of fortification is because this is the most profitable and sustainable strategies to increase iron consumption as well as it considered a good way to prevent and control this micronutrient deficiency in all populations [31, 32]. After four years of flour fortification strategy implementation, the prevalence of anemia slightly decreased to 31.5% in 2006, but then increased to 38.1% in 2008. The severe, moderate, and mild forms of anemia accounted for 1.3%, 18.3%, and 18.5% respectively in 2008 [11].

In terms of IDA, the rate of anemic women who were ID were 63.9% in 2006 and 59.1% in 2008. These data suggested that the iron-fortification program was having insufficient impact in WRA due to multifactor among others: 1 – The low bioavailability of EEI bioavailability because of its low solubility in gastric juice [33, 34]; 2 – Dietary behaviors consists of a lot of bad habits, it contains many iron absorption inhibitors such as phytic acid and polyphenols [35]; 3 – High rate (exceeds 70%) of *Helicobacter pylori* infection among Moroccan population [36, 37].

However, dietary quality affects absorption of non-heme iron from the gastrointestinal tract [38]. A variety of food factors impact the availability of iron for absorption and transport; the net effect of inhibitors and activators of iron intake can be used to describe food quality in terms of high or low bioavailability [38, 39]. Moreover, in Morocco, it is well documented that tea and coffee consumption inhibits non-heme iron absorption due to their high polyphenol content [40, 41]. The mean per capita annual consumption is estimated at 2,380 g for tea and 1,010 g for coffee, with tea alone accounting for more than 60% of hot drink consumption [42]. In the same context, even for NaFeEDTA which known for its high bioavailability [43], tea can decrease its bioavailability by more than 88% [35].

On the other hand, as the findings of this survey supported the fact that the fortification of flour with EEI did not have a significant effect on the reduction of the prevalence of anemia in WRA, it was recommended to replace the form of iron used for wheat flour fortification by one more bioavailable. The NaFeEDTA form has been chosen for this purpose. The choice was based on bioavailability study conducted in anemic and non-anemic Moroccan women. Indeed, Lazrak et al. [35] founded that fractional iron absorption from bread fortified with NaFeEDTA was equal to 36.7% and 16.7% in the case of anemic and non-anemic women, respectively. Making the fortification of flour with iron-NaFeEDTA mandatory by a Moroccan government decree No. 2-19-144 [44]. In addition to the change of the iron fortifier, the Moroccan MH has implemented various strategies based on the promotion of a diversified diet rich in or enriched with micronutrients, avoid drinking tea with meals and the promotion of public health measures (improvement of vaccination, improvement of hygiene conditions etc.) [14].

The second major result of this study is that the IDA was more prevalent in urban area than in rural areas. Generally, in developing countries, poverty, education level, socioeconomic differences, dietary pattern, are the main factors influencing IDA [45, 46], The long time working or crowded hours and poor eating habits can be one of the causes of this situation [45, 47]. Indeed, in Morocco according the last general census the employment rate of women was 22.3% [48] and the urban population is attracted by the convenience of ready-to-eat food products which are increasingly accessible and highly promoted. Eating out is also becoming more common, which encourages the consumption of foods that are higher in sugar and fat and with low nutritive added value [49]. Like our findings, an analysis of the Haiti Demographic and Health Survey revealed that women living in urban areas are more expected to anemia compared to living in rural areas [50].

CONCLUSIONS

In conclusion, this study highlights the persistent prevalence of anemia, ID, and IDA among WRA, particularly in urban areas. Addressing this issue requires targeted interventions, such as enhancing dietary iron intake through awareness-raising campaigns, optimizing fortification programs through the widespread use of NaFeEDTA flour fortification. However, given that we are now in 2025, it is clear that the goal of a 50% reduction in anemia prevalence among women of reproductive age has not been achieved. Comprehensive measures addressing socioeconomic and nutritional factors remain essential for sustainable improvement.

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Conflict of interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN DIET HEALTH QUALITY AND THE LEVEL OF FUNCTIONAL FITNESS AND QUALITY OF LIFE AMONG POLISH WOMEN AGED 60+

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ABSTRACT

Introduction. Healthy lifestyle is a key factor in improving health and quality of life at any stage of ontogenesis.
Objective. The aim of the study was to assess the relationship between of the health quality of diet and the level of functional fitness as well as quality of life among older women.
Material and Methods. The study was conducted among 201 women aged 60-85 who signed up for the "Active Healthy Senior" project (at the University of Physical Education in Kraków). In the research, the following were used: 1) Dietary Habits and Nutrition Beliefs Questionnaire (KomPAN); 2) Fullerton Functional Fitness Test; and 3) WHOQOL-BREF questionnaire. Relationships between the variables were evaluated using Spearman's R signed rank correlation coefficients in the IBM SPSS 21 program.
Results. In the study, it was demonstrated that along with an increase in the pro-healthy diet index (pHDI-8), agility increased, and with an increase in the non-healthy diet index (nHDI-8), agility and dynamic balance decreased ($p < 0.01$). When there was an increase in pHDI-8, the general perception of quality of life and all domains of quality of life: somatic, psychological, social and environmental, increased ($p < 0.01$). On the other hand, along with the increase of nHDI-8, the psychological domain regarding quality of life experienced a decrease ($p < 0.01$). However, the strength of the demonstrated relationships was low.
Conclusions. Significant (but weak) correlations were found between the health quality of the diet and indicators of functional fitness and quality of life among older women. Thus, health quality of a diet can be one of the predictors of functional fitness and quality of life in elder women.

Keywords: *older adult women, health quality of diet, functional fitness, quality of life, health promotion programme*

INTRODUCTION

Promoting healthy and active aging in Poland is one of the operational goals for the National Health Programme, the strategic objective of which is improving health and quality of life. An element related to the improvement of health potential, further preventing chronic diseases as well as postponing involutional changes is, *inter alia*, maintaining a healthy lifestyle which includes a rational model of nutrition and undertaking recreational physical activity [1]. An active, pro-health lifestyle optimizes various dimensions of holistically-understood health, including physical aspects and psychosocial aspects. On the other hand, mistakes regarding nutrition and hypokinesia are significant factors in the complex etiology of degenerative diseases, including those cardiometabolic, etc. [2, 3].

Within this context, it is necessary to highlight the importance of a significantly varied and balanced diet, which is rich in products having high nutritional density. At the same time, the consumption of high-energy density products, rich in saturated fatty acids, cholesterol, trans polyunsaturated fatty acids and simple sugars, should be limited. Dietary choices define the health quality of a diet, which is a function

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of the implementation of quantitative and qualitative nutritional recommendations, taking into account individual and environmental conditions [2]. Another element of creating health is physical activity, with the features of health training, including endurance and resistance as well as balance, mobility and agility exercises [3, 4]. The positive impact of physical activity on health has been established in various studies. This includes physical fitness, functional fitness, body mass and composition, cognitive functions, mental well-being and social integration of older women [2-4]. Aging is related, among others, with changes in body composition, as well as functional efficiency, which is understood as the aptitude to perform activities of daily living in a safe and effective manner activities [5-7].

Previous studies in this area were particularly concerned with the relationship between physical activity and somatic indicators, functional efficiency and quality of life among seniors [3, 4, 7]. Furthermore, in Finnish research, it has been shown that modifiable risk factors connected with lifestyle, including physical inactivity, increase the risk of depression in older adults [8]. In other studies carried out among this age group, the significance of physical activity with regard to improvement in physical fitness as well as mental state has also been confirmed [9], as well as the optimisation of somatic indicators and increasing life quality, which is defined as subjective assessment of a life situation in relation to culture, the value system, goals, expectations and interests of an individual [10-12]. In our previously published research, relationships were indicated between life satisfaction, eating habits and functional fitness [13] and between dietary behaviors and BMI and functional fitness among senior women [14]. Previous studies have also confirmed the positive impact of participation in a health training program over a 9-month period on BMI, functional capacity and quality of life of women aged 60+ [15].

Despite the crucial importance of behavioral factors for health and quality of life, numerous studies have shown the prevalence of nutritional irregularities and low physical activity in adults and the elderly. Trends in the area have been confirmed in studies carried out among perimenopausal women [16] as well as other groups of senior females in Poland [17, 18]. A low level diet related health quality has also been demonstrated in various regions of the world [19, 20].

The "Active Healthy Senior" project, implemented at the University of Physical Education in Kraków, fits in with the postulate of promoting a healthy lifestyle. The aim of the programme is to improve the psychophysical fitness among these individuals through participation in recreational activities (health training) and lectures on the modified determinants of the aging process as well as the possibility of delaying involutional changes. The programme was described in our earlier publication [13].

In reference to the subject and scope of the presented classes to activate older adults from the metropolitan (Kraków) environment, research was conducted to assess levels concerning the quality of diet-related health, indices of functional fitness and quality of life, and the relationship between of diet health quality and the level of indices of functional fitness and indicators of life quality with reference to the females taking part in the "Active Healthy Senior" project at the time of its commencement. The following research questions have been posed: i) What are the indices of diet health quality and functional fitness and quality of life among elderly women? ii) What are the relationships between diet health quality and the functional fitness as well as quality of life among women who signed up for the "Active Healthy Senior" programme.

MATERIAL AND METHODS

Participants and procedures

The study was conducted among women who agreed to sign up for participation in the "Active Healthy Senior" project, which was implemented at University of Physical Education in Kraków (POWR.03.01.00-00-T225/18). The programme was intended for people aged 60+ and was implemented in 2019-2022. The study was organised after obtaining participants' written informed consent for participation, and the trial was conducted in accordance with the principles proposed by the Declaration of Helsinki. The research protocol was approved by the Bioethics Committee of the District Medical Chamber in Kraków (No. 166/KBL/OIL/2018).

The study comprised a group of 201 women between the age of 60 and 85 (Mean = 66.5, SD = 5.4). The sample was dominated by women with higher (59.3%) and secondary education (36.6%), while fewer had basic vocational education (4.1%). Most of the participants were retired (85.5%), while there were fewer economically-active women (14.5%). Former teachers accounted for the highest percentage (12.4%). The majority of women lived in Kraków (89.7%), less often in smaller centres. The majority resided in a shared household with their spouses (46.9%) or ran a single household (42.2%), and the remaining women lived with their children (10.9%). They assessed their financial situation as average (79.4%), less often as below average (10.5%) and above average (10.1%). In terms of health assessment, women declared the presence of chronic diseases, including hypertension (39.9%), obesity (29.3%), type 2 diabetes (13.6%), osteoporosis (13.6%), lipid disorders (7.6%), neoplastic disease (5.5%) and depressive conditions (3.5%). The presented data regarding sociodemographic and

health characteristics were obtained on the basis of declarations made by the surveyed women (as part of a personal questionnaire).

Instruments

Indices of healthy diet quality

In order to assess nutrition, the Dietary Habits and Nutrition Beliefs Questionnaire (KomPAN), developed by the Human Nutrition Science Committee of the Polish Academy of Sciences, was used [21]. The frequency of product consumption was evaluated on a six-point ordinal scale. The original ranks were then converted into real numbers expressing the daily frequency of food consumption (as times/day), according to the formula: 'never' (0), 'once-three times a month' (0.06), 'once a week' (0.14), 'a few times a week' (0.5), 'once a day' (1) and 'a few times a day' (2) [21].

Totalling the values that determined daily consumption frequency with regard to specific product groups, allowed to note that two indicators of diet quality were calculated: the pro-healthy diet index-8 (pHDI-8), concerning food consumption with possibly positive influence effect on health, and the non-healthy diet index-8 (nHDI-8), indicating food consumption possibly detrimental to health. The pHDI-8 is related to the frequency of consuming eight product groups: whole-meal bread, milk, fermented milk drinks, cottage cheese, fish dishes, dishes from legume seeds, fruit and vegetables. The nHDI-8 is determined by the frequency of consuming eight groups of products: fast foods, fried foods, sweets, instant soups, canned meats and others, sweetened carbonated or non-carbonated beverages, energy drinks and alcoholic beverages [22]. The pHDI-8 and nHDI-8 were calculated by summing the frequency of consuming (times/day) the corresponding eight food groups. Then, the raw results (total frequency of consumption) were converted into a point scale (0-100 points), according to the formula: pro-healthy diet index (pHDI-8, in points) = (100/16) × the sum of the frequency of consuming the eight food groups (times/day); non-healthy diet index (nHDI-8, in points) = (100/16) × the sum of the frequency of consuming the 8 food groups (times/day) [22]. The interpretation of the indices is such that the higher the index value, the greater the intensity of the favourable or unfavourable nutritional features for one's health. The values of the pHDI-8 and nHDI-8 expressed as the sum of the daily consumption frequency of 8 product groups (times/day) are within the range of 0-16. The values of the pHDI-8 and nHDI-8 within the range of 0-5.33 are defined as low, between 5.34-10.66 as moderate, and 10.67-16.00 as high. After conversion to a point scale, 0-33 is assessed as low, 34-66 as moderate, and 67-100 is considered high [22].

The overall Diet Quality Index (DQI) was also calculated as the sum of all components of the pHDI-8 index (with a positive sign) and all components of the nHDI-8 index (with a negative sign), according to the formula: DQI = (100/16) × sum of frequency of 8 healthy food groups (times/day) + (-100/16) × sum of frequency of consumption of 8 unhealthy food groups (times/day). The DQI ranges from -100 to 100 points, with a range of 26 to 100 points interpreted as high intensity of healthy diet traits [21].

Indices of functional fitness

Physical fitness was evaluated using the Senior Fitness test (Fullerton Functional Fitness Test) [6]. The test consists of 6 fitness tests that allow to indirectly assess the strength of the upper and lower body, agility, complex motor coordination and balance, as well as aerobic endurance. Further trials of the Fullerton test include: 1) 'Arm Curl'; 2) 'Back Scratch'; 3) '30-Second Chair Stand'; 4) 'Sit-and-Reach'; 5) 'Up-and-Go 2.44 m'; 6) '2-Minute Step in Place' [6]. For women aged 60-85, the standards for the individual tests are: "Arm Curl" (10-19 no of reps); "Back Scratch" (-5.5 - +1.5 cm); 3) "30-Second Chair Stand" (9-17 no of reps); 4) "Sit-and-Reach" (-2 - +5 cm); 5) "Up-and-Go 2.44 m" (8.7-4.4 s); 6) "2-Minute Step in Place" (60-107 no of steps) [6]. Functional fitness measurements were carried out in accordance with standards determining their proper conduct, i.e. after instruction and a warm-up, including stretching exercises. These measurements were conducted by the study authors, specialists in the field of health training.

Quality of life

Quality of life was judged using the WHOQOL-BREF questionnaire. The scale is used to assess the quality of life of healthy and ill people (for cognitive and clinical purposes). It contains 26 questions implemented to evaluate quality of life profile in terms of 4 dimensions: physical (somatic), psychological, social and environmental. The questionnaire also includes 2 questions that are analysed separately: question 1 – on individual general perception of quality of life, and question 2 – on individual perception of one's own health. The scoring of the questions ranges from 1 to 5 and has a positive direction (the greater the number of points, the better the quality of life). The analysis of the results only included the question concerning quality of life self-assessment. The results are presented as raw values and converted into a scoring scale of 0-100 points, in accordance with WHO recommendations [23].

Statistical analyses

Descriptive statistics (M – mean, SD - standard deviation, Me – median, Q75 – upper quartile, Q25 –

lower quartile, Min, Max) were calculated using the IBM SPSS 21 statistical package. The distribution of variables was assessed using the Shapiro-Wilk test. Due to the nature of the variable distribution, the median was used as a measure of the central tendency. The relationships between the analysed variables were assessed using Spearman's R signed rank correlation coefficients, assuming the significance level of $p < 0.05$.

RESULTS

Health quality of women's diet

The median of the pro-healthy diet index was 33 points, while the non-healthy diet index totalled 3.5 points. The obtained results indicate a low level of both indices concerning the health quality of the diet (Table 1).

Assessing the level of pro- and non-healthy diet indices, it was demonstrated that the study group is dominated by women with a low (52.3%) and moderate (46.7%) level of the pHDI-8 and a low level of the nHDI-8 (99.0%) (Table 2).

The overall diet quality index (DQI-16), as a compilation of pHDI-8 and nHDI-8 indices, showed a high level of healthy diet among women (Me = 29.5 points; Q25 = 21.1; Q75 = 37.4; Min = -20; Max = 88.4). This group was dominated by women (64.8%) characterized by a high level of healthy diet (DQI above 26 points).

Women's indices of functional fitness

Measurements of functional fitness indicators for the Fullerton test indicated that women in the loaded arm flexion test obtained a result of 17 repetitions, in the attempt of 'Back Scratch', the so-called 'safety pin' test, the result of -4 cm was achieved, in the '30- Second Chair Stand' attempt, 15 repetitions was the result, in the attempt to bend forward in a sitting position, the result of 6 cm was obtained, in the test, get up and walk, the result was 4.9 seconds, and in the test of 2-min walk in place, the result totalled 110 steps (Table 3).

