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INNOVATIVE TOOLS IMPLEMENTATION TO UPDATE FOOD COMPOSITION DATABASES

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LIST OF ABBREVIATIONS

%En: percentage contribution to total energy intake;
3-dDR: 3-day Dietary Record;
4-dDR: 4-days Dietary Record;
7-dDR: 7-day Dietary Record;
AI: Adequate Intake;
ANSES: French Agency for Food, Environmental and Occupational Health and Safety;
AR: Average Requirement;
BDA v.98 v.08 v.15 v.22: *Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia*; Food Composition Database for Epidemiological Studies in Italy, in its 1998, 2008, 2015 and latest version (2022, object of the present work), respectively;
BMI: Body Mass Index;
CCP: Critical Control Point;
ChM: Choline Moiety;
CI: Confidence Intervals;
CoFID: Composition of Food's Integrated Dataset
CREA-NUT: Italian Council for Agricultural Research and Economy- Food and Nutrition;
CSP: Constraints Satisfaction Problem;
DHA, Docosahexaenoic acid.
DM: Dry Matter;
DP: Degree of Polymerization;
DRV: Dietary Reference Value;
DTU: Technical University of Denmark;
EFSA: European Food Safety Authority;
ENDB: European Nutrient Database;
EPA: Eicosapentaenoic acid;
EPIC: European Prospective Investigation into Cancer and Nutrition;
ESFRI: European Strategy Forum on Research Infrastructures;
EU: European Union;
EuroFIR: European Food Information Resource;
FAO: Food and Agriculture Organization of the United Nations;
FCD: Food Composition Data;
FCDB: Food Composition Database;
FF-Nystose, 1F-β-fructofuranosylnystose;
FFM: Fat Free Mass;
FFQ: Food Frequency Questionnaire;
FM: Fat Mass;
FOS: Fructo-Oligosaccharides;
GC: Gluten Containing;
GF: Gluten Free;
GOS: Galacto-Oligosaccharides;
HIIT: High Intensity Interval Training;

HPAE-PAD: High-Performance Anion-Exchange chromatography coupled to Pulsed Amperometric Detection;
IEO: *Istituto Europeo di Oncologia*; European Institute of Oncology;
INFOODS: International Network of Food Data Systems;
IOTF: International Obesity Task Force;
IRCCS: *Istituto di Ricovero e Cura a Carattere Scientifico;*
ITFs: Inulin-Type Fructans;
MAR: Mean Adequacy Ratio;
MICT: Moderate Intensity Continuous Training;
MOEA: Multi-Objective Evolutionary Algorithm
MUFAs: Monounsaturated Fatty Acids;
NAR: Nutrient Adequacy Ratio;
NL: Nutritional Label
NSGA: Non-dominating Sorting Genetic Algorithm;
OB: Obesity;
OR: Odds Ratio
OW: Overweight;
PHIME: Public Health Impact of long-term, low-level, Mixed element Exposure in susceptible population strata;
PRI: Population Reference Intake;
PUFAs: Polyunsaturated Fatty Acids;
RDA: Recommended Dietary Allowance;
RF: Retention factor;
RI: Reference Intake range for macronutrients;
SD: Standard Deviation
SDT: Suggested Dietary Target;
SE: Standard Error;
SFAs: Saturated Fatty Acids;
SM: Sphingomyelin
SOP: Standard Operating Procedure;
UI: User Interface;
USDA: United States Department of Agriculture;
$V'O_2$: pulmonary oxygen uptake;
YF: Yield Factor.

ABSTRACT

It is well known that dietary assessment in nutritional epidemiology presents some criticalities mainly related to: 1- the tools used to collect dietary habits (food records, 24-hour recalls or food frequency questionnaires) and 2- the tools used to convert food intake into nutrient intake— i.e., Food Composition Databases (FCDBs). In order to adequately represent the foods consumed by the given study population, FCDBs should be constantly updated. Therefore, the main objectives were to present the update of the compiled FCDB for epidemiological studies in Italy (*Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia*; BDA), and to propose innovative tools/procedures to improve FCDBs in terms of coverage in foods and food components. The present thesis is divided into 6 main sections.

Food and nutrient intakes: Three dietary assessment studies were conducted to evaluate nutritional adequacy in different populations and to identify BDA-related criticalities. Modern diets include a wide variety of manufactured foods whose composition is unknown, except from label information. Direct use of food composition data from nutritional labels (NLs) in nutritional research without further calculation and/or imputation procedures may result in many missing data, potentially leading to underestimation of nutrient intakes. We found that ~6%, 23%, and 38% of the total food list (excluding repeats) in the diets of centenarians, obese adults, and children, respectively, were manufactured foods not yet included in the BDA. As a result, significant amounts of missing nutrient data were found, potentially altering nutritional assessment results. Relative to the entire list of food entries, >40% of the data were missing for some vitamins, minerals, fatty acids, aminoacids and sugars.

Standard update of the BDA: The present update focused on the food categories: “Cereals and cereal-based products”, “Bread, crispbread, rusks”, “Sugar and confectionery”, and “Cakes”. Compared to the latest release, 51 additional food components and 141 new food items were compiled. Nine food items were deleted and 151 items were updated. Compared to the literature sources used to compile the current food categories, this BDA update (BDA v.22) borrowed fewer macronutrient values from Italian and international FCDBs and derived more macronutrient values from food label data than the previous BDA version.

Update of a gluten free FCDB: Due to the lack of comprehensive analytical data, a published gluten free (GF) FCDB was updated based on label information (ingredient list and NL) from manufactured foods. A modified label-based recipe approach was applied to 630 products. Food products were classified in items and categories, according to the BDA standard coding system. The final database included 101 GF items representing foods available in the Italian market, 91 food components, and no missing data. Moisture content was determined for 88 GF manufactured products and 93 corresponding gluten containing (GC) products from the food groups: “Bread and substitutes”, “Filled pasta”, “Biscuits”, and “Cakes and desserts”. The determination of water content was performed to obtain analytical composition data and to indirectly verify the accuracy of information from NL for its use in FCDBs. We found that the water content calculated from NL was generally higher than the analytical value (mean difference: 2.2±3.3 g/100g), implying that

the macronutrient composition might be underestimated in the label-based recipe calculation approach used to compile FCDBs. However, the extent of underestimation is likely to be minimal. In addition, the analysis showed that GF products —particularly breads and substitutes—generally have higher water content than the corresponding GC products, likely due to the use of additives, water, and hydrocolloids to compensate for the lack of gluten structure. When comparing the labelled nutrient composition of 88 GF and 93 GC products, we found significantly lower protein content in GF than GC foods.

Old *vs.* updated FCDB: A pilot study was conducted using the food records of ten 18-month-old infants enrolled in the public health impact of long-term, low-level, mixed element exposure in susceptible population strata (PHIME) study. The records were analysed using two different versions of the same study-specific FCDB based on the BDA. The first version was compiled using data as reported in NLs—without compiling missing nutrient data— and the second version was compiled imputing missing nutrient data using the recipe calculation approach. The magnitude of the intake underestimation ranged from 0% for macronutrients to 33±21% for vitamin E.

Development of a technological tool to impute ingredients' weight: To assist the compiler in calculating recipes based on labels, a tool was developed implementing a non-dominating sorting genetic algorithm (NSGAII) to determine the optimal weight of ingredients. Although the tool made it possible to standardise the decision-making process and achieve results equivalent to those of the manual trial-and-error approach, the process still remains user-dependent and very time-consuming.

Update of the case-control FCDB for components of interest: Biologically active compounds, not yet included in the standard BDA update, are gaining interest in nutritional epidemiology. In the present work the BDA-based case-control FCDB was updated for choline, sphingomyelins, and prebiotic content, using different compiling methods. Choline and Sphingomyelins content of foods was borrowed from other FCDBs and literature tables using standard food matching procedures. The content of inulin-type fructans and 5 prebiotic compounds was determined analytically in a selected sample of Italian plant- and cereal-based foods and then matched to the FCDB food items. Finally, the updated FCDB was used to assess prebiotic intakes in a population of 1953 colorectal cancer cases and 4154 controls. The results suggest an inverse association between dietary galacto-oligosaccharide intake and colorectal cancer risk.

In conclusion, the use of the BDA to convert food consumption to nutrient intake allowed the identification of the main critical issues that highlight the need for accessible, reliable, quality-documented, and complete food composition data. To overcome the identified criticalities, standard and innovative approaches were implemented to update the BDA, the GF-FCDB, and the case-control FCDB, thus providing novel food composition data for their use in nutritional epidemiology. In addition, food labels have been shown to have great potential in the food composition field. The approaches used in this thesis provide useful guidance for further development of electronic platforms for food composition data management within local and international projects such as METROFOOD-RI.

1. INTRODUCTION

1.1. FOOD COMPOSITION DATA AND DATABASES

The understanding of the influence that the diet exerts on human health provided the basis of the science of nutrition since early times. However, to study this association in the first place is essential to develop an extensive knowledge of the chemical composition of foods. Food composition tables and databases have been conceived to collect detailed information on the concentration of energy, nutrients, and other relevant components of foods, usually from a particular country.

A first attempt to describe the nutritional composition of food was dated in 1818 (Percy and Vaquelin, 1818). The publication collected mainly water and dry matter data of some foods in the form of a "nutrition scale". The earliest food composition table in the currently known format has been published in Germany many years later, in 1878 (König, 1878). One of the most widely known and most complete early tables was the one created by the United States of America in 1896 (Atwater and Woods, 1896). It collected the composition of raw and processed food, reporting data of nearly 2600 analyses expressed as: "refuse", water, protein, fats, carbohydrates, ash, and "fuel value". In Europe, United Kingdom (1921), Italy (1946) and the Netherlands (1941) were also pioneers of the food composition field. Moreover, in 1949 the Food and Agriculture Organization of the United Nations (FAO) produced the "Food composition tables for international use", the first attempt to harmonise and collect food composition data (FCD) in an international database (Church, 2006). Ever since, FCD have been published worldwide in printed tables and, more recently, in online databases.

At present, although printed tables are still produced, most FCD are stored in digital form in food composition databases (FCDBs) because of the ease with which large amount of data can be organised, accessed and updated. FCDBs continue evolving, parallelly with the knowledge on the chemical composition of foods, and the mechanisms by which specific bioactive compounds, foods, and dietary patterns exert influence on health and disease (Delgado et al., 2021). There are currently several publicly available international and national FCDBs worldwide. Globally, about three quarters of all countries have FCD stored in national tables (Traka et al., 2020). In Europe 33 countries dispone of at least one country-specific FCDB (FAO- INFOODS, 2021). Given the high variability of scope and details of FCDBs, data may be presented in a non-standard form. As a result, these inconsistencies between datasets make comparison between different countries and use of country-specific data for international purposes very difficult. Multiple international networks have been established to develop international standards and promote cooperation in the food composition field, aiming to improve the overall quality, availability, reliability and use of FCD at national and international level.

The International Network of Food Data Systems (INFOODS) was established in 1984, and co-sponsored by the FAO. INFOODS represents a network linking agriculture, biodiversity, food systems, health and nutrition. It aims to promote the cooperation, acquisition and dissemination of adequate and reliable data on the composition of foods in appropriate forms in order to meet the needs of various national and international users, and at last to achieve better nutrition worldwide. Its activities include the provision of guidelines, standards, quality criteria, compilation tools, databases, capacity development tools, policy advice, advocacy tools, and technical assistance to FCDB compilers at country-level.

Recently, an international infrastructure for promoting metrology in food and nutrition has been established to provide high-level metrology services in food and nutrition for the enhancement of food quality and safety. The METROFOOD-RI project (https://www.metrofood.eu) originated from the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap 2018 and its main objectives are to enhance quality and reliability of measurements, to encourage interaction between the various stakeholders, and to create a common and shared base of data, information and knowledge supporting research in food and nutrition, while focusing on emerging needs. It combines two strictly interconnected components: a physical (further divided in "Metro" and “Food” sides) and an electronic infrastructure. At present, the Consortium involves 48 Institutes from 18 European Countries (15 Member States and 5 Associated Countries), and is now undertaking its Preparatory Phase, supported by the H2020 INFRADEV-02-2019 CSA METROFOOD-PP project. University of Udine participates in the METROFOOD-RI activities as a linked third party, being involved in the physical infrastructure, but particularly in the electronic infrastructure activities, with regard to data exchange and quality data management systems.

At a European level, the European Food Information Resource (EuroFIR) is an international, member-based, non-profit Association under Belgian law originated in 2009 from the EuroFIR Network of Excellence on food composition databank systems funded by the European Commission’s Sixth Framework Programme under the ‘Food Quality and Safety Priority’ to facilitate collaboration on the development and application of unified, reliable and accessible food information. In 2005, the network comprised 48 partners from academia, research organizations and enterprises (Finglas et al., 2017), while EuroFIR AISBL currently counts 64 partner institutes from all over the world (www.eurofir.org). Its mission is to enhance awareness and understanding of the value of FCD encouraging wider applications for both research and commercial purposes, and to facilitate improved data quality, storage and access. It also provides tools for compilers (FoodCASE) and collects in a web app (FoodEXplorer) all the partner institution’s FCD. Currently, a total of 39 national FCDBs are indexed in FoodEXplorer (https://www.eurofir.org), having to comply with specific harmonization and international cooperation standards (Finglas et al., 2017).

Harmonization is essential to allow international cooperation in the food and nutrition field because FCDBs may be considerably different from each other. They may differ in terms of foods examined, number of nutrients and compounds whose content have been reported for each food item, and in terms of how data are presented, depending on the primary target use of the FCDB. Databases also differ in the methods used to gather and handle data. Indeed, reflecting the methodology adopted, data may be of different quality. FCD may be categorised in:

- Original analytical data from literature or unpublished laboratory reports.

- Imputed values estimated from analytical values of similar foods or another form of the same food;
- Calculated values derived from the contribution of each ingredient in complex recipes corrected for preparation factors (yield and retention) using specific algorithms;
- Borrowed values taken from other FCDB or table and adequately referenced. Values may be adapted to meet the dry matter or macronutrient content of the given food item, if needed;
- Presumed values, such as logical zero, assigned to a specific food or food category according to similar food items or regulations (Greenfield and Southgate, 2003).

Based on which type of data a FCDB predominantly contains, FCDBs are commonly described as analytical or compiled databases. However, the majority of FCDBs display analytical data obtained from a set of food items sampled to be representative of the foods consumed in that region, integrated with imputed, calculated or borrowed values from literature or other FCDBs.

Besides standard national and international FCDBs, some specific-purpose FCDBs have been published over the years, on those classes of bioactive compounds that are gaining more and more research interest. A bioactive compound is a compound that occurs in nature and that can interact with one or more compounds of the living tissue, by showing an effect to human health (Biesalski et al., 2009). Some of the major bioactive compound FCDB and tables include, for example: the eBASIS database (Plumb et al., 2017) and the Phenol-Explorer database (Rothwell et al., 2013), which are linked to EuroFIR; and the United States Department of Agriculture (USDA) isoflavone (Bhagwat and Haytowitz, 2015a), flavonoids (Bhagwat and Haytowitz, 2016), proanthocyanidins (Bhagwat and Haytowitz, 2015b), and choline (Patterson et al., 2008) food composition tables. Moreover, a huge amount of data on some bioactive compounds content in foods is published in the literature but those data are very difficult to use in epidemiological research, due to the lack of standardization regarding definition, food descriptors, units of measure and methods of analysis.

1.1.1. HOW TO DEVELOP A FOOD COMPOSITION DATABASE

To obtain a reliable and comprehensive FCDB, the following criteria have been defined by Greenfield and Southgate (Greenfield and Southgate, 2003):

1. Representability: FCD included in the database should represent the best available estimate of the composition of food, in its most common form.
2. Quality: To assure data quality it is essential to have a quality management system. Both data production (food sampling and analysis), data compilation (collection, aggregation, compilation and dissemination) and managing (database management systems) requires a quality assurance approach. At this regard, EuroFIR developed a quality management framework to guarantee the quality of the entire process (Castanheira et al., 2009; Westenbrink et al., 2016), which is reported in **Figure 1**. Generally, original analytical data produced by reliable and matrix-specific methods should be preferred. Moreover, the overall quality of each data may be summarised using a quality score. EuroFIR designed the QE-SCIREP which considered for the quality evaluation the complete set of metadata of the original source (scientific literature or laboratory reports).

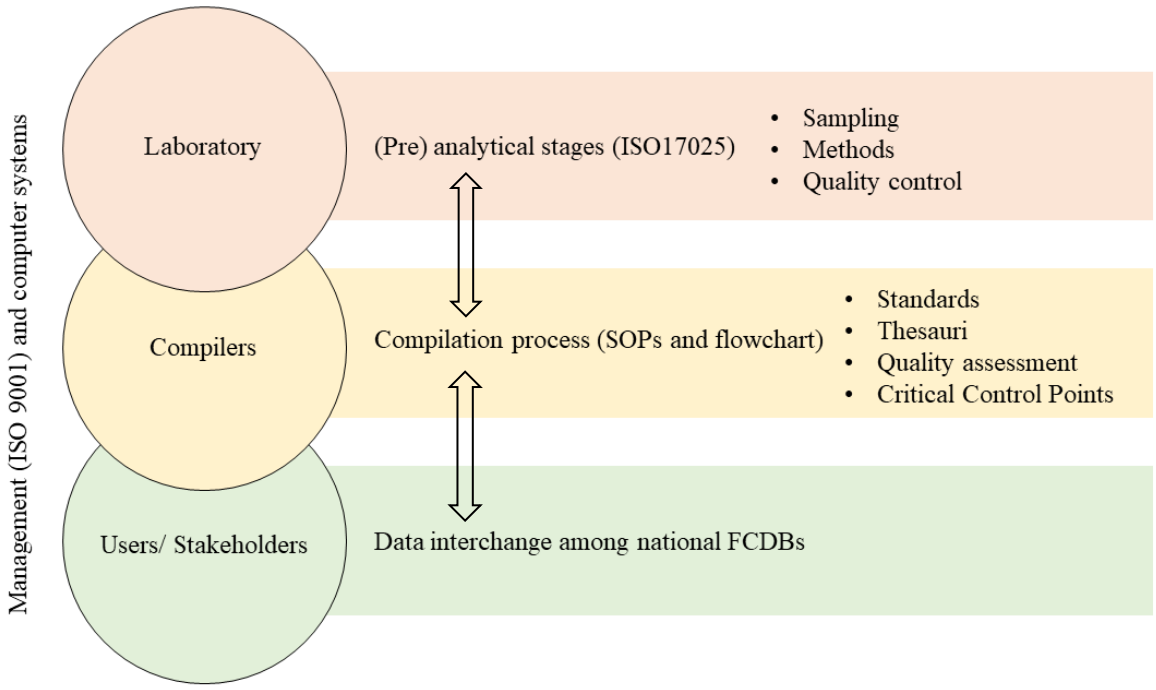

Figure 1. Overview of EuroFIR quality framework. *Modified from* Westenbrink et al., 2016. *Abbreviation: SOPs, standard operating procedures; FCDBs, food composition databases.*

3. Comprehensive coverage of foods: the objective of all FCDBs is to cover as completely as possible the foods eaten by the population of interest. However, due to the great amount of food items, the extreme variability of the food preparations forming the human diet, and the continuous development of new processed food products, it is impossible to store such

amount of data. As a result, it is fundamental to set a priority order, where at least all foods commonly consumed by the population should be included in the final food list.

4. Comprehensive coverage of nutrients: as many as possible nutrients and/or bioactive food components that are believed to be important in human nutrition should be included. To set a priority order, the list of nutrients to include should be prepared, for example, based on the current scientific knowledge in nutrition, the major health-related issues of the population of interest, feasibility of suitable analytical methods and existing data availability. Energy, macronutrients, and water content are essential data in published FCDBs. In particular, water content /dry matter (DM) information is essential when data from different sources are being compared or combined. Indeed, variations in water content generally determine variation in the content of all other compounds.
5. Clear food description: foods should be easily identified. There is a general consensus on the importance of the nomenclature, description, and classification of foods (Durazzo and Lucarini, 2021). In fact, a correct use of FCD, as well as the comparison and the exchange of data from different databases, requires precise identification of foods. While different purpose-specific food classification systems have been developed over time to group foods with similar characteristics (i.e., for the classification of commercial products, for regulatory purposes, for dietary monitoring), in FCDBs there is the need to give a precise and punctual description of the food without aggregating them in food groups (Ireland and Møller, 2015). Description systems ranged from detailed food name, classification, and description in free text format, to structured thesauri (i.e., a controlled indexing language with a hierarchical organization) and international standard coding systems. The most used system to describe and classify foods in FCDBs are: the LanguaL^{TM} multilingual thesaurus using faceted classification by INFOODS (Møller and Ireland, 2013), and the FoodEx2 description and classification system including a detailed list of individual food items aggregated into food groups and broader food categories and complemented with facet descriptors by the European Food Safety Authority (EFSA) (European Food Safety Authority, 2011).
6. Consistency and unambiguous expression: units of measure, calculation factors, and rounding procedures should be clearly stated and data should be consistent.
7. Documentation: nutrient-level information should be given on the source of data. For example: analytical data should be presented together with the sampling procedures and analytical method; calculated or imputed data should be presented together with the calculation or imputation method; and borrowed data should be presented together with the original reference. In fact, in accordance with the EuroFIR standards, the key to a good quality FCDB is to provide a full reference documentation.
8. Easiness of use: information provided in the database should be easily accessible and legible.
9. Compatibility to other databases: data should be presented in conformity to existing international standard to allow international cooperation and comparability of the data. An example is the use of the INFOODS tagnames to identify the correct food component, frequently described using solely the corresponding AOAC method code without any detailed explanation (FAO- INFOODS, 2013). In addition, EuroFIR provided standard

codes for components and methods separately, which may be more flexibly applied (Westenbrink et al., 2016).

10. Few missing data: FCDBs should have as few as possible missing data (at the nutrient- and food-level). When a FCDB is used to estimate nutrient intake, missing data can significantly distort nutrient intake evaluation. For this reason, it may be useful to include imputed or borrowed data when analytical data are not available, especially regarding nutrients of particular epidemiological interest.

Despite ideally FCDBs should have few nutrient missing data and contain the complete list of foods consumed by the population of interest, nutrient composition and the food list are not truly comprehensive in most FCDBs due to limited resource availability. All FCDBs contain data on macronutrients, but do not include all nutritional or biologically active compounds that are supposed of having an impact on health. Moreover, the list of food items is generally restricted to the traditional preparations and the most common foods, generally derived from national food consumption surveys.

1.1.2. THE IMPORTANCE OF FOOD COMPOSITION DATA

FCD are the fundamental evidence base for nutrition science. Indeed, they are extensively used in the public health domain, and essential to a wide spectrum of users ranging from international organizations to private individuals, as summarised in **Figure 2**.

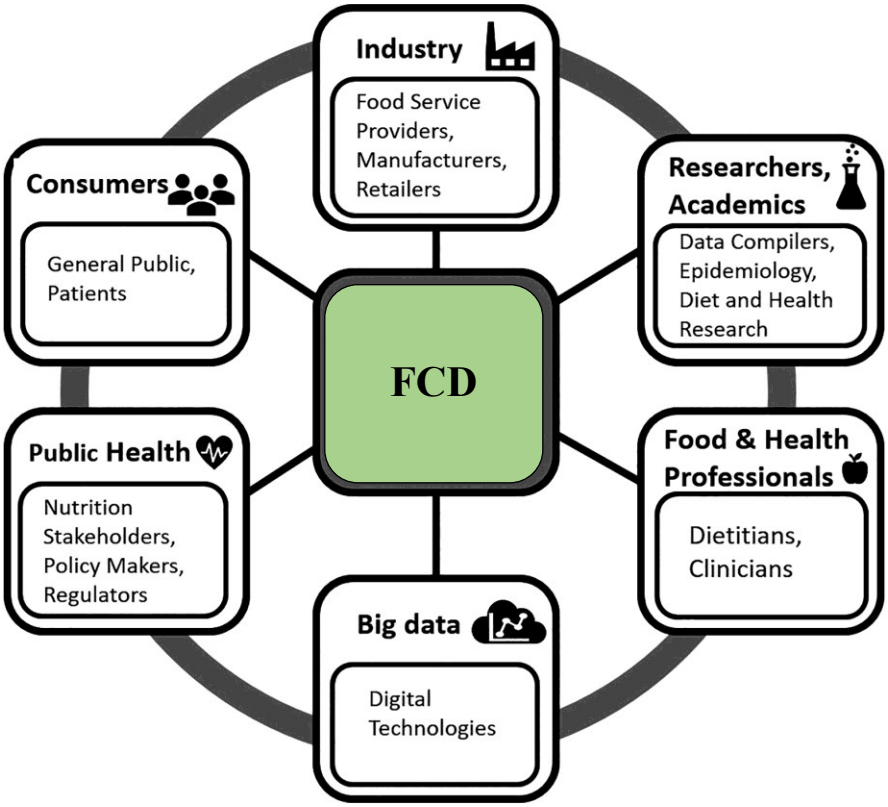

Figure 2. The different users and uses of food composition data. *Modified from Traka et al., 2020. Abbreviation: FCD, food composition data.*

At an international and national level, the nutritional composition of foods may be essential to develop food assistance programmes and policies, to assess the nutrient intake of the population through food consumption surveys, to develop new agricultural strains and cultivars, and to construct epidemiological hypothesis to correlate patterns of disease with dietary habits. At a regional level, information on food composition is needed to provide nutritionally adequate foods to the population in collective catering and health care services, to educate the population on healthy diets, and to improve nutritional value of food products by the food industry (Rand et al., 1991; Williamson, 2006). Regarding industry, since it is required by the European Union (EU) to include nutritional label (NL) information on pre-packed foods (1169/2011 Reg UE, 2011), and since laboratory analysis of nutrient composition may be expensive and complex, the calculation of food composition using FCDBs is permitted and largely used by means of online calculation tools (Traka et al., 2020). At an individual level, FCD is essential to nutritionists, dietitians and clinicians to examine diets, develop therapeutic diets, and to counsel individuals with respect to their personal needs. Finally, with the widespread use of technology, mobile apps, and internet

resources, also general consumers have increased their awareness on the importance of nutrient content information. However, it must be considered that at present the use of some of the leading apps presents some critical issues in the accuracy of its nutritional assessment, showing a general trend of total energy and fat intake underestimations, compared to the standard method. Moreover, most of them showed lack of transparency, not clearly presenting the source of FCD or robustness of the supporting science (Tosi et al., 2021).

As a result, inaccurate FCD may result in incorrect policies and nutritional guidelines, but more probably in misleading food labelling and health claims in food packaging, distorted consumer perception and inadequate food choices, particularly concerning pre-packaged foods with added salt, fats, and/or sugars (Delgado et al., 2021).