Women's quality of life

Among the areas regarding the quality of life scale, the surveyed women obtained the highest scores in the environmental (Me = 69.0) and social (Me = 69.0) strata, then the psychological domain (Me = 63.0), and lower at the somatic level (Me = 56.0). The overall perception of the quality of life of women was 4.0 (Table 4).

Table 1. Values of pro-healthy (pHDI-8) and non-healthy diet (nHDI-8) indices among older women (descriptive statistics)

	N	Mean ± SD	Median (Q25 - Q75)	Min - Max
pHDI-8 (times/day)	199	5.4 ± 1.9	5.3 (4.2 - 6.6)	0.0 - 14.1
nHDI-8 (times/day)	199	0.7 ± 0.9	0.6 (0.2 - 1.1)	0.0 - 6.5
pHDI-8 (points)	199	33.6 ± 12.3	33.0 (26.4 - 41.5)	0.0 - 88.4
nHDI-8 (points)	199	4.6 ± 5.5	3.5 (1.2 - 6.6)	0.0 - 40.4

Table 2. Level of pro-healthy (pHDI-8) and non-healthy diet (nHDI-8) indicators among older women (%)

Level of index	N	Pro-Healthy Diet Index (pHDI-8)	Non-Healthy Diet Index (nHDI-8)
Low	199	52.3	99.0
Moderate	199	46.7	1.0
High	199	1.0	0.0

Table 3. Functional fitness for older women (Fullerton test) (descriptive statistics)

Fullerton test trials	N	Mean ± SD	Median (Q25- Q75)	Min - Max
Arm Curl (number of repetitions)	200	17.1 ± 3.3	17.0 (13 -17)	7.0 - 28.0
Back Scratch (cm)	200	-5.4 ± 8.3	-4.0 (15 - 19)	-36.5 - 29.5
30-Second Chair Stand (number of repetitions)	199	15.1 ± 2.9	15.0 (2.5 - 17.5)	8.0 - 27.0
Sit-and-Reach (cm)	200	6.1 ± 12.9	6.0 (-5 - 4)	-27.0 - 31.0
Up-and-Go (sec)	200	5.0 ± 0.8	4.9 (5 - 5)	3.3 - 9.9
2-Minute Step in Place (number of steps)	199	109.4 ± 14.8	110 (101 - 118)	29 - 158

Correlations between diet quality and women's indices of functional fitness

Assessment of the correlations between indicators of diet health quality and the level of functional fitness indicates that agility and dynamic balance decreased along with the increase in pHDI-8 ($p < 0.01$), while with the increase of nHDI-8, agility and dynamic balance decreased ($p < 0.01$). However, the correlation rates are low (Table 5).

Correlations between diet quality and women's quality of life

Assessment of the correlations between the health quality indicators of diet and the intensity of the quality of life indicates that with the increase in pHDI-8, the overall perception of the quality of life also increased ($p < 0.01$), as well as all domains of quality of life: somatic ($p < 0.01$), psychological ($p < 0.01$), social ($p < 0.01$) and environmental ($p < 0.01$). On the other hand, with the increase in nHDI-8, the psychological domain of the quality of life decreased ($p < 0.01$). However, the strength of these relationships is low (Table 6).

DISCUSSION

Among women who signed up for the "Healthy Active Senior" programme, significant associations were found between higher health diet quality and better functional fitness and higher quality of life.

In the discussed research, a low level of the pro-healthy and non-healthy diet indices has been shown among older adult women. More than half of the women achieved a low, and less than half, a moderate

Table 4. Level of quality of life indices (WHOQOL) among older women (descriptive statistics)

WHOQOL domains	N	Mean ± SD	Median (Q25 - Q75)	Min - Max
WHOQOL 1 (overall quality of life)	198	3.8 ± 0.6	4.0 (3 - 4)	2.0 - 5.0
Somatic domain (raw results)	198	22.5 ± 2.7	22.0 (21 - 24)	15.0 - 30.0
Somatic domain (0-100 scale)	198	55.8 ± 10.0	56.0 (50 - 63)	31.0 - 81.0
Psychological domain (raw results)	198	21.3 ± 2.3	21.0 (20 - 23)	15.0 - 27.0
Psychological domain (0-100 scale)	198	61.6 ± 10.6	63.0 (56 - 69)	38.0 - 88.0
Sociological domain (raw results)	198	10.7 ± 1.3	11.0 (10 - 11)	5.0 - 13.0
Sociological domain (0-100 scale)	198	65.0 ± 10.7	69.0 (56 - 69)	19.0 - 81.0
Environmental domain (raw results)	198	29.0 ± 3.7	29.0 (26 - 31)	18.0 - 38.0
Environmental domain (0-100 scale)	198	68.6 ± 10.7	69.0 (63 - 75)	31.0 - 94.0

Table 5. Relationship between indicators of diet health quality and indices of functional fitness among older women (Spearman's R) (N=199)

Fullerton test trials	pHDI-8	nHDI-8
Arm Curl (number of repetitions)	0.06	0.01
Back scratch (cm)	0.06	0.09
30-Second Chair Stand (number of rep)	0.09	0.06
Sit-and-Reach (cm)	0.16*	0.12
Up-and-Go (sec)	-0.07	-0.19*
2-Minute Step in Place (number of steps)	0.05	-0.02

*$p < 0.01$

Table 6. Relationship between indicators of diet health quality and indices of quality of life among older women (Spearman's R) (N=198)

WHOQOL domains	pHDI-8	nHDI-8
WHOQOL-Q1	0.19*	0.04
Somatic domain (raw results)	0.19*	-0.07
Psychological domain (raw results)	0.17*	-0.15*
Sociological domain (raw results)	0.15*	-0.02
Environmental domain (raw results)	0.23*	-0.07

*$p < 0.01$

level of the pHDI-8. At the same time, almost all women achieved a low level of the nHDI-8. However, the general diet quality index indicated a high level of a healthy diet. Low values of the pro-healthy diet index indicate a limited frequency of consuming products recommended in the diet (i.e. whole-meal bread, dairy products, including fermented ones, fish, fruit and vegetables), which could reduce the supply of e.g. dietary fibre, calcium, omega-3 PUFAs and food antioxidants, ingredients important in the prevention of various diet-related diseases. In turn, low values of the non-healthy diet index indicate a low frequency of consuming less recommended and contraindicated products in the diet (i.e. fast food, sweets as well as sweetened and energy drinks), which could limit the supply of salt, trans isomers and simple sugars, ingredients that increase the risk of developing various chronic diseases, including those cardiovascular. The authors of a various publications suggest the pro-health nature of a diet rich in fruit, vegetables, milk and dairy products, legume and oil seeds, in opposition to processed foods, sweets, high-fat products and alcohol [24, 25]. A low level of the pro-healthy diet index was also described in other population groups in Poland, including adolescents and adults [26] and women from southern Poland [27]. Low values of health quality indicators of the diet were also described among people aged 23-80 from Warmia and Mazury (north-eastern Poland). The level of the pHDI-8 was 3.6 times/day and was lower than in the studied group from Kraków, while nHDI-8 was 1.2 times/day and was higher than in our research [22].

The evaluation of functional fitness among women participating in the "Healthy and Active Senior" programme, based on the Fullerton test, showed that women most often achieved results within the norm for women aged 60-85, presented in the methodological section [6]. In other studies among older adult women, a different level of physical fitness and its individual domains was demonstrated in the Fullerton test. Higher values of some indicators of functional fitness than those achieved in the present study were obtained among University of the 3rd Age students (60-75 years) in Włocławek (Poland) [28]. Lower values of some indices of functional fitness found among the studied women from the Kraków population compared to the above-mentioned studies (from Włocławek, Poland) may result from the fact that women enrolled in the "Healthy Active Senior" programme were at the beginning of the physical activation programme. Cognitively interesting in this context would are the results of tests repeated after completing the programme. Comparing the results before and after implementing the health training programme, it was found that the while the functional fitness indicators, including the tests: '30-Second Chair Stand', '30-Second Arm Curl', 'Back Scratch' and 'Two-Minute Step-in-Place' increased [15]. In other studies on the functional fitness of women aged 60-74 from the University of the 3rd Age in Warsaw (Poland), no significant age-related differences were shown, and the values of some samples oscillated around mean values similar to those obtained in our own research [29].

Our research in the area of quality of life assessment among women from the "Healthy Active Senior" programme allowed to demonstrate the highest results in the environmental and social domains, and the lowest in the somatic area. The obtained results indicate that the surveyed women were most satisfied with their functioning in the environment and interpersonal relationships, less in terms of their mental state, and the lowest assessment of the physical dimension of life, which can be explained, among others, by declared chronic diseases. In other studies among women with excess body mass, lower values were noted for the quality of life indices in all domains of the WHOQOL scale, with a similar overall perception of quality of life [30].

In our research, significant (however weak) dependencies were also demonstrated between quality of diet health as well as levels of some areas of functional fitness and the quality of life among the women registered in the "Healthy Active Senior" programme.

With regard to the correlation between diet quality and the indicators of functional fitness, it was found that along with the increase in the quality of the pro-healthy diet index (pHDI-8), agility of the lower body increased, and with the decrease in diet quality (increase in nHDI-8), agility and dynamic balance decreased. However, the relationships shown were weak. The obtained regularities suggest the importance of a rational diet for the physical fitness of older adult women. Assuming that physical fitness is related to physical activity level, it can be concluded that people with more rational food choices are also more involved in undertaking physical activity, which is also indicated by other authors [31]. The research results obtained by other authors correspond with the presented trends. In systematic reviews, role of nutrition and physical activity have been confirmed in the etiopathogenesis of clinical frailty syndrome, which is characterised by loss of muscle strength and impairment of physical function associated with more frequent falls and hospitalisation in the 60+ age group [32]. In research on the role of diet in geriatric rehabilitation, it has been shown that there is a correlation between malnutrition and poor physical fitness as well as a weaker rehabilitative effects. At the same time, the importance of dietary intervention (additional supply of energy and protein)

for the improvement of physical fitness, including the strengthening of muscle mass and strength, and the role of a high-quality health diet in delaying the development of sarcopenia [33], have been confirmed.

In terms of the relationship between the diet quality as well as quality of life, it was observed that along with increasing quality of the pro-healthy diet index (pHDI-8), the overall opinion regarding quality of life and the intensity of all quality of life domains (somatic, psychological, social and environmental) increased, while the quality of nutrition (with an increase in nHDI-8), the psychological domain of quality of life decreased. However, the relationships shown were weak. The obtained regularities suggest the importance of a rational diet in improving quality of life, which is related to holistically understood health, as a balance and integration of all dimensions constituting a human being (physiological, psychological and sociological), and to factors that determine them, including those behavioural and environmental (physical and psycho-social). The correlations indicated above are consistent with the results of other studies in this area of research. The associations between indicators of nutritional status and the quality of life in older adult women have been demonstrated in various studies. In this respect, it has been indicated that with the increase in WHR, the quality of life of Iranian women decreased [34]. In other studies on Australian women, relationships have been found between indicators of diet quality, physical fitness and overall health. Correlations between the quality of diet and the physical and psychological domain of the quality of life among Australian women aged 60 and above have also been demonstrated [35]. In our previous research, positive associations were also confirmed between rational eating behaviours (in terms of the correct number of meals, consumption of wholegrain cereal products, vitamin D supplementation and proper hydration) with higher life satisfaction, which is an indicator of assessment regarding subjective quality of life [13]. Our previously published work also found that healthy eating habits (including natural dairy products as snacks and regular meals) were associated with higher quality of life, while unhealthy eating habits (including sweetened beverages and sweet dairy products as snacks) were associated with lower quality of life [36], which is consistent with the results of the discussed studies on the relationship between quality of life and the health quality of the diet of women aged 60+.

The limitations of the present study are principally associated with the nature of the group, including individuals that are interested in maintaining a healthy lifestyle who willingly took part in the health activation programme for older adults, restricting the potential of transferring the results of this study to the general population of individuals aged 60 and above. Another important limitation is the cross-sectional and descriptive nature of the study, as well as the lack of a control group. In following research, it would be possible to evaluate relationships between the analysed variables, but in a different configuration, e.g. between somatic features, functional efficiency and quality of life.

CONCLUSIONS

Among women who joined the "Healthy Active Senior" programme in Kraków (Poland), significant positive (but weak) relationships were found between the health quality of the diet and some areas of functional fitness and the all domains of quality of life. The results therefore suggest that diet health quality may be one of the predictive factors for the functional fitness and quality of life among women aged 60+ from a metropolitan population interested in an active lifestyle.

Conflict of interest

None declared.

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ORIGINAL ARTICLE

EVALUATION OF ANTHROPOMETRIC PARAMETERS BASED ON EMOTIONAL EATING

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ABSTRACT

Background. Emotions and moods are important regulators of food intake. While initially excessive intake, especially of unhealthy foods, was associated with negative emotions, now the emphasis is also on positive ones.

Objective. The aim of the work was to evaluate the emotional behavior of a selected group of the Slovak population in relation to nutritional behavior, as well as in relation to body composition, and to identify correlations between emotional eating and body composition.

Material and Methods. One hundred and eighty volunteers were involved in the study. To assess the emotional aspects of eating, we used a questionnaire developed within the EATMOT project. Body composition was analyzed using a bioimpedance device InBody 970 (multi-frequency bioelectrical impedance/MF-BIA).

Results. The results showed that participants who relieve stress by eating had significantly the lowest values of parameters related to muscle mass (SLM, FFM, SMM, BMR). Participants who consume food that corrects their body weight had significantly the lowest values of fat parameters and in most cases the highest values of parameters related to muscle mass. The analysis showed a strong correlation between question Q_1 and Q_6 ($r = 0.649$; $P < 0.001$), Q_8 ($r = 0.636$; $P < 0.001$) and Q_9 ($r = 0.651$; $P < 0.001$). The questions mentioned form block 1, in which food represents a form of escape. A strong correlation was also confirmed between Q_6 and Q_8 ($r = 0.658$; $P < 0.001$) and a moderate one with Q_7 ($r = 0.488$; $P < 0.001$). A strong correlation was also found in the case of Q_8 and Q_9 ($r = 0.575$; $P < 0.001$) and a moderate one with Q_5 ($r = 0.491$; $P < 0.001$). We did not find any significant differences between block 1 and block 2 (positive emotions) ($P > 0.05$). The values of anthropometric parameters in block 1 were significantly different from the values corresponding to question Q_2. As expected, participants in Q_2 had lower values of fat parameters and higher values related to muscle mass than participants in block 1.

Conclusions. Emotional eating has a significant impact on body composition. However, it should be clearly pointed out that emotional eating is not only associated with negative feelings, but also with positive ones. We can eat not only stress and depressive states, but also feelings of happiness and well-being. The results showed that the values of anthropometric parameters did not differ significantly between those who associate food with negative emotions and those who associate its consumption with positive emotions. However, it was clearly confirmed that those who choose food consciously in relation to the sustainability of adequate body weight also achieved the most optimal values of anthropometric parameters.

Keywords: *emotions, food, weight, obesity, InBody*

INTRODUCTION

An indispensable requirement for life is to satisfy the needs of the organism, especially in terms of energy and nutritional intake. The energy and nutrients that we consume through food are essential for life processes and functions of various structures, organs and the course of metabolic processes. The primary regulator of food intake is the hunger and satiety center, as well as the physiological and nutritional needs of the organism [1]. However, personalized food choice is also influenced by other factors. The relationship between nutrition and health has been described and confirmed by numerous studies [2-7, 8]. For this reason, nutritional recommendations at national and global levels are oriented towards the intake of health-promoting foods, with optimal energy and nutritional content, antioxidant effect, as well as adequate glycemic load [9]. The goal of a rational diet is to ensure the prevention of non-communicable diseases of a civilization nature [10]. Despite this, the health status of the population is constantly deteriorating.

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This is a consequence of lifestyle and changing conditions for a full life. This is clearly related to our daily diet and the choice of food not only based on the needs of the body, but also based on psychological aspects, economic situation, environmental awareness and other factors [3, 11-13].

Emotions are important regulators of food intake. While initially excessive intake, especially of unhealthy foods (foods high in fat, refined sugars, high in energy or high in glycemic index) was associated with negative emotions, now the emphasis is also on positive ones [14, 15]. Some consumers tend to prefer food according to emotions and the most common consequence of this is overeating related to compensation for negative or positive feelings [14, 16, 17]. Specific emotions such as anger, fear, sadness and joy influence eating responses throughout the entire food intake process [18, 19]. Such food and eating behavior also have a significant impact on our body weight and body composition.