Depending on the use, different FCDBs may be preferred. For example, for an international use, it is preferred to use a regional database, whose FCD are relative to the food consumed in that geographical area. At present, FAO and INFOODS have produced regional databases for Latin America, Africa, East Asia, Near East, Pacific Island countries, and Southern Asia (FAO-INFOODS, 2021). Other regional databases have been created over time from the collaboration of different institutes of a specific region in order to produce large-scale epidemiological data. For example a standardised European Nutrient Database (ENDB) was created within the European Prospective Investigation into Cancer and Nutrition (EPIC) study (Slimani et al., 2007). Globalization have blurred the lines between regional food consumption habits, and consequently between FCDBs. Thus, facilitating data interchange (Traka et al., 2020). However, ideally each country should develop and use its own database collecting composition data on the foods and recipes most consumed in that area. Country-specific data are needed to avoid food missing data when analysing food and nutrient intakes in national surveys and/or national epidemiological studies. Indeed, recipes may change substantially among countries. Some countries have unique food products, foods preparations or processing procedures. Moreover, the greatest difference may be because of the variability of the level of certain nutrients, particularly in plant products because of the differing cultivars, climates, and agricultural and technological practices (Greenfield and Southgate, 2003). The composition of vegetables, for example, may vary because of the different soil's composition and many other external conditions; the fat content of meats may vary because of different breeding techniques; and the formulation of foods available on the market may vary from country to country based on the marked needs and the customer preferences.

1.1.3. NATIONAL FOOD COMPOSITION DATABASES

Given the importance of comprehensive and country-specific FCDBs, most countries have developed their own FCDB collecting data on common foods and locally eaten preparations. Indeed, national FCDBs were meant to meet local requirements and specific aims. Some of the major and most comprehensive national FCDBs and their characteristics are reported in **Table 1**.

The Standard Reference Legacy database maintained by USDA (USDA, 2018) is the largest and more comprehensive national FCDB. It comprises data on 7793 food items and up to 150 food components. It is the main source of FCD in the United States of America and provides the foundation for other national and international FCDBs used in private and public sectors. Moreover, USDA also developed the Food and Nutrient Database for Dietary Studies (FNDDS) (USDA, 2020a) based on the standard reference data, which is used for the assessment of nutrient intake in national surveys.

In Europe, the main source of FCD is the McCance and Widdowson's Composition of Foods Integrated Dataset (CoFID), which stores data of 2887 food items and up to 187 food components (Public Health England, 2021). The database was developed to support the national survey on diet and nutrition, and it is based on analytical data. Ad hoc standard sampling procedures were developed in order to ensure that the foods analysed were representative of those consumed by the UK population, and when literature data are used, preference was given to food similar to those consumed in the UK. Finnish and French databases ("Fineli" and "Ciqual", respectively) stored data on an outstanding list of food items but on a more limited set of nutrients (ANSES, 2020; Finnish institute for health and welfare, 2019).

In Italy, two databases have been developed, concurrently updated, and published online to be publicly available: the Italian food composition tables by the Council for Agricultural Research and Economy (CREA-NUT, 2019), and the Italian Food Composition Database for Epidemiological Studies (*Banca Dati di Composizione degli Alimenti per Studi Epidemiologici in Italia*; BDA) by the European Institute of Oncology (IEO) (Gnagnarella et al., 2015). The first is founded on analytical data. In the CREA-NUT tables (available at https://www.alimentinutrizione.it), about 80% of the values are analytical data obtained from ad hoc sampling procedures and traditional preparations made in an experimental kitchen to specifically identify the composition of the main foods consumed in Italy: 18% are literature data, mainly national, and about 2% are calculated or estimated values from similar foods. On the other hand, the BDA (available at http://www.bda-ieo.it) is a compiled database characterised by its specific epidemiological destination of use. Indeed, its main goal is the minimization of the amount of food and nutrient's missing values, thus storing different types of FCD, mainly borrowed from other national or international sources. All FCDBs provide composition data together with their metadata, such as the number of samples analysed, sampling location, limits of detection, date of collection, analytical or imputation method, source codes, as applicable.

Table 1. *Some of the main country-specific food composition databases (available online) and their general characteristics.*

Country	Last update (y)	FCDB name	Organization	N° items	N° max food components	Source	Website
USA	2018	Standard Reference Legacy (SR)	United States Department of Agriculture (USDA)	7,793	150	(USDA, 2018)	fdc.nal.usda.gov
USA	2018	Food and Nutrient Database for Dietary Studies (FNDDS)	United States Department of Agriculture (USDA)	7,083	64	(USDA, 2020a)	fdc.nal.usda.gov
Canada	2015	Canadian Nutrient File (CNF)	Health Canada	5,690	152	(Health Canada, 2015)	www.canada.ca
New Zealand	2018	New Zaeland FOODfiles™	Plant & Food Research	2,767	363	(Plant & Food Research, 2018)	www.foodcomposition.co.nz
Australia	2018	Australian Food Composition Database (ex NUTTAB)	Food Standards Australia New Zealand	1,534	256	(Food Standards Australia New Zaeland, 2018)	www.foodstandards.gov.au
UK	2021	McCance and Widdowson's The Composition of Foods Integrated Dataset (CoFID)	Public Health England	2,887	187	(Public Health England, 2021)	www.gov.uk
The Nederlands	2021	Nederlands Voedingsstoffenbestand (Nevo)	National Institute for Public Health and the Environment (RIVM)	2,207	135	(RIVM, 2021)	www.rivm.nl
Denmark	2019	Frida	Technical University of Denmark (DTU)	1,180	209	(DTU, 2019)	frida.fooddata.dk
Finland	2019	Fineli	Finnish institute for health and welfare	4,232	74	(Finnish institute for health and welfare, 2019)	fineli.fi
France	2020	Ciqual	French Agency for Food, Environmental and Occupational Health & Safety (ANSES)	3,185	67	(ANSES, 2020)	ciqual.anses.fr
Norway	2020	Matvaretabellens	Norwegian Food Safety Authority	1,878	57	(Norwegian Food Safety Authority, 2020)	matvaretabellen.no

Czech Republic	2020	Czech Food Composition Database	Institute of Agricultural Economics and Information (IAEI)	934	99	(Czech Centre for Food Composition Database, 2020)	www.nutridatabaze.cz
Italy	2019	Tabelle Di Composizione Degli Alimenti	Council for Agricultural Research and Economy (CREA-NUT)	900	120	(CREA-NUT, 2019)	www.alimentinutrizione.it
Italy	2015	Food Composition Database for Epidemiological Studies in Italy (BDA)	European Institute of Oncology (IEO)	978	91	(Gnagnarella et al., 2015)	www.bda-ieo.it

1.2. INTERNATIONAL STANDARDS TO COMPILE FOOD COMPOSITION DATABASES

In the food composition field, the term "compilation" is used to describe the activities of collecting, selecting, evaluating, and managing FCD in a FCDB. The compilation process is divided in 4 main steps (Greenfield and Southgate, 2003; Rand et al., 1991):

- Data source: FCD are collected from different sources after establishing the list of foods and components to be included (e.g., analytical data, other FCDBs or tables, scientific literature, laboratory reports).
- Archival database: FCD are evaluated and collected in their original format in a preliminary form. Data are documented, and a food code is assigned to each food item. Not all database management systems separate the archival from the reference database.
- Reference database: FCD are checked, compiled and managed in the database. This is the central step of the compiling process. Calculations, aggregations, completion of missing data, and evaluation procedures are implemented at this stage.
- User database: the final FCDB where all data have been completed and checked to be published.

Constant effort to update FCD and information is essential for the sustainability and reliability of existing FCDBs, as well as for the development of component-specific databases. FCD need to be improved continuously to meet the needs of stakeholders, as new and more accurate analytical methods develop and eating habits change. New fresh, ambient, frozen, and processed foods are introduced in the market every year, some of them are removed from the market, and the nutrient content of existing foods change over time. Many foods have been reformulated in line with government public health initiatives, and home preparation and cooking habits have also changed through time (Public Health England, 2021). New food items and components need to be included in FCDBs also in response to the emerging research interest.

However, the pursuit of scientifically robust data cannot prescind from the need of cost-effective strategies to compile and update existing FCDBs. One of the major issues is in fact the lack of suitable resources in the FCD field (Finglas et al., 2017). Frequently, datasets are incomplete due to the lack of resources to analytically analyse all food and compounds of interest. Thus, FCDBs are often compiled using not only direct methods —where the values are the results of analysis specifically carried out for the compiling of the FCDB— but also indirect methods. Indirect methods include borrowing data from other sources —FCDBs, published, or unpublished literature—, calculating, and imputing data (Greenfield and Southgate, 2003; Rand et al., 1991). Calculations are generally used to derive nutrient values of cooked or dried foods based on the nutrient values of raw foods, as well as to derive nutrient values of mixed dishes based on the nutrient values of their raw ingredients. Imputations, such as the use of logical zero, aggregating data, and borrowing values from similar foods, are generally used when no other data source is available. The compiler should store in the FCDB all the details and decision-making information

(e.g., formulas, rules, methods' descriptors) of the imputation/calculation process (Becker et al., 2007; Charrondiere et al., 2002).

Standardization and harmonization of FCD from different sources with distinct metadata are essential to ensure data interchange and efficient data linking (Delgado et al., 2021). As a result, standard procedures, guidelines and tools have been developed worldwide. In the past decades, several projects (e.g., INFOODS and EuroFIR) with the aim of improving the quality and exchange of data between national FCDBs have been carried out. Standardization of FCDBs affects not only compilers, but also significantly impacts their usage and application in nutritional research. Primary issues in the harmonization process include the definition and classification of foods and foods components, analytical methods, recipe calculations, quality evaluation, and delivery of data to users and stakeholders. In detail, EuroFIR provides standard operating procedures (SOPs) for the overall compiling process, guidelines for imputing component values, and a standard quality framework (Becker et al., 2007; EuroFIR AISBL, 2019a, 2019b). The EuroFIR steps in the compilation process are briefly described in **Table 2**. Critical control points (CCP) for which a SOP is required were identified and described in detail within the EuroFIR technical manual (EuroFIR AISBL, 2019a; Westenbrink et al., 2009). Moreover, EuroFIR develops tools for compilers, for example, by collecting all partner institute's FCD in an European Food Data Platform: the FoodEXplorer tool (EuroFIR AISBL, 2020; Finglas et al., 2014).

Table 2. *The steps in the food compilation process.*

STEP	DESCRIPTION
1	Decision on which foods and nutrients need to be added / updated
2	Collection and/or production of original data
3	**Identification of relevant foods, nutrients, background information**
4	Use of data, archive rejected data and document decision making
5	**Attribution of Quality (Index) to original data**
6	**Coding of original data**
7	Original data entry/import
8	**Check on original data entry**
9	Decide whether data are correct or not
10	Storage of original computerised data
11	**Physical storage of original data**
12	Extraction of all original data for each food-component pair
13	**Selection of the original data to be further used to determine aggregated data**
14	**Selection of algorithms to calculate means, recipes, imputed nutrients**
15	**Application of algorithms to produce aggregated and compiled data**
16	**Validation of aggregated and compiled data**
17	Correct errors and/or inconsistencies identified during validation
18	**Determine confidence code of the aggregated and compiled data**
19	Storage of aggregated and compiled computerised data
20	**Selection of aggregated and compiled data to be published**
21	Storage of data selected for dissemination
22	Dissemination

Modified from EuroFIR AISBL, 2019a. Steps that require standard operating procedures are highlighted in bold typeface.

On the other hand, INFOODS provides standard terminology, tagnames, classification systems and tools (available at: www.fao.org), and produces specific guidelines for the compilers (Charrondiere et al., 2016), described in **Table 3**.

Table 3. *FAO-INFOODS guidelines for food compilers.*

GUIDELINE	DESCRIPTION AND OBJETIVES
Guidelines for Food Matching, version 1.2 (2012)[1]	As food matching procedures are critical to obtaining high quality estimations of nutrient intake, the guidelines are intended to assist users in selecting the most appropriate foods to match to foods reported in food consumption surveys or to a food from another FCDB during the compiling process, including when filling missing data.
Guidelines for Converting Units, Denominators and Expressions, version 1.0 (2012)[2]	The objectives of these guidelines are to make users aware of possible difficulties in conversions procedures, to provide to compilers a comprehensive list of those procedures, and to encourage researchers to publish all necessary data in order to make their data suitable for the development of standardised FCDB.
Guidelines for Checking Food Composition Data prior to the Publication of a User Table/Database, version 1.0 (2012)[3]	The guidelines are intended to describe the internal checks and documentation needed prior to the FCD publication.

[1](FAO/INFOODS, 2012a); [2](FAO/INFOODS, 2012b); [3](FAO/INFOODS, 2012c). Abbreviations: FCD, Food Composition Data; FCDB, Food Composition Database.

1.2.1. RECIPE CALCULATIONS

Although analytical values are the best source of data, it is often necessary to complete a FCDB with, calculated data, imputed data, or data borrowed from other sources. Given the abundance and variability of preparations of the same food within the Italian population and the rapidly changing food offer from the market, calculated values are sometimes of equally acceptable quality as analysed values when considering composite dishes. Indeed, the need for additional nutritional data on cooked and composite foods in FCDBs has recently emerged in the nutrition field (Marconi et al., 2018).

A standard procedure for calculating nutrient composition based on ingredients' weight and cooking method used to prepare a specific recipe has been formulated by EuroFIR (Vásquez-Caicedo et al., 2008; Westenbrink et al., 2016) based on pre-existing guidelines (Bognár and Piekarski, 2000; Heli Reinivuo and Laitinen, 2007; Unwin, 2000). Moreover, EuroFIR is currently working on a technological tool to manage food composition and consumption data (FoodCASE) which also propose a pre-formed scheme for recipe calculations (Presser et al., 2018).

The main steps to calculate the nutritional profile of a composite food are the following (Greenfield and Southgate, 2003):

- Recipe selection according to the food habits of the population of interest and recipe naming. The selection should also consider possible variations in ingredient list and/or cooking method.
- Collection of recipe information and ingredients' weight in grams of edible portion, including added water and fats.
- Assurance of a complete nutrient composition of all ingredients in the database. When too many missing nutrient values are present in an ingredient, it is required to evaluate if this would have a major impact on the nutrient values of the final food item or not.
- If appropriate, selection of food- and cooking-specific Yield (YF) and Retention (RF) Factors.
- Calculation of the final nutrient composition of the recipe referring back to 100g of edible portion.
- Compiling of the documentation. All recipes included in the FCDB should be linked to the ingredient list and weights, to a comprehensive food description, and to the YF and RF together with their sources.

Many composite dishes are cooked. To account for the nutrient and weight changes that occur during the cooking process, calculations need to be performed. YF is used to adjust the nutrient composition of a cooked dish for losses and gains of water and/or fats during processing. It is a coefficient that express the weight change, where 1 indicates that there is no weight change due to the cooking method; 0.90, for example, means that the food lost 10% of its weight during cooking; and 3.00 indicates that the weight of the food increased during cooking from 100g to 300g (that is the case of most cereals and legumes). The YF is calculated as follows (Bognár, 2002; Vásquez-Caicedo et al., 2008):

$$YF = \frac{[prepared\ dish\ (g)]}{[total\ qualtity\ of\ ingredients\ (ready\ to\ cook)(g)]}$$

If the analytical determination of the YF is not feasible, it is possible to borrow the value from the available literature for similar foods. The most comprehensive YF tables are those published by the USDA (Roseland et al., 2014; USDA, 1975).

On the other hand, RF is used to adjust the nutrient composition of a cooked dish for nutrient losses due to cooking and/or processing methods. The amount of nutrient retained after cooking is closely related to the moisture/fat changes, and depend on several factors such as temperature, time, and pressure. Thus, RF are grouped by cooking method: cooking by moist heat, cooking by dry heat, and cooking with fats or oils. The standard equation to calculate RF is the following (Vásquez-Caicedo et al., 2008):

$$RF = \frac{[nutrient\ content\ per\ 100g\ of\ dish]}{[nutrient\ content\ per\ 100g\ of\ ingredients\ (ready\ to\ cook)]}\ x\ YF$$

RF may vary between 0 and 1 or between 1 and 100, when expressed as percentage of retention. For example, a nutrient RF of 0.90 for a specific vitamin would mean that the given food loses 10% of its vitamin content during cooking. Determination and appropriate selection and use of YF and RF for nutrient calculation in cooked foods is fundamental to calculate a reliable nutrient composition. The most used factors available in the literature are those for vitamins and minerals reported by Bognár (Bognár, 2002), and by USDA (Bell et al., 2006), currently published in an updated versions (USDA, 2007). However, at present, the available RF does not cover a comprehensive number of foods. The latest collection of RF values has been published by Vásquez-Caicedo and colleagues among the EuroFIR project, with the aim to standardise their use in European FCDBs and give clear indication on the best-match rules for borrowing RF from similar food groups (Vásquez-Caicedo et al., 2008).

However, different methods have been proposed for nutrient composition calculation (FAO/INFOODS, 2021):

- The raw ingredient method, where nutrient values of raw ingredients are simply summed and reproportioned to 100g of the final food item. This method may be used if the final composite dish is raw, or if the ingredients used to calculate the recipe are already cooked.
- The ingredient and/or the total recipe method, where RF and YF are both applied at the ingredient level, or at the recipe level, respectively.
- The mixed method, which is the one adopted by EuroFIR (Vásquez-Caicedo et al., 2008; Westenbrink et al., 2016). In this method the YF is applied at the recipe level and the RF at the ingredient level, as summarised in **Table 4**. For each nutrient, firstly all RFs are applied per ingredient, then the adapted nutrient composition of the ingredients is summed up (in their relative proportion) to obtain the nutrient content of the final dish. The nutrient composition obtained is finally adapted for water/fat loss or gain by the YF and reproportioned to 100g of edible part. This method has the advantages that the yield factor corresponds to the real loss of the final dish and that the nutrient retention factors for simple

foods (food ingredients) are generally more available than the RF for complex composite dishes.

Table 4. *Application of nutrient retention factors and yield factor using the mixed approach.*

Ingredient	raw weight (g)	factor	Raw nutrient (g/100g)	Cooked nutrient	Cooked dish (g)
Ingredient A	A	RF_A	X_A	$Y_A = \frac{XA \cdot A \cdot RFA}{(A+B+C) \cdot YF}$	
Ingredient B	B	RF_B	X_B	$Y_B = \frac{XB \cdot B \cdot RFB}{(A+B+C) \cdot YF}$	
Ingredient C	C	RF_C	X_C	$Y_C = \frac{XC \cdot C \cdot RFC}{(A+B+C) \cdot YF}$	
Total Recipe	A+B+C	YF	$X_A+X_B+X_C$	$Y_A+Y_B+Y_C$	(A+B+C) ·YF

Abbreviations: YF, yield factor; RF, retention factor.

1.3. NUTRITIONAL EPIDEMIOLOGY AND FOOD COMPOSITION DATA USE

Nutritional epidemiology is defined as the application of epidemiological methods to study how diet is related to health and disease in humans at the population level (Thornton and Villamor, 2015). It studies the exposure to specific nutrients, foods, food groups, and dietary pattern in relation to several health outcomes, collecting data on large sample of people and implementing statistical methods to estimate the extent to which a factor influences risk of disease in a population. Epidemiological studies focusing on nutrition, usually measure long-term dietary exposure, in particular those related to chronic diseases such as cancer, cardiovascular disease, and obesity, which are likely to occur over extended periods of time —or the entire lifespan.

Cancer is one of the most studied diseases in nutritional epidemiology. One of the major epidemiological study on cancer conducted in Italy is the case-control study on cancer (La Vecchia et al., 1987), conducted in several Italian centres in three main data collection periods: 1985-91, 1992-96, and 2008-10 (Rosato et al., 2016). 110 journal papers are currently indexed on Scopus. The most cited papers include the study of the influence of the Mediterranean diet on cancer risk (Bosetti et al., 2003; Turati et al., 2014), the association between flavonoids, breast cancer (Bosetti et al., 2005), and colorectal cancer (Rossi et al., 2006), the role of acrylamide exposure on human cancers (Pelucchi et al., 2011), and, more recently, the association between dietary inflammatory index and various types of cancers (Shivappa et al., 2015, 2014). In case-control studies, information about the habitual earlier diet is obtained from diseased subjects compared with control subjects without the disease. Case-control studies are, together with cohort studies, the major source of information in nutritional epidemiology, where clinical controlled trials are likely to display several limitations or to be non-feasible at all (Willet, 2013). However, also in case-control study some biases are likely to occur. One of the major bias compared to cohort studies is the retrospective dietary investigation; subjects may not recall or distort their food habits of prior the illness. Moreover, generally modest relative risks are likely to be found in case-control studies due to possible biases in the selection of the control group, in the recall, or in other methodological aspects. As a result, it is crucial to define and use reliable methods to collect dietary data and estimate nutrient intake.

Nutritional epidemiology set the basis for targeted policies not only on diet, but also on overall health status. Thus, it is of fundamental importance also to assess other environmental and lifestyle confounding variables. Interventions and/or studies on obesity prevention, as well as those on health promotion in children, cannot prescind from the study of physical activity and physical fitness status. In Italy the prevalence of obese and overweight youth is one of the highest across Europe (Ahrens et al., 2014). Results from a study on a large sample of primary school children in the Friuli-Venezia Giulia region (Fiori et al., 2020) showed that the prevalence of overweight (OW) and obesity (OB) (24.6%) was lower than in previous Italian studies (Lauria et al., 2019; Sacchetti et al., 2012), but in line with the prevalence observed in 2019 (29.8%) by the national surveillance system "*OKkio alla salute*" (Lauria et al., 2019). Furthermore, the level of physical fitness of these children was generally low compared to other European children (Fiori et al., 2021;

Vaccari et al., 2021), and it has been found to be significantly related to children's weight status (Fiori et al., 2020).

Finally, epidemiological methods are also frequently applied in research to estimate nutrient intake and dietary habits of populations of interest. Nutritional monitoring and surveillance are intended to discern trends in a population over time or to compare the diet of specific subgroups of a population. For example, epidemiological methods may be applied to monitor the diet of a representative sample of the national population (Sette et al., 2013), to assess the adherence to the Mediterranean Diet (Gnagnarella et al., 2018), the total antioxidant capacity of the diet (Pellegrini et al., 2007), or the nutrient intake of children at different ages (Concina et al., 2021; Rosi et al., 2021; Verduci et al., 2019).

1.3.1. DIETARY ASSESSMENT

The most challenging aspects in nutritional epidemiology are the collection and the management of dietary information, as well as the development of practical methods to measure diet in a reliable but relatively inexpensive way (Emmett et al., 2019; Willet, 2013). Depending on the study design and objectives, the researcher should select and use appropriate methodologies. The main tools to assess food and nutrient intakes are dietary records, food frequency questionnaires (FFQs), and 24-hour recalls.

The 24-hour recall method is an interview conducted by a trained interviewer, which inquire detailed information about the total dietary consumption (food and drinks) of the past 24-hour period. The strength of this method is the support of an experienced interviewer to recall type and quantity of food consumed in a relatively short and close period. However, major sources of error may be the participant's memory and the difficulty to recall portion sizes. The quality and level of detail of the data collected through the 24-hour recall method is thus crucially dependent from the training and personal skills of the interviewer (Willet, 2013). Moreover, some aids may be used to recall portion sizes: food atlas, plastic food models, standard household measures. In particular, photographs and text description of portion sizes have been found to be useful to accurately estimate portion sizes in multiple studies (De Keyzer et al., 2011; Lucassen et al., 2021).

The dietary record method, also called food diary, is an auto-compiled list of foods and drinks consumed over a longer period: generally, from 3 to 7 days. Food intake is supposed to be recorded at the time the food is consumed by the participant. Contrarily to the 24-hour recall method, the dietary record method minimises the memory bias. Dietary record is, in fact, referred as the gold standard method for dietary data collection (Willet, 2013). However, participants should be trained to correctly record, weigh, or determine volumes of the food/beverage they ate/drank. Reviewing the record with the participant after data collection is desirable to capture missing details that may have been omitted, such as sauces, added salt or sugar, drinks, and snacks. One possible bias of dietary record may be the so-called *reactivity.* Participants may tent to eat foods which are more socially acceptable while keeping the diary, and the energy intake calculated from food records may be significantly underestimated (Willet, 2013). Moreover, it must be considered that the food diary is generally time-consuming and require a high motivation of the participant. Traditionally, food record are paper-based methods. However, technological enhancement of dietary assessment has been studied in the past years to lower the cost, reduce errors and time needed to fill in the data and analyse it (Thompson et al., 2010). Currently, several mobile apps to monitor food intake are available on the market. However, a recent study (Tosi et al., 2021) showed that the leading nutrition apps present critical issues in assessing the intake of energy and nutrients. As a result, further research on efficacy and use of apps to monitor food intake is needed.

The primary strength of dietary record and 24-hour recall is the collection of the actual intake in absolute and not relative way. Being open-ended, they also are capable to collect additional detail on preparation, food processing method, and occasion of consumption. On the other hand, some possible limitations that these 2 methods may have in common are the need of participants' motivation to reliably recall or report food/beverages consumed, and the intentional or unintentional underreporting. Furthermore, in epidemiological investigations, the interest is

frequently focused on the habitual or past diet rather than the current daily food intake, and the food consumed in few days could not substantially represent the habitual diet of the subject. As a result, a common tool in nutritional epidemiology is the FFQ that aimed to assess the usual diet of an individual. It consists in a dietary structured food list and a frequency response section to collect how often the food/beverage was eaten/drunk. FFQs are generally inexpensive, they can be easily self-administered, and the collected data are usually readily computer processed. Contrarily to dietary records and 24-hour recalls, FFQs originate from the rationale that in epidemiological research average long-term diet is more important than the diet of few specific days in terms of dietary exposure. However, FFQs are less precise in the measure of food/beverage intake and subject to the memory bias. Contrarily to dietary records, the list of food/beverage is limited to the willing and/or aims of the researcher, and therefore, it has to be carefully chosen. Furthermore, energy intake over- or underestimation is common, due to the difficulty to calculate an accurate weekly or monthly intake from FFQs. Validity and reproducibility studies are essential to obtain good quality data from FFQs.

Then, food consumption data collected through different tools has to be translated in nutrient data. Up-to-date FCDBs are essential resources to convert food consumption in nutrient intakes, as well as appropriate food matching procedures and a good software for nutrient calculation. Completeness of nutrients is of extreme importance when a database is used to compute nutrient intakes, as well as the uniformity of the method used to obtain specific food composition values (Willet, 2013). However, when using a FCDB to assess nutrient intakes it is important to be aware of some possible limitations (Greenfield and Southgate, 2003). These may include:

- The great variability in the composition of raw and processed foods, for example due to climate, soil, cultivar, brand, but also cooking or processing methods. For example, some labile nutrients (e.g., vitamin C and folates) or compounds may be added or removed during processing of the food.
- The variability in the composition of food through time. Due to limited resources slowing the update process, which is continuously needed, some values may be inevitably old and not truly representative of the current composition of foods. This is particularly true for manufactured pre-packaged products, whose formulation and nutrient composition may be extremely fast changing due to national fortification policies, customers' demand, and marketing strategies.
- The incomplete coverage of foods, nutrients, or bioactive compound of health interest (i.e., the presence of missing values) or the use of an incompatible database.
- The methodological limitations in detecting food consumption and in software usage.

In epidemiology, to reliably assess nutrient intakes and their possible health implications, it is of fundamental importance to track the origin of the data and the missing values. When missing values occur in FCDBs, in nutrient intake estimation it is applied a nutrient content value of 0 to those foods. This might result in systematic underestimation of nutrient intakes. This is particularly important for new food components of interest, which may have many missing values throughout the database, or when several nutrient label data are used in the nutritional assessment (Ocké et al., 2021; Willet, 2013). Studies of the relationship between diet and health have led to increased interest in specific biologically active compounds and constituents of foods which are not traditionally included in the FCDB variables of interest. In this case it is important to select

appropriate sources of data from multiple tables, databases, or scientific literature: considering the scope of the FCDB, the geographical area of origin of the data, the methods of analysis, and the population to be studied.