The aim of our research work was to evaluate the emotional behavior of a selected group of the Slovak population in relation to food intake and nutritional behavior, as well as in relation to selected anthropometric indicators of body composition, and to identify correlations between answers to individual questions in relation to selected anthropometric parameters.

MATERIAL AND METHODS

Study design

A total of 207 volunteers were included in the study, but twenty-seven of them were excluded due to insufficient or missing data or due to the presence of a serious illness. Exclusion criteria included age < 18 or > 50 years, BMI > 40 kg/m^2, the presence of serious diseases of a physical or psychological nature, use of medications affecting body weight, physiological obstacles such as pregnancy or suspected pregnancy, performance of professional sports, contraindications for bioimpedance measurement, increased physical activity immediately before the measurement, recent weight loss, increased intake of coffee, alcohol or fats ≤ 8 hours before testing and diuretics 7 days before testing. The study was conducted from March to September 2024. The selection of volunteers was random and voluntary. Before inclusion in the study, the volunteers were informed about the research protocol, which contained details about the research carried out with the objectives, methodological procedure, possible risks in the case of withholding important information regarding health status (risks in the case of an electrical device implanted in the body on the heart or in the case of pregnancy) and the volunteer's consent to inclusion in the study.

Body composition

Body composition was analyzed using the InBody 970 (MF-BIA; InBody Corporation, Seoul, South Korea), which measured the impedance of five body segments at 1, 5, 50, 250, and 500 kHz and 1, 2, and 3 MHz. We determined the values of the following anthropometric parameters and indices: Body Mass Index (BMI, kg.m^{-2}), Soft Lean Mass (SLM, kg), Fat Free Mass (FFM, kg), Skeletal Muscle Mass (SMM, kg), Body Fat Mass (BFM, kg), Percentage Body Fat (PBF, %), Waist Circumference (WC, cm), Waist-Hip Ratio (WHR), Visceral Fat Area (VFA, cm^2). Before the measurement, participants were asked to exclude and refrain from drinking large amounts of water, not to consume alcohol 24 hours before testing, to avoid food with a high sugar, salt or fat content for 12 hours before testing, to refrain from intense physical activity for at least 12 hours beforehand. In addition to informed written consent, all participants also signed consent to the processing of personal data. The study was conducted with the approval of the Ethics Committee of the Specialized Hospital of St. Zoerardus Zobor in Nitra, Slovakia (protocol no. 20230512/2) according to the guidelines of the Declaration of Helsinki.

Emotional eating questionnaire

To assess the emotional aspects of the volunteers' eating habits, we used a questionnaire developed within the EATMOT project by Ferrão et al. [20] and modified by Bacârea et al. [18]. The questionnaire consisted of nine questions to which the volunteer could answer with the following five options, namely: 1-totally disagree, 2-disagree, 3-neither agree nor disagree, 4-agree, 5-totally agree. The questions were as follows: Q_1 – food helps me cope with stress; Q_2 – I usually eat food that helps me control my weight; Q_3 – I often consume foods that keep me awake and alert (such as coffee, coke, and energy drinks); Q_4 – I often consume foods that help me relax (such as some teas, and red wine); Q_5 – food makes me feel good; Q_6 – when I feel lonely, I console myself by eating; Q_7 – I eat more when I have nothing to do; Q_8 – for me, food serves as an emotional consolation; Q_9 – I have more cravings for sweets when I am depressed. Questions Q_1, Q_6, Q_8, Q_9 create a block related to food as an escape. Questions Q_4 and Q_5 create a block for which food is typically associated with a sense of well-being [18].

Statistical analysis

We used Microsoft Office Excel 2016 (Los Angeles, CA, USA) in combination with XLSTAT (version 2019.3.1) for data processing. We performed statistical analysis using the computer software STATISTICA 13 (TIBCO Software, Inc., Palo Alto, CA, USA) and MedCalc software (MedCalc® Statistical Software Ltd, Ostend, Belgium, version 23.0.2). The normality

of the variable distribution was checked by the Shapiro-Wilk test. We used the paired t-test if the data were normally distributed, if the distribution was not normal, the Wilcoxon signed rank test was used. We performed descriptive analysis using mean ± standard deviation. To evaluate the relationship between variables, we used Spearman›s correlation analysis and expressed it graphically with color scales through correlograms. The level of statistical significance was set as $P < 0.05$.

RESULTS AND DISCUSSION

Based on the above, we had to exclude twenty-seven people from the research group for objective reasons. The final number of volunteers was 180 (135 female, 45 male). The average age of the volunteers was 23.2 ± 4.6 years (min. 20 years, max. 49 years). One hundred and twenty-five participants had urban residence, fifty-five rural, one hundred and forty-seven participants were studying at university at the time of the research, and thirty-three were actively employed. The research group was non-obese in terms of input anthropometric data and average values with optimal values of body mass index, body fat mass, percentage of body fat, waist circumference, waist-to-hip ratio and visceral fat area. However, according to personalized BMI, thirty-three participants were undernourished, forty-two were overweight, and nine were obese grade 1. According to visceral fat area, thirty-two participants had above-limit values, and seventy-five exceeded the optimal waist value. More detailed information is provided in Table 1.

Table 2 shows the absolute and relative frequency of responses to the research questions. In relation to the first question focused on coping with stress through eating, the volunteers answered mostly disagreeing (40%) or neutrally (40%). However, stress eats up to 20%. The sixth question, linking food and loneliness, was answered with a negative response by a relatively

Table 1. Descriptive characteristics of the study group

Parameters N = 180	Mean	SD	Mode	Minimum	Maximum
Age (years)	23.2	4.6	22.0	20.0	49.0
Height (cm)	168.2	8.4	169.3	155.8	190.0
Weight (kg)	65.2	13.2	55.3	48.8	107.7
Body Mass Index (BMI, $kg.m^{-2}$)	22.9	3.2	22.2	18.2	32.8
Basal Metabolic Rate (BMR, kcal)	1433	244	-	1120	2198
Soft Lean Mass (SLM, kg)	46.3	10.7	43.1	32.7	79.8
Fat Free Mass (FFM, kg)	49.2	11.3	39.5	34.7	84.6
Skeletal Muscle Mass (SMM, kg)	27.3	6.9	-	18.8	49.0
Body Fat Mass (BFM, kg)	16.0	6.1	12.0	6.4	42.7
Percentage Body Fat (PBF, %)	24.5	7.5	18.0	9.4	41.8
Waist Circumference (WC, cm)	81.5	10.2	73.3	66.9	129.4
Waist-Hip Ratio (WHR)	0.9	0.1	0.9	0.8	1.2
Visceral Fat Area (VFA, cm^2)	68.2	33.2	51.4	16.9	234.7

Table 2. Absolute and relative frequency of responses

Questions	Totally disagree N (%)	Disagree N (%)	Neither agree nor disagree N (%)	Agree N (%)	Totally agree N (%)
Q_1	27 (15)	45 (25)	72 (40)	27 (15)	9 (5)
Q_2	9 (5)	27 (15)	72 (40)	57 (31.7)	15 (8.3)
Q_3	27 (15)	42 (23.3)	42 (23.3)	54 (30)	15 (8.3)
Q_4	21 (11.7)	57 (31.6)	45 (25)	54 (30)	3 (1.7)
Q_5	0	0	36 (20)	90 (50)	54 (30)
Q_6	36 (20)	63 (35)	57 (31.7)	18 (10)	6 (3.3)
Q_7	12 (6.7)	36 (20)	36 (20)	78 (43.3)	18 (10)
Q_8	33 (18.3)	54 (30)	69 (38.3)	12 (6.7)	12 (6.7)
Q_9	24 (13.3)	48 (26.7)	30 (16.7)	60 (33.3)	18 (10)

large number of respondents (55%), neutral by 31.7% and affirmative by 13.3%. As in the previous questions, the vast majority also answered the question regarding the association between food and emotional comfort (Q_8) with a negative response (48.3%) or neutral (38.3%). A total of 13.4% agreed. The last question of the first block associate's food and especially sweets with depressive states (Q_9). In this case, the frequency of answers has changed compared to questions Q_1, Q_6 and Q_8. Forty percent answered with a negative response, 16.7% with a neutral response, but 43.3% with an affirmative response. It follows from the above that depression and similar states cause an increased appetite for sweets in almost half of consumers. Block 2, including questions Q_4 and Q_5, connects food with pleasant feelings and well-being. In the fourth question, participants expressed whether they consume foods that help them relax more often. 43.3% disagreed, 25% were neutral, and 31.7% agreed. The fifth question addresses good feelings related to food consumption. We did not find any disagreeing answers for this question. Twenty percent were neutral, and 80% agreed. The remaining questions Q_2, Q_3, and Q_7, not included in the blocks, were evaluated as follows. Question 2 addresses the issue of choosing foods that help with body weight correction. Twenty percent disagreed, 40% were neutral, and 40% agreed. The next question in order, the third, addresses the issue of using foods that have a stimulating effect. 38.3% disagreed, 23.3% were neutral and 38.3% agreed with their use. The seventh question concerns the issue of increased food consumption when consumers feel bored or do not perform any activity. We recorded the second highest proportion of affirmative responses for this question (53.3%); 26.7% disagreed and 20% were neutral. Based on the above, we can conclude that a relatively large proportion of participants agree with questions regarding food consumption during depressive states or when feeling bored. However, in many cases there was a relatively high proportion of neutral responses, with either affirmative or negative responses prevailing, depending on the question. More details are provided in Table 2.

Table 3 shows the values of anthropometric parameters according to the prevalence of answers. We evaluated each question individually with possible answers and the corresponding values of anthropometric parameters. For the first question regarding food and stress, we found significant associations in relation to body weight, SLM, FFM and SMM. These were mostly differences between the disagreeing and agreeing opinions, with the disagreeing group, which rejects stress eating, achieving higher values of the mentioned parameters. It is possible that the mentioned group consisted of physically and sports-active individuals who respond to stressful situations by increasing physical activity. However, this needs to be examined in more detail. For the second question, we recorded the highest number of anthropometric parameters for which significant differences were observed, mostly in favor of the group that agreed with the relationship between conscious food selection for body weight correction. This was demonstrated by the highest values of parameters related to muscle mass and the lowest values of fat parameters. Statistically significant differences were found in the case of body weight, SLM, FFM, SMM, BFM, PBF, WC, WHR and VFA. In the third question regarding the use of stimulating foods and drinks such as coffee, energy drinks and others, we found significant differences in the case of weight, BMI, BFM, WC and VFA. In the fourth and fifth questions, we did not find significant differences between the answers. In the sixth question, a statistically significant difference was found only in the body weight values, with higher values being found in the disagreeing group compared to the agreeing group. The seventh question related to higher food intake during a feeling of boredom brought more striking results, as we found significant differences in the case of all anthropometric parameters, except for SLM, FFM, SMM. Paradoxically, however, higher values, especially for fat parameters, were achieved by the group of participants who strictly disagreed with the question. Question eight was statistically significant only in the case of body fat percentage and question nine in the WHR index.

In the following section, we were interested in the differences in the values of anthropometric parameters according to the affirmative answers to individual questions. We only considered respondents who answered the questions agree or totally agree. The results showed that participants who stress eat with food (Q_1) had statistically significantly the lowest values of parameters related to muscle mass (SLM, FFM, SMM, BMR). On the contrary, participants who consume food that corrects their body weight (Q_2) had significantly the lowest values of fat parameters and in most cases the highest values of parameters related to muscle mass. Detailed results are presented in Table 4.

Table 5 presents the results of the correlation analysis and the relationships between individual questions. The analysis showed a strong correlation between question Q_1 and Q_6 ($r = 0.649$; $P < 0.001$), Q_8 ($r = 0.636$; $P < 0.001$) and Q_9 ($r = 0.651$; $P < 0.001$). The questions form a block in which food is a form of escape and it is therefore expected that the questions will correlate with each other. Furthermore, we found a strong correlation between Q_6 and Q_8 ($r = 0.658$; $P < 0.001$) and a moderate one with Q_7 ($r = 0.488$; $P < 0.001$). A strong correlation was also found in the

Table 3. Values of anthropometric parameters according to the prevalence of answers

		Totally disagree	Disagree	Neither agree nor disagree	Agree	Totally agree			Totally disagree	Disagree	Neither agree nor disagree	Agree	Totally agree
Weight (kg)	Q_1	64.6	70.9[a]	69.3	59.2[b]	61.3	BFM (kg)	Q_1	15.1	17.0	18.1	16.1	14.2
	Q_2	62.7	57.3[a]	71.1[b]	65.7	71.2[b]		Q_2	20.0	16.3	19.5[a]	13.9[b]	14.6
	Q_3	58.4[a]	72.2[b]	69.0	66.6	62.8		Q_3	13.1[a]	20.5[b]	17.2	16.7	13.2
	Q_4	73.2	68.4	65.0	64.1	74.4		Q_4	16.5	19.2	15.5	15.1	27.8
	Q_5			63.8	67.7	67.7		Q_5			17.5	17.4	15.4
	Q_6	65.6	72.0[a]	64.8	59.3[b]	63.3		Q_6	15.9	19.3	15.9	14.3	14.6
	Q_7	83.6[a]	70.9	67.4[b]	63.2[b]	62.8[b]		Q_7	28.8[a]	16.7[b]	17.3[b]	15.6[b]	14.0[b]
	Q_8	71.6	68.1	66.0	60.5	59.8		Q_8	19.7	14.7	17.7	15.8	14.6
	Q_9	69.0	67.0	63.9	68.8	62.6		Q_9	17.0	15.5	14.6	18.8	17.6
BMI ($kg.m^{-2}$)	Q_1	22.3	24.0	24.1	22.2	21.7	PBF (%)	Q_1	23.1	23.5	26.0	27.1	23.4
	Q_2	22.9	22.1	24.5	22.6	23.6		Q_2	31.3[a]	27.8[a]	27.1[a]	20.9[b]	21.5
	Q_3	20.4[a]	24.8[b]	24.5[b]	23.2[b]	22.1		Q_3	22.8	27.6	24.9	25.1	21.5
	Q_4	23.3	23.8	23.3	22.8	27.6		Q_4	21.5	27.5	23.6	24.2	37.3
	Q_5			23.7	23.7	22.6		Q_5			27.0	25.7	22.5
	Q_6	22.6	24.5	23.3	21.7	21.5		Q_6	23.3	26.7	24.7	23.7	23.4
	Q_7	28.8[a]	23.6[b]	23.5[b]	22.7[b]	21.9[b]		Q_7	34.8[a]	23.3[b]	25.8	24.6[b]	21.9[b]
	Q_8	24.6	23.3	23.5	22.0	21.3		Q_8	26.7	21.2[a]	27.1[b]	25.8	24.7
	Q_9	23.9	22.9	22.7	24.0	22.8		Q_9	23.4	23.2	23.2	27.0	27.9
SLM (kg)	Q_1	46.6	50.7[a]	48.2	40.6[b]	44.3	WC (cm)	Q_1	81.1	85.9	84.5	79.2	77.7
	Q_2	40.1	38.6[a]	48.6[b]	48.8[b]	53.4[b]		Q_2	82.1	78.7[a]	87.9[b]	79.7[a]	82.0
	Q_3	42.6	48.6	48.9	47.0	46.7		Q_3	76.1[a]	88.0[b]	84.1	83.6	78.1
	Q_4	53.5	46.3	46.7	46.1	43.7		Q_4	85.7	85.5	81.3	80.5	93.6
	Q_5			43.5	47.3	49.2		Q_5			82.4	83.9	82.4
	Q_6	46.8	49.7	46.2	42.4	45.8		Q_6	81.3	87.6	81.6	77.4	78.2
	Q_7	51.6	51.1	47.2	44.9	45.9		Q_7	100.5[a]	85.5[b]	83.4[b]	80.1[b]	79.0[b]
	Q_8	48.9	50.3	45.5	42.0	42.5		Q_8	87.1	82.7	83.6	78.2	76.6
	Q_9	49.0	48.5	46.5	47.1	42.3		Q_9	84.1	82.5	78.6	86.4	80.1
FFM (kg)	Q_1	49.5	53.9[a]	51.2	43.1[b]	47.1	WHR	Q_1	0.86	0.88	0.87	0.85	0.84
	Q_2	42.7	41.0[a]	51.6[b]	51.8[b]	56.6[b]		Q_2	0.86	0.86	0.90[a]	0.84[b]	0.84
	Q_3	45.3	51.7	51.9	49.9	49.6		Q_3	0.83	0.89	0.86	0.87	0.83
	Q_4	56.8	49.2	49.5	48.9	46.6		Q_4	0.88	0.88	0.85	0.85	0.92
	Q_5			46.3	50.2	52.3		Q_5			0.86	0.87	0.86
	Q_6	49.7	52.8	49.0	45.0	48.7		Q_6	0.85	0.89	0.86	0.84	0.84
	Q_7	54.8	54.3	50.1	47.6	48.8		Q_7	0.97[a]	0.88[b]	0.87[b]	0.85[b]	0.85[b]
	Q_8	51.9	53.4	48.3	44.6	45.2		Q_8	0.88	0.86	0.87	0.84	0.83
	Q_9	52.0	51.5	49.4	50.0	45.1		Q_9	0.86	0.86	0.83[a]	0.89[b]	0.85
SMM (kg)	Q_1	27.5	30.3[a]	28.4	23.6[b]	26.0	VFA (cm^2)	Q_1	65.0	71.9	80.1	68.7	57.7
	Q_2	23.2	22.3[a]	28.7[b]	28.9[b]	31.8[b]		Q_2	88.3	72.2	87.3[a]	55.6[b]	59.7
	Q_3	24.9	28.7	29.0	27.8	27.6		Q_3	54.0[a]	91.9[b]	73.6	71.8	53.9
	Q_4	31.8	27.2	27.6	27.2	25.6		Q_4	69.3	86.6	62.9	64.3	133.6
	Q_5			25.5	27.9	29.2		Q_5			75.3	76.7	64.6
	Q_6	27.6	29.5	27.2	24.8	27.0		Q_6	65.9	85.6	68.7	59.2	58.3
	Q_7	30.6	30.3	27.9	26.4	27.1		Q_7	135.4[a]	74.7[b]	74.5[b]	65.4[b]	55.4[b]
	Q_8	28.9	30.0	26.7	24.5	24.8		Q_8	85.2	62.7	78.2	66.2	59.5
	Q_9	29.1	28.7	27.5	27.7	24.7		Q_9	70.0	64.2	60.6	85.9	75.7