Moreover, despite in the epidemiological research it is crucial to estimate nutrient intakes, it is important not to rely exclusively on results on one specific chemical compound, but to consider the whole complexity of the diet. Some important variables to consider are: the food matrix, the degree of processing, the relationship between food compounds and their bioavailability, the whole dietetic pattern, and other non-dietary factors. Thus, standard applications of FCDBs may reflect a reductionist view of foods (as the sum of nutrients) (Delgado et al., 2021; Fardet and Rock, 2020), while maximal insight into the relationship between diet and disease may be obtained by examining dietary habits both at nutrient- and food-level (Willet, 2013). Epidemiological results on the association between a specific nutrient and its health effects, may be of difficult interpretation and application. Epidemiological analysis based on foods, instead of nutrients, are generally most useful to dietary recommendations, because the general population may easily change their nutrient intakes by adjusting their food choices (Willet, 2013).

1.3.2. MANUFACTURED FOODS AND NUTRIENT LABEL DATA

A considerable increase in the number of manufactured complex foods available in the market has been observed over the years, as well as their consumption by the general population as an alternative to more traditional homemade preparations. The consumption of such products has been estimated to account for up to 60% of the total food intake, but these are rarely included in FCDBs (Greenfield and Southgate, 2003). For these complex food products, standard FCDBs may not be the most accurate source of information to determine nutritional data. Most databases present some analytical data derived from commercial products in its more "typical" and "average" form. However, it is complex and very time and cost-consuming to keep a FCDB up to date due to the extremely rapid change of the market (Traka et al., 2020). The increasing need of branded FCD, has prompted to the development of new methods of data collection. As an example, the USDA Global Branded Food Products Database (USDA, 2021), was created to collect information received from a number of food industry data providers, presented in accordance to the USDA standards, and it is generally updated twice a year to reflect the actual changes in the food market. Companies voluntary submit nutrient data, serving sizes, description, and ingredient list in a dedicated synchronization network. Then, USDA standardises the reported values by calculating nutrient values per 100 grams. However, the final FCDB reports only data of the main food components, and it present a large amount of missing nutrient data. In Europe, other databases have been developed collecting data from food industry data providers. For example, the Dutch LEDA branded food database actually collects compositional data from approximately 100,000 branded foods (Westenbrink et al., 2021). Recently, data mining methods have also been implemented to collect FCD on branded products by means of the retailer websites [e.g., FoodDB (Harrington et al., 2019), Open Food Facts (https://world.openfoodfacts.org)]. However, despite these approaches are cheaper and faster than the traditional FCDB compiling process and represent a great opportunity to increase the capacity for capturing nutritional information of branded foods, they generally lack transparency and/or accuracy and presents huge amounts of nutrient missing data. Nutritional values presented online do not require to disclose their origin and/or update status (Traka et al., 2020). Moreover, such amount of data cannot be analytical verified. Thus, the real changes in manufactured food composition remain unknown, leading to possible unrepresentative estimates of nutrient intake when used in nutritional epidemiology. Indeed, unrepresentative FCD can have a systematic impact: a reduction in the intake of those nutrients for which nutrient levels are reduced in new manufactured products would not be noticed (Westenbrink et al., 2012).

Moreover, an important issue in using label data in nutritional epidemiology is the large amount of missing nutrient data. Pre-packaged foods' NLs can only provide information on limited nutrients, contrarily to the broader range of nutrients available in FCDBs. The mandatory information in Europe include energy and 6 nutrients, such as carbohydrates, sugars, protein, fats, saturated fatty acids, and salt (1169/2011 Reg UE, 2011), while all the micronutrient composition is generally missing. Few exceptions may occur for fibre content, polyols, starch, polyunsaturated and monounsaturated fatty acids, and some micronutrients, as applicable (1169/2011 Reg UE, 2011). Micronutrient composition can be reported in the NL only if the value is greater than 15% of the reference daily intake value and greater than 7.5% of the reference daily intake value for

beverages (1169/2011 Reg UE, 2011), meaning that micronutrient information is rarely available for manufactured foods. As an example, in the LEDA branded food database collecting FCD provided by manufacturers, coverage for mineral (excluding sodium/salt, which is mandatory) and vitamin values, was found to be less than 2% (Westenbrink et al., 2021). Nutritional declaration is mandatory since 2016, while previously it was optional unless a nutrition or health claim was stated. However, the background of NL data and their quality is not always known. The European regulation on nutritional labelling require the FCD reported in the label to be the "average value that best represent the amount of the nutrient which a given food contains, and allows for natural variability, seasonal variability, patterns of consumption and other factors which may cause the actual value to vary" (1169/2011 Reg UE, 2011). Thus, values may be derived by different methodologies (Italian Ministry of Health, 2016; 1169/2011 Reg UE, 2011):

- Chemical analysis on a representative sample of the food by the manufacturer;
- Calculations from the known or actual average value of the used ingredients;
- Calculations based on generally established and accepted data.

As a result, when using label data, attention is needed to check for inconsistencies and to fill-in missing values. EuroFIR reports 2 possible approaches to include manufactured food products in FCDB (EuroFIR AISBL, 2019b): compilers can enter each branded food product and its declared nutrient values into the FCDB and use these in data aggregation to create a general food item with a mean macronutrient composition representative of what found on the market, or use ingredients to construct a food entity with an averaged recipe in order to calculate missing component values. A third approach may be used combining the two methods: compilers may use aggregated label information for macronutrients and recipe calculation to impute the missing values from the nutrient labelling.
As an example, the approach adopted by McCance and Widdowson (Public Health England, 2021) to compile the UK FCDB was to perform analytical determinations on a sample of the main branded foods sold on the national market, obtaining actual analytical data on macro and micronutrients together with a complete documentation on the sampling procedures and the description of the analysed products (if necessary, including brand names). However, this approach may be very expensive and time-consuming, considering the extremely rapid change in the marketed foods and their formulation. Another approach to complete missing nutrients from a complex manufactured product whose NL is available, is the mapping with generic foods (Carter et al., 2016). However, the mapping approach was found to have some limitations (Ocké et al., 2021). The mapping consists in the matching of branded food data to pre-existing data from similar products in the former FCDB. This approach may include errors, in particular if the formulation of the products is peculiar. This is the case of dry products, fortified products, or specifically formulated foods for medical purposes such as gluten free (GF) products, whose ingredient list and nutritional characteristics are not comparable with the corresponding gluten containing (GC) ones (Babio et al., 2020; Cornicelli et al., 2018; Fry et al., 2018; Myhrstad et al., 2021).

Another approach (EuroFIR AISBL, 2019b) is based on recipe calculations. The main issue with this approach is the missing information about ingredients' weight in the food label. According to the European legislation, all that is known is the descending order of ingredients by weight, and in some cases, the ingredient's percentage of weight in respect to the final product (1169/2011 Reg UE, 2011). Some authors have applied the recipe approach on a selection of GF

products to create a FCDB to allow epidemiological research on GF dietary patterns in Austria (Missbach et al., 2015) and in Italy (Mazzeo et al., 2015). These databases were built based on label information collected from the main products available on the market in that region at that time, considering both the ingredient list and the NL. However, micronutrient imputation procedures may be very time consuming. For example, due to the remarkable advancement in food technologies, GF products are constantly reformulated so that the mapped values as well as the calculated ones result rapidly outdated.

1.4. THE FOOD COMPOSITION DATABASE FOR EPIDEMIOLOGICAL STUDIES IN ITALY

The Italian FCDB for epidemiological studies in Italy (BDA) was firstly published in 1998 from the need to develop a suitable and complete tool for epidemiological studies (Salvini et al., 1998). Indeed, worldwide the relationship between dietary habits and health was emerging from epidemiological studies, but there were limited and inadequate tools to convert national dietary information in qualitative and quantitative data on energy, macro- and micronutrient intake. Italian tables (Carnovale and Miuccio, 1989; Fidanza and Versiglioni, 1989) were insufficient to cover the wide range of foods consumed by Italian subjects participating in epidemiological studies (Franceschi et al., 1993) or national surveys (Turrini et al., 1991), and they lacked micronutrient and/or bioactive compounds whose association to health outcomes was emerging in epidemiological research. Moreover, there was the urgency to track the data sources and the missing values to correctly read the epidemiological findings and minimise underestimations in the nutrient intake assessment. As a result, BDA structure and methodology reflected its primary objective. Particular attention was paid to select representative sources, and to provide a nutritional profile of each food included in the final food-list as complete as possible. Thus, the BDA is defined as a compiled database, meaning that the FCD were derived from pre-existing sources. The database was compiled according to a well-documented methodology from its first edition, using FCD from, in order of priority: the National Nutrition Institute (INN) of Rome (currently CREA-NUT) (Carnovale and Miuccio, 1989), other national (Fidanza and Versiglioni, 1989) and international tables or databases (mainly from UK, USA, France, Germany), and from the literature.

In the following years, the methodology has evolved, adapting to the international EuroFIR guidelines (Becker et al., 2007). In 2012, EuroFIR reviewed and certified the BDA compilation process to verify the adherence to standards and operating procedures that ensure quality and validity of FCD. Within this certification process, the BDA group has thus identified all compilation procedures (EuroFIR AISBL, 2019a; Westenbrink et al., 2009), summarised in an ad hoc flow chart, reported in **Figure 3**.

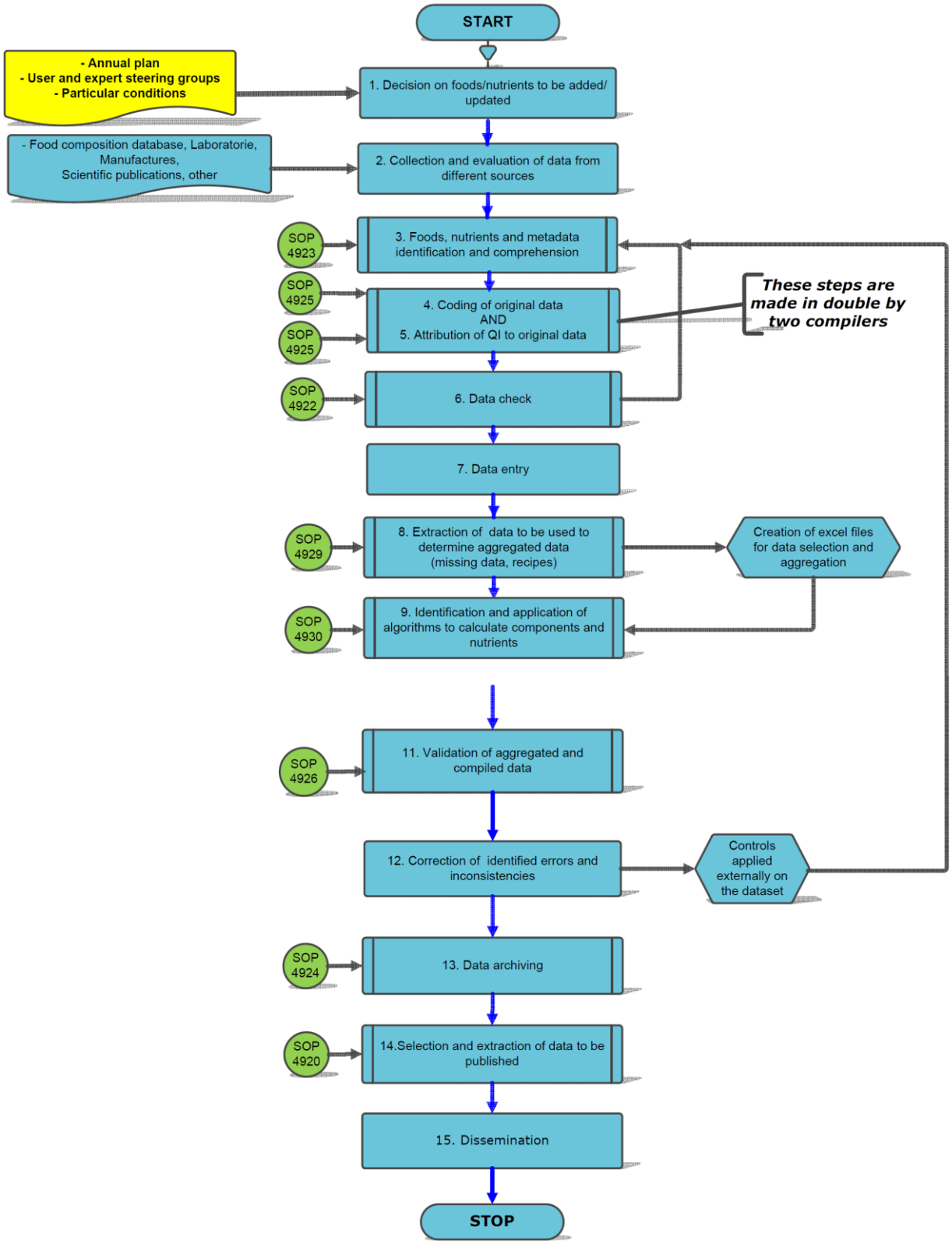

Figure 3. The BDA flow chart describing its compilation process, in accordance to EuroFIR standards. *Abbreviation: SOP, standard operating procedure.*

As in all FCDBs, the continuous change in food habits, preparations and preferences in the Italian population, and the change in the composition of foods from the food market, made the continuous revision and update of BDA FCD mandatory. Since 1998, which version comprised 778 food items and 37 food components, two major updates were achieved, in 2008 and 2015 (**Table 5**). Main data sources remained CRA-NUT tables (EX-INRAN 2000 edition), followed by additional non-national databases and scientific papers, as appropriate (Gnagnarella et al., 2004).

Table 5. *BDA versions and their main characteristics.*

BDA Version	Food items (N)	Updated food items (N)	Energy and edible part (N)	Food components (N)
1998	778	-	3	37
2008	935 (+157)	94	3	67 (+30)
2015	978 (+43)	137 (+43)	5 (+2)	86 (+19)

Data in parenthesis indicate the variation compared to the previous version.

In 2008, information on 30 nutrients was added for the following food categories: “Milk and dairy products”; “Meat and fish”; “Alcoholic and non-alcoholic beverages”; “Eggs”; “Fats and oils”. In 2015, additional and/or updated food composition information was published for: fresh fruit and berries, cooked or canned fruit, dried fruit and seeds; fruit flours, and fruit juices and drinks. The full list of components of the 2015 version of the BDA (BDA v.15) is reported in **Table 6**. Moreover, in the BDA v.15, the coding of food components has been modified, and computerised quality controls have been improved to assess appropriateness, completeness and accuracy of the data.

Table 6. *List of food components considered for the BDA v.15 update.*

Edible part	Edible part
Energy	Energy, recalculated; **Energy, recalculated with fibre**.
Macronutrients	Water, total protein, animal protein, plant protein, total lipids, animal lipids, plant lipids, cholesterol, available carbohydrates, soluble carbohydrates, starch, fibre, alcohol.
Minerals	Iron, sodium, potassium, phosphorus, zinc, **magnesium, copper, selenium, chloride, iodine, manganese, sulphur**.
Vitamins	Thiamine, riboflavin, niacin, vitamin C, vitamin B6, folic acid, **pantothenic acid, biotin, vitamin B12**, retinol equivalents, retinol, beta-carotene equivalents, vitamin E, vitamin D, **vitamin K**.
Fatty acids	Total SFAs, **C4:0-C10:0, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid**, total MUFAs, myristoleic acid, palmitoleic acid, oleic acid, **eicosanoid acid, erucic acid**, total PUFAs, linoleic acid, linolenic acid, **arachidonic acid, EPA, DHA**, other PUFAs.
Sugars	**Fructose, glucose, sucrose, maltose, lactose.**
Aminoacids	**Tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline, serine**.

Additional nutrient components are highlighted in bold typeface. Abbreviations: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The BDA in its latest version is currently published online at www.bda-ieo.it to be freely and easily available for the general population (**Figure 4**). The nutritional information of each food item is given by a table, that reports: the food numerical code, the full food item name and its descriptors (note, category), the database version (v.98, v.08 or v.15), and the list of components, each with indications of the value, the unit of measure, and the codes referring to the source type, name, and/or the imputing or calculating methodology. All nutrients available for the given food are expressed on 100g or 100mL of edible part.

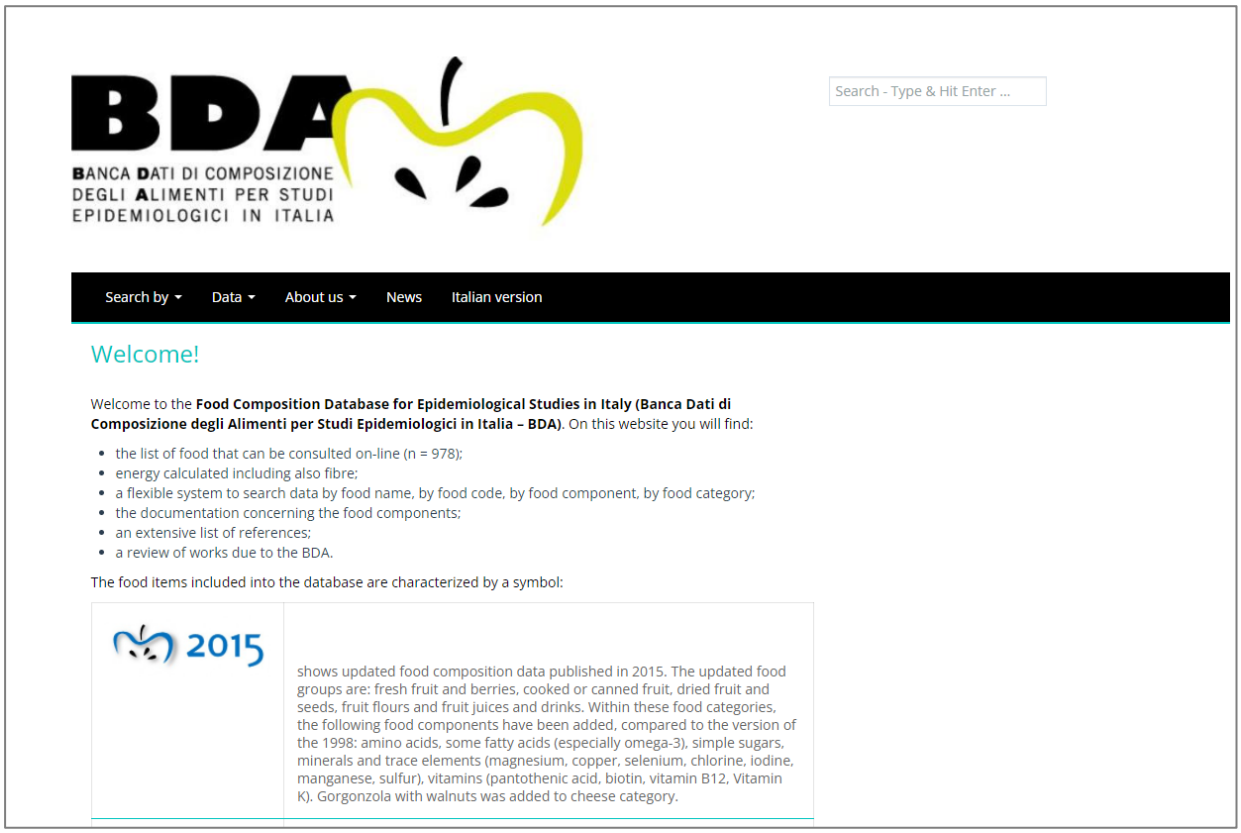

Figure 4. The BDA website homepage (English version).

1.4.1. THE CASE-CONTROL FOOD COMPOSITION DATABASE

When the BDA was to be developed, one of the involved large epidemiological study demanding a comprehensive country-specific FCDB was the Case-Control Study of Breast Cancer and Colorectal Cancer with Emphasis on the Role of Diet by La Vecchia and colleagues, conducted in several Italian centres (Salvini et al., 1998). The purpose of this study was to investigate the role of dietary and other risk factors in the aetiology of cancer in order to exploit differences in the extent of exposure and disease (La Vecchia et al., 1987). As the main objective was to investigate the association between nutrient/food intake and cancer, the availability of a high-quality and comprehensive FCDB was essential to minimise methodological biases and nutrient intake underestimations. For example, one of the hypotheses to tested was the protective role of beta-carotene and vitamin E in breast (Favero et al., 1998) and colorectal cancer (La Vecchia, 1998), but FCD for these nutrients were not available in the Italian tables (Salvini et al., 1996). Therefore, a first version of the FCDB was developed in 1996 for this case-control study to convert food intake derived from an Italian FFQ into nutrient intake (Salvini et al., 1996). The FFQ included questions on average weekly consumption of 83 foods and beverages, food groups and popular recipes divided into 7 sections: 1- bread and cereal-based meals; 2- meat, fish, cheese, eggs and by-products; 3- vegetables, potatoes and legumes; 4- fruits; 5- sweets, desserts and soft drinks; 6- milk, hot beverages and sweeteners, and 7- alcoholic beverages (Decarli et al., 1996).

In the first version of the study-specific FCDB, the energy and nutrient composition for the items of the FFQ were mainly derived from 302 simple foods published in the Italian BDA (Salvini et al., 1998, 1996). The FCDB has subsequently been periodically updated to include information on specific compounds of interest having a hypothesised role in the aetiology or prevention of cancer. The current version is based on the BDA as updated in 2015 (Gnagnarella et al., 2015).

2. AIMS

The main objectives of this work are to present the update of the BDA and to propose innovative tools to improve FCDBs comprehensiveness and reduce biases in its epidemiological application.

The work is divided into sections whose specific aims are described in detail:

1. First, to apply the **BDA** to assess food and nutrient intakes in 3 epidemiological studies involving different populations (children, obese adults, and centenarians). The main issues in using the BDA and the possible intervention strategies are highlighted.
2. To update the cereal, bread, sweets and cakes food categories of the **BDA**, using the standard protocol and a label-based recipe approach.
3. To update the cereal, bread, sweets and cakes **BDA** food categories with a new section for gluten free foods, based on the manufactured gluten-free products available on the Italian market. Dry matter analysis is presented to verify the reliability of data from NL for their use in FCDBs and to compare gluten free manufactured foods with their gluten containing counterparts.
4. To apply the label-based recipe approach in a pilot study on the FCDB used for the public health impact of long-term, low-level, mixed element exposure in susceptible population strata (PHIME) study, based on the **BDA**. The study highlights the differences in the assessment of nutrient intake in a population of 18-month-old children using the old *vs.* the updated version of the database.
5. To implement an algorithm that automates the label-based recipe approach minimizing calculations errors and supporting compilers in decision-making.
6. To update the **BDA**-based FCDB used to assess nutrient intake in a large Italian case-control study on cancer for specific components of epidemiological interest (choline, sphingomyelins, and prebiotics).
7. Finally, to apply the **BDA**-based FCDB in a case-control study assessing the association between dietary prebiotic consumption and colorectal cancer risk.

3. MATERIAL AND METHODS

3.1. BDA USE IN DIETARY ASSESSMENT

3.1.1. CHILDREN'S DIET

Scientific paper published in: Nutrients vol. 14, 515
Title: Adherence to Dietary Recommendations of 7-Year-old Children from a Birth Cohort in Friuli Venezia Giulia, Italy
Authors: Giordani E., Marinoni M., Fiori F., Concina F., Ronfani L., Dalmin P., Barbone F., Edefonti V.*, Parpinel M.*
January 2022

STUDY POPULATON

The study population consisted in the 7-year children from the follow up of the prospective Northern Adriatic Cohort (NAC-II) (Brumatti et al., 2021) whose dietary habits were assessed at 7 years (between 2014–2016). The cohort originated (between 2007–2009) with the enrolling of 900 pregnant women within the framework of the PHIME European Union project (Valent et al., 2013), with the aim of investigating the association between low-level mercury exposure from food consumption in pregnancy and child neurodevelopment at 18 months. Briefly, at the 7-year follow-up, parents of those children tested for the neurodevelopment outcomes at 18 months (N=632) were contacted for further dietary and neurodevelopment evaluation. The current paper considered dietary intake for the 381 children whose parents filled in the corresponding dietary record at 7 years of age. The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Institute for Maternal and Child Health —IRCCS Burlo Garofolo (CE/V-109-12/04/2010). All participating families were informed and consented to participate to the study.

ANTROPOMETRIC AND LYFESTYLE CHARACTERISTICS

Parents filled in a questionnaire assessing lifestyle of their 7-year-old children. Socio-demographic characteristics of both parents, including education level, marital status, and citizenship, were obtained from a questionnaire administered at delivery (Valent et al., 2013). Children's height and weight were measured from healthcare staff during the neuropsychological assessment at 7 years (Brumatti et al., 2021). Body Mass Index (BMI; kg/m^2) was calculated as: [weight (kg)/height^2 (m^2)]. Children were categorised as normal weight, underweight, overweight

or obese according to the cut-offs proposed by the International Obesity Task Force (IOTF) (Cole and Lobstein, 2012). The general characteristics of the parents and children were presented as frequency and percentage distribution for categorical variables, and as median, 25th and 75th centile for continuous variables with a non-normal distribution. Normality assumption was tested for each continuous variable using the Shapiro-Wilk test.

DIETARY ASSESSMENT AND ADEQUACY EVALUATION

Dietary data were collected using a 3-day dietary record (3-dDR) filled in at home by one parent instructed on how to record type and portion size of the food consumed by the child. Common kitchen utensils were suggested as an alternative to traditional kitchen scales to measure solids and fluids (e.g., teaspoon, glass); in this case, estimated equivalents in grams were also indicated to the parents. A researcher's telephone contact was provided whenever parents need clarification while filling in the 3-dDR. Intakes of 39 selected macro- and micronutrients were derived after uploading individual food information from the 3-dDRs in the Microdiet V4.4.1 software (Microdiet software - Downlee Systems Ltd., High, Peak, UK), which contains the **BDA** (Gnagnarella et al., 2015), integrated with information from nutrients collected from NLs when needed. For each nutrient, the Microdiet software provided total intakes over the observation period; we calculated daily intakes by dividing total intake by the number of collection days. Total energy intake was estimated by summing the mean daily intakes of single macronutrients, each multiplied by the corresponding energy conversion factor.

Individual nutrient intakes were compared with the Dietary Reference Values (DRVs) proposed by the Italian Society for Human Nutrition (SINU, 2014), when available. The DRVs include adequate intake (AI), reference intake (RI) range for macronutrients, average requirement (AR), population reference intake (PRI) and suggested dietary target (SDT) for the corresponding nutrient. Given the availability of anthropometric information for most of the children (N=350; ~92%), for protein intake the AR and PRI child-specific cut-offs using the individual weights and the age-specific DRVs for 7-year-old children were calculated (AR=0.8 and PRI=0.98). Standard evaluation of nutritional adequacy was carried out using median, 25th and 75th centile and percentage of children meeting the DRV requirements. Sex-specific median, 25th and 75th centiles were also provided, and the presence of potential sex differences was investigated using the two-sample Wilcoxon rank-sum (Mann-Whitney) test. Furthermore, the presence of potential inadequacy in individual protein intakes by comparing the observed intakes (g/day) with the corresponding AR and PRI (g/day) with the two-sample Wilcoxon rank-sum (Mann-Whitney) test was investigated.

Furthermore, the adequacy of individual diets at the nutrient- and overall-diet-level using the Nutrient Adequacy Ratio (NAR) and the Mean Adequacy Ratio (MAR) was evaluated, respectively (Hatløy et al., 1998). In detail, the NAR is defined as the ratio of each child's intake to the national DRV for the appropriate age category. The MAR is the sum of all (nutrient-specific) NARs divided by the total number of NARs. As any ratio, a NAR equal to 1 indicates that the corresponding subject meets the requirement fixed for that nutrient. A MAR equal to 1 indicates that the subjects meet the requirements for all the selected nutrients. To take into account inadequacy due to excess intake, the approach proposed by Atløy in children (Hatløy et al., 1998) was extended to those macro-and micronutrients for which a maximum desirable intake is available. In detail:

- for all micronutrients with one DRV indicating the minimum desirable intake (i.e., AI or AR), we truncated all NARs greater than 1 to 1 so that these nutrients could not compensate those with a NAR lower than 1 in the MAR calculation;
- for the remaining macro- and micronutrients indicating a maximum desirable intake (i.e., RI: protein, available carbohydrates, total fats, MUFAs, total PUFAs, PUFAs ω-3 and ω-6; SDT: soluble carbohydrates, SFAs, sodium, and chloride), we followed suggestions by Hilbig (Hilbig et al., 2015) and redefined NARs greater than 1 (inadequate intake by excess) to be equal to: 1 minus the exceeding amount. For example, when the original NAR was equal to 1.15, our modified NAR value is equal to 0.85.

To assess the importance of the individual nutrients in the MAR calculation, we also carried out an influence analysis where the single components were removed one at a time from the MAR definition. Index-based evaluation of adequacy was based on median, 25^{th} and 75^{th} centile of NAR and MAR. Statistical significance for all tests was set at 0.05. Stata (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.) was used for all statistical analysis.

3.1.2. OBESE ADULTS' DIET

Abstract presented at the Italian Society of Hygiene (SITI) National Congress
Title: Valutazione nutrizionale in un campione di volontari obesi inclusi in un programma di intervento nutrizionale e di attività sportiva
Authors: Fiori F., Vaccari F., Passaro A., D'Amuri A., Sanz J.M., Di Vece F., Capatti E., Magnesa B., Comelli M., Mavelli I., Grassi B., Bravo G., Avancini A., Marinoni M., Lazzer S., Parpinel M.
Lecce, November 2021

STUDY POPULATION

A total sample of 32 obese adult volunteers without further pathologies were recruited from the Exercise Physiology Laboratory of the University of Udine and randomly assigned to two physical activity intervention groups, one group following a Moderate Intensity Continuous Training (MICT, N=16) and the second group following a High Intensity Interval Training (HIIT, N=16) (Vaccari et al., 2020). After the inclusion visit, the subjects began a 3-month multidisciplinary weight management intervention, including physical activity, nutritional and lifestyle education, with a 4 month further follow up (**Figure 5**). Before the beginning, immediately after the completion of the programme, and at the end of the 4-month follow up, body composition and dietary intakes were evaluated, as well as physical capacity and fat oxidation rates (Vaccari et al., 2020). The Ethics Committee of the Friuli-Venezia-Giulia Region approved the study (protocol number 1764) and all participating subjects were informed and consented to participate.

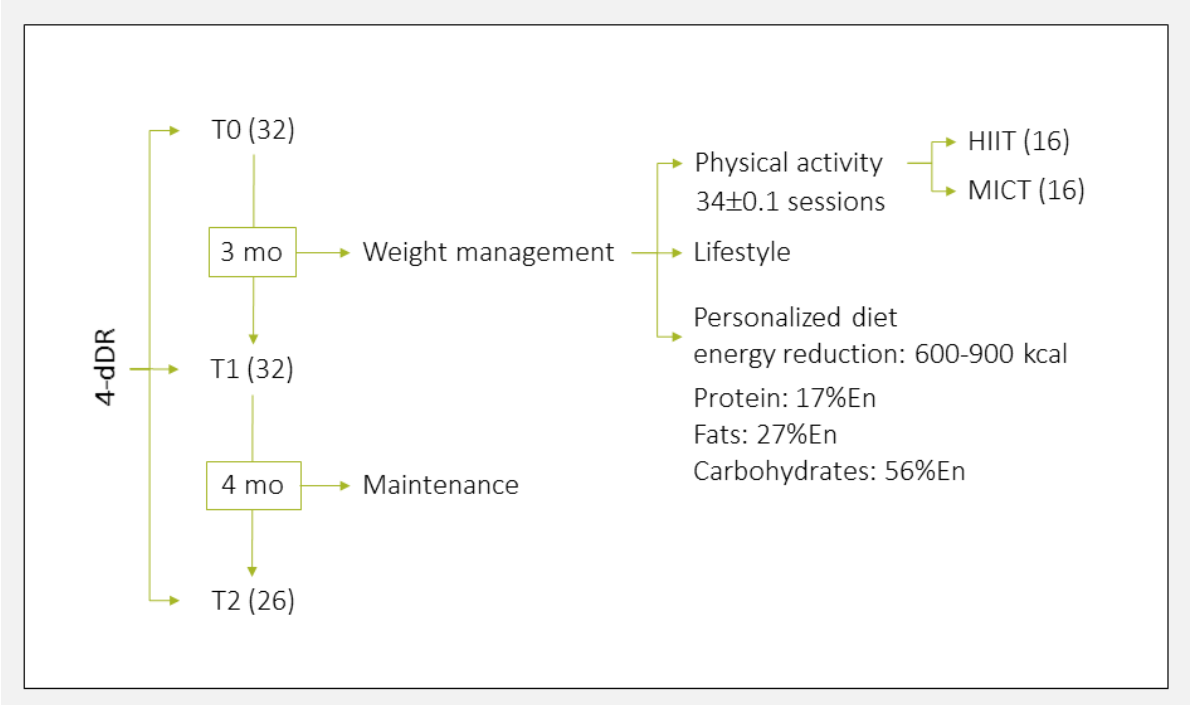

Figure 5. Schematic representation of the study design. *Abbreviation: mo, month; 4-dDR, 4-day dietary record; HIIT, high intensity interval training; MICT, moderate intensity continuous training; T0, before the intervention; T1, after the intervention; T2, after the follow-up period.*

PHYSICAL ACTIVITY INTERVENTION

The physical activity intervention consisted of a physical training programme including three endurance and strength training sessions per week under supervision. The MICT group followed a moderate intensity endurance training, set at a heart rate corresponding to 60% of the initial pulmonary oxygen uptake ($V'O_2$) peak, and the HIIT group followed a high intensity interval training, consisting of 10 minutes at 50% of $V'O_2$ peak, and 3 to 7 repetition at 100% of $V'O_2$ peak, interspersed by 1.5 minutes at 50%. Both the HIIT and MICT training sessions were modelled to obtain a similar energy expenditure per kg of fat-free mass (FFM) (20 kJ/kg FFM). All subjects were also advised to practice leisure physical activities during the weekend and holidays. During the 4-months follow up was suggested to all the subjects to perform three training session per week: one high intensity [90% hearth rate peak for less than 30 min], one medium intensity (~70-80% hearth rate peak for 30-50 min) and one low intensity (<70% hearth rate peak for more than 60 min).

ANTROPOMETRIC CHARACTERISTICS

Body mass was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardised wall-mounted height board. BMI was calculated as body mass (kg)/stature^2 (m^2). Waist, hip and wrist circumferences were measured to the nearest 0.1 cm by a measuring tape. Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy) according to the method of Lukaski and colleagues (Lukaski et al., 1986). Fat mass (FM) and FFM were calculated with equations derived either in obese people of different ages and BMI (fat-specific formulae), by utilizing a two-compartment model (Gray et al., 1989).

DIETARY ASSESSMENT AND INTERVENTION

Dietary data were collected using a 4-day dietary record (4-dDR) given to the subjects in 3 different occasions in order to analyse their diet before the beginning of the intervention (T0), at the end of the intervention (T1) and after the follow-up period (T2). The food diaries were given together with instructions on how to record type, quantity, and mode of consumption of foods over a 24-hour period on four separate days, including one at the weekend. The instructions included a table with a list of household implements that could be used at home to weigh foods and fluids, with an estimate of the equivalent in grams. Data extracted from food diaries were analysed using the Microdiet software (V2.8.6, Downlee Systems Ltd., High, Peak, UK) containing the **BDA** (Gnagnarella et al., 2015), integrated with information from NLs when there was missing data and the brand was specified in the diary. Sixteen food components were considered for nutritional analysis: total proteins, carbohydrates (available and soluble, starch, fibre), fats (total, saturated, monounsaturated and polyunsaturated fatty acids; oleic, linoleic and linolenic acid; cholesterol; eicosapentaenoic and docosahexaenoic acid) and sodium.

After collecting the food diaries on the basal dietary consumption, nutritional advice was provided and a normo-caloric balanced diet (approximately: protein, 17%En; fats, 27%En; available carbohydrates, 56%En) was delivered to the participants based on their total energy expenditure. The diet was formulated according to the Italian DRVs (SINU, 2014) and energy expenditure was calculated multiplying the basal metabolism, estimated using the Harris-Benedict

equation (Harris and Benedict, 1918), times the physical activity level, set at 1.3 as suggested by the Italian guidelines for obesity treatment (SIO-ADI, 2017). 6 weeks after the beginning of the intervention a reduced in energy and balanced diet was provided to the subjects based on their prior diet and personal feedbacks. The reduction ranged from 600 kcal to 900 kcal (2610–3766 kJ). A further food diary was delivered at 6 weeks to encourage the implementation of the reduced diet. During the 4-month follow up, instruction to follow nutritional advices was given to all subjects.

STATISTICAL ANALYSIS

The mean and standard error (SE) were calculated for each daily nutrient intake or percentage of macronutrients contribution to energy intake in all subjects who delivered the food diaries and completed the study. The nutrient intakes and percentages were compared with the Italian DRVs (SINU, 2014). To detect significant differences between the groups, a comparative analysis was conducted at each time and for each variable using Wilcoxon-Mann Whitney test. Friedman's test was used to detect significant differences in the mean distributions of each variable through the three moments. Statistical significance for all tests was set at 0.05. Analyses were conducted using SAS software version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

3.1.3. CENTENARIAN'S DIET

Abstract presented at the Italian Society of Gerontology and Geriatrics (SIGG) National Congress

Title: Valutazione della composizione dietetica in un gruppo di ultracentenari residenti in Lombardia

Authors: Azzolino D., Ferri E., Edefonti V., Parpinel M., Fiori F., Arosio B.

Online, December 2020

STUDY POPULATION

Within the PRIN project: "Ruolo della Epigenetica e della Genetica del DNA mitocondriale nella Longevità: studi su soggetti con più di 105 anni di età (semi-supercentenari)", a total sample of 123 centenarian living in the Lombardy region, in the north-west of Italy were enrolled. In the present work, 15 subjects not presenting any dysphagic disorder were examined to assess their food and nutrient intakes. Anthropometric and socio demographic data were collected by means of a questionnaire and the age of the subjects were confirmed by the inspection of the birth certificates or identity card. The Regional Ethics Committee approved the study and all participating subjects were informed and consented to participate.

DIETARY ASSESSMENT

Dietary data were collected using a 3-day dietary record (3-dDR) given to the subjects or their caregiver together with instructions on how to record type, quantity, and mode of consumption of foods over a 24-hour period on three separate days, including one at the weekend. Data extracted from food diaries were analysed using the Microdiet software (V2.8.6, Downlee Systems Ltd., High, Peak, UK) containing the **BDA** (Gnagnarella et al., 2015), integrated with information from NLs when there was missing data and the brand was specified in the diary. Energy and eleven food components were considered for nutritional analysis: protein, carbohydrates (available and soluble, fibre), fats (total, saturated, monounsaturated and polyunsaturated fatty acids; cholesterol), alcohol, vitamin D, and sodium. Data were presented as mean and SD.

3.2. BDA STANDARD UPDATE PROTOCOL

BDA v.22 standard update protocol follows the EuroFIR recommendations for quality FCD compiling (EuroFIR AISBL, 2019a). All FCD are currently maintained in a Microsoft Access database that allows the management and upload of the food and nutrient data.

The standard update protocol was divided in 3 main steps:

1. Examination and evaluation of possible substitutions/additions of items, within each food category, and actual update of each nutrient value in paper format (BDA v.98).
2. Data check by a second compiler, to verify source item choices, nutrient values, calculations, coding of the source, food, category and food note.
3. Upload of updated data and metadata (documentation in accordance to the EuroFIR standards) in Microsoft Access. All the codes linked to the old food items were modified adding "_1" to the original code, while the updated food items were coded as "_2". The filling of the data and metadata for each food component included, as applicable: Value, Value type, Acquisition type, Date, Confidence code, Number of analysis, minimum, maximum, SD, Method type, Method indicator, Original source code or imputation code, and Food notes.

Within the first step, firstly a consistency check was performed on food names in Italian and English, scientific food name, if applicable, and food notes/description, referring to the reported primary source of data. The primary source to check for novel published data are the Italian tables by CREA-NUT (CREA-NUT, 2019). If data for the given item was not available, other international FCDBs were screened for the most suitable data. **Table 7** shows the main sources used for the current BDA update in their priority order, and the reference code assigned to each borrowed value. It is always important to check the methodology reported by the authors/ compilators for each source nutrient value in order to select for their use in the BDA only comparable and quality-documented data (Castanheira et al., 2009; Westenbrink et al., 2016). All source names, codes, citations, and FCD source, were stored in paper and/or electronic format in a dedicated directory.

For food components of exclusive animal origin, zero values (i.e., logical zero) were systematically assigned to simple foods of plant origin, such as cereals in grains, flour and starch, or foods whose ingredients never include animal products such as jams and sweeteners. Components of natural origins include: animal protein and fats, cholesterol, vitamin B12, retinol, vitamin D, lactose and galactose. Logical zero were also assigned to the aminoacids, fatty acid, sugar, retinol and beta-carotene profile when the total protein, fats, soluble carbohydrates, and retinol equivalent values were equal to zero or trace values in the main source, respectively.

Table 7. *Export of the main food composition sources listed in priority order.*

REFERENCE CODE	COUNTRY	SOURCE NAME	WEBSITE	YEAR
FE	IT	Tabelle di composizione degli alimenti. Version 2019	http://www.alimentinutrizione.it	2019
ER	UK	McCance and Widdowson's The Composition of FoodS Integrated Dataset 2019	https://www.gov.uk	2019
EC	UK	McCance and Widdowson's The Composition of Foods. Seventh Summary edition.	Finglas *et al.* (book)	2015
FG	USA	U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019.	http://ndb.nal.usda.gov	2019
EP	DK	Frida Food Data. National Food Institute, Technical University of Denmark, version 3,5, 2019	http://frida.fooddata.dk	2019
FL	FI	Fineli Food Composition Database. National Institute for Health and Welfare, Nutrition Unit. Fineli. Finnish food composition database. Release 20 (June 27, 2019)	http://www.fineli.fi	2019
FH	FR	ANSES-CIQUAL French food composition table version 2020	https://ciqual.anses.fr	2020
FI	CH	Banca dati svizzera dei valori nutritivi (Versione online V6.3 02/03/2021)	http://www.valorinutritivi.ch	2019
EO	AU	Food Standards Australia New Zealand (2019). Release 1 (2019)	http://www.foodstandards.gov.au	2019
EM	NZ	FOODfiles™. New Zealand Food Composition Database 2018	http://foodcomposition.co.nz	2018
EL	SW	Swedish Food Composition Database Livsmedelsverket.	http://www7.slv.se	2017
DZ	CA	Canadian Nutrient File (CNF)	https://food-nutrition.canada.ca	2015
EH	NO	Norwegian Food Composition Database. Norwegian Food Safety Authority	www.matvaretabellen.no	2018
EI	NL	NEVO-Dutch Food Composition Database online version 2016/5.0	https://nevo-online.rivm.nl	2016

When the full composition was not available in any FCDB or table source, it was necessary to borrow data from a similar food item. Where similar food item is defined as an item having similar name, plant-origin, macronutrient and water composition, processing, and/or other descriptive information (FAO/INFOODS, 2012a). If needed, a reproportioning on dry matter, fats, protein, or soluble carbohydrates was implemented based on the macronutrient composition of the primary source *vs.* the secondary source used to compile missing data. For example, when there were significant differences in fat content, the values for fatty acids, fatty acid fractions and cholesterol have been adapted accordingly. If there were significant differences in water contents, then all nutrients have been adapted, as follows:

$$Nutrient\ value\ (F2)\ x\ [100 - water\ (F1)]\ /\ [100 - water\ (F2)]$$

where F2 = the secondary source food from which to borrow data, and F1 = the primary source food included in the BDA.

All values included in the database were well documented in terms of the origin of information and metadata. Food items were identified by an individual food code; each component value was matched to a bibliographic code and to the original source code (if borrowed from another FCDB). An additional code was matched to each nutrient value to indicate whether the component is shown exactly as reported in the original source or was recalculated.

When no other sources of data were found, the recipe method (Reinivuo and Laitinen, 2007; Vásquez-Caicedo et al., 2008) was implemented to compile missing data, in particular for some micronutrients, animal and plant fats/proteins. Recipes were created based on traditional recipes, DOP/IGP protocols, legislation documents, or manufactured products label information.

The nutrient composition of novel foods which were considered as common food choices by the Italian population but not found in any literature source, was mainly imputed by means of the label-based recipe method. Since the latest food consumption survey in Italy dates back to 2005-2006 (Leclercq et al., 2009), new food items were selected based on the European Food Safety Authority (EFSA) food consumption data, available online at: www.efsa.europa.eu (EFSA, 2021), and after a careful screening of supermarket and brand leaders' websites, as well as physical markets.

When no data was available for a given item, the mean nutrient data from NLs of foods whose commercial name corresponds to the food item were used for compiling. Decisions about the ingredient list and weights to use to create the recipe were made after a wide online screening of the food items available on the Italian market. The label information of a wide sample of manufactured products corresponding to the given food item (i.e., with similar name and description) was collected in a dedicated excel file. Then, the recipe was created from food ingredients already uploaded in the BDA, keeping the ingredient list as much simple as possible. Ingredients' weights were imputed based on the order and percentages reported in the label of the manufactured foods, and then referred back to 100g. The resulting composition was reproportioned on dry matter. Water content was borrowed from similar Italian or international source foods, or analytically determined by means of the standard AOAC gravimetric method at the University of Udine (see **chapter 3.3.2**).

Finally, before the uploading of data and metadata in Microsoft Access, a list of informatic checks and calculations were performed to control for possible errors, omissions, and to calculate energy (in kJ and kcal, with or without fibre) based on the macronutrient specific conversion factors (Greenfield and Southgate, 2003), where "P" refers to total protein, "F" to total fats, "AC" to available carbohydrates, "A" to alcohol, and "FI" to fibre content (g/100g):

$$E\ (kcal)\ =\ (P\ x\ 4)\ +\ (F\ x\ 9)\ +\ (AC\ x\ 3.75)\ +\ (A\ x\ 7)\ [+\ (FI\ x\ 2)]$$
$$E\ (kJ)\ =\ (P\ x\ 17)\ +\ (F\ x\ 37)\ +\ (AC\ x\ 16)\ +\ (A\ x\ 29)\ [+\ (FI\ x\ 9)]$$

To ensure the consistency of the values, the applied informatic checks were the following:

- Sum of all nutrient variables (those not part of other variables) ≤ 100;
- Total protein = animal protein + plant protein;
- total fats = animal fats + plant fats;

- Sum of fatty acids and cholesterol ≤ total fats;
- Sum of aminoacids ≤ total protein;
- Sum of single mono- and disaccharides = soluble carbohydrates;
- Sum of soluble carbohydrates and starch (times 1.1 for conversion in monosaccharide equivalent) = available carbohydrates;
- Retinol + 1/6 beta-carotene equivalents = retinol equivalents;
- Water content and edible part ranging from 0 to 100;
- Comparison with nutrient variables of similar food items

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3.3. GLUTEN FREE FOOD COMPOSITION DATABASE

3.3.1. THE PROTOCOL

An updated version of an Italian GF-FCDB firstly published by Mazzeo and colleagues (Mazzeo et al., 2015) was developed to represent the food composition of GF products available on the Italian market in 2020-2021 and to expand it to all food components included in the latest BDA update (Gnagnarella et al., 2015). The new database will be published in a specific GF section of the **BDA v.22**. The first version of the Italian GF-FCDB (Mazzeo et al., 2015) represented the composition (energy, macronutrients, cholesterol, 6 minerals and 9 vitamins) of 60 GF products present on the market in 2013. 17 and 26 new food items were further added in the database in 2015 and in 2018, respectively. To estimate the complete nutrient composition of single GF manufactured products, we used NL nutrient composition data and labelled ingredients list (1169/2011 Reg UE, 2011). The update was divided in 6 main steps.

STEP 1: Screening of brand leaders' websites and label data collection

To select novel GF foods to be included in the database and to verify the presence on the market of GF products considered for the creation of the old food items, a web-based and physical market search (GF specialised shops and supermarket chains in the province of Udine) was conducted. Similarly to the sampling methods used to generate analytical data, due to the lack of specific consumption surveys on the Italian coeliac population, the strategies used to select the brand to include in the screening were: market shares from retailers, brand notoriety, and retail availability (Mazzeo et al., 2015; Traka et al., 2020). Twelve brands were selected, and their websites were screened to collect all food label data on GF cereal-based products available. Contrarily to the previous sampling procedures (Mazzeo et al., 2015), in this work not only GF-specialised brands were selected (*Schär, Nutrifree, Giuliani, Piaceri Mediterranei, Agluten, Biaglut, Le Veneziane*), but also brand leaders from the general market who newly produce GF-specific product lines (*Barilla/Mulino Bianco, Galbusera, Bauli, Algida, Sanmontana*). When for an old food item, 0 to 2 products of the selected brands were found on the market (physical and/or online), other brands were considered in order to update that given item (*Cereal, Amadori, Pasta di Venezia, Viall*, *Nutrisì, Molino di Ferro, private labels*, etc.). Products that presented incomplete nutritional declaration within the brand or supermarket website were excluded. The food label data collection was performed between December 2019 and September 2021. Label data were collected in item-specific Microsoft Excel worksheets. Food items were then clustered in food groups: "Flour", "Pasta", "Filled pasta, ready-to-eat pasta, and gnocchi", "Savoury snacks", "Bread and substitutes", "Pizza", "Miscellaneous ready-to eat dishes", "Ice-cream", "Cakes and desserts", "Breakfast products", Biscuits", and "Sweet snack bars", further divided in food categories according to the BDA coding system (Gnagnarella et al., 2015).

STEP 2: Missing data

The nutrient composition of each ingredient was mainly derived from the BDA (Gnagnarella et al., 2015). When an ingredient was not present in the BDA (e.g., leavening agents, gums, and protein isolates), the composition of that ingredient was estimated from calculations, or borrowed from 2 international databases (Public Health England, 2021; USDA, 2018). For BDA v.15 ingredients presenting excessive amounts of missing data (particularly cereal and cereal-based products), missing values were compiled in accordance to the standard BDA methodology (**chapter 3.2**) in a dedicated Microsoft Excel file, tracking all data sources.

STEP 3: Recipe creation for each branded product

Within a food item, recipes were created for each branded product matching each ingredient with the corresponding BDA food (FAO/INFOODS, 2012a). The full composition (per 100g) of each ingredient was copied from the BDA to an item-specific Microsoft Excel worksheet (**Table 8**) and ingredient weights were imputed based on the ranked order stated in the ingredient list of the food label. The sum of all ingredients must be equal to 100g or >100g, considering the possible loss in water due to manufacturing and processing. Then, percentage contribution of each ingredient to the total weight of the recipe was calculated to allow a comparison with percentages of ingredients reported in in the label, when available. If water was present as a labelled ingredient, it was included in the ingredient list following the ranked order. If high amount of water was likely to be evaporated due to cooking procedures, water was added as an ingredient— named "evaporated water"— and not considered for percentage calculations. If water was not present in the labelled ingredient list, a maximum of 5% of water may be added as an ingredient (1169/2011 Reg UE, 2011). When all ingredients and weights were entered in the worksheet, a weighted mean was calculated for the branded food product considering each ingredient's nutrient composition and weight (light yellow line in **Table 8**, Recipe calculation: "*Buon mattino NUTRIFREE*"): " $= SUM.PRODUCT(nutrient\ X\ per\ 100g\ ingredeint; ingredient's\ weight)/100$".

Then we compared the resulted nutrient composition of the branded food (light yellow line in **Table 8**) with the one reported in NL of that given product (light green line in **Table 8**). The ingredients' weights were than manually adjusted using a trial-and-error approach until calculation results reflected the values of NL nutrients.

STEP 5: Retention Factors

When applicable, nutrient composition was adjusted for cooking losses, applying, at the ingredient level, the cooking- specific vitamin and mineral RF in a dedicated Microsoft Excel file. The applied RF were those published by Vásquez-Caicedo and colleagues (Vásquez-Caicedo et al., 2008). Adjusted ingredient compositions were then copy-pasted in the food item worksheet (grey lines in **Table 8**), when a cooking process was likely to be carried out.

STEP 6: GF item final composition

The described process was repeated for all branded foods collected within a single GF food item (light yellow and green lines in **Table 8**). Then, mean values were determined both for calculated composition values (recipe calculation) and NL values (dark yellow and dark green lines in **Table 8**, respectively). The final item nutrient composition was calculated combining mean NL values for protein, fats, SFAs, carbohydrates, sugars, and fibre (as applicable); NL-

derived values for sodium and chloride; and mean recipe calculation values for other nutrients. Further calculations were applied at this stage to adjust the overall nutrient composition profile:

- Animal and plant protein (mean recipe calculated value) were reproportioned on total proteins (mean label value).
- Animal and plant fats (mean recipe calculated value) were reproportioned on total fats (mean label value).
- Starch value was calculated by difference from available and soluble carbohydrates (mean label values).
- Single fatty acids (mean recipe calculated value) were reproportioned on total fats (mean label value).
- Single aminoacids (mean recipe calculated value) were reproportioned on total protein (mean label value).
- Single sugars (mean recipe calculated value) were reproportioned on soluble carbohydrates (mean label value).
- Water content was calculated as: "$100 - (total\ protein + total\ carbohidrates + ltotal\ lipids + fiber + sum\ of\ minerals\ in\ grams + sum\ of\ vitamins\ in\ grams)$". When water content was available from analytical determinations (see **chapter 3.3.2**) it was used, and the entire food item composition was reproportioned on dry matter. Data from branded foods analytical determination were aggregated in food items, according to the standard methodology.

STEP 6: Informatic checks and energy calculation

Finally, informatic checks and calculations were performed to control for possible errors, omissions, and to calculate energy (in kJ and kcal, with or without fibre) based on the macronutrient specific conversion factors as in the standard BDA protocol described in detail in **chapter 3.2**.

Statistical analysis

In the present thesis, the energy and nutrient composition per 100g (protein, fats, cholesterol, available carbohydrates, starch, soluble carbohydrates, fibre, water, sodium, SFAs, MUFAs, PUFAs) obtained through the described label-based approach was presented for each food group as mean and SD.