[a, b] – different symbols (post-hoc analyses) in a row indicate significant differences between groups; values without a symbol had no statistically significant difference

Table 4. Values of anthropometric parameters depending on the positive answers to the questions

Parameters/Qs	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6	Q_7	Q_8	Q_9
Age (years)	22.4	22.2[a]	23.5	25.3[b]	23.0	22.5	23.9	22.4	23.2
Weight (kg)	59.7	66.8	65.7	64.6	67.7	60.3	63.1	60.1	67.3
Body Mass Index (BMI, $kg.m^{-2}$)	22.1	22.8	23.0	23.1	23.3	21.7	22.5	21.6	23.7
Basal Metabolic Rate (BMR, kcal)	1321[a]	1511[b]	1446	1425	1472[b]	1362	1404	1340	1425
Soft Lean Mass (SLM, kg)	41.4[a]	49.8[b]	46.9	46.0	48.0[b]	43.2	45.1	42.2	46.0
Fat Free Mass (FFM, kg)	44.0[a]	52.8[b]	49.8	48.8	51.0[b]	45.9	47.9	44.9	48.8
Skeletal Muscle Mass (SMM, kg)	24.1[a]	29.5[b]	27.7	27.1	28.4	25.3	26.5	24.7	27.0
Body Fat Mass (BFM, kg)	15.7	14.0[a]	15.9	15.8	16.7	14.4	15.3[a]	15.2	18.5[b]
Percentage Body Fat (PBF, %)	26.3[a]	21.0[b]	24.3	24.9	24.5	23.6	24.1	25.2	27.2[a]
Waist Circumference (WC, cm)	78.8	80.2	82.4	81.2	83.3	77.6	79.9	77.4	84.9
Waist-Hip Ratio (WHR)	0.85	0.84[a]	0.86	0.85	0.87	0.84	0.85[a]	0.83	0.88[b]
Visceral Fat Area (VFA, cm^2)	66.1	56.5[a]	67.9	68.0	72.1	58.9	63.5[a]	62.9	83.5[b]

[a, b] – different symbols (post-hoc analyses) in a row indicate significant differences between groups; values without a symbol had no statistically significant difference

Table 5. Correlogram of mutual associations between questions

	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6	Q_7	Q_8	Q_9
Q_1		P = 0.9059	P = 0.0002	P = 0.4108	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001
Q_2	-0.008		P = 0.0055	P < 0.0001	P = 0.2701	P = 0.2285	P = 0.1765	P = 0.8904	P = 0.5958
Q_3	0.256	0.195		P = 0.4519	P = 0.1412	P < 0.0001	P = 0.4700	P = 0.0012	P < 0.0001
Q_4	0.058	-0.287	-0.053		P = 0.0186	P = 0.0390	P = 0.9571	P = 0.9470	P = 0.1052
Q_5	0.298	0.078	-0.104	-0.166		P < 0.0001	P < 0.0001	P < 0.0001	P = 0.0122
Q_6	0.649	-0.085	0.365	0.146	0.383		P < 0.0001	P < 0.0001	P < 0.0001
Q_7	0.424	0.096	0.051	0.004	0.286	0.488		P < 0.0001	P = 0.0010
Q_8	0.636	0.01	0.227	-0.005	0.491	0.658	0.386		P < 0.0001
Q_9	0.651	-0.038	0.29	-0.115	0.176	0.388	0.231	0.575	

Table 6. Values of anthropometric parameters depending on blocks and unclassified questions

Parameters/Blocks	Block 1	Block 2	Q_2	Q_3	Q_7
Age (years)	22.8	23.6	22.2	23.5	23.9
Weight (kg)	63.5	66.8	66.8	65.7	63.1
Body Mass Index (BMI, $kg.m^{-2}$)	22.7	23.2	22.8	23.0	22.5
Basal Metabolic Rate (BMR, kcal)	1379[a]	1458	1511[b]	1446	1404
Soft Lean Mass (SLM, kg)	43.9[a]	47.5	49.8[b]	46.9	45.1
Fat Free Mass (FFM, kg)	46.7[a]	50.4	52.8[b]	49.8	47.9
Skeletal Muscle Mass (SMM, kg)	25.7[a]	28.0	29.5[b]	27.7	26.5
Body Fat Mass (BFM, kg)	16.8	16.4	14.0	15.9	15.3
Percentage Body Fat (PBF, %)	26.2[a]	24.6[a]	21.0[b]	24.3	24.1
Waist Circumference (WC, cm)	81.3	82.7	80.2	82.4	79.9
Waist-Hip Ratio (WHR)	0.86	0.86	0.84	0.86	0.85
Visceral Fat Area (VFA, cm^2)	72.8[a]	71.0	56.5[b]	67.9	63.5

[a, b] – different symbols (post-hoc analyses) in a row indicate significant differences between groups; values without a symbol had no statistically significant difference

case of Q_8 and Q_9 ($r = 0.575$; $P < 0.001$) and a moderate one with Q_5 ($r = 0.491$; $P < 0.001$).

We were also interested in whether there were differences in the values of anthropometric parameters between the questions when they were combined into block 1 and block 2 and questions Q_2, Q_3 and Q_7. As can be seen in Table 6, we found some significant differences, but in most cases the values of the parameters in block 1 were statistically significantly different from the values belonging to question Q_2. As expected, Q_2 participants had lower values of fat parameters and higher values related to muscle mass than participants from block 1. However, we did not find statistically significant differences between block 1 and block 2 ($P > 0.05$).

As studies indicate, this issue is highly topical, and they bring diverse results depending on the factors monitored. When monitoring the mutual relationships between individual items, we have in several cases recorded the same results as other authors. Ferrão et al. [20] found a strong correlation between question Q_1 (food helps me cope with stress) and Q_6 (eating helps me with feelings of loneliness) with a score of 0.62, which is consistent with our findings. We also agree with the strong relationship between item Q_1 and item Q_9 (depression). Unlike the authors, we also found a strong correlation in the case of items Q_1 and Q_8 (emotional comfort). Our results also agree with the positive moderate correlation found between Q_1 and Q_7 (eating when bored).

For the first question regarding food and stress, we found significant associations with body weight, soft lean mass, fat-free mass and skeletal muscle mass. The group that refused to eat stress achieved higher values for the above parameters. As we have already mentioned above, we believe that the above group consisted of sports-active individuals who respond to stressful situations by increasing physical activity. These hypotheses are in line with the findings of other authors, according to whom physical activity is a beneficial association for emotional eaters [21, 22]. However, despite our findings, stressful situations can also influence consumer behavior in the opposite direction and can lead to overeating. The recent global Covid-19 pandemic has caused extensive changes in lifestyle and socio-economic areas. This has been manifested in isolation, limited movement and deterioration of diet. Several studies have provided evidence of the negative impact of the pandemic and its management on the body composition of the population, especially with an emphasis on the increase in the prevalence of overweight and obesity [23-25].

For question Q_2 (weight control), we recorded the highest number of anthropometric parameters for which significant differences were observed, mostly in favor of the group that agreed with the relationship between conscious and mindful food choices for body weight correction. This was demonstrated by the highest values of parameters related to muscle mass and the lowest values of fat parameters. Mindful eating expresses maximum physical attention to food consumption and emotional experience of food in a certain way, intentionally and in the present moment [26]. Such consumers are fully aware of feelings of satiety and adequately regulate food intake, avoiding inappropriate stimuli to eat, such as advertisements or emotions. However, in line with the results of Ferrão et al. [20], we found that item Q_2 had very weak correlations with all other items.

The question related to eating out of boredom (Q_7) yielded more striking results, as significant differences were found for all anthropometric parameters, except for soft lean mass, fat-free mass and skeletal muscle mass. Paradoxically, higher values, especially for fat parameters, were achieved by the group of participants who strongly disagreed with the question. Crockett et al. [27] found that inappropriate eating habits occurred in those with a tendency to boredom and emotional difficulties.

In relation to body weight, weight gain, unhealthy body composition (in favor of fat components) and overeating, not only negative emotions should be considered, but also positive emotions related to mood improvement. A meta-analysis by Evers et al. [28] showed that positive emotions led to increased eating. The fact is that emotional eaters can respond to both negative and positive emotions, but weight gain is mostly associated only with negative emotions [11, 29, 30]. Bacârea et al. [18] found that people who answered yes to questions Q_1, Q_6, Q_8 and Q_9 (block 1) had significantly higher BMI. However, when examining BMI in relation to the questions included in block 2 (Q_4 and Q_5) or in relation to item Q_2, they did not observe any significant associations. According to their results, weight gain was associated with block 1, but not with block 2 or Q_2. However, this does not match our findings. We did not find statistically significant differences between block 1 and block 2 ($P > 0.05$). In our case, we only found statistically significant differences in parameter values in block 1 compared to the values corresponding to question Q_2. As expected, participants in Q_2 had lower values of fat parameters and higher values related to muscle mass than participants in block 1.

According to our findings, participants who stress eat with food (Q_1) had statistically significant lowest values of parameters related to muscle mass (SLM, FFM, SMM, BMR). Conversely, participants who consume food that corrects their body weight (Q_2) had significantly lowest values of fat parameters and in most cases highest values of parameters related to

muscle mass. This is in line with the findings of authors Keller et al. [31], according to which consumers who choose food that regulates their body weight do not tend to increase their body mass index. Another study confirms that emotional overeating is associated with increased body mass index, overweight and obesity [32].

The limitations of our study lie in the low representation of older people and male participants. However, this is a pilot publication that focuses on the relationship between the components of emotional eating and selected indicators of body composition in the Slovak population.

CONCLUSIONS

Based on the results obtained, we can conclude that our emotions and emotional eating have a significant impact on the body composition of consumers. Our results showed that the values of anthropometric parameters did not differ significantly between those who associate food with negative emotions and those who associate its consumption with positive emotions. However, it was clearly confirmed that those who choose food consciously in relation to maintaining adequate body weight also achieved the most optimal values of anthropometric parameters.

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Conflict of interest
There were no conflicts of interest.

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ORIGINAL ARTICLE

SUGAR-SWEETENED BEVERAGE CONSUMPTION AND RISK OF VISCERAL FAT ACCUMULATION AMONG UNIVERSITY STUDENTS IN THAILAND

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ABSTRACT

Background. Increased consumption of sugar-sweetened beverages (SSBs) is associated with obesity and metabolic health risks.

Objective. This study determined the relationship between SSB intake and body composition, with a focus on visceral fat accumulation among Thai university students.

Material and Methods. A cross-sectional study was conducted with 387 university students aged 19-22 years. Dietary intake was assessed using a 3-day, 24-hour dietary recall conducted on three consecutive day to quantify SSB consumption. Body composition metrics, including body mass index (BMI), fat mass, and visceral fat levels (VFL), were measured using bioelectrical impedance analysis. Statistical analyses, including t-tests and linear regression, were used to identify the associations between SSB intake and body composition.

Results. Sweetened tea, particularly freshly prepared iced milk tea, was most frequently consumed. High sugar consumption from SSB (≥ 24 g/day) was significantly associated with increased fat mass (16.9 ± 9.9 vs. 14.8 ± 7.8 kg, p = 0.021), BMI (22.6 ± 5.0 vs. 21.3 ± 4.2 kg/m², p = 0.007), and VFL > 9 (83.3% vs. 16.7%, p = 0.013). Sugar intake increased progressively across BMI categories: underweight (25.21 g/day), normal-weight (28.78 g/day), overweight (32.18 g/day), and obese (34.00 g/day). Participants with a VFL above 9 consumed over 40 g/day of SSB-derived sugar. At VFL exceeding 10, males had an average BMI of 30.06 ± 2.40 kg/m², whereas females exhibited a dramatically higher BMI of 41.20 ± 3.27 kg/m².

Conclusion. Excessive SSB consumption, particularly sweetened tea, is strongly associated with higher visceral fat and unfavorable body composition in young adults. Public health interventions targeting reduced SSB intake are urgently required to address obesity and metabolic health risks. Further longitudinal studies are recommended to confirm causality and inform dietary guidelines.

Keywords: *sugar-sweetened beverages (SSBs), visceral fat accumulation, body composition assessment, adolescent health, metabolic health risks, dietary assessment*

INTRODUCTION

Obesity has been reported as a critical global public health problem, contributing significantly to the prevalence of non-communicable diseases (NCDs) such as type 2 diabetes, cardiovascular disease, and metabolic syndrome [1]. Among the dietary factors driving obesity, sugar-sweetened beverage (SSB) consumption has been a major concern because of its high simple sugar (sucrose, fructose) content (readily absorbable sugars) and widespread accessibility [2]. Therefore, public health strategies worldwide have prioritized reducing sugar intake (to less than 24 g/day of added sugar) to combat obesity and its associated health risks [3]. However, the recent COVID-19 pandemic, accompanied by quarantine measures, has complicated public health efforts by encouraging unhealthy changes in dietary habits, prompting increased reliance on energy-dense beverages, including sugar-sweetened beverages (SSBs) [4, 5].

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Moreover, periods of lockdown and social distancing have been associated with reduced physical activity, elevated stress levels, and disrupted daily routines, all of which have been linked to unhealthy eating and drinking patterns [6].

Adolescents and young adults may be particularly susceptible to these changes as they often experience heightened psychological stress and exhibit a greater propensity to consume convenience-oriented food and beverage products. Sugar-sweetened beverages (SSBs) contain large amounts of sucrose, which are digested into glucose and fructose. Fructose undergoes rapid metabolism in the liver, where it is converted into lipids. This process contributes to fat accumulation in the liver and visceral organs, leading to adverse health outcomes such as insulin resistance. Additionally, fructose metabolism in the liver can be converted into lipids that promote rapid proliferation of cancerous cells, as shown in animal models [7]. Thailand presents a compelling case study for examining the health impacts of sugar-sweetened beverage (SSB) consumption due to its elevated baseline levels of intake. In particular, the Bangkok Metropolitan Region demonstrates a significantly higher consumption rate compared to other parts of the country, including the southern region, where consumption levels tend to be more moderate. These regional disparities underscore the importance of considering local cultural norms, availability of beverage products, and socioeconomic determinants in dietary behavior analysis [8]. Thailand provides a unique context for addressing this research gap. According to a 2019 national survey, 34% of Thai residents aged 15 and older consumed SSBs at least three times per week, and 14.3% consumed them daily, far exceeding global averages and the World Health Organization recommendation for free sugar intake of less than 24 g/day [9]. University students represent a particularly high-risk subgroup within this population due to their distinct dietary practices and transitional lifestyle behaviors. As they gain autonomy over food choices during the transition to adulthood, many rely increasingly on convenient high-sugar beverages. The widespread availability and affordability of SSBs on university campuses further exacerbate this issue. Moreover, academic and social stressors, coupled with high levels of sedentary behavior related to academic demands, place this demographic at heightened risk for weight gain, adverse metabolic outcomes, and related comorbidities. Although substantial evidence links SSB consumption to obesity, research on the specific effects of SSB intake on body composition, particularly the accumulation of visceral adipose tissue (VAT), remains less well documented [10]. This gap in evidence is particularly relevant in young adults from low- and middle-income countries (LMICs), including Thailand, where dietary patterns are strongly influenced by cultural and environmental factors. Addressing this knowledge deficit is critical for the development of effective, context-specific public health strategies.

This small-scale, cross-sectional study aimed to (1) quantify total sugar intake from SSB consumption, including taxed and non-taxed beverages, among Thai university students and (2) examine the association between sugar intake from SSB and body composition metrics. Specifically, this study was designed as a small-scale survey to gather data on these factors from university students in Thailand. This study determined SSB consumption patterns and their impact on visceral adipose tissue (VAT) in a population vulnerable to pandemic-related lifestyle disruptions. By examining the relationship between SSB intake and VAT, this study highlighted the metabolic risks associated with dietary behaviors among Thai university students. The findings contribute to post-pandemic dietary research and inform evidence-based public health interventions, supporting Thailand's obesity prevention efforts, while providing actionable insights for similar global contexts to mitigate long-term health risks in young adults.

MATERIAL AND METHODS

Study design and participants

This cross-sectional study was conducted between July and October 2022 at Walailak University, Nakhon Si Thammarat, Thailand. Walailak University was chosen as the study site because of its diverse student population, which includes individuals from varied socioeconomic and cultural backgrounds, making it an ideal setting for investigating dietary and lifestyle behaviors. The university also represents a microcosm of the broader Thai adolescent and young adult demographic, particularly in terms of dietary transitions and lifestyle changes influenced by urbanization and academic pressures. Ethical approval for the study was granted by the Walailak University Ethics Committee (WUEC-22-207-01) and all participants provided written informed consent prior to participation. Eligible participants were undergraduate students aged 19-22 years who met the following criteria: (1) enrolled in a full-time academic study (6-8 hours per day), (2) no underlying medical conditions that could influence metabolic health, and (3) regular consumption of sugar-sweetened beverages (SSBs) at least three times per week. Exclusion criteria included physical, mental, or cognitive limitations that might affect the reliability of the questionnaire. A target sample size of 387 students was calculated using the Taro

Yamane formula (e = 0.05) to ensure adequate statistical power and representativeness. Systematic random sampling was used to reduce selection bias, while ensuring proportional representation across academic disciplines.

Data collection and measurements

Setting-specific context and questionnaire development

Walailak University was selected not only for its diverse demographics, but also for its location in a semi-urban area of southern Thailand, where dietary patterns often reflect a mix of traditional and modern influences. The availability of low-cost, high-sugar beverages on and around campus further highlights the relevance of studying SSB consumption in this setting. Data were collected through face-to-face interviews using a pre-validated questionnaire (index of item-objective congruence = 0.89). The questionnaire was tailored to reflect the common dietary habits and beverage consumption patterns among Thai university students, particularly in a semi-urban context. The key components are as follows: (1) Demographics: Age, gender, income, and academic background, (2) Lifestyle Factors: Physical activity, smoking, and alcohol use, (3) SSB Consumption: Types, frequency, and volume of beverages consumed.

Dietary intake assessment

A 3-day, 24-hour dietary recall was used to quantify sugar-sweetened beverage (SSB) consumption over three consecutive days, as previously described by Schröder et al. [11]. Students provided detailed information about the types, brands, and quantities of beverages consumed, as well as the context of consumption (e.g., alone, socially, with meals). To ensure accuracy, nutritional labels and previously published LC-MS/MS data [12] were used to estimate the sugar contents of both labeled and unlabeled beverages. Trained interviewers provided guidance to minimize reporting bias.

Body composition analysis

Body composition metrics were assessed using bioelectrical impedance analysis (BIA) (TANITA Model BC-418MA, Tokyo, Japan) in a controlled environment at the School of Public Health to ensure participant convenience while maintaining standardized conditions. All assessments were performed by a single trained research assistant who received standardized training to ensure consistency in measurement procedures. This same assessor was responsible for all measurements to minimize variability. To enhance measurement accuracy and reduce potential confounding factors, participants adhered to strict pre-assessment conditions, including conducting measurements in the early morning following an overnight fast (≥ 8 hours), wearing light clothing, and emptying their bladders before assessment. Additionally, participants were required to abstain from vigorous physical activity for at least 12 hours and avoid alcohol consumption for 24 hours before the assessment. Measurements were taken after five minutes of rest in a standing position to ensure fluid stabilization, while room temperature was maintained between 22°C and 24°C to minimize temperature-related variations in bioelectrical impedance. Female participants were scheduled to avoid measurements during menstruation to account for potential fluid retention effects. The potential error rate in body composition assessment could arise from both device-related and human-related factors. The TANITA Model BC-418MA is a validated BIA device, though BIA generally has an error margin of ± 3-8%, with variability influenced by hydration status, body temperature, and compliance with pre-measurement protocols. Human-related error, such as slight variability in participant posture, electrode contact, or adherence to pre-assessment instructions, was minimized by strictly following a standardized protocol. These precautions were implemented to ensure measurement consistency and enhance the accuracy of body composition assessments. The BIA provides detailed metrics, including body fat percentage (BFP%), fat mass (FM), fat-free mass (FFM), muscle mass (MM), basal metabolic rate (BMR), visceral fat levels (VFL),visceral fat levels (VFL): A score ≤ 9 was considered healthy, while ≥ 10 indicated increased metabolic risk. To enhance accuracy, participant-specific characteristics (age, sex, and height) were inputted into the BIA system. body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Statistical analysis

Descriptive statistics (means, standard deviations, and percentages) were used to summarize demographic, dietary, and body composition data. The statistical methods used were as follows:

- *Chi*-square and Fisher's Exact Tests: For Associations between categorical variables (e.g. SSB consumption levels and BMI categories).
- Independent t-tests: To compare the mean differences in SSB intake and body composition metrics between groups.

Statistical significance was set at $p < 0.05$, and all analyses were performed using GraphPad Prism (version 10). Limitations related to the cross-sectional nature of the study and potential biases in self-reported dietary data were addressed through methodological rigor and the contextual focus provided by Walailak University.

RESULTS

Demographic and body composition characteristics of participants

This small-scale cross-sectional study recruited 387 Thai university students to investigate the relationship between sugar-sweetened beverage (SSB) consumption, lifestyle behaviors, and body composition. Participants were classified into two groups based on their sugar intake from SSBs: high sugar intake (> 24 g/day) and low sugar intake (≤ 24 g/day) (Table 1). The results revealed significant differences in the body composition metrics between these groups. Participants in the high SSB intake group demonstrated significantly higher mean body mass index (BMI) (22.6 ± 5.0 vs. 21.3 ± 4.2, p = 0.007, t-test) and fat mass (16.9 ± 9.9 kg vs. 14.8 ± 7.8 kg, p = 0.021, t-test). Elevated visceral fat levels (VFL > 9) were notably more prevalent in the high SSB group (83.3% vs. 16.7%, p = 0.013, *Chi*-square test), indicating a heightened metabolic risk associated with consuming more than 24 g/day of sugar. Additionally, participants whose energy intake from SSBs exceeded 10% of their basal metabolic rate (BMR) had significantly higher total energy requirements (1326.9 ± 245.5 kcal vs. 1239.5 ± 208.9 kcal, p < 0.001). Differences in body composition appeared to be associated with SSB consumption. Lifestyle behaviors also differed significantly between the two groups. Alcohol was more common among participants with high SSB intake (61.4% vs. 38.6%, p = 0.037, *Chi*-square test), whereas regular physical activity was slightly less frequent in this group (48.4% vs. 51.6%, p = 0.010, *Chi*-square test). These findings suggest that excessive SSB intake may be associated with unhealthy lifestyle behaviors, including alcohol consumption and reduced physical activity.

SSB consumption and visceral fat risks

Excessive consumption of sugar-sweetened beverages (SSBs) was associated with obesity-related risks, including elevated fat mass and visceral adiposity. To further elucidate this association, distinct SSB consumption patterns were analyzed, and their relationship with obesity-related risks was examined. As illustrated in Figure 1, there were significant variations in sugar-sweetened beverage (SSB) consumption patterns among the participants; freshly prepared iced milk tea was identified as the most frequently consumed SSB, followed by carbonated, freshly prepared iced milk coffee, and fruit juice drinks. Participants with elevated visceral fat levels (VFL > 9) consumed nearly double the amount of added sugar from SSBs compared to those in the normal VFL group (49.7 g/day vs. 26.9 g/day). Notably, sweetened tea accounted for a substantial proportion of this sugar intake, with high-risk individuals consuming an average of 42.2 g/day compared with 21.3 g/day in the lower-risk groups. Participants categorized as high-sugar

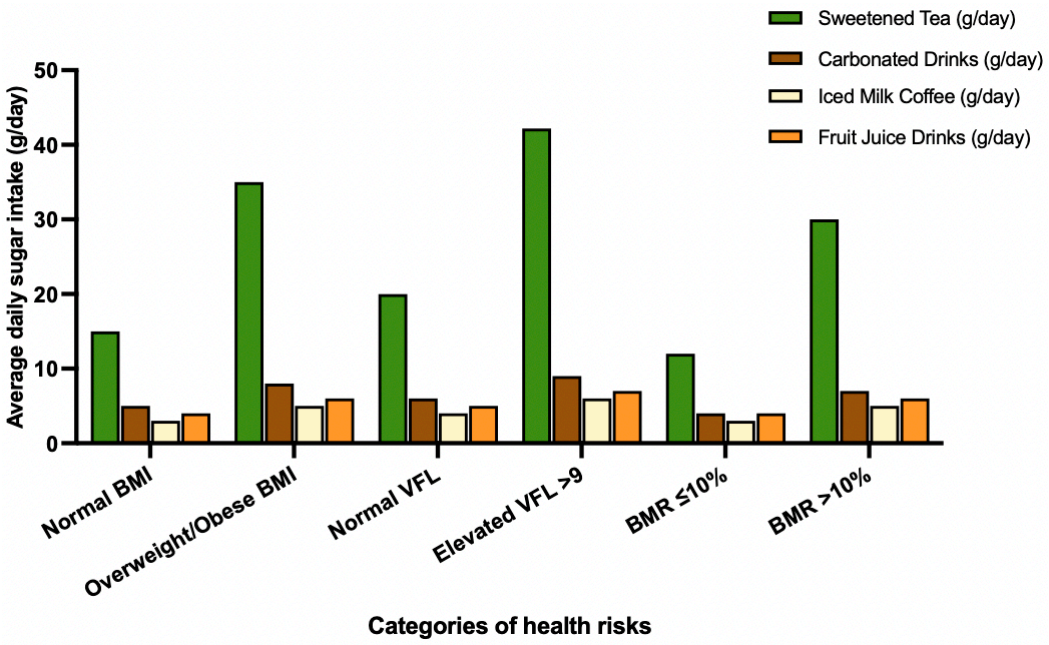


Figure 1. Average daily sugar intake from different SSB types across health risk categories among Thai university students (n = 387). The bar graph shows the average daily sugar intake (g/day) for four categories of sugar-sweetened beverages (SSBs): sweetened tea, carbonated drinks, iced milk coffee, and fruit juice drinks. Participants were categorized based on BMI (normal vs. overweight/obese), visceral fat level (VFL; normal vs. elevated VFL > 9), and the percentage of basal metabolic rate (BMR) derived from sugar (≤ 10% vs. > 10%). Data were analyzed using GraphPad Prism (version 10).

Table 1. The association between sociodemographic factors, body composition metrics, and sugar consumption

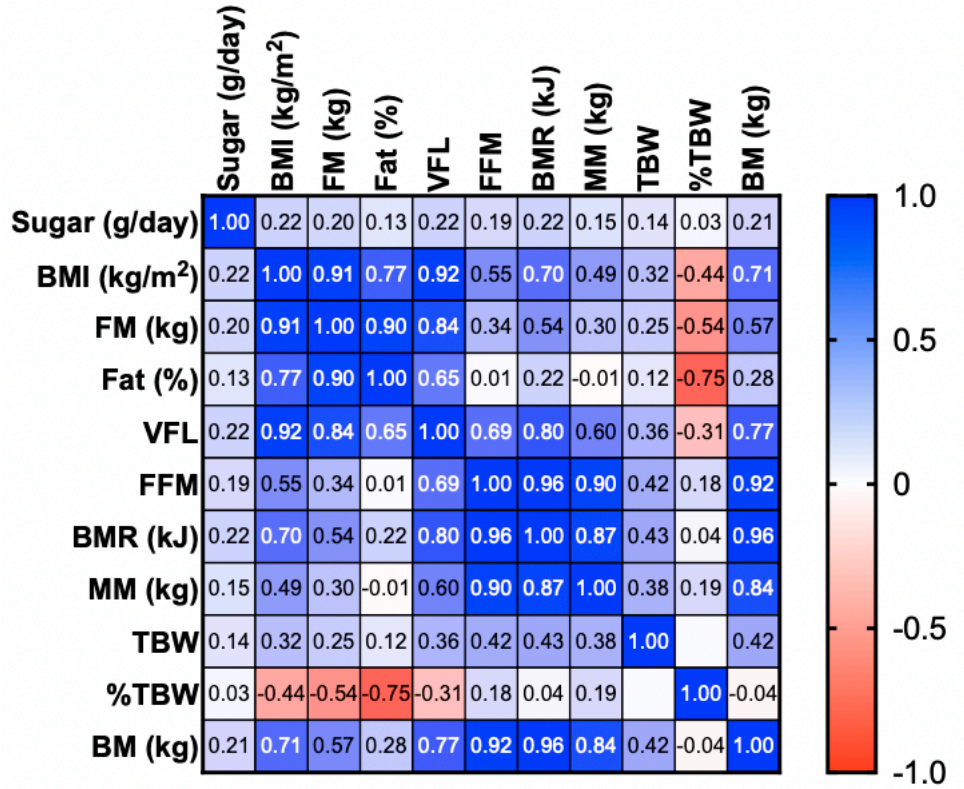
Variables	All participants n (%) or mean ± SD	Sugar-sweetened beverages consumption n (%) or mean ± SD		p-value
		> 24 g/day (n = 215)	≤ 24 g/day (n = 172)	
Age[a], years	21.52 ± 2.8	20.91 ± 2.5	21.01 ± 2.1	0.249
Gende[rb])				
– Male, n	65 (16.8)	45 (69.2)	20 (30.8)	0.472
– Female, n	322 (83.2)	170 (52.8)	152 (47.2)	
Income[b)] (Bath)				
– < 5,000 (150 USD), n – 5,000-10,000, n – > 10,000 (286 USD), n	150 (38.8) 197 (50.9) 40 (10.3)	83 (55.3) 107 (54.3) 25 (62.5)	67 (44.7) 90 (45.7) 15 (37.5)	0.535
Exercise[b)], n	192 (49.6)	93 (48.4)	99 (51.6)	0.017*
Smoking[b)], n	9 (2.3)	3 (33.3)	6 (66.7)	0.185
Alcohol[b)], n	158 (40.8)	97 (61.4)	61 (38.6)	0.037*
BMI[b)] (kg/m^2)				
– Underweight, n	92 (23.7)	39 (42.4)	53 (57.6)	0.033*
– Normal, n	163 (42.0)	92 (56.4)	71 (43.6)	
– Overweight, n	51 (13.4)	33 (64.7)	18 (35.3)	
– Obesity, n	81 (20.9)	51 (63.0)	30 (37.0)	
BMI[a)], kg/m^2	22.1 ± 4.7	22.6 ± 5.0	21.3 ± 4.2	0.007*
BMR				
– ≤ 10% BMR[b)], n	254 (65.6)	174 (68.5)	80 (31.5)	< 0.001*
– BMR[a)], kJ	5389.0 ± 977.3	5552.0 ± 1027	5186 ± 874	< 0.001*
Fat				
– Risk[b),c)], n	251 (64.9)	106 (42.2)	145 (57.8)	0.142
Fat mass[a)], kg	16.0 ± 9.0	16.9 ± 9.9	14.8 ± 7.8	0.021*
VFL				
– VFL > 9 (Risk)[b),d)], n	18 (4.7)	15 (83.3)	3 (16.7)	0.013*
– Visceral fat level[e)] (Medium (Q2, Q3))	3 (1, 5)	3 (2, 6)	2 (2.5)	0.002*
Total body water[a)], %	52.2 ± 26.1	51.1 ± 4.3	50.9 ± 4.0	0.6898
Bone mass[a)], kg	2.7 ± 0.5	2.4 ± 0.5	2.2 ± 0.4	< 0.001*
Muscle mass[a)], kg	39.2 ± 8.4	40.6 ± 8.7	37.7 ± 7.2	< 0.001*

[a)] Difference in mean were determined by a t-test, with *p-value < 0.05; [b)] Difference in percentage were determined by a *Chi*-square test, with *p-value < 0.05; [c)] Body Fat Percentage: mormal male (14-20%), normal female (17-24%); [d)] For individuals under 30 year of age, a normal visceral fat level is less than 9; BMI – body mass index, BMR – basal metabolic rate, VFL – visceral fat level; [e)] For non-normally distributed variables (e.g., visceral fat level), Mann-Whitney U tests were applied ($p < 0.05$).

consumers (> 24 g/day) exhibited significantly higher mean BMI and fat mass, demonstrating an association between SSB consumption and increased abdominal fat. Additionally, participants whose sugar-derived energy intake surpassed 10% of their basal metabolic rate (BMR) consumed significantly more sugar from SSBs **(**48.9 g/day**)** than those within the recommended threshold (17 g/day). This finding highlights that sweetened tea, as the primary contributor to excessive sugar intake, was associated with an elevated body fat percentage and a higher likelihood of being overweight or obese.