Table 8. *Example of the calculation worksheet for the gluten free breakfast biscuits (food item code: 50) from 2 of the branded products included in the given item.*

BDA ingredient / Manufactured product name	**Weight**	**Percentage**	**Total protein**	**Animal protein**	**Plant protein**	**Total fats**	**Animal fats**	**Plant fats**	**Cholesterol**	**Available carbohydrates**	**Starch**	**Soluble carbohydrates**	**Fibre**	**Alcohol**	**Water**	**Iron**	**Calcium**	**Sodium**
	g	%	g	g	g	g	g	g	mg	g	g	g	g	g	g	mg	mg	mg
"*Buon mattino NUTRIFREE*"																		
Water	3	3%	0	0	0	0	0	0	0	0	0	0	0	0	99.9	0	10	2
Potato starch	40	38%	1.4	0	1.4	0	0	0	0	91.5	91.5	0	0	0	16.1	0.3	10	31
Corn flour	16	15%	8.7	0	8.7	2.7	0	2.7	0	81.5	80	1.5	2.6	0	12.5	1.8	6	1
Sugar (sucrose)	15.5	15%	0	0	0	0	0	0	0	104.5	0	104.5	0	0	0.5	0.3	1	1
Sunflower seed oil	15	14%	0	0	0	99.9	0	99.9	0	0	0	0	0	0	0	0.1	0	0
Starch	10	10%	0.4	0	0.4	0.2	0	0.2	0	100.1	100.1	0	0	0	9	0.4	13	9
Honey	2.4	2.3%	0.6	0	0.6	0	0	0	0	80.3	0	80.3	0	0	18	0.5	5	11
Egg, whole	1	1%	12.4	12.4	0	8.7	8.7	0	371	0	0	0	0	0	77.1	1.5	48	137
Skimmed milk, powder	1	1%	33.1	33.1	0	0.9	0.9	0	22	56.2	0	56.2	0	0	5	0.9	1323	550
Salt	0.3	0%	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3	10	39300
Leavening agents	0.5	0%	5.2	0	5.2	0	0	0	0	37.8	37.8	0	0	0	6.3	0	1130	11800
Recipe calculation:	104.7	100%	**2.49**	0.456	2.03	**15.53**	0.10	15.44	3.93	**78.53**	59.60	**18.93**	**0.42**	0	13.70	0.55	26.23	**197.71**
Data from nutritional label:			**2.1**			**16**				**78**		**19**	**0.8**					**172**
[…]																		
"*Gran Risveglio SCHAR*"																		
[ingredient list]																		
Recipe calculation:	109.4	100%	**4.58**	1.38	3.20	**15.9**	1.11	14.83	28.10	**75.22**	56.63	**18.59**	**0.81**	0	18.02	0.9	41.63	**303.99**
Data from nutritional label:			**4.6**			**16**				**75**		**19**	**1.2**					**300**
[…]																		
MEAN VALUE FROM RECIPES			4.95	1.14	3.79	15.80	4.20	11.59	26.25	72.69	51.46	20.51	1.72	0.00	17.44	1.32	56.01	469.36
MEAN VALUE FROM LABELS			4.83			14.53				66.46		18.49	2.59					474.91
Reproportioning on label values and water calculation				1.11	3.70		3.87	10.67			47.97				9.80			
50-Breakfast biscuits GF-FCDB			**4.83**	**1.11**	**3.70**	**14.53**	**3.87**	**10.67**	**26.25**	**66.46**	**47.97**	**18.49**	**2.59**	**0.00**	**9.80**	**1.32**	**56.01**	**474.91**

Ingredients with a percentage contribution to the recipe stated in the food label are underlined. Matching nutrient values from the recipe calculation and the nutritional label are highlighted in bold typeface. Grey lines report ingredients for whom the composition has been adjusted applying food- and cooking-specific retention factors. Mean values from recipes (dark yellow line) and labels (dark green line) were calculated as the mean values from the composition of all products included in the given food item (light yellow lines and light green lines, respectively). The black line reports the final composition of the gluten free food item (per 100g). Note that the nutrient and product list is not complete in this example.

3.3.2. MOISTURE DETERMINATION

Ninety-three gluten containing (GC) products and 88 corresponding GF products were chosen for moisture determination based on a web-based screening (as described in **chapter 3.3.1)** and the actual availability on the physical and/or online marketplace.

GC and GF food products were selected based on the BDA food items of the "cereal and cereal based products" food group (Gnagnarella et al., 2015) and the corresponding GF-FCDB food group. Concurrently, foods were selected by matching each GC product to the corresponding GF product, as applicable, based on food name and label description. Matching for brand was mostly not possible, since very few manufacturers produced both GC and GF food lines, including few GF products (e.g. *Barilla/Mulino Bianco, Galbusera, Bauli, Nestlè, Mondelez*). Moreover, these products were rarely matching in terms of food type and description. For GF products, a brand priority order was established based on consumer preferences and brand leader products availability (Pellegrini, 2016). On the other hand, brand leaders in the GC food market are highly differentiated, thus the main brand leaders were identified for each food group.

The physical market screening included all the major supermarket chains located in the province of Udine, Italy, and the specialised GF shops, health shops, and pharmacies. Online shopping was required to purchase some GF products not available in physical markets (e.g., traditional Italian cakes such as "*colomba*", "*panettone*" and "*pandoro*").

The food groups considered in this analysis are reported in **Table 9**, together with the number of analysed branded products. For each GF-FCDB food item, we purchased approximately 3±1 product from different manufacturers (as appropriate). Discrepancies in the number of GF and GC products analysed within each food group were due to the lack of some correspondences in branded products available on the GF and general market.

Table 9. *Food groups considered for moisture determination and number or purchased GF and GC products.*

FOOD GROUPS	GF PRODUCTS (N)	GC PRODUCTS (N)
Bread and substitutes	27	29
Filled pasta	5	8
Pizza	2	2
Biscuit	14	14
Cakes	40	40

Abbreviations: GF, gluten free; GC, gluten containing.

The analytical determination of moisture in the selected branded GF and GC products was performed in the University of Udine laboratories of the Department of Agri-Food, Environmental and Animal Sciences, in collaboration with Prof. Sonia Calligaris, Prof. Nicoletta Pellegrini, and Dr. Marilisa Alongi. Moisture determination procedures are summarised in **Figure 6**.

Since handling and sample preparation are the greatest potential sources of error, precautions were taken to minimise inadvertent moisture losses or gains that may occur during these steps (Nielsen, 1998). The aluminium pans were firstly oven treated (105°C/30min) to prepare them for use and cooled in a functioning silica gel desiccator. From each product the analysis was conducted

in triplicate. The samples were minced and weighted by means of an analytical scale in the oven-treated aluminium pans, and the tare weight was recorded.

According to Greenfield and Southgate (Greenfield and Southgate, 2003), drying of the sample was performed using a vacuum oven set at 72° for about 12 hours, which is the preferred method for analysing water content of foods rich in sugars. This method, applying relative low temperatures for long time periods, allows a complete removal of water (but also volatiles) without decomposition of the food matrix.

Finally, dried samples were weighted and the water loss in grams, as well as the original product percentage of moisture were calculated, as follows:

$$Moisture\ (\%\ or\ g/100g) = \frac{[net\ sample\ (g)]}{[gross\ dry\ sample\ (g) - tare\ (g)]} \times 100$$

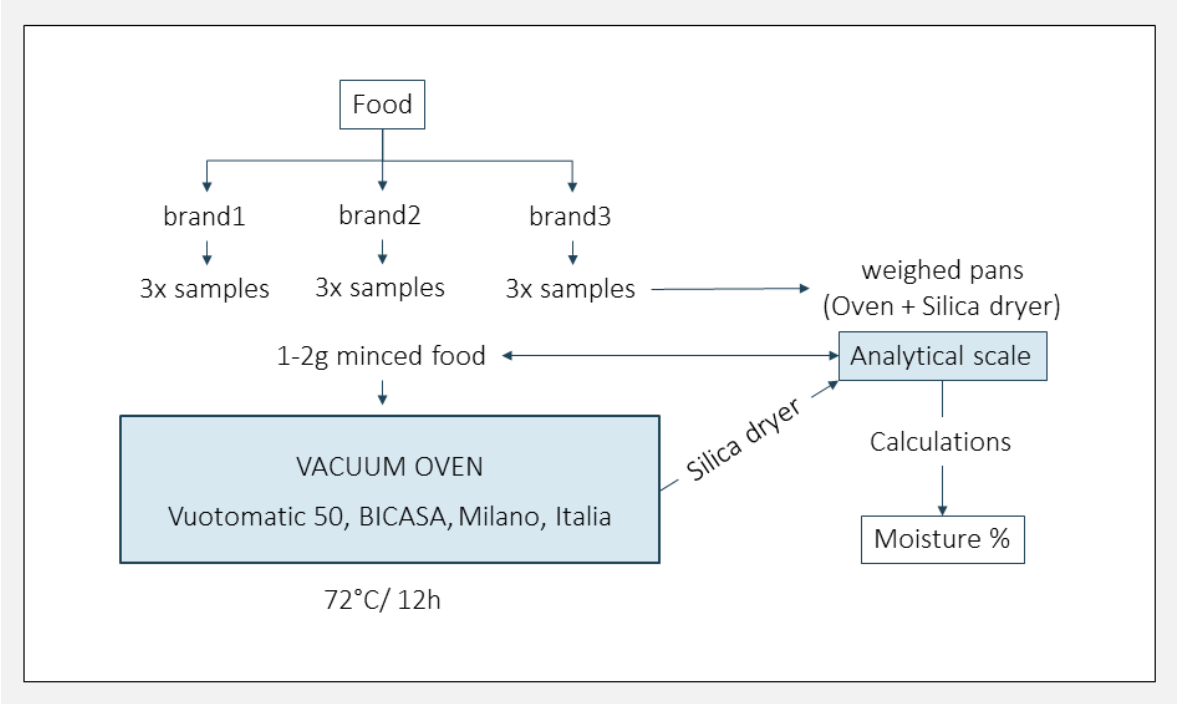


Figure 6. Schematic representation of the analytical procedure.

Finally, the mean and SD values of the 3 food samples were calculated, as well as mean and SD values of each food type (i.e., GF-FCDB/BDA food item, as applicable).

To identify the level of accuracy of NL data, a comparison was made between analytical values of water content and calculated values from NL:

$$Water\ by\ difference = 100 - [protein\ (g/100g) + lipids\ (g/100g) + carbohydrates\ (g/100g) + fibre\ (g/100g) + salt\ (g/100g)]$$

Differences between water content calculated by difference from NL and measured through the analytical method were investigated using the Wilcoxon signed-rank test. GF and GC comparison regarding differences between analytical and calculated water content of foods

($Water\ by\ difference - Water, analytically\ determinated$) were investigated using the two-sample Wilcoxon rank-sum (Mann-Whitney) test. Water content of the whole sample of GF and GC products, as well as their analytical *vs.* NL-derived product-specific difference in water content, was expressed as mean, SD, median, and 25^{th} –75^{th} centiles. The Stata software (StataCorp. 2013. Stata Statistical software: Release 13. College Station, TX; StataCorp LP.) was used for statistical analysis. Statistical significance for all tests was set at 0.05.

3.3.3. GLUTEN FREE *VS.* GLUTEN CONTAINING PRODUCTS

For the 88 GF products and 93 corresponding gluten containing (GC) products whose water content was analysed, label data were also manually collected and stored in a Microsoft Excel worksheet. Seven products were excluded for the present analysis due to lack of correspondence among product type between GF and GC market: excluded products were GC "*friselle*" (N=2), GC cream-filled croissants (N=2), GC refrigerated pizza dough (N=1), GF non-refrigerated filled-pasta with spinaches and ricotta cheese (N=2), GF plain muffin (N=1), and GF sugar-free cakes (N=2). The remaining products were grouped in the following food groups to obtain a statistically appropriate numerosity: "Bread and substitutes", "Filled pasta", "Biscuits", and "Cakes and desserts".

Mean, SD, median, and 25^{th} –75^{th} centiles were calculated for aggregated data from each food group. NL differences between GF and GC food products (energy, protein, fats, SFAs, available carbohydrates, sugars, salt and fibre) were investigated using the two-sample Wilcoxon rank-sum (Mann-Whitney) test for non-normal distributed variables and the two-sample t test for normal distributed variables, as applicable). Statistical significance for all tests was set at 0.05. The Stata software (StataCorp. 2013. Stata Statistical software: Release 13. College Station, TX; StataCorp LP.) was used for all statistical analysis.

3.4. A PILOT STUDY ON BABYFOOD COMPOSITION DATA

Abstract presented at the Italian Society of Human Nutrition (SINU) National Congress
F. Fiori, F. Concina, P. Gnagnarella, G. Carioni, M. Parpinel (2020). Update of "babyfoods" and "snacks" categories from the food composition database used for the analysis of infants diet at 18 months of age in PHIME study. NMCD. NUTRITION METABOLISM AND CARDIOVASCULAR DISEASES, vol. 30, p. 539.
Genova, November 2019

The present pilot study originated from the PHIME study, whose design was described in **chapter 3.1.1**. The subjects were a limited sample of 18-month old infants belonging to the NAC-II cohort whose parents or caregiver completed the 7-day dietary record (7-dDR) (Concina et al., 2016, 2021; Valent et al., 2013). Dietary records from 10 infants were chosen randomly from the database for their inclusion in this analysis.

Dietary data were collected using a food diary provided to mothers with instructions on how to record type, quantity, and method of feeding over a 24-hour period on 7 days (Concina et al., 2021; Valent et al., 2013).

Data extracted from the 10 selected food diaries were analysed using the Microdiet V2.8.6 software (Microdiet software – Downlee Systems Ltd., High, Peak, UK), which contains the **BDA** (Gnagnarella et al., 2015). Originally, the study-specific FCDB was integrated with macronutrient information from NLs (i.e., for commercial products, follow-on formula) collected and stored in digital or paper form between 2009 and 2014 (Concina et al., 2021). Then, the study-specific FCDB was updated using the label-based recipe approach to fill missing nutrient data on manufactured products. For each manufactured product present in the food record, a recipe was created to estimate the missing FCD from NLs. The methodology adopted was the one described in **chapter 3.3.1**, with the difference that in the present work the composition of the exact brand reported in the food diary was used for nutritional evaluation as it is, without calculating the mean composition of multiple brands, in an aggregated food item. Ingredient list and NL considered for the recipe simulation were those collected from the food package for the original database between 2009 and 2014, thus representing the manufactured product composition at that particular time. **Figure 7** shows an example of the label-based recipe creation.

Item	ALI56: Kinder Fetta al Latte FERRERO (E)	Ingredient List:	latte fresco pastorizzato (40%), olio di palma, zucchero, farina di frumento, latte scremato in polvere, miele (5%), burro anidro, uova in polvere, cacao magro, cruschello di frumento, agenti lievitanti (difosfato disodico, carbonato acido di sodio, carbonato d'ammonio), emulsionanti (mono e diglicendi degli acidi grassi), aromi, sale.										
		Grams	%	Protein	Fats	CHO	Sugars	SFAs	MUFAs	PUFAs	Vit. B2	Vit. B6	Starch	Sodium
1602_2	**Latte di vacca, intero pastorizzato**	49	**40%**	3.3	3.6	4.9	4.9	2.11	1.10	0.12	0	0	0	50
908_2	Olio di palma	22.5	19%	0	99.9	0	0	47.10	38.92	12.58	0.18	0.06	0	0
2021_1	zucchero (saccarosio)	18	15%	0	0	104.5	104.5	0.00	0.00	0.00	0	0	0	1
12_1	Farina di frumento, tipo 00	5.8	5%	11	0.7	78	1.7	0.16	0.08	0.43	0	0	76.3	3
1622_2	latte di vacca, scr. in polvere	5.8	4.8%	33.1	0.9	56.2	56.2	0.63	0.23	0.05	0.03	0.15	0	550
2017_1	**Miele**	5.8	**5%**	0.6	0	80.3	80.3	0.00	0.00	0.00	1.8	0.6	0	11
1806_2	uovo di gallina, intero, in polvere	6	5.0%	51.9	36.4	0.4	0.4	14.04	18.33	5.83	0.04	0.02	0	573
3000_2	cacao amaro, in polvere	4	3.3%	20.4	25.6	11.5	0	14.34	9.47	1.02	0.02	0	11.5	950
30039_1	Crusca di grano	0.5	0.4%	14.1	5.5	26.8	3.8	0.93	0.73	2.86	1.26	0.46	23	28
17-355 UK	agenti lievitanti	0.5	0.4%	5.2	0	37.8	0	0	0	0	0.3	0.07	37.8	11800
8021_1	sale da cucina	**0.3**	0%	0	0	0	0	0.00	0.00	0.00	0.36	1.38	0	39300
Recipe simulation		121.2	100.0%	8.23	27.57	34.46	29.27	13.10	10.80	3.32	0.29	0.13	5.19	306.87
Nutritional label				8.4	27.3	34.5	29.2	16.6			0.28			257
Calculations									10.69	3.29			5.30	
KINDER FETTA AL LATTE				8.40	27.30	34.50	29.20	16.60	10.69	3.29	0.28	0.13	5.30	257.0

Figure 7. Example of a label-based recipe calculation Microsoft Excel worksheet. *Food ingredient names are reported as in the BDA Italian version. Abbreviations: CHO, carbohydrates; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; vit., vitamin.*

To quantify differences in nutrient intakes estimated by means of the 2 versions of the database (original *vs.* updated), a comparison was performed on 29 food components: total proteins, carbohydrates (available and soluble, starch, and fibre), fats (total, SFAs, MUFAs and PUFAs; oleic acid, linoleic acid and linolenic acid; cholesterol), minerals (sodium, potassium, calcium, iron, zinc) and vitamins (vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E expressed as a-tocopherol equivalent, retinol, vitamin A, niacin, and folate).

For each infant, the mean daily intake of macro- and micronutrients was calculated on a 7-day observation basis. The difference (%) between each nutrient intake estimated with the 2 databases was calculated and expressed as mean and SD.

3.5. IMPLEMENTATION OF AN ALGORITHM TO IMPUTE INGREDIENTS' WEIGHT

In order to automatise the procedures described in **chapters 3.3** and **3.4** to calculate the nutrient composition of manufactured products based on food label information (i.e., ingredient list and NL), a tool has been developed in collaboration with Dr. Stefano Burigat from the Department of Mathematics, Computer Science and Physics of the University of Udine.

The tool is based on the idea that identifying the correct ingredients' weight in order to further estimate nutrient composition based on label information can be formalised as a multi-objective optimization problem. Optimization can be defined as the process of identifying the best solution from among the set of all feasible solutions (Miettinen, 1999). The criteria that are used to compare solutions are known as objectives. Single-objective optimization uses a single criterion while multi-objective optimization employs two or more criteria. Generally, there is no single optimal solution to multi-objective problems because the different objectives can conflict with each other. As a consequence, decision-makers are responsible for exploring the set of potential solutions to identify the most appropriate one(s) based on their needs.

A multi-objective optimization problem with M objectives can be formalised as:

$$\begin{aligned} \underset{\mathbf{x}\in\Omega}{\text{minimize}} \quad & F(\mathbf{x}) = (f_1(\mathbf{x}), f_2(\mathbf{x}), \ldots, f_M(\mathbf{x})) \\ \text{subject to} \quad & c_i(\mathbf{x}) = 0, \ \forall i \in \mathcal{E}, \\ & c_j(\mathbf{x}) \leq 0, \ \forall j \in \mathcal{I}. \end{aligned}$$

where **x** is the vector of decision variables that are manipulated during the optimization problem

$$\mathbf{x} = \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_L \end{bmatrix}$$

For our purposes, decision variables will be defined as assuming real values in closed intervals. The multi-objective optimization problem may include zero or more equality and inequality constraints c(x) that inform the optimization process as to which solutions are infeasible or impractical.

As an example, suppose we have the following labelled ingredient list, in order: milk (40%); palm oil; sugar; wheat flour; skimmed milk, powder; honey; butter; egg; cocoa. The NL reports: fats, 27.3 g/100g; of which SFAs, 16.6g/100g; carbohydrates, 34.5g/100g; of which sugars, 29.2 g/100g; protein, 8.4 g/100g; salt, 0.64 g/100g —sodium, 256 mg/100g. The full nutrient composition of the ingredients (**Table 10**) was derived from the **BDA** (Gnagnarella et al., 2015).

Table 10. *BDA macronutrient and sodium composition (per 100g) of the labelled ingredient list.*

Food code	BDA item	Variable	Fats	SFAs	Carbohydrates	Sugars	Protein	Sodium
			g	g	g	g	g	mg
1602_2	Milk, whole, pasteurised	**x1**	3.6	2.11	4.9	4.9	3.3	50
908_2	Palm oil	**x2**	99.9	47.10	0	0	0	0
2021_1	Sugar (sucrose)	**x3**	0	0.00	104.5	104.5	0	1
12_1	Wheat flour (type 00)	**x4**	0.7	0.16	78	1.7	11	3
1622_2	Skimmed milk, powder	**x5**	0.9	0.63	56.2	56.2	33.1	550
2017_1	Honey	**x6**	0	0.00	80.3	80.3	0.6	11
1900_2	Butter	**x7**	83.4	48.78	1.1	1.1	0.8	7
1806_2	Egg, whole	**x8**	36.4	14.04	0.4	0.4	51.9	573
3000_2	Cocoa, powder	**x9**	25.6	14.34	11.5	0	20.4	950

Abbreviation: SFAs, saturated fatty acids.

The unknown values we need to find to estimate the nutrient composition are the weights of the ingredients, which will be our decision variables: x1 = weight of milk, x2 = weight of palm oil, x3 = weight of added sugar, and so on.

The objectives (functions) to be optimised will be defined as follows:

$$f1(x) = \frac{3.6 * x1 + 99.9 * x2 \ + 0.0 * x3 \ + \ 0.7 * x4 + \cdots + \ 25.6 * x9}{100} - 27.3$$

$$f2(x) = \frac{2.11 * x1 + 47.10 * x2 \ + \ 0.0 * x3 \ + \ 0.16 * x4 + \ \ldots + 14.34 * x9}{100} - 16.6$$

...

$$f6(x) = \frac{0.05 * x1 + 0.0 * x2 \ + \ 0.001 * x3 \ + \ 0.003 * x4 + \ \ldots + 0.95 * x9}{100} - 0.256$$

There is one function for each nutrient reported in the NL. Each function is built by summing the proportion of each ingredient which is made of that nutrient and subtracting the weight of the nutrient reported in the label. The proportions are derived from the nutrient composition of the ingredients. For example, the nutrient composition table shows that 3.6% of the milk is fat, hence the coefficient 3.6 for variable x1 in function f1(x). Optimizing this function means minimizing it so that the sum of the fat contribution of each ingredient equals the amount of fat reported in the label.

An additional, slightly different, objective concerns water. Part of the water that was present in the raw ingredients evaporates during the preparation process. This is another unknown variable, x0, that needs to be subtracted from the water objective, leading to the following formula for water:

$$f7(x) = \frac{w1 * x1 + w2 * x2 \ + \ w3 * x3 \ + \ w4 * x4 + \ \ldots + w9 * x9}{100} - wl - x0$$

where w1, …, w9 are the coefficients representing the proportion of water in each ingredient, wl is the amount of water reported in the label (if such value is available) and x0 is the amount of evaporated water.

Constraints can be defined by the decision-maker based on the data in the NL and other needs. One set of constraints derives from the order of the ingredients in the NL, which are reported in descending order of weight, hence:

$$x1 \geq x2$$
$$x2 \geq x3$$
$$\ldots$$
$$x8 \geq x9$$

One constraint derives from the fact that the sum of the weights of the ingredients must equal 100 (g) plus the weight of evaporated water:

$$x1 + x2 + x3 + x4 + \cdots + x9 = 100 + x0$$

Another constraint that the decision-maker might define to guide the optimization problem is to force the sum of the objectives to be lower than a threshold t:

$$Math.abs(f1) + Math.abs(f2) + Math.abs(f3) \ldots + Math.abs(f7) < t$$

This is because the optimization process is supposed to find a solution that minimises each function independently from the others but does not necessarily minimise their sum, which might be preferable for the decision-maker.

Other constraints might derive from the availability of information in the NL about the percentage of one or more ingredients in the finished product.

Once a nutrient composition problem has been formalised as a multi-objective optimization problem like in the previous example, it is possible to use different approaches to solve it and find a set of optimal solutions (i.e., assignments of values to the decision variables that minimise the functions while complying with the constraints). The approach used in our tool is based on a multi-objective evolutionary algorithm (MOEA) (Coello et al., 2007) that searches for multiple Pareto optimal solutions in a single run. More specifically, we used the NSGA-II algorithm (Deb et al., 2002), one of the first and most widely used MOEAs.

Evolutionary algorithms (EAs) are a class of search and optimization algorithms that are inspired by natural evolution processes (Holland, 1975). The first step of EAs is an initialization process that generates the initial search population. Next, EAs enter a loop that includes selecting parent individuals from the search population, applying a recombination operator to generate offspring, and finally updating the search population with these offspring using a replacement strategy. The loop is repeated until a fixed number of objective function evaluations are applied. After termination, the EA reports the set of optimal solutions discovered during the search.

We developed the tool as a JavaFX application with a graphical user interface that allows the decision-maker to easily insert the parameters of the optimization problem, refine them, and visualise the solutions (**Figure 8**).

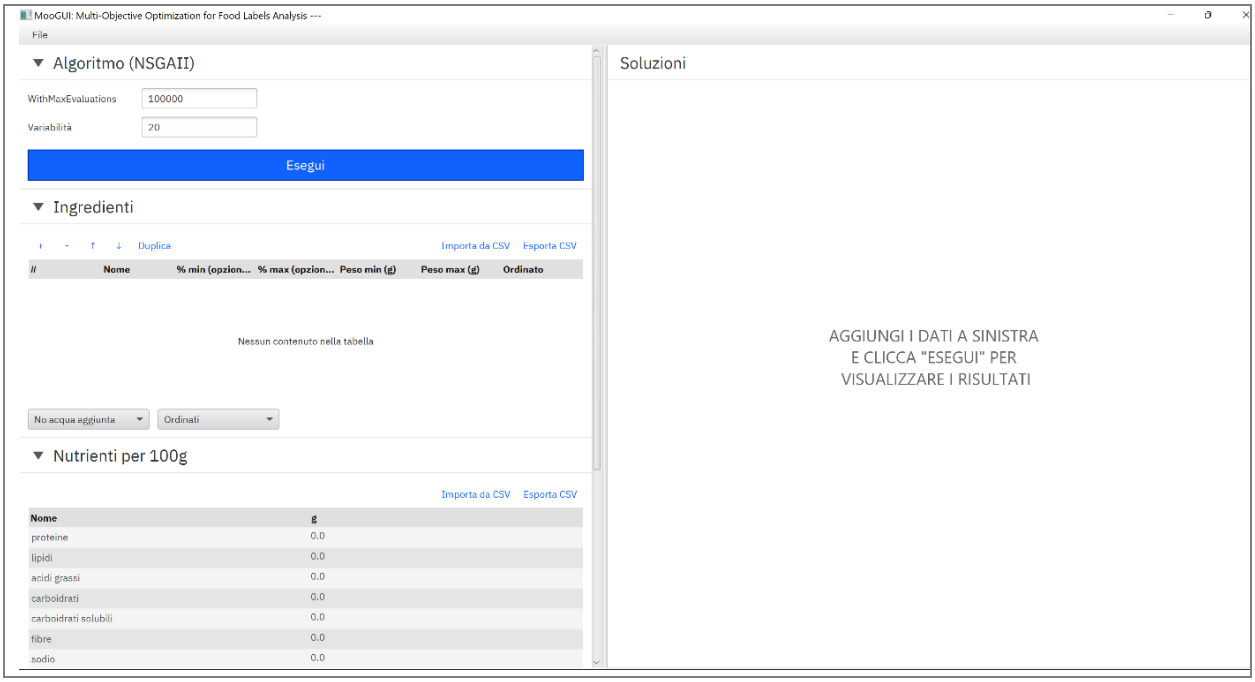


Figure 8. User interface of the JavaFX application (Italian version).