Associations between sugar intake and body composition metrics

Given the significant association between daily sugar intake from SSBs and various body composition measures, the relationship between SSB-derived sugar intake and specific body composition metrics was further investigated. As depicted in Figure 2,



	Sugar (g/day)	BMI (kg/m²)	FM (kg)	Fat (%)	VFL	FFM	BMR (kJ)	MM (kg)	TBW	%TBW	BM (kg)
Sugar (g/day)	1.00	0.22	0.20	0.13	0.22	0.19	0.22	0.15	0.14	0.03	0.21
BMI (kg/m²)	0.22	1.00	0.91	0.77	0.92	0.55	0.70	0.49	0.32	-0.44	0.71
FM (kg)	0.20	0.91	1.00	0.90	0.84	0.34	0.54	0.30	0.25	-0.54	0.57
Fat (%)	0.13	0.77	0.90	1.00	0.65	0.01	0.22	-0.01	0.12	-0.75	0.28
VFL	0.22	0.92	0.84	0.65	1.00	0.69	0.80	0.60	0.36	-0.31	0.77
FFM	0.19	0.55	0.34	0.01	0.69	1.00	0.96	0.90	0.42	0.18	0.92
BMR (kJ)	0.22	0.70	0.54	0.22	0.80	0.96	1.00	0.87	0.43	0.04	0.96
MM (kg)	0.15	0.49	0.30	-0.01	0.60	0.90	0.87	1.00	0.38	0.19	0.84
TBW	0.14	0.32	0.25	0.12	0.36	0.42	0.43	0.38	1.00		0.42
%TBW	0.03	-0.44	-0.54	-0.75	-0.31	0.18	0.04	0.19		1.00	-0.04
BM (kg)	0.21	0.71	0.57	0.28	0.77	0.92	0.96	0.84	0.42	-0.04	1.00

Figure 2. The heatmap displays the Pearson correlation coefficients (r) between sugar-sweetened beverage (SSB) consumption and body composition metrics. Sugar intake showed a weak positive correlation with BMI (r = 0.22), fat mass (r = 0.20), fat percentage (r = 0.13), and visceral fat levels (VFL; r = 0.22). Strong correlations were observed between BMI and fat mass (r = 0.91), BMI and % of fat (r = 0.77), and fat mass and VFL (r = 0.84), indicating the interconnected nature of adiposity measures. The color intensity indicates the strength and direction of the correlation, with red representing a positive correlation and blue representing a negative correlation. Data were analyzed using GraphPad Prism (version 10).

the correlation matrix revealed a weak positive relationship between sugar intake and obesity-related measures. The correlation coefficients were 0.22 for fat mass, 0.20 for BMI, 0.13 for fat percentage (visceral fat), and 0.22 for visceral fat levels (VFL). BMI displayed a strong positive correlation with fat mass (r = 0.91) and a moderate correlation with % fat (r = 0.77), whereas fat mass showed a robust correlation with % fat (r = 0.90) and a moderate correlation with VFL (r = 0.84). Higher SSB consumption was associated with slight increases in BMI, fat mass, and VFL, particularly among individuals whose sugar-derived energy intake exceeded 10% of their BMR. Stronger correlations were observed in body composition measures, emphasizing the link between adiposity and fat distribution. These findings highlight the importance of reducing SSB intake and incorporating comprehensive body composition assessments to mitigate metabolic health risk.

Figure 3A shows a significant mean difference between sugar-sweetened beverage (SSB) consumption and body mass index (BMI) categories. Daily SSB-derived sugar intake progressively increased across BMI categories, with underweight individuals (BMI < 18.0 kg/m²) consuming an average of 25.21 g/day, normal-weight participants (BMI 18.0-22.9 kg/m²) consuming 28.78 g/day, overweight individuals (BMI 23.0-29.9 kg/m²) consuming 32.18 g/day, and obese individuals (BMI ≥ 30.0 kg/m²) consuming the highest amount at 34.00 g/day. This trend suggests that a higher SSB intake is closely associated with increased BMI, potentially contributing to body weight gain and fat accumulation. Additionally, Figure 3B shows a strong positive correlation (r = 0.77) between BMI and body fat percentage, with each 1-unit increase in BMI corresponding to a 1.4% increase in body fat percentage. This observational study highlighted a positive association between daily sugar intake from SSBs and BMI, suggesting a potential link between higher SSB consumption and increased body weight and fat accumulation.

Gender-specific differences in visceral fat and BMI associated with SSB consumption

As previously reported, given the potential influence of sex-specific physiological and metabolic processes as well as potential variations in consumption behaviors, sex-specific differences in the associations between SSB consumption, visceral fat, and BMI were further investigated. In males (Figure 4A, mean-connecting plot), a visceral

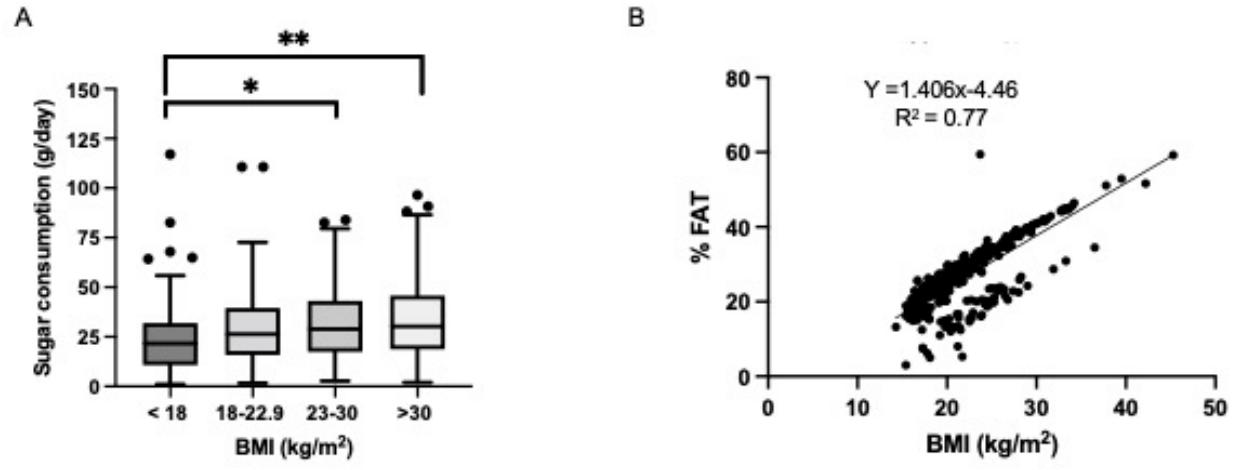


Figure 3. Relationship between sugar consumption, BMI, and body fat percentage in Thai university students (n = 387). (A) Sugar consumption (g/day) across BMI categories (<18, 18-22.9, 23-29.9, ≥ 30 kg/m²). Data are presented as box-and-whisker plots showing the median, interquartile range (IQR), and minimum/maximum values. *p < 0.05, **p < 0.01 (one-way ANOVA with Tukey's post hoc test). (B) Correlation between BMI (kg/m²) and body fat percentage (%). The line represents the linear regression fit, with the regression equation and the coefficient of determination indicated ($R^2 = 0.77$). Data were analyzed using GraphPad Prism (version 10).

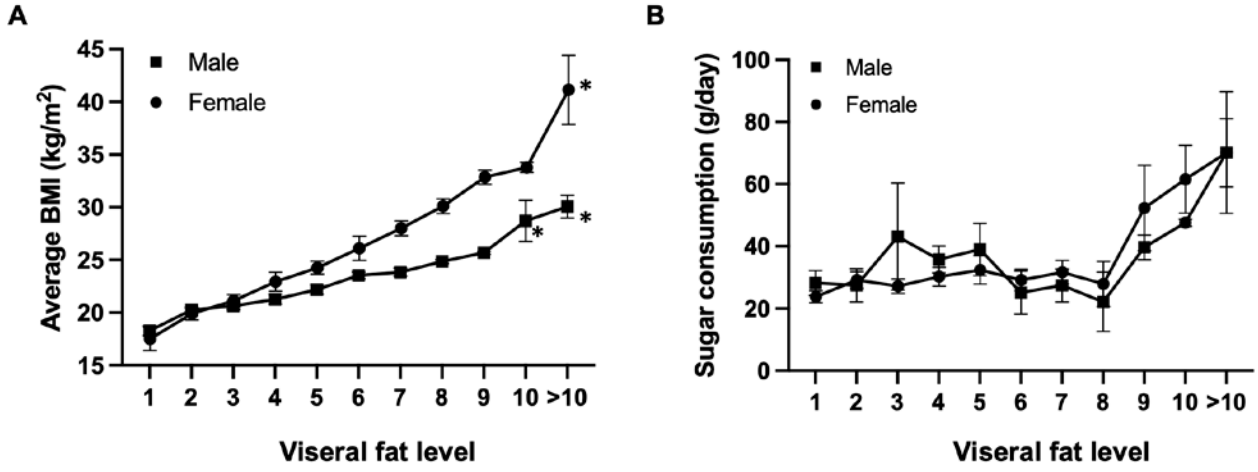


Figure 4. Mean-connecting plot of sex-specific associations between visceral fat level (VFL), body mass index (BMI) (A), and SSB-derived sugar consumption (B). *p < 0.05, (t-test).

fat level (VFL) of 10 or higher was associated with significantly elevated BMI values: 28.72 ± 4.67 kg/m² (p = 0.0159, t-test) for VFL = 10 and 30.06 ± 2.40 kg/m² (p = 0.0159, t-test) for VFL > 10, compared to a BMI of 25.70 kg/m² for a normal VFL of 9. In females, BMI also increased with higher VFL, reaching 32.91 ± 0.67 kg/m² (p = 0.0667, t-test) for VFL = 10 and 41.20 ± 3.27 kg/m² (p = 0.0061, t-test) for VFL > 10. This suggests that visceral fat accumulation is associated with an increased BMI, indicating a potential risk factor for obesity-related health issues. As illustrated in Figure 4B, the mean-connecting plot shows that students with a normal visceral fat rating (VFL = 9) consume approximately 30 g/day less sugar from SSBs, while individuals with a VFL greater than 9 tend to increase their sugar consumption from SSBs, exceeding 40 g/day for both males and females. Although this upward trend was not statistically significant, it was particularly pronounced in females, with consumption exceeding 70 g/day among those with the highest VFL. This observation underscores the potential association between excessive SSB consumption and increased visceral fat accumulation, particularly among females. Given their potentially heightened susceptibility to the adverse effects of excessive SSB consumption on visceral adiposity, further focused investigations of this demographic are warranted.

DISCUSSION

The rising prevalence of obesity and metabolic disorders has been strongly associated with sugar-sweetened beverage (SSB) consumption, which is

a key source of added sugars in modern diets. Although significant research has examined the impact of SSB intake on weight gain and metabolic health, this study sought to address critical gaps in understanding how specific SSB consumption patterns influence body composition metrics and their relationship with unhealthy lifestyle behaviors, particularly in young adults. This study identified a significant association between high sugar consumption from SSBs (> 24 g/day) and adverse body composition metrics, including increased body mass index (BMI) and visceral fat levels. Notably, individuals with elevated visceral fat levels (VFL ≥ 10) consumed nearly twice the amount of sugar from SSBs than those with normal VFL. Participants who derived more than 10% of their daily energy needs (basal metabolic rate, BMR) from sugar exhibited a three-fold higher sugar intake than those who consumed less than 10% of their BMR from sugar. This higher sugar consumption was further linked to significantly elevated overall calorie intake (1326.93 ± 245.45 kcal vs. 1240.45 ± 208.89 kcal, $p < 0.001$). These findings align with those of previous studies highlighting the metabolic risks associated with SSB consumption. Park et al. [13] reported that sweetened tea, carbonated beverages, sweetened coffee, and fruit drinks are the most frequently consumed SSBs, consistent with the results of the present study. Sweetened tea, particularly freshly prepared iced green tea and sweetened coffee, were identified as prominent contributors to SSB consumption. These beverages often surpass traditional sodas in terms of consumption frequency [14] and present unique challenges for sugar reduction efforts, especially when prepared in small shops where sugar content is difficult to regulate [15, 16]. The cultural significance, perceived health benefits (particularly for green tea), and widespread appeal of sweetened beverages underscores the need for targeted public health interventions. These interventions should prioritize reducing sweetened tea and coffee consumption as part of broader strategies to reduce dietary sugar intake. High SSB consumption was associated with clustering unhealthy habits, including increased alcohol consumption, which may exacerbate metabolic risks associated with excessive sugar intake [17]. Addressing these intertwined behaviors is critical for mitigating obesity-related health risks. Although this study provides valuable insights, it is essential to note its cross-sectional design, which precludes causal inferences. The observed associations reflect correlation rather than causation, emphasizing the need for longitudinal studies to confirm these findings and to better understand the temporal relationship between SSB consumption and body composition changes. Additionally, variations in sugar content due to unregulated preparation methods in small shops may introduce measurement variability, highlighting the importance of further studies to more accurately quantify sugar intake. This study underscores the significant metabolic risks associated with high SSB consumption, particularly its effect on BMI and visceral fat accumulation. Targeted public health strategies should promote healthier beverage alternatives, such as water, unsweetened drinks, and reduced-sugar options, while addressing broader lifestyle behaviors associated with excessive sugar intake. Longitudinal research is necessary to strengthen the evidence base and guide effective interventions to curb the rising prevalence of obesity and related metabolic disorders.

This study identified sweetened tea (particularly freshly prepared iced green tea) as the primary source of dietary sugar across all participant groups, with a significant contribution among overweight and obese individuals. These findings emphasize the need to focus on public health strategies to reduce sweetened tea consumption and mitigate obesity-related risks [18]. Conversely, lower SSB consumption among underweight and normal-BMI individuals may reflect healthier dietary patterns or higher physical activity levels [14]. However, additional research is warranted to further explore these associations and examine the causal pathways involved. Considering the potential role of caffeine in obesity, its combined effects with dietary sugars in beverages warrant further investigation. Global caffeine consumption remains high, with coffee and tea serving as predominant sources [19]. The patterns of caffeine intake are influenced by sociocultural factors and vary widely across regions. For instance, a 2015 U.S. survey reported an average daily caffeine intake of 164.5 mg, primarily derived from coffee [19]. In contrast, rural Thai populations demonstrated significantly higher intakes (302.5 mg/day), driven predominantly by sweetened tea consumption among working-age adults (15-59 years). This trend underscores the role of cultural preferences for sweetened caffeinated beverages in contributing to the rising obesity rates in specific populations. The complexity of the factors that influence obesity must also be acknowledged. Anthropometric changes often reflect the interplay of reduced physical activity, increased sedentary behaviors (exacerbated during the COVID-19 lockdowns), dietary shifts, and greater reliance on refined carbohydrates in fruits and vegetables [4, 19, 20]. Significant differences in lifestyle behaviors further underscored these relationships. For example, this study found that higher alcohol consumption among high-SSB consumers (61.4% vs. 38.6%, $p = 0.037$) highlights the clustering of unhealthy habits with excessive SSB intake. Recognizing the multifaceted nature of obesity is crucial in designing effective public health interventions. To combat the growing prevalence

of obesity and related health risks, comprehensive public health strategies must prioritize the reduction of added sugars from sources such as sweetened tea. Promoting healthier beverage options, such as water and unsweetened alternatives, while addressing societal and behavioral dynamics that drive excessive SSB consumption is essential. Targeting cultural and behavioral factors influencing dietary patterns can bolster efforts to mitigate the metabolic risks posed by high sugar and caffeine intakes [3, 18]. By addressing these interconnected factors through targeted public health strategies, meaningful progress can be made to reduce the global burden of obesity and its associated health complications. Long-term interventions must consider regional and cultural dynamics to ensure sustainable dietary behavioral changes.

This study investigated the relationship between sugar intake from sugar-sweetened beverages (SSBs) and body composition, with a focus on BMI and body fat percentage. The findings revealed a significant association between higher daily sugar intake from SSBs and increased BMI. For instance, participants classified as underweight (BMI < 18.0 kg/m²) consumed an average of 25.21 g/day of sugar from SSBs, while those categorized as overweight or obese (BMI > 25.0 kg/m²) exhibited significantly higher consumption levels, ranging from 32.18 to 34.00 g/day. These results align with prior research, such as the work of Malik et al. [16], who established a strong link between excessive SSB consumption and an increased risk of overweight and obesity among children and adolescents. In addition to its association with BMI, SSB consumption is associated with higher body fat percentage. Specifically, a 1.4% increase in body fat percentage was observed for every unit increase in BMI. This is consistent with the findings of English et al. [20], who reported significant increases in both body fat percentage and waist circumference among adolescents with high SSB intake over a two-year period, even in the absence of notable BMI changes [21]. These results highlight the disproportionate impact of SSB intake on adiposity, reinforcing its role as a key contributor to metabolic health risk. Our correlation analysis revealed modest yet significant associations between daily sugar intake from SSBs and obesity-related body composition measures, as evidenced by the correlation coefficients of 0.22 (fat mass), 0.20 (fat percentage), and 0.22 (visceral fat rating). While these correlations were modest, they underscored the role of SSB consumption in slight elevations in visceral fat, a critical determinant of metabolic health risks, including type 2 diabetes and cardiovascular diseases [16]. Stronger correlations were observed within body composition metrics, such as the robust association between BMI and fat mass (r = 0.91), and a moderate correlation between fat percentage and visceral fat rating (r = 0.84). These interconnected pathways highlight the complexity of adiposity development and emphasize the need to evaluate multiple body composition metrics to fully understand associated health risks. Sex-specific differences in SSB consumption and their impact on body composition were notable. Males with a borderline high visceral fat level (VFL = 10) had an average BMI of 28.72 ± 4.37 kg/m², while females exhibited a significantly higher mean BMI of 33.83 ± 0.47 kg/m² at the same VFL. Similarly, males consumed 47.60 ± 2.56 g/day of free sugar from SSBs, while females consumed considerably more (62.23 ± 19.03 g/day). This finding suggests potential sex-specific susceptibility to the adverse effects of SSB consumption on visceral fat accumulation. High SSB intake was also associated with the clustering of unhealthy behaviors. For instance, participants who derived more than 10% of their daily energy needs (BMR) from sugar exhibited elevated BMI, fat mass, and VFL compared to those adhering to dietary guidelines. This clustering reinforces the role of excessive SSB consumption in abdominal fat accumulation, which is a well-documented precursor of metabolic disorders [22]. These findings underscore the importance of dietary interventions targeting SSB reduction to combat central obesity and mitigate associated health risks. Promoting the substitution of high-sugar beverages with healthier alternatives such as water or unsweetened options is critical for addressing the increasing prevalence of metabolic disorders. Special attention should also be directed toward sex-specific differences in SSB consumption patterns and their implications for body composition, particularly among females, who demonstrated higher sugar intake levels and BMI at comparable visceral fat levels.

Although this study provides valuable insights, its cross-sectional design limits the ability to infer causality. The observed associations between SSB consumption and body composition reflect a correlation rather than causation, necessitating longitudinal studies to confirm these findings and elucidate the underlying mechanisms. The variability in sugar content due to the preparation methods, particularly in sweetened beverages, also warrants further investigation to enhance the accuracy of dietary assessments.

CONCLUSIONS

This study highlighted a significant association between daily sugar intake from SSBs and increased BMI, body fat percentage, and visceral fat levels, reinforcing the metabolic risks posed by high SSB consumption. These findings contribute to a growing body of evidence supporting the need for public health

strategies focused on reducing SSB consumption and promoting healthier dietary behaviors. By addressing the complex interplay between behavioral, dietary, and biological factors, future research can guide the development of effective nutrition policies to mitigate obesity-related health risks and improve metabolic outcomes.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding sources

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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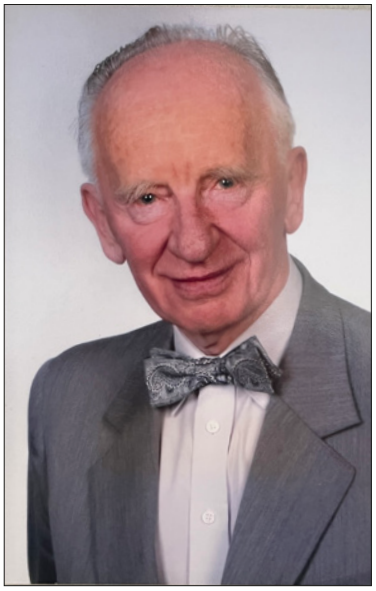
PAMIĘCI PROFESORA DR HAB. WIKTORA BOHDANA SZOSTAKA

(1933-2025)



Z głębokim żalem przyjęliśmy wiadomość o śmierci w dniu 1 marca 2025 roku Pana Profesora dr hab. med. Wiktora Bohdana Szostaka, długoletniego dyrektora Instytutu Żywności i Żywienia (IŻŻ) i kierownika Zakładu Żywienia Klinicznego IŻŻ.

Prof. dr hab. med. Wiktor Bohdan Szostak ukończył studia lekarskie na Akademii Medycznej w Warszawie w 1957 roku. Stopień doktora nauk medycznych uzyskał na Wydziale Lekarskim tej uczelni w 1967 roku a doktora habilitowanego nauk medycznych w 1971 w Centrum Medycznym Kształcenia Podyplomowego (CMKP), na podstawie pracy „Badania nad lipazą lipoproteinową w powiązaniu z hipertriglicerydemią samoistną i otyłością". W roku 1978 otrzymał tytuł profesora nadzwyczajnego, a w 1987 tytuł profesora zwyczajnego. Był specjalistą II stopnia w zakresie chorób wewnętrznych, miał także podspecjalizację z dietetyki.

Zainteresowania naukowe Profesor Szostak wykazywał już w latach studenckich działając w studenckim kole naukowym. Rozwijał je pracując w II Klinice Chorób Wewnętrznych Studium Doskonalenia Lekarzy (później przekształconego na CMKP), a następnie w Klinice Gastroenterologii i Przemiany Materii pod kierunkiem Profesora Edwarda Rużyłło. Zajmował się tam m.in. metodami badania stężeń lipidów w surowicy krwi w celu ich wykorzystania w badaniach skriningowych.

W tym okresie przebywał dwukrotnie na stypendiach naukowych Światowej Organizacji Zdrowia (WHO) na Uniwersytecie w Goeteborgu, gdzie zajmował się badaniami nad uwalnianiem lipazy lipoproteinowej z tkanki tłuszczowej pod wpływem heparyny w hipertriglicerydemii i otyłości. Badania z tego zakresu były podstawą rozprawy habilitacyjnej Profesora Szostaka. W ten sposób lipidologia stała się również Jego pasją. Można powiedzieć, że był pierwszym lipidologiem w Polsce. Zainteresowania te przekazał swoim uczniom, którzy pod Jego kierunkiem obronili prace doktorskie lub z Jego inspiracji – przygotowali prace habilitacyjne.

Dorobek naukowy Profesora Szostaka, w postaci prac oryginalnych i poglądowych oraz rozdziałów w podręcznikach, dotyczy metabolizmu lipoprotein, hiperlipoproteinemii, patogenezy miażdżycy oraz roli żywienia i farmakoterapii w leczeniu zaburzeń lipidowych. Te zagadnienia były też tematami Jego wykładów podczas szkoleń podyplomowych lekarzy, a należy podkreślić, że był On bardzo cenionym wykładowcą.

W 1971 roku Profesor Szostak rozpoczął pracę w Instytucie Żywności i Żywienia na stanowisku zastępcy dyrektora. Dzięki staraniom Profesora w Instytucie powstała Poradnia Chorób Metabolicznych, formalnie powołana przez Wydział Zdrowia Rady Narodowej m.st. Warszawy. Poradnia jako pierwsza w Polce przyjmowała pacjentów z zaburzeniami lipidowymi oraz z otyłością z całego kraju.

Przy Poradni rozpoczęło też działalność standaryzowane laboratorium analityczne. Z działalnością Poradni wiązało się rozwinięcie poradnictwa dietetycznego. W tamtym czasie w Poradni pracowało 10 lekarzy, przyjmowano ponad 10 tysięcy pacjentów rocznie.

W roku 1976 Profesor Szostak został dyrektorem IŻŻ. Funkcję tę pełnił do roku 1991. Jednocześnie był kierownikiem Zakładu Żywienia Klinicznego Instytutu. Ponadto kierował kilkuletnim resortowym programem „Optymalizacja żywienia człowieka" oraz częścią rządowego programu „Optymalizacja spożycia białka przez człowieka".

Z Jego inicjatywy w 1985 roku w Instytucie powołano Klinikę Chorób Metabolicznych i Gastroenterologii na bazie Oddziału Gastroenterologii Szpitala Bródnowskiego (obecnie Mazowieckiego Szpitala Bródnowskiego).

Bardzo ważnym osiągnięciem Profesora Szostaka było nadanie Instytutowi statusu WHO Collaborating Center for Nutrition nadzorowanego przez Biuro Europejskie WHO w Kopenhadze. Warto podkreślić, że Instytut jako WHO Collaborating Center organizował, przed 1989 r., spotkania ekspertów z dziedziny żywienia z obu stron „żelaznej kurtyny".

Profesor Szostak był również inicjatorem i propagatorem Narodowego Programu Profilaktyki Cholesterolowej. Jedną z form Jego działalności było redagowanie kwartalnika pod nazwą List Informacyjny „Profilaktyka cholesterolowa", który zawierał zbiór krótkich artykułów przygotowanych na podstawie literatury światowej z własnymi komentarzami. List był przesyłany m.in. członkom Sekcji Żywienia i Przemiany Materii Towarzystwa Internistów Polskich, której Profesor był przewodniczącym.

Należy również wspomnieć, że przez kilka lat z inicjatywy Profesora w Instytucie działały studia doktoranckie z prawem nadawania stopnia doktora nauk medycznych, które zaowocowały kilkoma obronionymi doktoratami.

Profesor Szostak był członkiem honorowym medycznych towarzystw naukowych, Komitetu Żywienia Człowieka PAN, rad naukowych innych instytutów oraz licznych komisji i zespołów.

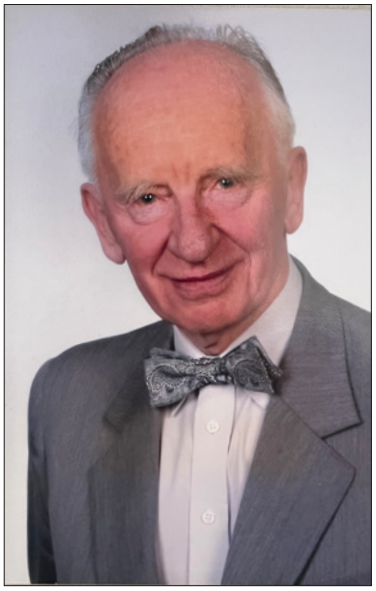
Został odznaczony Krzyżem Kawalerskim Orderu Odrodzenia Polski i Złotym Krzyżem Zasługi.

Można z przekonaniem stwierdzić, że Pan Profesor był wybitną osobowością, człowiekiem wielu talentów, jako badacz, nauczyciel, wykładowca i organizator.

Prof. dr hab. med. Barbara Cybulska

IN MEMORIAM: PROFESSOR WIKTOR BOHDAN SZOSTAK

(1933-2025)



It is with deep regret that we have received the news of the death on 1 March 2025 of Professor Wiktor Bohdan Szostak, MD, PhD, Director of the Institute of Food and Nutrition (IFN) and Head of the Clinical Nutrition Department of the IFN for many years.

Professor Wiktor Bohdan Szostak graduated from the Medical Academy of Warsaw in 1957. He obtained the degree of Doctor of Medicine from the Faculty of Medicine of this academy in 1967 and the postdoctoral degree from the Medical Centre for Postgraduate Education (MCPE) in 1971, on the basis of the dissertation entitled 'Studies on lipoprotein lipase in relation to spontaneous hypertriglyceridemia and obesity'. He was awarded the title of associate professor in 1978 and the title of professor in 1987. He was a second-degree specialist in internal medicine and also had a subspecialization in dietetics.

Professor Szostak showed his scientific interests as early as his student years when he was active in a student scientific club. He developed his interests while working at the 2nd Department of Internal Medicine of the Medical College (later renamed MCPE), and then at the Department of Gastroenterology and Metabolic Diseases under the direction of Professor Edward Rużyłło. There he dealt, among other things, with methods of examining serum lipid concentrations for use in screening studies.

During this period he was twice on World Health Organization (WHO) research fellowships at the University of Gothenburg, where he investigated heparin-mediated lipoprotein lipase release from adipose tissue in hypertriglyceridaemia and obesity. Research in this area formed the basis of Professor Szostak's habilitation thesis. Thus, lipidology also became his passion. It can be said that he was the first lipidologist in Poland. He passed this interest on to his students who defended their doctoral theses under his supervision or prepared their habilitation theses inspired by him.

Professor Szostak's scientific output, in the form of original and review papers and chapters in monographs, concerns lipoprotein metabolism, hyperlipoproteinemia, the pathogenesis of atherosclerosis and the role of nutrition and pharmacotherapy in the treatment of lipid disorders. These issues were also the topics of his lectures during postgraduate training of doctors, and it should be noted that he was a highly regarded speaker.

In 1971, Professor Szostak began working at the Institute of Food and Nutrition as Deputy Director. Thanks to his efforts, an Outpatient Clinic for Metabolic Diseases was established at the Institute, formally appointed by the Department of Health of the National Council of the Capital City of Warsaw. The Outpatient Clinic was the first in Poland to admit patients with lipid disorders

and obesity from all over the country. A standardised analytical laboratory also began operating at the Outpatient Clinic. The development of dietary counselling was associated with the Outpatient Clinic's activities. At that time, 10 physicians worked at the Outpatient Clinic, receiving more than 10,000 patients a year.

In 1976, Professor Szostak became director of the IFN. At the same time, he was head of the Institute's Department of Clinical Nutrition. In addition, he directed the several-year departmental programme 'Optimisation of Human Nutrition' and part of the government programme 'Optimisation of Human Protein Intake'.

On his initiative, in 1985, the Metabolic Diseases and Gastroenterology Clinic was established at the Institute on the basis of the Gastroenterology Department of the Bródno Hospital (now the Mazovian Bródno Hospital).

Professor Szostak's very important achievement was to grant the Institute the status of WHO Collaborating Center for Nutrition supervised by the WHO Regional Office for Europe in Copenhagen. It is worth noting that the Institute, as a WHO Collaborating Center, organised, before 1989, meetings of nutrition experts from both sides of the 'Iron Curtain'.

Professor Szostak was also the initiator and promoter of the National Cholesterol Prevention Programme. One of the forms of his activities was editing a quarterly magazine called the Information Letter 'Cholesterol Prophylaxis', which contained a collection of short articles prepared on the basis of world literature with his own comments. The letter was sent, among others, to members of the Nutrition and Metabolism Section of the Polish Society of Internal Medicine. Professor was a chairman of this society.

It should also be mentioned that for several years, on Professor Szostak's initiative, a doctoral programme with the right to confer the degree of Doctor of Medical Sciences was operating at the Institute, which resulted in several defended doctoral theses.

Professor Szostak was an honorary member of medical scientific societies, the Human Nutrition Committee of the Polish Academy of Sciences, scientific councils of other institutes and numerous committees and teams.

He was awarded the Knight's Cross of the Order of Polonia Restituta and the Golden Cross of Merit.

It can be stated with conviction that Professor Szostak was an outstanding personality, a man of many talents as a researcher, teacher, speaker, and organiser.

Professor Barbara Cybulska, MD, PhD

NORMY ŻYWIENIA DLA POPULACJI POLSKI 2024

Eksperci Narodowego Instytutu Zdrowia Publicznego PZH – Państwowego Instytutu Badawczego w 2024 roku znowelizowali normy żywienia dla populacji Polski. Prace te realizowane były w ramach Narodowego Programu Zdrowia na lata 2021-2025.

Znowelizowane normy uwzględniają najnowsze osiągnięcia nauki o żywieniu człowieka, której rozwój sprawia, że istnieje potrzeba systematycznej aktualizacji norm.

Normy żywienia mają szerokie zastosowanie w praktyce, ponadto stanowią punkt wyjścia do dalszych badań. Normy określają jaka ilość energii oraz niezbędnych składników odżywczych jest wystarczająca do zaspokojenia zapotrzebowania organizmu osób zdrowych w populacji. Uwzględniają różnice w zapotrzebowaniu organizmu zależne od wieku, płci, stanu fizjologicznego, aktywności fizycznej oraz masy ciała. Wyrażone są w przeliczeniu na jedną osobę na dobę.