3.6. THE CASE-CONTROL FOOD COMPOSITION DATABASE: UPDATE PROCEDURES ON VARIABLES OF INTEREST

3.6.1. CHOLINE AND SPHINGOMYELINS

The present work focused on the update of the **BDA**-based case-control FCDB (Salvini et al., 1996) for choline and sphingomyelins (SMs). The compiling method used was the indirect method (Greenfield and Southgate, 2003).

Firstly, a literature and/or internet search was performed in order to find available choline and SMs FCD. Then, for either SMs and choline content, each food item included in the case-control FCDB, was matched to a food from one of the selected data sources, according to a defined priority order and seeking for the soundest match (similarity of food name, description, macronutrients). Decisions on food matching and imputations were made case by case following international guidelines (FAO/INFOODS, 2012a). When an exact match between a food item of the case-control FCDB and a food item from the data source was not possible, different approaches were used. If a SMs/choline value from the same food in a different form was available in the data source, a reproportioning was applied on dry matter. For choline content obtained from the USDA National Nutrient Database for Standard Reference (USDA, 2018) data was recalculated based on the reported imputation method (dry matter, proteins, non-fat solids, fats, as appropriate). If a simple match by description or botanical family could not be found, the mean SMs /choline value obtained from similar foods or from the entire food group or category was assigned to that given food item. For complex foods with multiple ingredients, choline and SMs content were calculated by percent weight contribution of each ingredient.

Finally, a quality code was assigned to each match based on the FAO/INFOODS guidelines on food matching (FAO/INFOODS, 2012a). Quality code A was assigned when food and descriptors from the specific FCDB match with food and descriptors from the FCD source and with analytical data in the original source. Quality code B was assigned:

- when the food from our FCDB was matched to the same food in a different form (reproportioned if needed);
- when the food from our FCDB was matched to a food of similar botanical origin,
- when the mean value of multiple foods was used to compile the FCDB;
- when the exact match was found in another FCDB, but the source reported non-analytical data.

Quality code C was assigned when data from the closest match possible was used.

3.6.2. PREBIOTICS

To update the case-control FCDB (Salvini et al., 1996) for prebiotic composition of foods, Inulin-Type Fructans (ITFs), Fructo-Oligosaccharides (FOSs) and Galacto-Oligosaccharides (GOSs) were determined in 7, 78 and 78 food sources, respectively. There were: 32 vegetables, 15 fruits, 9 pulses, 22 cereals and cereal-based products. Foods were selected based on those reported in a food frequency questionnaire (FFQ) used to assess dietary habits in subjects participating in an Italian multicentric case-control study (Decarli et al., 1996; Franceschi et al., 1993).

Food sampling and prebiotic determinations was conducted in a certified laboratory for food analysis by Neotron SPA. Foods were purchased in the Modena province from mass retail channels (i.e., supermarkets). from 17 May to 24 June 2021.

INULIN-TYPE FRUCTAN DETERMINATION

ITFs in fresh sample were determined using an internal analytical method based on AOAC 997.08 procedure. The limit of quantification was 0.005 g/100g. The analyses were conducted on 7 food sources such as: fresh onion, garlic, banana, leek, jerusalem artichoke, artichoke and shallow.

Freeze-dried samples (1-5g), containing about 1 g of ITFs, were weighted into a 100mL of baker and extracted about 40mL of hot water (~ 60°C) with an immediately pH check and mild agitation. If necessary, it was adjusted with 0.05 M potassium hydroxide or 0.05 M hydrochloric acid. Final volume was made up to 50 mL with deionised water. The mixture was incubated in a shaking water-bath at a temperature equal to 85±2°C for 15 minutes (M_1). Extracted sample solution (3-5g) was weighted directly into a 25mL volumetric flask which contained 0.01 g of rhamnose as internal standard, the diluted to 25mL with deionised water and set aside for direct analysis (assay A_0).

- First hydrolysation: 15 g homogenised mixture (M_1) were transferred into a 100mL screw cap bottle and equal amount of 0.2M acetate buffer was added (pH should be between 4.5±0.5). If necessary, it was adjusted with 0.05M potassium hydroxide or 0.05M hydrochloric acid. Sufficient amount of amyloglucosidase was added and incubated for 30 min in shaking water bath at 60±2°C. After cooling at room temperature, 10g of first hydrolysate was weighted and set aside for analysis (assay A_1).
- Second hydrolysation: sufficient amount of inulinase solution was added to the remaining part of the first hydrolysate, considering the amount of fructans present, and the enzyme concentration (*Fructozyme*). Then it was incubated for 30 min in shaking water bath at 60±2°C. After cooling at room temperature, weight measurement was taken and set aside for analysis (assay A_2).

Assay A_0, A_1 and A_2 were injected into a high-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAE-PAD), with 2.0 g of glucoheptose internal standard solution.

Sugar content (glucose, fructose, sucrose, maltitol and galactose) analysis in the three different assays was performed using a Dionex ICS 6000 apparatus equipped with an autosampler and a

pulsed electrochemical detector. Elution of carbohydrates was performed at room temperature on a Dionex Carbopac PA1 column (4x250 mm) equipped with pre-column Carbopac PA1 (4×50 mm). Flow rate was 1.3 mL/min. The injection volume was twenty-five microliters. Gradient elution was applied using three solvents: 0.15 M sodium hydroxide in water (eluent A), 0.5 M sodium acetate and 0.15 M sodium hydroxide in water (eluent B) and water (eluent C). All mobile phases were sparged and pressurised with nitrogen to prevent adsorption of atmospheric carbon dioxide. Elution conditions were: 0–13 minutes isocratic elution with 8% of eluent A and 92% of eluent B, followed by 14.5 minutes gradient elution with 26% eluent A and 74% eluent C; 1.5 minutes isocratic step followed by 26 minutes gradient elution with 88% eluent A, and 12% eluent B; 2 minutes of column equilibration. The washing of the column was performed at 100% of eluent B for 5 minutes followed by 6 minutes of column equilibration. The injection volume was twenty-five microliters.

ITFs content was calculated using the following formula:

$$ITFs = k \cdot (Gi + Fi)$$

where:

k = 0.91 for the expression as inulin in chicory;

k = 0.925 for expression as oligofructose;

$Gi = inulin\ from\ glucose\ = GA2 - GA1 - GS - GLact - GMal$

GA2 = Glucose from assay A_2

GA1 = Glucose from assay A_1

GS = Glucose from sucrose= Sucrose from assay $A_0/1.9$

GLact = Glucose from lactose

GMal = Glucose from maltitol

$= (maltitol\ from\ assay\ A1 - \ maltitol\ from\ assay\ A2)/1.9.$

$Fi = inulin\ from\ fructose\ = FA2 - FS - FA0$

FA2=Fructose from assay A_2

FS=Fructose from sucrose=sucrose from assay $A_0/1.9$

FA0=Fructose from assay A_0.

FRUCTO-OLIGOSACCHARIDES AND GALACTO-OLIGOSACCHARIDES DETERMINATION

FOSs and GOSs in fresh samples were determined according to Manali Aggrawal and Jeff Rohrer method (Aggrawal and Rohrer, 2015). The following molecules were quantified: raffinose (GOS), stachyose (GOS), nystose (FOS), kestose (FOS) and 1F-β-fructofuranosylnystose (FOS). The limit of quantification was 0,002–0,02 g/100g, based on the food matrix.

Four hundred grams of food were homogenised with a blender blade. One gram of homogenised sample was put into a plastic flask and 200 mL of sodium hydroxide 0.0025 M was added. The solution was shacked with Geno/Grinder for 5 minutes and filtered through a 0.45-micron PVDF filter. Samples were injected into a HPAE-PAD.

Carbohydrate analyses were performed with a Dionex ICS 6000 apparatus equipped with an autosampler and a pulsed electrochemical detector. Elution of carbohydrates was performed at room temperature on a Dionex Carbopac PA200 column (3x250 mm), equipped with pre-column Carbopac PA200 (3×50 mm). Flow rate was 0.3 mL/min. Gradient elution was applied using three

solvents: 0.15 M sodium hydroxide in water (eluent A), 0.5 M sodium acetate and 0.15 M sodium hydroxide in water (eluent B) and water (eluent C). All mobile phases were sparged and pressurised with nitrogen to prevent adsorption of atmospheric carbon dioxide. Elution conditions were: 0–12 minutes isocratic elution with 50% of eluent A and C, followed by 24 minutes gradient elution with 14% eluent A, 36% eluent B and 50% eluent C. The washing of the column was performed at 80% of eluent B and 20% of eluent C for 5 minutes followed by 6 minutes of column equilibration. The injection volume was twenty-five microliters.

3.7. APPLICATION OF THE PREBIOTICS DATA IN NUTRITIONAL EPIDEMIOLOGY

Scientific paper submitted to:
International Journal of Cancer
Title: Dietary prebiotic fibres and colorectal cancer risk: the PrebiotiCa study
Authors: Turati F., Concina F., Rossi M., Fiori F., Parpinel M., Taborelli M., Giacosa A., Crispo A., Pagan E., Rosato V., Garavello W., Negri E., La Vecchia C.
December 2021

STUDY POPULATION

The present work originated from an Italian case-control study on colorectal cancer conducted in six Italian areas (Milan, Genoa, Pordenone/Gorizia, Forlì, Latina, and Naples) in the period 1992–1996 (Franceschi et al., 1995). The study protocol was approved by the local ethical committees, and all participants signed an informed consent.

The case-control study included:

- 1953 histologically confirmed colorectal cancer cases diagnosed no longer than one year before the interview and with no previous cancer diagnosis (1225 colon cancers and 728 rectal cancers, 1125 men and 828 women, median age 62, range 19-74 years);
- 4154 controls (median age 58, range 19–74 years). Controls were subjects with no history of cancer and admitted to the same hospitals as cases for acute, non-neoplastic conditions unrelated to tobacco, alcohol, hormonal or digestive tract diseases and to long-term modifications of diet; 21% were admitted for traumas, 26% for other orthopaedic disorders, 24% for surgical conditions, 18% for eye diseases, and 11% for other illnesses.

DATA COLLECTION

A structured questionnaire was administered by trained interviewers to cases and controls. Data on socio-demographic characteristics, anthropometric measures, physical activity, lifetime smoking habits, alcohol-drinking habits, personal medical history, and family history of cancer was collected.

A reproducible (Franceschi et al., 1995) and valid (Decarli et al., 1996) interviewer-administered FFQ was used to assess study participants' habitual diet during the 2 years prior to cancer diagnosis (for cases) or hospital admission (for controls). The FFQ collected data on the average weekly consumption of 78 foods, food groups or complex recipes. Intakes lower than once a week, but at least once a month, were coded as 0.5 per week. Additional questions were asked to assess fat intake and other general habits. From the FFQ, the intakes of selected nutrients, food components, and total energy were derived using the Italian **BDA** (Gnagnarella et al., 2015), integrated with data from laboratory analysis (**chapter 3.6.2**) following standard matching procedures.

STATISTICAL ANALYSIS

We derived the odds ratios (OR) of colorectal cancer with the corresponding 95% confidence intervals (CI) according to quintiles (derived among controls) of fibre prebiotic intake by unconditional multiple logistic regression models, adjusted for multiple variables (age, sex, study centre, years of education, body mass index, occupational physical activity, smoking, alcohol intake, age at menopause and use of hormone replacement therapy, diabetes, aspirin use, family history of colorectal cancer, and total energy intake). Prebiotics were included into the model as continuous variables, with a measurement unit equal to the difference between the upper cut points of the 4th (i.e., the 80° percentile) and the 1st quintiles (i.e., the 20° percentile).

All the analyses were performed using the SAS software, version 9.4 (SAS Institute, Inc., Cary, NC, USA).

4. RESULTS AND DISCUSSION

4.1. BDA USE AND ITS LIMITATIONS

4.1.1 CHILDREN'S DIET

Scientific paper published in: Nutrients vol. 14, 515
Title: Adherence to Dietary Recommendations of 7-Year-old Children from a Birth Cohort in Friuli Venezia Giulia, Italy
Authors: Giordani E., Marinoni M., Fiori F., Concina F., Ronfani L., Dalmin P., Barbone F., Edefonti V.*, Parpinel M.*
January 2022

LIFESTYLE AND ANTHROPOMETRIC CHARACTERISTICS

In total, 381 children (females: 48.3%; males: 51.7%) whose parents filled in 3-dDR were included in the present study. Only 6.3% of the mothers were foreign citizens. Almost 82% of the mothers and ~70% of the fathers had a high school diploma (45.1% and 47.0%, respectively) or a higher educational level (38.6% and 22.0%, respectively) (data not shown). Children's median age was 7.1 (7.1–7.2) years (**Table 11**). Approximately 72.9% of the children were normal weight, whereas 19.1% and 5.4% were overweight and obese, respectively. Most of the children (79.5%) practiced extra-curricular sport activities from 1 to 3 days per week, with a 15.2% practiced sport more than 4 days per week. Percentages of males and females, prevalence of overweight, as well as percentages of mothers and fathers with a high school diploma were similar to those reported in the national survey "*OKkio alla SALUTE*" on 8–9 year-old children in 2016, for the Friuli Venezia Giulia region (Pani and Carletti, 2016) and at the national level (Nardone et al., 2018). Prevalence of obesity in our sample was in line with data from Friuli-Venezia Giulia (Fiori et al., 2020; Pani and Carletti, 2016; Vaccari et al., 2021), but lower than the national-level data (Nardone et al., 2018). This likely reflects the high frequency of practicing sports detected in our sample.

Table 11. *Children's lifestyle and anthropometric characteristics at 7 years of age (N=381).*

	N	%
Sex		
Male	197	51.7
Female	184	48.3
Weight status[1]		
Underweight	9	2.6
Normal weight	255	72.9
Overweight	67	19.1
Obese	19	5.4
Extra-curricular sport or play activities		
Never	15	3.9
1-3 days/week	303	79.5
>4 days/week	58	15.2
Not reported	5	1.3

MACRONUTRIENT INTAKE AND NUTRITIONAL ADEQUACY

Descriptive statistics [median (25^{th}–75^{th} centile)] of the observed intakes of energy and macronutrients per day are reported in **Table 12**, together with the corresponding Italian DRVs.

Table 12. *Distribution of energy and macronutrient intakes of 7-year-old children in the overall sample (N=381).*

	Median	25^{th}	75^{th}	DRVs
Energy (kJ/d)	6291.8	5593.2	6982.2	
Energy (kcal/d)	1503.0	1336.2	1668.0	
Protein (g/d)	55.6	47.2	64.3	
Protein (%En)	14.8	13.2	16.5	12 – 18 %En (RI)[1]
Total fats (g/d)	52.2	42.6	61.7	
Total fat (%En)	31.3	27.4	35.1	20 – 35 %En (RI)
Saturated fatty acids (g/d)	20.5	16.5	24.5	
Saturated fatty acids (%En)	12.2	10.6	14.0	< 10 %En (SDT)
Monounsaturated fatty acids (g/d)	18.0	14.5	22.1	
Monounsaturated fatty acids (%En)	10.8	9.3	12.6	10 – 15 %En (RI)[2]
Oleic acid (g/d)	16.5	13.4	20.1	
Polyunsaturated fatty acids (g/d)	5.2	4.1	6.6	
Polyunsaturated fatty acids (%En)	3.1	2.5	3.9	5 – 10 %En (RI)
Arachidonic acid (mg/d)	146.3	95.6	219.7	
Linoleic acid (g/d)	3.9	3.1	5.2	
PUFAs ω-6 (%En)	2.4	2.0	3.2	4 – 8 %En (RI)
Alpha - linolenic acid (g/d)	0.6	0.4	0.7	
EPA+DHA (mg/d)	61.0	23.7	208.3	250 mg/d (AI)
PUFAs ω-3 (%En)	0.4	0.3	0.5	0.5 – 2.0 %En (RI)
Cholesterol (mg/d)	185.3	143.0	224.8	
Available carbohydrates (g/d)	197.6	163.7	223.9	
Available carbohydrates (%En)	51.8	48.3	56.6	45 – 60 %En (RI)
Soluble carbohydrates (g/d)	72.5	59.0	87.5	
Soluble carbohydrates (%En)	19.4	16.4	23.0	< 15 %En (SDT)
Fibre (g/1000kcal/d)	7.0	5.7	8.7	8.4 g/1000 kcal (AI)

[1] Reference Intake calculated by difference: RI (protein)=100%- RI (total fats)- RI (available carbohydrates); [2] Reference Intake calculated by difference: RI(MUFAs)=RI (total fats)- RI(PUFAs)- SDT(SFAs). Abbreviations: d, day; DRVs, dietary reference value; %En: percentage contribution to total energy intake; PUFAs: polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The median daily energy intake of the overall sample was 1503 kcal (1336–1668), with a statistically significant difference by sex ($p<0.05$). Overall, the median percentage contribution of protein, total fats, and available carbohydrates to daily energy intake was found to be in line with the recommendations. Most of the children from our sample met the Italian DRVs and showed a NAR index equal to 1 for those macronutrients (**Figure 9**).

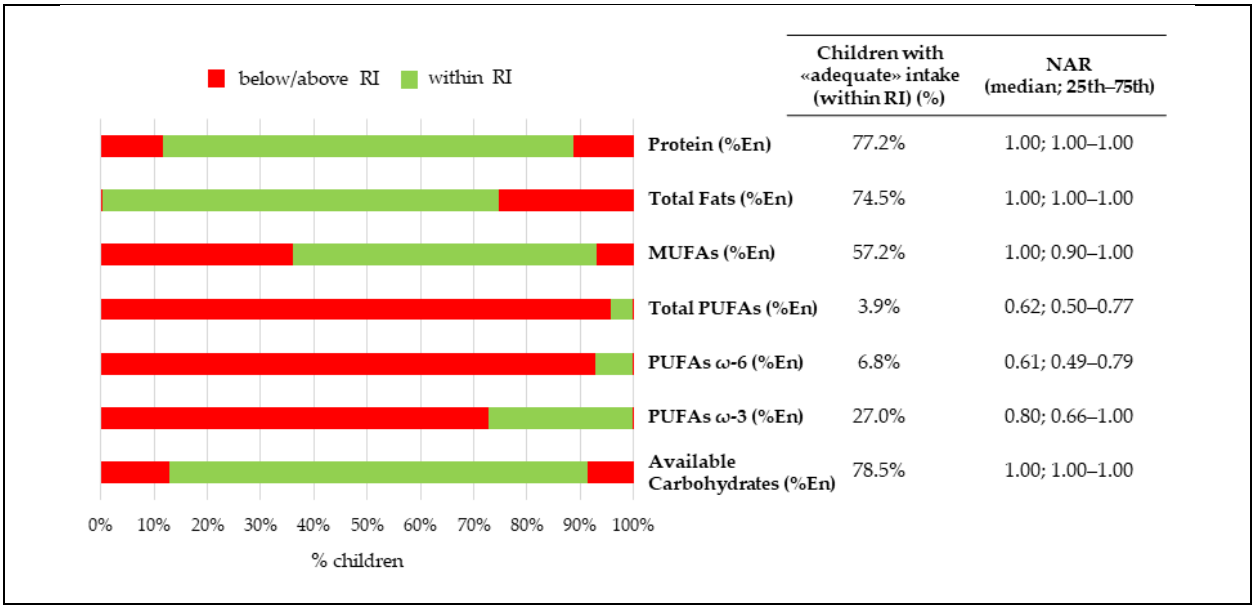


Figure 9. Nutritional adequacy relative to the reference intake. NAC-II, 2014-2016 (N=381). *NAR was based on the RI range. Children having intakes equal to the cut-off values were considered to be adequate for that specific nutrient. Abbreviations: RI, reference intake range for macronutrients; NAR, nutrient adequacy ratio; %En, percentage contribution to total energy intake; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.*

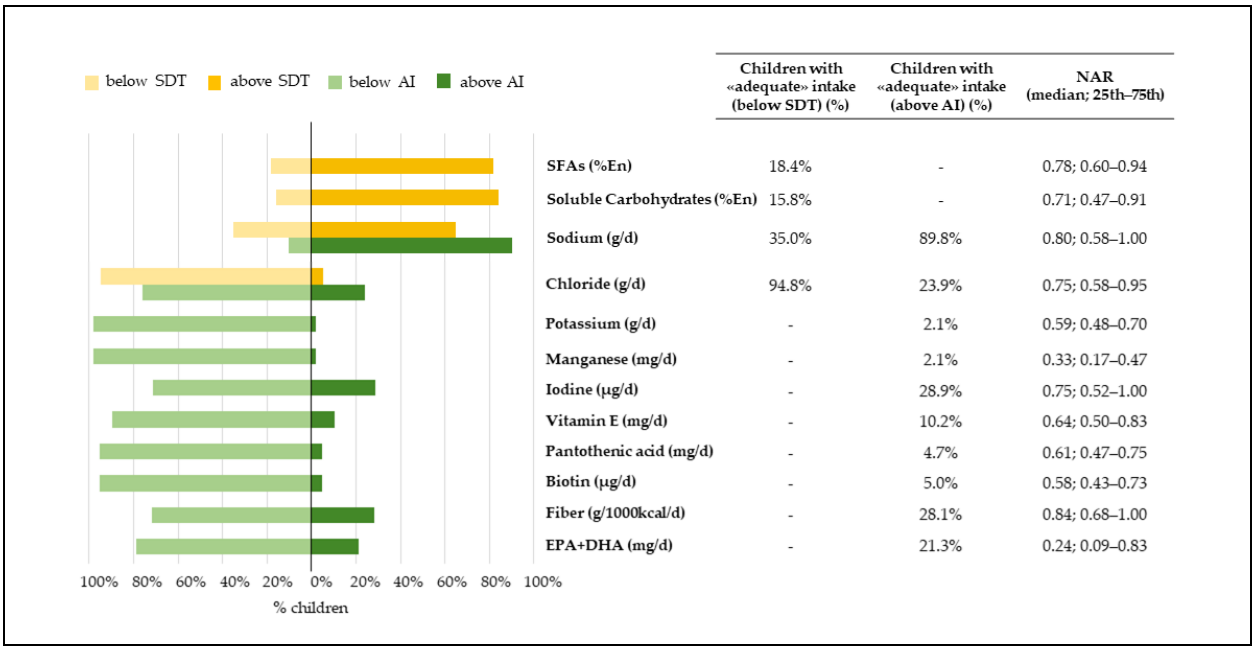
Although apparently reassuring, this hides a substantial unbalance of the overall diet towards total fats and protein. The median %En from total fats (31.3 %En; 27.4–35.1) was close to the upper limit of the RI range (20–35 %En) and the median %En from available carbohydrates (51.8 %En; 48.3–56.6) was close to the lower limit of the recommendations (45–60%). No child was below the RI lower limit for total fats, and 1 out of 4 children (25.2%) exceeded the upper RI limit.

Regarding protein intake, 100% of the enrolled children reached their AR; 99.7% of the children reached their PRI too. However, by calculating child-specific PRI cut-offs for protein, ~63% of the children at least doubled their recommended PRI and ~11% at least tripled it. From a different perspective, the median protein intake of our sample is 55.6 g/d, which is comparable to the daily protein requirement of an adult woman of 60 kg of weight (54 g/day) (SINU, 2014). The described unbalance towards protein and total fats has been already documented in most of the other Italian (Rosi et al., 2021; Verduci et al., 2019) studies on primary school children, except for one older Italian study (Verduci et al., 2007) where available carbohydrates of 8-year-old children reached 60% of total energy intake.

In line with other Italian data (Piccinelli et al., 2011; Rosi et al., 2021; Verduci et al., 2019), we observed:

- intakes of total PUFAs below the RI lower limit in 96% of the children [19.4 %En (16.4–23.0)];

- an excess intake of SFAs [12.2 %En (10.6–14.0)], with 82% of children being above the SDT (**Figure 10**);
- most children exceeded the SDT for soluble carbohydrates (84.2%) [72.5 %En (59.0–87.5)].



	Children with «adequate» intake (below SDT) (%)	Children with «adequate» intake (above AI) (%)	NAR (median; 25th–75th)
SFAs (%En)	18.4%	-	0.78; 0.60–0.94
Soluble Carbohydrates (%En)	15.8%	-	0.71; 0.47–0.91
Sodium (g/d)	35.0%	89.8%	0.80; 0.58–1.00
Chloride (g/d)	94.8%	23.9%	0.75; 0.58–0.95
Potassium (g/d)	-	2.1%	0.59; 0.48–0.70
Manganese (mg/d)	-	2.1%	0.33; 0.17–0.47
Iodine (µg/d)	-	28.9%	0.75; 0.52–1.00
Vitamin E (mg/d)	-	10.2%	0.64; 0.50–0.83
Pantothenic acid (mg/d)	-	4.7%	0.61; 0.47–0.75
Biotin (µg/d)	-	5.0%	0.58; 0.43–0.73
Fiber (g/1000kcal/d)	-	28.1%	0.84; 0.68–1.00
EPA+DHA (mg/d)	-	21.3%	0.24; 0.09–0.83

Figure 10. Nutritional adequacy relative to the adequate intake and suggested dietary target. NAC-II, 2014-2016 (N=381). *NAR was based on the SDT and AI. Children having intakes equal to the AI cut-off value were considered to be adequate for that specific nutrient, while children having in-take equal to the SDT cut-off value were considered to be inadequate. Abbreviations: AI, adequate intake; SDT, suggested dietary target; d, day; NAR, nutrient adequacy ratio.*

No child from our sample had a soluble carbohydrates intake <5 %En, 4 children were < 10 %En *vs.* 60 (15.8%) < 15 %En, which corresponds to the Italian SDT. In addition, when analysing individual intakes of soluble carbohydrates with more stringent cut offs, in our study, 15% of the enrolled children derived at least 25% of their total energy intake from soluble carbohydrates, against recommendations of the Italian Society of Human Nutrition (SINU, 2014), who considered intakes >25% to be at risk for adverse effects on health.

Furthermore, within the PUFAs dietary profile the median %En from PUFAs, PUFAs ω-6, and PUFAs ω-3 was below the lower limit of the RI range in most of the children. The corresponding median NARs were far from 1 and equal to 0.62 (0.50–0.77), 0.61 (0.49–0.79), and 0.80 (0.66–1.00), respectively, thus suggesting substantial inadequacy (**Figure 9**). Median intake of the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (mg/day) was below the AI.

MICRONUTRIENT INTAKE AND NUTRITIONAL ADEQUACY

Descriptive statistics [median (25^{th}–75^{th} centile)] of the observed intakes of micronutrients per day are reported in **Table 13**, together with the corresponding Italian DRVs.

Table 13. *Distribution of micronutrient intakes of 7-year-old children in the overall sample (N=381).*

	Median	25^{th}	75^{th}	DRVs
Sodium (g/d)	1.7	1.3	2.1	1.1 g/d (AI); 1.5 g/d (SDT)
Potassium (g/d)	1.8	1.4	2.1	3 g/d (AI)
Calcium (mg/d)	537.8	409.6	706.3	900 mg/d (AR); 1100 mg/d (PRI)
Magnesium (mg/d)	83.5	65.5	105.8	130 mg/d (AR); 150 mg/d (PRI)
Phosphorus (mg/d)	819.0	693.8	966.9	730 mg/d (AR); 875 mg/d (PRI)
Iron (mg/d)	5.9	4.8	7.2	5 mg/d (AR); 13 mg/d (PRI)
Zinc (mg/d)	6.3	5.3	7.4	7 mg/d (AR); 8 mg/d (PRI)
Selenium (µg/d)	18.5	13.0	28.2	30 µg/d (AR); 34 µg/d (PRI)
Copper (mg/d)	0.4	0.2	0.5	0.4 mg/d (AR); 0.6 mg/d (PRI)
Chloride (g/d)	1.3	1.0	1.7	1.7 g/d (AI); 2.3 g/d (SDT)
Manganese (mg/d)	0.4	0.2	0.6	1.2 mg/d (AI)
Iodine (µg/d)	75.0	51.8	104.6	100 µg/d (AI)
Vitamin B1 (mg/d)	0.7	0.6	0.9	0.6 mg/d (AR); 0.8 mg/d (PRI)
Vitamin B2 (mg/d)	1.1	0.8	1.3	0.7 mg/d (AR); 0.8 mg/d (PRI)
Niacin (mg/d)	9.6	7.7	12.2	9 mg/d (AR); 12 mg/d (PRI)
Pantothenic acid (mg/d)	2.1	1.6	2.6	3.5 mg/d (AI)
Vitamin B6 (mg/d)	1.3	1.0	1.5	0.7 mg/d (AR); 0.9 mg/d (PRI)
Biotin (µg/d)	11.5	8.6	14.7	20 µg/d (AI)
Folate (µg/d)	160.6	127.5	199.7	210 µg/d (AR); 250 µg/d (PRI)
Vitamin B12 (µg/d)	2.4	1.8	3.3	1.3 µg/d (AR); 1.6 µg/d (PRI)
Vitamin A (µg/d)[1]	603.7	438.8	853.4	350 µg/d (AR); 500 µg/d (PRI)
Vitamin C (mg/d)	63.2	40.7	98.4	45 mg/d (AR); 60 mg/d (PRI)
Vitamin D (µg/d)	1.1	0.7	1.5	10 µg/d (AR); 15 µg/d (PRI)
Vitamin E (mg/d)[2]	5.1	4.0	6.6	8 mg/d (AI)

[1] Expressed as retinol equivalents; [2] expressed as alpha-tocopherol equivalents. Abbreviations: d, day. DRVs, dietary reference values.

Median sodium intake (1.7 g/day; 1.3–2.1) reached the AI (1.1 g/day), but it exceeded the SDT (1.5 g/day). Ninety percent of the children had a sodium intake above the AI; however, only 35% had an intake not exceeding the SDT cut-off value, as reflected by a median NAR smaller than 1 [NAR: 0.80 (0.58–1.00)] (**Figure 10**). Even if underestimation of sodium is likely to occur in dietary records, our median sodium intake was in line with the one reported by Rosi and colleagues (Rosi et al., 2021), whereas Verduci and colleagues (Verduci et al., 2019) reported a lower median intake.

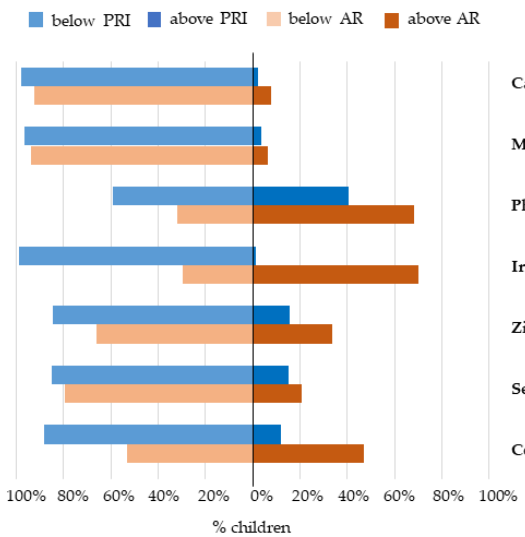
Except for iron, copper, and phosphorus, intake of other minerals was generally inadequate (i.e., median intake lower than the corresponding AR) in our sample. Similar conclusions were reached for iron, phosphorus, sodium, calcium, potassium, and zinc, in one or more of the available Italian studies (Rosi et al., 2021; Verduci et al., 2019). However, generally higher mean/median intakes were observed in comparison with previous European studies (Glynn et al., 2005; Zaragoza-Jordana et al., 2018), as well as with the older Italian INRAN-SCAI study (Piccinelli et al., 2011). Downgrading evidence on zinc inadequacy from standard DRV-based analysis, the

NAR-based approach revealed a modest deviation of zinc intake from the DRVs in most children (median NAR=0.90) (**Figure 11, A**). This indicates that dietary inadequacy was not severe in our sample, as also hypothesised for zinc deficiency in serum of European children (Gibson et al., 2008).

Seven out of 12 available vitamins showed a median adequate intake, with five of them (vitamin B2, vitamin B6, vitamin B12, vitamin A, and vitamin C) even reaching the PRI. Among others, the worst degree of inadequacy was observed for folate and vitamin D. Folate median intake was below the AR and 80% of children did not reach it (**Figure 11, B**). In Italy and Europe, mean/median intake of folate in primary school children was similarly low (Glynn et al., 2005; Rosi et al., 2021; Zaragoza-Jordana et al., 2018). An inadequate intake of vitamin D was observed in our sample: no child met the AR, and the median intake of children was 11%, as compared to the AR cut-off value (median NAR=0.11). This is alarming, but in line with dietary data of other Italian (Piccinelli et al., 2011; Rosi et al., 2021; Verduci et al., 2019) and European studies (Glynn et al., 2005; Zaragoza-Jordana et al., 2018). Evidence of serum deficiency of vitamin D was also reported in paediatric population in Italy (Rutigliano et al., 2021; Vierucci et al., 2014), suggesting increased sun exposure and dietary intake has to be reached.

We did not observe substantial variation in nutrient intake between males and females (data not shown). Most of the significant differences were found for macronutrients, with the higher available carbohydrates intake in males likely reflected in their higher energy intake, as also found in Verduci and colleagues (Verduci et al., 2019).

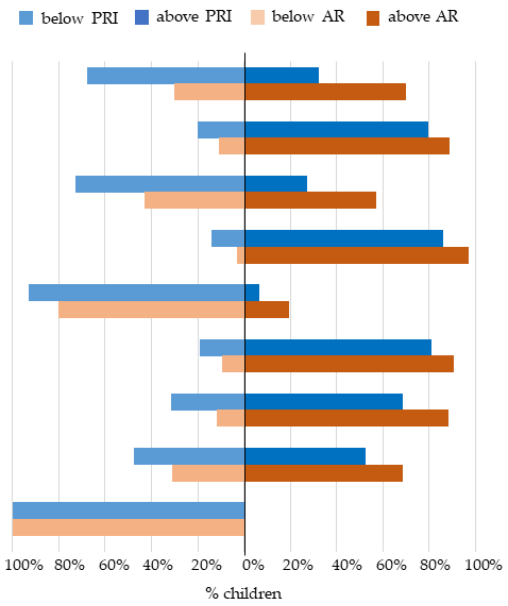
(A)



	Children with «adequate» intake (above PRI) (%)	Children with «adequate» intake (above AR) (%)	NAR (median; 25th–75th)
Calcium (mg/d)	2.1%	7.6%	0.60; 0.46–0.78
Magnesium (mg/d)	3.4%	6.3%	0.64; 0.50–0.81
Phosphorus (mg/d)	40.7%	68.2%	1.00; 0.95–1.00
Iron (mg/d)	1.3%	70.3%	1.00; 0.97–1.00
Zinc (mg/d)	15.5%	33.9%	0.90; 0.75–1.00
Selenium (µg/d)	15.0%	20.7%	0.62; 0.43–0.94
Copper (mg/d)	11.8%	47.0%	0.96; 0.60–1.00

Figure 11.A. Nutritional adequacy of minerals relative to the average requirement and population reference intake (N=381). *NAR was based on the AR. Children having intakes equal to the cut-off value were considered to be adequate for that specific nutrient. Abbreviations: PRI, population reference intake; AR, average requirement; NAR, nutrient adequacy ratio; d, day.*

(B)



	Children with «adequate» intake (above PRI) (%)	Children with «adequate» intake (above AR) (%)	NAR (median; 25th–75th)
Vitamin B1 (mg/d)	32.3%	69.8%	1.00; 0.95–1.00
Vitamin B2 (mg/d)	79.8%	89.0%	1.00; 1.00–1.00
Niacin (mg/d)	27.3%	57.2%	1.00; 0.85–1.00
Vitamin B6 (mg/d)	86.1%	97.1%	1.00; 1.00–1.00
Folate (µg/d)	6.8%	19.7%	0.76; 0.61–0.95
Vitamin B12 (µg/d)	81.1%	90.6%	1.00; 1.00–1.00
Vitamin A (µg/d)	68.5%	88.2%	1.00; 1.00–1.00
Vitamin C (mg/d)	52.5%	68.8%	1.00; 0.90–1.00
Vitamin D (µg/d)	0.0%	0.0%	0.11; 0.07–0.15

Figure 11.B. Nutritional adequacy of vitamins (B) relative to the average requirement and population reference intake (N=381). *NAR was based on the AR. Children having intakes equal to the cutoff value were considered to be adequate for that specific nutrient. Abbreviations: PRI, population reference intake; AR, average requirement; NAR, nutrient adequacy ratio; d, day.*

Finally, in our population, no children reached the optimal MAR value of 1.00, targeting adequacy on all the available nutrients. Overall, the median MAR was 0.75 (0.69–0.79). In the influence analysis, median values of the MAR after removal of one component at a time ranged from 0.74 to 0.76. No statistically significant differences were found in median MAR values between females and males.

CONCLUSIONS AND FCD-RELATED LIMITATIONS

The current study evaluated nutritional adequacy in 381 7-year-old children from Friuli Venezia Giulia, Italy, who were enrolled within a cohort study aimed at evaluating the effects of low-level mercury exposure during pregnancy on infant neurodevelopment at 18 months and later ages. Results revealed an inadequate intake of key nutrients, as highlighted by standard analyses and the NAR indexes, and suboptimal adequacy of the overall dietary profile, as expressed by the MAR index equal to 0.75 with no child reaching the optimal adequacy value of 1.

However, despite the present work presented a comprehensive description of dietary intake following different approaches (i.e., the traditional comparison with DRVs, and the NAR and MAR modified indexes implementation), it has to be noticed that the application of the BDA in its 2015 version may have led to underestimations of some micronutrient intakes. Among the 39 nutrients compared with DRVs, 26 did not show missing values in the BDA. On the other hand, regarding vitamins: biotin and pantothenic acid present some missing data in the BDA (34% and 33% of the total BDA items, respectively) regarding cereal-based products and vegetables (which are however poor sources of these vitamins), pulses, cakes and snacks. Regarding minerals: chloride, magnesium, manganese, selenium and iodine present some missing data in the BDA

(32%–33% of the total BDA items). Vitamin K was excluded from the analysis because fraught with so many missing values (86% of the total BDA items) to likely not providing a reliable estimate of its intake.

Moreover, the use of the limited nutrient composition reported in the food label for complex commercial products (~4.2 % of the total food entries; 37.7% of the reported food items without repetitions) may have led to inaccurate estimates of a few macronutrients and/or underestimation of micronutrient intakes, due to their high number of nutrient missing values. This means that the nutrient composition of 37.7% of the total food list including all the different food items reported by the subjects (without considering repetitions) was incomplete. Accordingly, considering the food list without repetitions we observed missing values ranging from 36.9% (4.0% of the total food entries) to 91.4% (92.8% of the total food entries), for calcium and vitamin K, respectively. The only nutrients presenting less than 1% missing values were those reported in NLs (protein, fats, carbohydrates, SFAs, sugars, and sodium). As a result, many nutrient intakes may be underestimated. In particular, more than 50% of missing values were observed in the food list (>38% considering the total food entries) mainly for those nutrient also presenting some missing values in the BDA: sulphur (not included in the nutritional evaluation), copper, chloride, magnesium, manganese, selenium, iodine, biotin, pantothenic acid, vitamin B12, vitamin K, arachidonic acid, EPA and DHA.

4.1.2. OBESE ADULTS' DIET

Abstract presented at the Italian Society of Hygiene (SITI) National Congress
Title: Valutazione nutrizionale in un campione di volontari obesi inclusi in un programma di intervento nutrizionale e di attività sportiva
Authors: Fiori F., Vaccari F., Passaro A., D'Amuri A., Sanz J.M., Di Vece F., Capatti E., Magnesa B., Comelli M., Mavelli I., Grassi B., Bravo G., Avancini A., Marinoni M., Lazzer S., Parpinel M.
Lecce, November 2021

17 male and 15 female volunteers aged 38.6±1.4 (40.1±1.8 and 37.3±2.3, HIIT and MICT, respectively) were included in this analysis. All subjects were obese without further pathologies and 29 of 32 declared to have previously attempted to lose weight without long term results, with a mean weight oscillation range during adulthood of 32.3±3.1 and 36.1±3.1 kg, for HIIT and MICT groups respectively (data not shown). All subject completed at least 90% of 34±0.1 sessions of physical training and compiled at least the T0 and T1 food records (Vaccari et al., 2020).

BASAL NUTRIENT INTAKE

In the present sample, protein intake at T0 was high (72.0±6.9 g/d and 90.5±8.9 g/d in the HIIT and MICT group. respectively) but lower than the intake observed in another Italian population of obese subjects (Ricci et al., 2011). If considering protein intake in grams per kg, the intake exceeded the AR and the PRI in solely 56% and 28% of the subjects, respectively, because of their substantial body mass. Mean available carbohydrates contribution to energy intake was near to the RI lower limit. Moreover, 44% of the subjects had an intake below the RI. Soluble carbohydrates contribution to energy intake was generally high: 56% of the subjects had intakes above the SDT. Fibre consumption was inadequate both if expressed in grams per day (SDT) or in grams per 1000kcal (RI) in 75% and 78% of the subjects, respectively. Total fats and SFAs contribution to the energy intake were above the RI upper limit in 50% and 72% of the subjects, respectively, and PUFAs contribution to energy intake was below 5% (RI) in 69% of the subjects. Cholesterol intake was in line with the recommendations in 66% of the subjects and the sum of EPA and DHA reached adequate levels in solely 23% of the subjects.

Mean energy and all macronutrient intakes resulted lower than those reported in another Italian study on obese subjects, except for PUFAs, SFAs, and cholesterol whose intakes in grams were comparable (Ricci et al., 2011). However, in their sample, subject with a BMI>40 were the most represented ones, and the mean BMI was higher.

Finally, sodium intake exceeded the SDT in 84% of the subjects, and alcohol consumption was below the SDT for the majority (81%) of the subjects. Only 2 subjects exceeded the limit in the MICT group and 5 in the HIIT group.

POST-INTERVENTION ANTROPOMETRIC CHARACTERISTICS

Anthropometric characteristics and body composition measured at T0, T1 and T2 are reported in **Table 14**. Since no significant differences were found between the groups at the three moments,

HIIT and MICT were considered together in the time dependent analysis (Friedsman's test). A significant improvement in body weight, BMI, body composition, waist and hip circumferences was observed in the subjects after the multidisciplinary intervention ($p<0.001$). Despite no significant differences were found between the groups, at the follow up the HIIT group maintained better the weight and FM loss, compared to the MICT group. Surprisingly, at T2 circumferences reduced and FFM increased in both groups, in respect to T1.

Table 14. *Anthropometric characteristics and body composition of 32 obese volunteers grouped by physical activity intervention (HIIT and MICT) at T0, T1, and T2.*

	T0 (n=32)		**T1 (n=32)**		**T2 (n=21)**		***p-value***
	HIIT (N=16)	**MICT (N=16)**	**HIIT (N=16)**	**MICT (N=16)**	**HIIT (N=10)**	**MICT (N=11)**	
Weight (kg)	103.4±7.3	107.1±4.3	97.5±7.4	101.0±4.5	98.0±3.0	103.8±5.4	<0.001*
BMI (kg/m^2)	35.1±2.4	36.1±1.3	30.7±2.5	32.7±1.7	29.8±3.5	32.7±2.4	<0.001*
Waist circumference (cm)	114.1±7.5	113.0±3.5	99.6±7.9	105.9±5.4	94.9±11.1	103.7±7.4	<0.001*
Hip circumference (cm)	114.3±8.0	119.5±5.4	106.9±8.3	113.6±5.4	102.3±11.8	111.3±7.6	<0.001*
FFM (kg)	65.1±2.9	69.4±3.9	64.7±2.7	68.6±4.1	69.3±3.9	71.0±5.0	<0.001*
FM (kg)	38.4±2.1	37.7±2.7	32.9±2.5	32.4±2.3	28.8±2.0	32.5±3.0	<0.001*

The Wilcoxon test was applied to detect any statistical differences between HIIT and MICT groups. The Friedsman's test was applied to detect any statistical differences trough time. * $p<0.05$. Abbreviations: HIIT, high intensity interval training; MICT, moderate intensity continuous training; T0, before the intervention; T1, after the intervention; T2, after the follow-up period; BMI, body mass index; FFM, fat free mass; FM, fat mass.

Results are mixed regarding the different effects of HIIT and MICT in improving body composition (Fisher et al., 2015; Keating et al., 2014), but it is known that a better improvement and better health outcomes may be achieved with combined intervention (i.e., diet and physical activity) (Moredich and Kessler, 2014; Nazare et al., 2013; Valente et al., 2011). Moreover, it seems that nutrition education associated with a dietetic prescription should be considered important in effective weight-loss interventions (Mahdavi et al., 2016).

POST-INTERVENTION ENERGY AND NUTRIENT INTAKE

Improvement in circumferences, body composition and body mass were accompanied by a reduction in energy intake and an improvement in nutrient intakes. **Table 15** shows energy and nutrient contributions to energy intake of 32 obese volunteers at the three times the diet was evaluated. The energy and nutrient intake at T0 were considerably variable among the subjects, especially fat profile. However, the only significant difference between the two groups was found in the amount (in grams) of available ($p=0.015$) and soluble ($p=0.008$) carbohydrates (i.e., sugars).

Energy intake decreased from T0 to T1 (-402±100 kcal/day), and then increased again at T2 (+225±122 kcal/day) ($p=0.002$). In the HIIT group, the mean follow-up energy intake value overcame the initial one. Nevertheless, energy intake was initially higher in the MICT group in respect to the HIIT group, although the difference was not significant ($p=0.0865$), and the intervention helped to align the two groups by diminishing the difference over time.

Table 15 *Daily nutrient intakes (mean ±SE) of 32 obese volunteers grouped by physical activity intervention (HIIT and MICT) at T0, T1, and T2, compared to the Italian DRVs.*

	T0 (N=32)		**T1 (N=32)**		**T2 (N=21)**		**DRVs**	**p-value**
	HIIT (n=16)	**MICT (n=16)**	**HIIT (n=16)**	**MICT (n=16)**	**HIIT (n=10)**	**MICT (n=11)**		
Energy (kcal/d)	1845±125	2273±182	1574±118	1741±147	1854±167	1938±152		**0.002***
Energy (kJ/d)	7720±521	9509±763	6584±492	7285±614	7759±697	8110±636		**0.002***
Protein (%En)	15.5±1.0	16.0±0.7	19.2±1.4	19.2±1.2	16.8±1.2	18.4±1.6		**0.003**
Protein (g/d)	72.0±6.9	90.5±8.9	71.4±4.6	82.6±8.2	77.81±9.42	85.42±6.03		0.919
Protein(g/kg/d)	0.69±0.06	0.86±0.09	0.74±0.05	0.83±0.08	0.79±0.08	0.87±0.07	0.71 (AR); 0.9 (PRI)	0.292
Available carbohydrates (%En)	44.9±1.4	45.3±2.2	46.1±1.5	45.3±2.0	45.7±2.6	47.4±2.4	45-60 (RI)	0.218
Available carbohydrates (g/d)	**217.1±12.6***	**274.4±22.2***	192.2±15.6	205.8±15.2	217.10±12.82	248.9±24.27		**0.002***
Soluble carbohydrates (%En)	14.9±0.9	16.8±0.9	14.5±1.0	16.0±1.2	14.7±1.3	16.2±1.4		0.846
Soluble carbohydrates (g/d)	**66.3±4.2***	**94.9±8.4***	55.0±4.3	68.3±6.4	64.98±5.03	76.81±6.45	<15 (SDT)	**0.002***
Fibre (g/d)	19.1±2.3	21.39±1.8	18.6±1.5	17.6±1.3	19.84±7.14	18.83±2.84	>25 (SDT)	0.687
Fibre (g/1000kcal/d)	10.6±1.1	9.9±1.0	12.05±0.72	10.99±0.83	11.02±1.21	10.18±1.42	12.6-16.7 (RI)	0.216
Total fats (%En)	35.9±1.6	36.8±2.2	30.8±2.1	33.3±1.5	32.4±2.0	33.2±2.1	20-35 (RI)	**0.009***
Fats (g/d)	74.3±6.3	92.8±9.4	54.7±5.3	66.3±7.7	67.74±7.88	71.67±7.75		**0.003***
SFAs (%En)	11.6±0.6	12.5±0.9	9.3±0.8	10.7±0.9	10.3±1.0	10.8±0.8	<10 (SDT)	**0.034***
MUFAs (%En)	14.4±0.6	13.8±1.3	14.0±1.2	14.3±0.9	13.1±0.9	12.4±1.7		**0.093***
PUFAs (%En)	4.5±0.4	4.2±0.3	3.9±0.3	4.6±0.3	4.1±0.3	3.8±0.6	5-10 (RI)	**0.048***
Linoleic acid (g/d)	7.76±1.06	8.02±0.82	5.43±0.57	6.92±0.61	6.39±0.73	5.94±0.77		0.064
Linolenic acid (g/d)	0.93±0.08	1.08±0.11	0.75±0.06	0.89±0.11	0.81±0.13	0.79±0.13		**0.009***
EPA+DHA (g/d)	0.27±0.11	0.28±0.08	0.23±0.07	0.44±0.17	0.40±0.19	0.32±0.16	0.25 (AI)	0.687
Cholesterol (mg/d)	210±26	294±38	208±35	249±43	211±38	230±27	<300 (SDT)	0.830
Sodium (mg/d)	3065±234	3398±393	2339±216	2227±173	3026±196	2682±254	<2000 (SDT)	**0.008***
Alcohol (%En)	3.7±1.0	1.9±0.7	3.8±2.1	2.2±0.7	5.1±3.2	1.0±0.4		0.846
Alcohol (g/d)	10.7±3.3	6.7±2.5	10.7±6.4	6.1±2.0	17.06±11.67	2.61±0.92	<20M; <10F (SDT)	0.500

The Wilcoxon test was applied to detect any statistical differences between HIIT and MICT groups. The Friedsman's test was applied to detect any statistical differences trough time. Significant differences were highlighted in bold typeface; * $p<0.05$. Abbreviations: d, day; %En, percentage contribution to total energy intake; HIIT, high intensity interval training; MICT, moderate intensity continuous training; T0, before the intervention; T1, after the intervention; T2, after the follow-up period; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DRVs, dietary reference values; AI, adequate intake; AR, average requirement; PRI, population reference intake; RI, reference intake range for macronutrients; SDT, suggested dietary target.

Changes in macronutrient contribution to energy intake were reported graphically in **Figure 12** (**A** and **B**) and compared with the Italian DRVs. At T1 it was observed a general mild tendency to balance the macronutrient contribution to the energy intake in order to meet the DRVs and the composition of the proposed diet, except for PUFAs. Indeed, at T1 the mean PUFAs contribution to energy intake remained lower than the SDT in 75% of the subjects. Moreover, it significantly decreased trough time ($p=0.048$), as well as the linolenic acid intake ($p=0.009$).

On the contrary, the greatest improvement was found in total fats and SFAs contribution to energy intake in both HIIT and MICT groups. Total fats contribution to energy intake was significantly reduced from PRE to POST (-3.5±2.2% and -5.0±2.5%, in MICT and HIIT groups, respectively) even if it slightly increased from T1 to T2 (+0.8±1.2% and +1.9±3.1%) ($p=0.009$). Therefore, the intervention succeeded in reaching the RI in 62% and 52% of the subjects at T1 and T2, respectively, and in lowering the daily grams of fats consumed ($p=0.002$). SFAs and SFAs contribution to energy intake decreased ($p<0.001$ and $p=0.034$, respectively) but remained higher than the DRVs in 47% of the subjects at T1 and 43% at T2.

Soluble carbohydrates contribution to energy intake was constant for the whole period of the study, close to the SDT limit. Fibre intake in grams and in g/1000kcal did not change at T1, while sodium intake was significantly reduced ($p=0.008$) even if it remained higher than the SDT in 63% of the subjects at T1 and 86% at T2.

Finally, despite protein intake in grams and g/kg did not change at T1, a significant increase in protein contribution to energy intake ($p=0.003$) was observed in the present sample. Protein contribution to energy intake increased between T0 and T1 (+3.7±1.3 in the HIIT group and +3.3±1.2 in the MICT group) and partially decreased between T1 and T2 (-1.7±1.7 in the HIIT group and -1.4±2.1 in the MICT group), remaining higher than at T0.