Normy żywienia dla populacji Polski uwzględniają zalecenia m.in. ekspertów Europejskiego Urzędu Bezpieczeństwa ds. Żywności (EFSA), National Academies of Sciences, Engineering, and Medicine ze Stanów Zjednoczonych oraz wyniki najnowszych badań krajowych i zagranicznych.

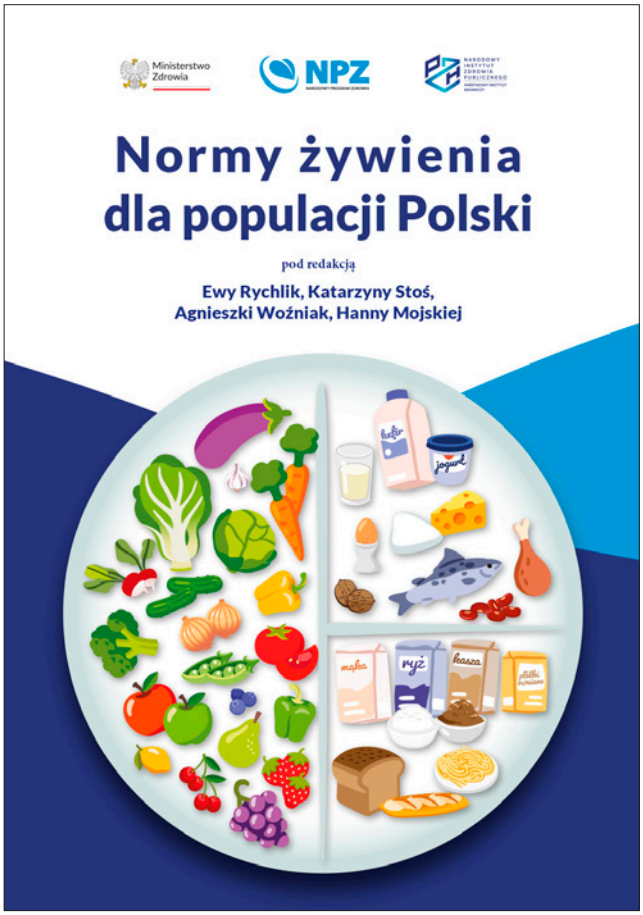
Poprzednia aktualizacja norm żywienia w Polsce została przeprowadzona w roku 2020. Obecnie zaktualizowano przede wszystkim normy na energię, białko i tłuszcz, ponadto do określenia wartości norm dla osób dorosłych wykorzystano najnowsze dane antropometryczne dla populacji polskiej. Po raz pierwszy opracowano normy na molibden. Znowelizowano normy na biotynę dla wszystkich grup populacyjnych. W monografii przedstawiono także wyniki najnowszych prac EFSA dotyczących górnych tolerowanych poziomów spożycia (UL).

Monografia zawiera również informacje, które będą pomocne w korzystaniu z norm żywienia i ich zastosowaniu w praktyce. Dotyczą one oceny i planowania spożycia, wartości referencyjnych zamieszczanych na opakowaniach żywności oraz stosowania suplementów diety.

Uaktualnione normy będą przydatne przy prowadzeniu badań naukowych, w kształceniu studentów uniwersytetów medycznych, przyrodniczych, rolniczych i innych szkół z kierunkami związanymi z nauką o żywieniu człowieka oraz przy planowaniu działań mających na celu poprawę zdrowia Polaków. Powinny też być pomocne w pracy lekarzy, dietetyków oraz osób planujących żywienie indywidualne i zbiorowe. Warto też, żeby zapoznał się z nimi każdy, kto interesuje się zdrowym żywieniem. Zachęcamy Państwa do zapoznania się z monografią, którą można pobrać bezpłatnie.

Elektroniczna wersja monografii została opublikowana na stronach:

- Narodowego Instytutu Zdrowia Publicznego PZH – Państwowego Instytutu Badawczego https://www.pzh.gov.pl/normy-zywienia-2024/
- oraz Narodowego Centrum Edukacji Żywieniowej https://ncez.pzh.gov.pl/abc-zywienia/zasady-zdrowego-zywienia/normy-zywieniowe-2024/



Zadanie realizowane ze środków Narodowego Programu Zdrowia na lata 2021-2025, finansowane przez Ministra Zdrowia.

DIETARY REFERENCE VALUES (DRVs) FOR POLISH POPULATION 2024

Experts from the National Institute of Public Health NIH – National Research Institute have revised the Dietary Reference Values (DRVs) for Polish population in 2024. This work was carried out as part of the National Health Programme 2021-2025.

The revised DRVs take into account the latest achievements in the science of human nutrition, the development of which makes it necessary to systematically update the DRVs.

The DRVs have a wide application in practice and provide a baseline for further research. The DRVs define what energy value and amount of essential nutrients is sufficient to meet the requirements in a healthy population. They take into account differences depending on age, gender, physiological state, physical activity and body weight. They are expressed per person per day.

The Polish DRVs take into account the recommendations of experts from the European Food Safety Authority (EFSA), the National Academies of Sciences, Engineering, and Medicine from the United States and the results of the latest national and international research.

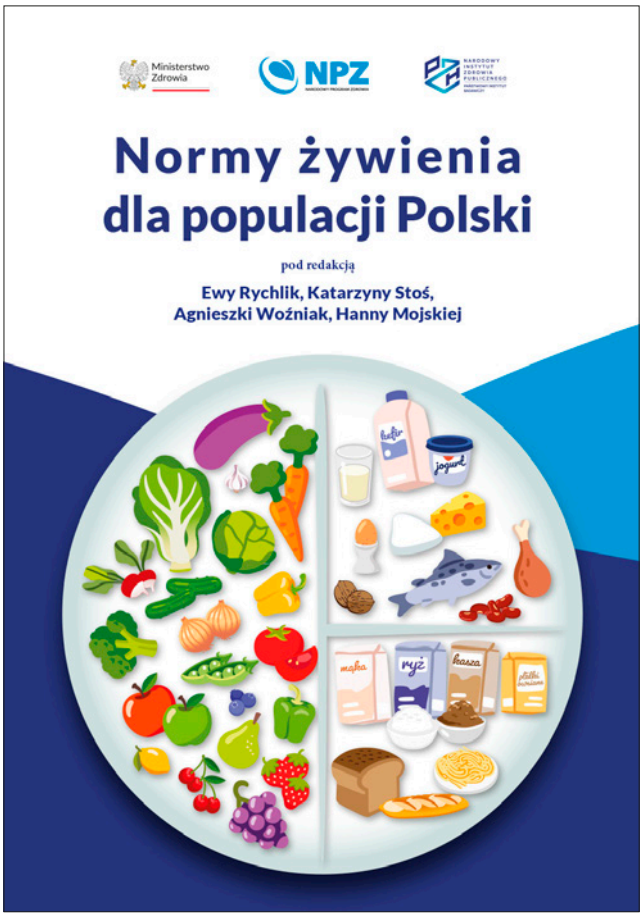
The previous update of the Polish DRVs was carried out in 2020. Now, the values for energy, protein and fat have been updated, in particular, and the latest anthropometric data for the Polish population have been used to determine the values for adults. DRVs for molybdenum have been developed for the first time. Values for biotin for all population groups have been revised. The monograph also presents the results of recent EFSA work on Tolerable Upper Intake Levels (ULs).

The monograph also contains information that will be helpful in using the DRVs and applying them in practice. This information relates to intake assessment and planning, reference values on food packaging and the use of dietary supplements.

The updated DRVs will be useful in conducting scientific research, in education of students at medical and agricultural universities, universities of life sciences and other schools with specialisations in human nutrition, and in planning activities to improve the health of Polish population. They should also be helpful in the work of physicians, nutritionists and those planning nutrition at individual level and mass catering. It is also worth reading for anyone interested in healthy eating. We encourage you to read the monograph, which can be downloaded free of charge.

An electronic version of the monograph has been published on the following websites:

- National Institute of Public Health NIH – National Institute of Public Health https://www.pzh.gov.pl/normy-zywienia-2024/
- and the National Centre for Nutrition Education https://ncez.pzh.gov.pl/abc-zywienia/zasady-zdrowego-zywienia/normy-zywieniowe-2024/



The task carried out with the funds of the National Health Programme 2021–2025, financed by the Minister of Health.

Rocz Panstw Zakl Hig 2025;76(1):93-95
https://roczniki.pzh.gov.pl/

INSTRUCTION FOR AUTHORS

Scope of the Journal

The journal Roczniki Państwowego Zakładu Higieny - Annals of the National Institute of Hygiene is the peer-reviewed scientific journal that publishes original research articles, reviews, short communications and letters to the Editor.
The journal is devoted to research studies on food and water safety, nutrition, dietetics, environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences.

General rules for manuscript submission

All manuscripts in English should be submitted to the Editorial Office exclusively online through the editorial system (https://www.editorialsystem.com/rpzh/). Submissions by e-mail will not be accepted.

1. Submitted manuscripts must be an original work, not have been previously published nor be under consideration for publication in another journal.
2. Only high scientific quality articles in English complying with the scope of the journal will be considered for publication.
3. The journal requires that authors provide an ORCID IDs when submitting a manuscript for publication.
4. If the article is accepted for publication, the Corresponding Author will receive 'License to Publish Statement' document via the editorial system. The signed document should be submitted to the Editorial Office. Without the submission of the signed document, it will not be possible to publish the article in a journal.
5. The Editorial Office reserves the right to make editorial corrections to the manuscript that do not influence the substantive content of the article, without prior notification to the author.

Manuscript arrangement

Manuscripts submitted for publication should be prepared in doc, docx, rtf formats using Microsoft Word Times New Roman 12 font and 1.5 space between lines on A4 format.
Manuscript should include: (1) title page, (2) text of the manuscript, (3) Tables, (4) Figures, (5) Appendices. A complete manuscript should not exceed 16 pages, including text, tables, figures and references.

Abstract should not exceed more than 300 words. The original research article should consist of the following sections: Background, Objective, Material and Methods, Results, Discussion and Conclusions. The abstract of a review article does not need to be divided into sections.

Key words. 5-7 words or short phrases according to the MeSH (Medical Subject Headings) catalogue available at www.nlm.nih.gov/mesh/meshhome.html.

Original research article should be divided into the sections: Introduction/Background, Material and Methods, Results, Discussion, Conclusions, Acknowledgements, Conflict of interest and References.

Review article should include: Introduction/Background, Conclusions, Acknowledgements, Conflict of interest and References. The remaining section titles depend on the topic of the article.
Introduction/Background should contain the scientific rationale and the aim of the study or in the case of a review the purpose of the article. Only references directly related to the paper should be cited.

Material and Methods should provide detailed information on the subject of the study, methods, reagents, apparatus and techniques used in sufficiently exhaustive way to enable readers to repeat the experiments or observations. For generally known methods references should be given together with name of the methods or statistical analysis used in the study. For new or substantially modified methods detailed descriptions are to be added. In the case of experimental studies on laboratory animals, the information should be provided

on the approval by a local Ethics Commission. In the case of clinical studies in humans that they have been performed with the ethical standards according to the Helsinki Declaration.

Results should be presented in a logical sequence in the text, the same applies to the tables and figures. The data from the tables and figures should not be repeated in the text, where only the most important observations from the studies are to be summarized.

Discussion should emphasize the new and important aspects of the results and a comprehensive interpretation of the results obtained against the background of results obtained by other authors. Quotations should be restricted to those with immediate relevance to the author's findings.

Conclusions should be stated in points or descriptively and should be logically connected with the aims stated in the introduction. Statements and conclusions not derived from own observations should be avoided. If a hypothesis is proposed it must be stated clearly.

Acknowledgements. One or more statements should specify: (1) persons who contributed substantially to the study but cannot be regarded as authors, such as technical assistants, statisticians, data collectors etc. Their assistance should be acknowledged for the sake of transparency. It must be clear that they are not responsible for the final version of the article. The consent of all the persons named in the acknowledgements must be obtained; (2) all sources of financial and material support, which should specify the nature of the support. The recommended form is: "This work was supported by …….: (name of the organization, project number etc.)".

Conflict of interest. Information regarding conflict of interest should be disclosed. If there is no conflict, authors should declare that.

References should be presented according to the Vancouver referencing style. References should be cited in the order in which they first appear in the text. If the same publication is cited again the same number should be given. The journal title should be abbreviated in accordance with MEDLINE. Each position in the list of references should start from the new line and contain: consecutive number, author's (authors') surname(s) and initials of name(s), full title of the paper, official journal title abbreviation (according to the List of Journals Indexed in Index Medicus), year, volume, number, the first and the last page number. If the cited article or book has a DOI (Digital Object Identifier), it should be included in the references. The titles of the cited papers in other language than English should be translated into English. In the text of the manuscript only the number referring to the cited publication listed in references should be given in square brackets. The list of references should include only those that are cited in the text of the manuscript.
The journal Roczniki Państwowego Zakładu Higieny – Annals of the National Institute of Hygiene should be cited as: Rocz Panstw Zakl Hig.

The examples of reference style are given below:

Journal article with 1-6 authors:

1. Mertens E, Colizzi C, Peñalvo JL. Ultra-processed food consumption in adults across Europe. Eur J Nutr. 2022;61(3):1521-1539. doi: 10.1007/s00394-021-02733-7.

Journal article with more than 6 authors:

2. Osei-Kwasi HA, Boateng D, Danquah I, Holdsworth M, Mejean C, Terragni L, et al. Acculturation and Food Intake Among Ghanaian Migrants in Europe: Findings From the RODAM Study. J Nutr Educ Behav. 2020;52(2):114-125. doi: 10.1016/j.jneb.2019.09.004.

Book:

3. Kerner S, Chou C, Warmind M. Commensality: From Everyday Food to Feast. London: Bloomsbury Publishing PLC; 2015. ISBN 9780857857361.

Book chapter:

4. Lucas BL, Feucht SA. Nutrition in childhood. In: Mahan LK, Escott-Stump S, editors. Krause's Food & Nutrition Therapy. 12th ed. St. Louis, MO: Saunders Elsevier; 2008. p. 222–245. ISBN 9780808923787.

Internet source:

5. World Health Organization. GHE: Life expectancy and healthy life expectancy [Internet]. Geneva: World Health Organization; 2024. [cited 2024 Jan 19] Available from: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-life-expectancy-and-healthy-life-expectancy.

Legislative acts:

6. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance). OJ L 354, 31.12.2008, p. 16–33. Available from: http://data.europa.eu/eli/reg/2008/1333/oj.

Tables should be prepared in separate file(s) in doc, docx, rtf, odt formats and numbered using Arabic numerals. The title should be place directly above each table. Tables should always be cited in the text in consecutive numerical order. Each column in tables should have a brief heading, more extensive explanation should be given below the table, if necessary. The number of tables should be limited to the necessary minimum.

Figures and photographs should be prepared in separate file(s) in jpg, gif, tif, png, docx formats and numbered in Arabic numerals according to the sequence of their appearance in the text. The titles should be placed below the figures. Photographs must be of high quality.

Tables, figures or photographs should be cited in the text in consecutive numerical order: (Table 1), (Figure 1), (Photo 1).

Abbreviations, symbols, units. Generally known and used abbreviations may be left unexplained, others must be explained at the first use in the text. Metric SI units are recommended, however also other generally used units are accepted.

Peer-review process

- Roczniki Państwowego Zakładu Higieny – Annals of the National Institute of Hygiene uses the double blind peer-review model in the process of reviewing manuscripts. It means that authors and reviewers remain anonymous and don't know each other's identities.
- The manuscripts submitted for publication undergo in the Editorial Office the initial evaluation in terms of compliance of the subject matter with the profile and formal requirements of the journal.
- The manuscripts that do not meet the journal's requirements are not qualified for the further publishing process, and the authors are informed. Manuscripts that meets these requirements and receives a positive initial evaluation is directed for peer-review evaluation to at least two independent reviewers, experts in the field of the subject matter of the manuscript.
- The reviewers must ensure independence of evaluation and no conflict of interest. They should be affiliated in different institution than the authors.
- The review form is available to reviewers after logging into the Editorial System.
- The review is in writing form and contains the reviewers' final conclusions as to whether the article should be published in its current form, after minor corrections, after major changes, or not it should be published.
- The Editor-in-Chief, on the basis of the reviews received, decides whether the manuscript will be accepted for publication, sent back to the corresponding author for correction, or rejected. The author receives a written review and information on the status of the submitted manuscript and its qualification for publication.
- The author should respond to all comments and remarks made by reviewers and submit a corrected version of the manuscript. This version of manuscript is re-checked before the Editor-in-Chief makes the final decision on acceptance it for publication.

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It will be published only in the online version.

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