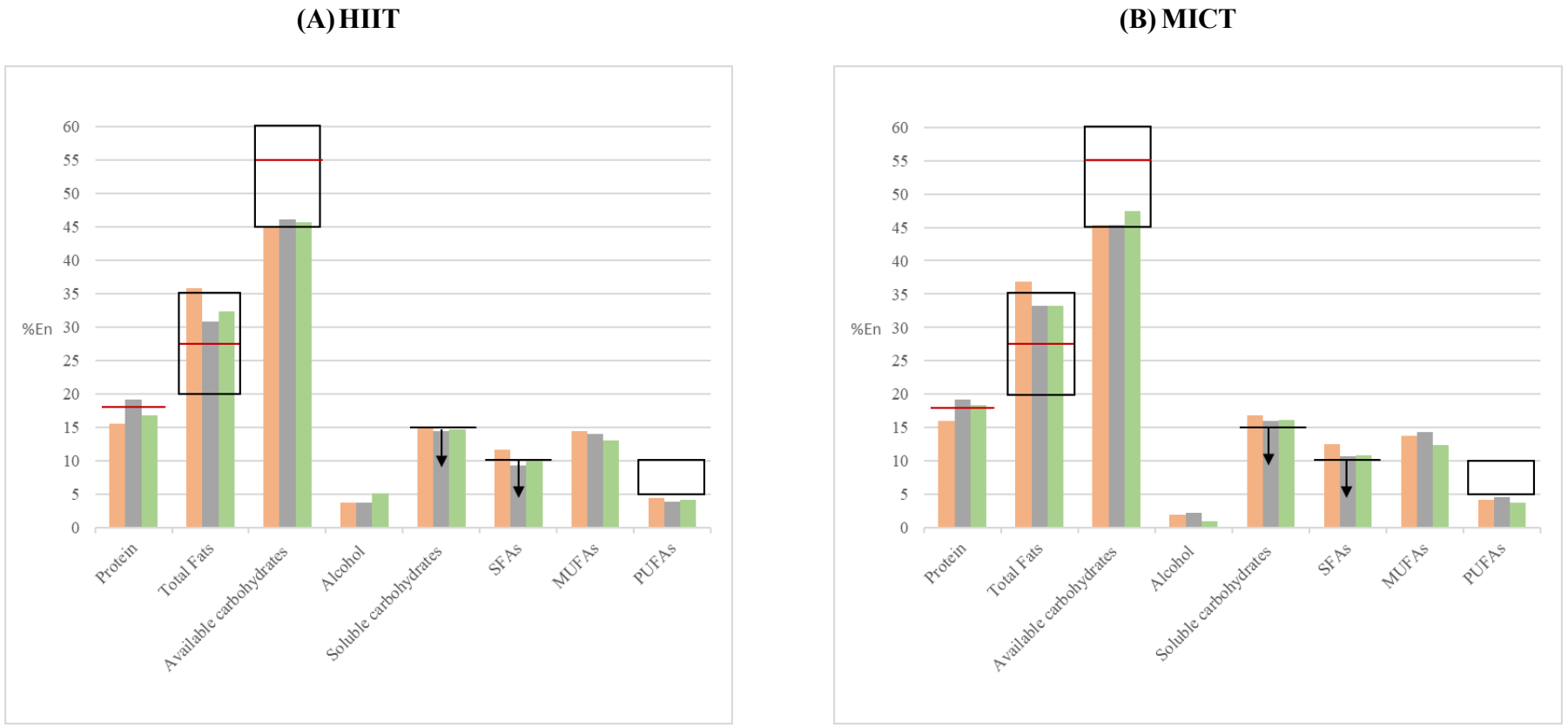


Figure 12. Macronutrient contribution to energy intake among obese people grouped by physical activity intervention [(A): HIIT; (B): MIICT] at each time the diet was evaluated (T0, ■; T1, ■; and T2, ■). *Data was compared with Italian dietary reference values (—) and the proposed diet (—). Abbreviations: HIIT, high intensity interval training; MICT, moderate intensity continuous training; T0, before the intervention; T1, after the intervention; T2, after the follow-up period; SFAs, saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids.*

CONCLUSIONS AND FCD-RELATED LIMITATIONS

In conclusion, the intervention succeeded in reducing body circumferences and body mass similarly in the two groups. Macronutrient intake at baseline were unbalanced through total fats, SFAs and soluble carbohydrates. Sodium intake was overall excessive, while fibre and PUFAs were generally inadequate. Dietary assessment showed that, despite some qualitative changes in the diet were observed, after the follow up period their energy intake increased again, as well as their total body weight. Interestingly, at the follow up visit we observed no changes in FM and an increased FFM, highlighting the beneficial effect of the present intervention in the medium term.

In this nutritional analysis, the major limitations derived from the dietary assessment method. Despite the food record is the gold standard, in the case of obese subjects it may lead to significant under- or misreporting. Moreover, those subjects consumed frequently manufactured and ready-to eat food products which are generally not included in the BDA (7.5% of the total food entries at T0; 23.4% of the food items at T0). For those products whose composition is not known, the nutrient composition reported in the NL was uploaded in the Microdiet software. However, for all these products micronutrient information was missing, thus not allowing to reliably estimate some of the analysed nutrient intakes: that may be the case of non-mandatory nutrients information (1169/2011 Reg UE, 2011) such as MUFAs, PUFAs, Linoleic acid, Linolenic acid, EPA, DHA, cholesterol, and eventually fibre.

4.1.3. CENTENARIAN'S DIET

Abstract presented at the 65° Italian Society of Gerontology and Geriatrics (SIGG) National Congress
Title: Valutazione della composizione dietetica in un gruppo di ultracentenari residenti in Lombardia
Authors: Azzolino D., Ferri E., Edefonti V., Parpinel M., Fiori F., Arosio B.
Online, December 2020

Fifteen subjects, of which 12 women, filled in the 3-dDR. Mean age and BMI were equal to 105±1 year and 22.3±3 kg/m^2, respectively (**Table 16**). Female/male ratio was 4:1 in the present population, while the national ratio has been reported to be 4.54:1 (Vasto et al., 2012).

Table 16. *Anthropometric and socio-educational characteristics (mean ±SD) of 15 centenarian subjects.*

Age (years)	105.0±1.5
Education (years)	8.3±4.7
Body mass index (kg/m^2)	22.3±2.79
Waist circumference (cm)	88.7±8.14
Arm circumference (cm)	22.0±3.7
Calf circumference (cm)	27.7±5.5

Table 17 shows the 25 most consumed foods and their frequency of consumption (i.e., number of entries) in a total of 42 days among the 3-dDR. The aggregated food diaries recorded the consumption of 174 different food items (971 total food entries). Foods consumed by the present sample were simple foods: milk, fruits and vegetables, potatoes, broth, beef, pasta, bread, rice and eggs. Extra virgin olive oil was the main dressing used (7.4% of the total food entries), followed by butter (2.6%). The most consumed beverages included, in order: water, coffee, red wine, and tea. Similarly, another study on a centenarian sample in Sicily reported a high intake of seasonal plant food and extra virgin olive oil and an infrequent intake of sweetened beverages and/or pre-packaged snacks (Vasto et al., 2012). However, contrarily to what found in other centenarian populations (Davinelli et al., 2012; Vasto et al., 2012) in our sample we observed frequent red meat and refined cereals intakes.

Table 17. *Number of entries of the most consumed foods among 42 days of record (971 total food entries).*

Food item	N entries
Extra virgin olive oil	72
Water	71
Coffee	39
Sugar (sucrose)	35
Whole milk	29
Butter	25
Zucchini	25
Carrot	23
Red wine	23
Cooked fruit, without sugar	22
Vegetal broth	21
Potatoes	21
Beef	20
Biscuits	18
Tea	18
Pasta	16
Onion	15
Bread	14
Rice	14
Grana cheese	12
Kiwi	12
Egg, whole	12
Celery	11
Apple	10
Spinach	10

Mean energy and nutrient intakes are reported in **Table 18**, and macronutrient contribution to energy intake is shown in **Figure 13**. Mean energy intake was low (1275±401 kcal/day), as reported in other studies (Davinelli et al., 2012; Vasto et al., 2012). Percentage contribution to energy intake of sugars, PUFAs, SFAs were inadequate in most subjects (80%, 87%, and 47%, respectively), compared to the Italian DRV (SINU, 2014). Moreover, mean cholesterol intake was below the SDT (<300 mg) but mean vitamin D and fibre intakes were far below the DRVs (20µg/day and 25g/day, respectively).

Table 18. *Energy and nutrient intakes (mean ±SD) compared with the Italian dietary reference values in a sample of 15 centenarian subjects.*

Energy (kcal/d)	1275±401
Energy (kJ/d)	5336±1679
Carbohydrates (g/d)	167.1±46.7
Sugars (g/d)	76.3±30.6
Protein (g/d)	55.1±14.0
Fats (g/d)	44.1±19.0
Cholesterol (mg/d)	223±124
Saturated fatty acids (g/d)	16.1±8.2
Monounsaturated fatty acids (g/d)	17.3±7.9
Polyunsaturated fatty acids (g/d)	5.3±2.2
Fibre (g/d)	15.3±6.0
Alcohol (g/d)	4.5±7.1
Vitamin D (µg /d)	1.7±2.3

Abbreviation: d, day.

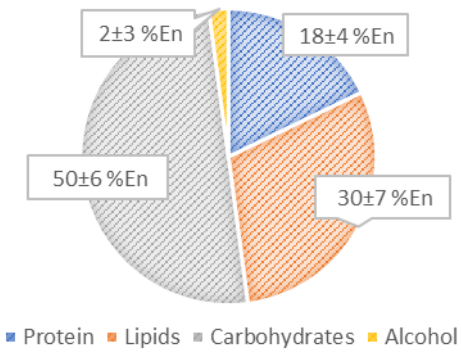


Figure 13. Percentage contribution on macronutrient to total energy intake.

CONCLUSIONS AND FCD-RELATED LIMITATIONS

In conclusion, the overall evaluation of the diet of the present sample of centenarian subjects showed a very repetitive food choice and a limited food variety, resulting in poor nutrient intakes. Moreover, their energy intake was generally low.

However, the nutritional analysis presents some limitations. It has to be considered that dietary records were generally filled in by caregivers, who may not be fully aware of the subject total food consumption. Moreover, the filling in of the food records was in most of the cases inaccurate, with lacking information on portion sizes and recipe preparations. In case of missing information, standard recipes and standard portion sizes were used, leading to possible biases. The poor quality of the 3-dDR made it necessary to restrict the nutritional assessment mainly to macronutrients.

An additional source of biases included missing data in the BDA. Moreover, the use of NLs for the conversion of complex commercial products (2.6% of the total entries; 5.7% of the food items) may have led to inaccurate estimates of a few macronutrients and/or underestimation of micronutrient intakes. Considering the entire list of entries, about 52% of missing values (50% considering the food list without repetitions) were found in the aminoacidic profile, 53% (52% without repetitions) in the single sugar profile, leading to the impossibility to reliably assess those intakes. Micronutrient and missing values ranged from 1.7% (1% without repetitions) to 86.2% (90% without repetitions), for sodium and vitamin K, respectively. Fatty acids ranged from 6.3% (3% without repetitions) of missing values for oleic acid, to 52.9% (42% without repetitions) for behenic acid.

4.1.4. BDA LIMITATIONS IN ASSESSING NUTRIENT INTAKE

A proper use of FCDBs, the selection of the most appropriate method to collect food intake, and a good software to analyse them, are key aspects in nutritional research (Willet, 2013). However, it is of fundamental importance to be aware of the methodological limitations to avoid inaccuracies in nutrient intake estimation and interpretation of the results. Indeed, in **chapter 4.1.1**, **4.1.2**, and **4.1.3** we observed that nutrient intake underestimations were possibly derived from non-modifiable methodological and subject-dependent biases, but also from FCD-related limitations.

From the BDA application, we observed that missing data may be crucial to reliably assess micronutrient intakes. Moreover, the lack of new manufactured products in the BDA may also represent an important bias leading to the possible underestimations. According to the European regulation (1169/2011 Reg UE, 2011) only 6 nutrients have to be reported in the NL. Thus, when frequently consumed, the use of NL information of manufactured products in nutritional research may lead to important nutrients underestimations. Indeed, nutritional research requires values for all nutrients and foods consumed by the population to avoid the possibility to treat missing values as zero, and the unsuitability of old FCD may substantially affect epidemiological results. Several authors have compared nutrient composition and/or intake estimated by means of different (old *vs.* updated or country-specific vs other country) databases, highlighting significant differences on various nutrients (Orešković et al., 2015; Parpinel et al., 2005; Probst and Mamet, 2016; Van Puyvelde et al., 2020; Westenbrink et al., 2012).

To avoid FCD-related biases there is the need to:

- Periodically update the BDA (and/or the study specific FCDB) compiling missing nutrient data, updating the composition of old foods, and including novel foods which can be easily accessible to the Italian population.
- Develop strategies and tools to reliably use food label information in nutritional research.

4.2. RESULTS FROM THE STANDARD UPDATE OF THE CEREAL, SUGARS, AND CEREAL-BASED BDA FOOD GROUPS

To address the constant changes in food consumption habits of the Italian population and in the availability of foods on the Italian market, the BDA (Gnagnarella et al., 2015) has been updated in the period 2018–2022 with the aim to reliably allow epidemiological analysis in the food and nutrition field.

The present update (BDA v.22) regarded the following food groups: "Cereal and cereal-based products", "Bread crispbread and rusks", "Sugar and confectionery", and "Cakes", further divided in 24 food categories (**Table 19**).

Table 19. *List of the updated BDA food groups and categories.*

CODE	FOOD GROUPS/FOOD CATEGORY
8	CEREAL AND CEREAL-BASED PRODUCTS
8001	cereals in grains
8002	cereal flours and starch
8003	cereal flakes, pop corns, bran (also in tablets), popped
8006	Pasta
8007	filled pasta ("ravioli" and "tortellini")
9	BREAD, CRISPBREAD, RUSKS
9001	grissini, crackers, salted snacks, "crostini"
9002	bread, toasted bread, pizza, focaccia, bread and pizza dough
14	SUGAR AND CONFECTIONERY
14000	Nougat
14001	chocolate candies, chocolate bars and spreads
14002	candied fruits
14003	sugar and honey
14004	candies, liquorice, sugar-coated almonds
14005	jams, marmalade
14006	ice creams, ice pops
14007	artificial sweeteners
14008	Syrups
15	CAKES
15001	Snack cakes, brioches, tarts (no filling)
15002	melba toasts
15003	puddings, spoon-desserts
15004	pastries (excluding dry pastries)
15006	cakes and cake-mixes
15007	cookies, biscuits and dry pastries
15008	Snack cakes, brioches, tarts, doughnuts (filled or coated)
15009	chocolate based snacks (e.g., "*mars", "kit-kat"*)

Such food groups included food items in their 1998 version (BDA v.98), whose composition data may be outdated and not representative of the actual food composition. Furthermore, BDA v.98 food items presented missing data for all the nutrient components introduced in the 2008

(BDA v.08) and 2015 (BDA v.15) versions. **Table 20** shows the different number of food components comparing the BDA v.98 and BDA v.22.

Table 20. *Difference [N (%)] between food components included in the BDA v.98 and in the current update (BDA v.22).*

Food components	1998	2022	Difference	Added food components
Edible part	1	1	-	
Energy	2	4	2 (100%)	Energy, recalculated with fibre (kcal and kJ)
Macronutrients	13	13	-	
Minerals/trace elements	6	13	7 (117%)	Mg, Cu, Se, Cl, I, Mn, S
Vitamins	11	15	4 (36%)	Pantothenic acid, biotin, vit. B12, vit. K
Fatty acids	7	21	14 (200%)	Sum of C 4:00 to C 10:00 fatty acids, lauric, myristoleic, palmitic, stearic, arachidic, behenic, myristoleic, palmitoleic, eicosanoid, erucic, arachidonic acids, EPA, DHA
Aminoacids	-	18	18	Tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline, serine
Soluble Carbohydrates	-	6	6	Glucose, fructose, galactose, saccharose, maltose, lactose
Total	**40**	**91**	**51 (128%)**	

Energy calculations were performed considering also fibre content of foods. Moreover, added food components were mainly micronutrients (7 minerals and 4 vitamins) and fatty acids (14). Aminoacidic (18) and soluble sugars (6) profiles were newly introduced for the present food groups.

As a result, the update regarded a total number of 283 food items, of which 141 (~50%) were newly added items (**Table 21**), mainly derived from Italian sources or label-based recipes calculations. The food category for which major changes were applied were: “Cereals in grains” (+209% items, compared to the BDA v.98), “Ice creams, ice pops” (+240% items), “Syrups” (+200% items), “Cookies, biscuits and dry pastries” (+209% items), “Snack cakes, brioches, tarts (no filling)” (+167% items), “Snack cakes, brioches, tarts, doughnuts (filled or coated)” (+200% items). The category “Chocolate-based snacks (e.g., mars, kit-kat)” was newly created including 6 items. The great change in the number of items is suggestive of the significant change in the Italian food market occurred in the past decades. New foods have been proposed on the national market, particularly novel manufactured branded products, and/or food products and preparations borrowed from different cultures.

Table 21. *List of food categories, number of items present in the published BDA v.98 and in the current updated version (BDA v.22), newly added items [N (%)].*

Code	Food category: English name	1998	2022	New Items
8001	Cereals in grains	11	34	23 (209%)
8002	Cereal flours and starch	15	24	9 (60%)
8003	Cereal flakes, pop corns, bran (also in tablets), popped	12	17	5 (42%)
8006	Pasta	4	10	6 (150%)
8007	Filled pasta ("ravioli" and "tortellini")	2	5	3 (150%)
9001	Grissini, crackers, salted snacks, "crostini"	9	16	7 (78%)
9002	Bread, toasted bread, pizza, focaccia, bread and pizza dough	20	32	12 (60%)
14000	Nougat	1	1	-
14001	Chocolate candies, chocolate bars and spreads	6	14	8 (133%)
14002	Candied fruits	3	6	3 (100%)
14003	Sugar and honey	3	4	1 (33%)
14004	Candies, liquorice, sugar-coated almonds	7	10	3 (43%)
14005	Jams, marmalade	7	5*	3 (43%)
14006	Ice creams, ice pops	5	15†	12 (240%)
14007	Artificial sweeteners	4	7	3 (75%)
14008	Syrups	2	6	4 (200%)
15001	Snack cakes, brioches, tarts (no filling)	3	7‡	5 (167%)
15002	Melba toasts	5	5	-
15003	Puddings, spoon-desserts	5	5	-
15004	Pastries (excluding dry pastries)	4	5	1 (25%)
15006	Cakes and cake-mixes	7	12	5 (71%)
15007	Cookies, biscuits and dry pastries	11	23	12 (109%)
15008	Snack cakes, brioches, tarts, doughnuts (filled or coated)	5	14‡	10 (200%)
15009	Chocolate based snacks (e.g., "*mars", "kit-kat*")	0	6	6
	Total	**151**	**283**	**141 (93%)**

*5 food items from the BDA v.98 were deleted; †2 food items from the BDA v.98 were deleted; ‡1 food item from the BDA v.98 was deleted.

The literature sources used to update macronutrient data from the given food categories are presented in **Figure 14**, in comparison with those used in the BDA v.98. The main difference was the increased use of manufactured food label data instead of national and international data from food composition tables or FCDBs. This decision was taken to ensure the representativity of FCD included in the BDA to the actual food market offer. Indeed, most macronutrient data reported in the Italian and UK sources were found to be out of date and/or not in line with the mean value from the NLs found on the current Italian market.

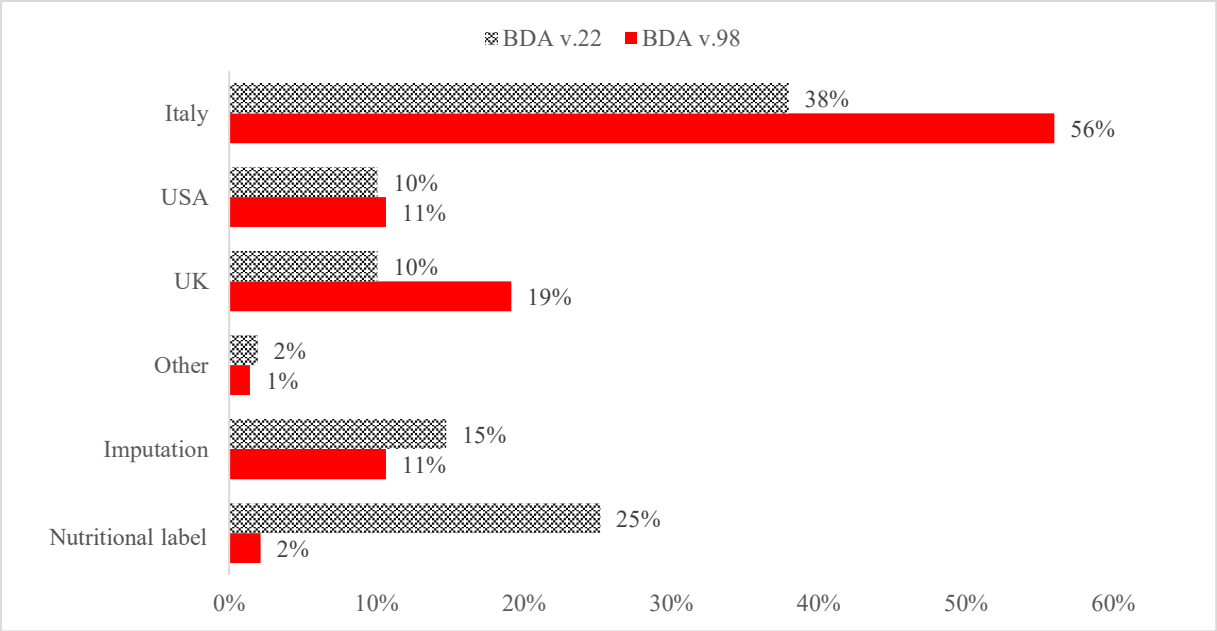


Figure 14. Different sources used to compile macronutrient data in the BDA v.98 and BDAv.22 for the food groups "Cereal and cereal based products", "Bread, crispbread, rusks", "Sugar and confectionery" and Cakes". *A total of 25 food items of the BDA v.22 were not considered in this analysis because their data are still in phase 2 of the update standard protocol (i.e., not yet uploaded in the Microsoft Access database). The corresponding food items in the BDA v.98 were also excluded from the analysis, as applicable (N=10).*

To give an example, the food category "Ice creams and ice-pops", which contained only 5 food items in its 1998 version, was mainly updated using the label-based recipe approach. Due to the lack of Italian FCD regarding food items of this food category, in the BDA v.22, 60% of the food components were calculated based on food label information (53%) or derived from standard recipes (7%), and 40% were borrowed from French data (ANSES, 2020). Also, the snack cakes (code: 15001 and 15008) and the jams (code: 14005) changed substantially due to the variations in the product availability in the Italian food market. The items: "cakes, commercially prepared, sponge cake type"; "mini cakes, commercially prepared, filled"; "jam (apricot, fig, quince, peach, plum)"; "jam (sour cherry, cherry, grape)"; "jam, orange; jam, apricot"; "jam, plums"; were excluded from the final food list in BDA v.22. Commercially prepared tarts, muffins, manufactured brioches, and "*krapfen"* were added in the snack cakes food category, as well as different items representing reduced sugar, or artificially sweetened jams for the jam and marmalade category.

Another important update regarded the "Cereal in grains" category, which represents a crucial improvement in the BDA. Particularly, for "Cereal in grains", additional cereals and pseudocereals were included in their raw and cooked form: "rice, red; "rice, white, basmati"; "emmer wheat"; "buckwheat"; "millet; "millet, whole";" rice, type *venere*"; "teff"; "spelt"; "rye"; "kamut"; "quinoa"; "amaranth"; and existing BDA v.98 cereals were included in their cooked form: "rice, white polished, cooked"; "rice brown, cooked"; "rice, white parboiled, cooked"; "barley, pearl, cooked". Indeed, an increased interest in alternative cereals has been raising in the general population, as well as their marketing potential from the manufacturers' point of view, leading to an increasing number of alternative cereal-based foods available on the Italian market.

When comparing nutrient values of the 4 main food groups considered for the present update between the two versions of the BDA, we observed similar macronutrient composition, except for

sodium, total fats and SFAs (**Figure 15, A, B,** and **C,** respectively). This indicates a general trend in lowering added salt in composite sweet foods (mainly in the "Bread, crispbread and rusks" food group), similarly to what found by previous authors (Santos et al., 2019). On the other hand, total fats and SFAs content increased, particularly in the "Cakes" and "Sugar and confectionery" food groups).

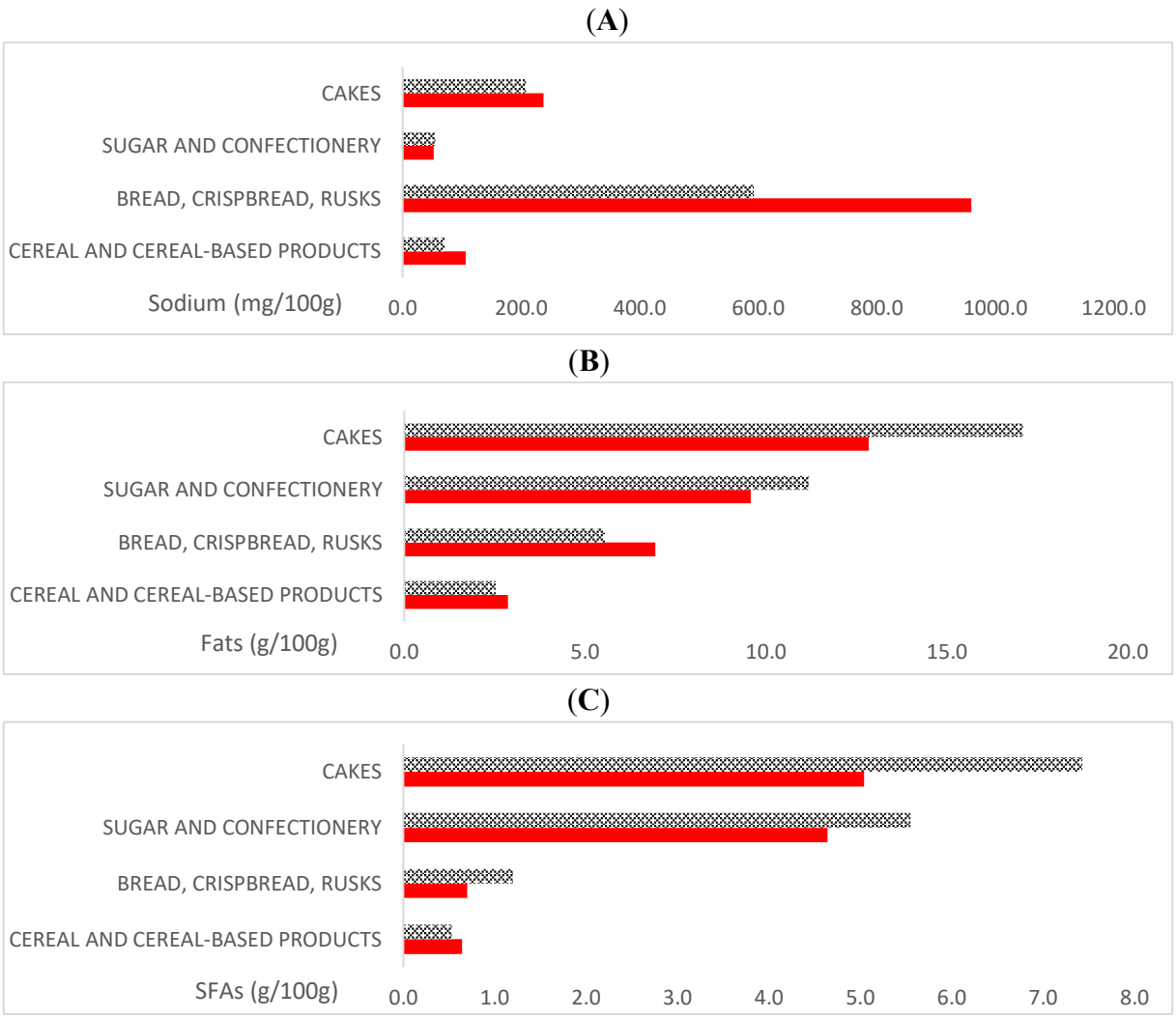


Figure 15. Mean content of Sodium (A), Fats (B), and SFAs (C) in the food groups: "Cereal and cereal-based products", "Bread, crispbread, rusk", Sugar and Confectionery" and "Cakes" in the 2 versions of the BDA (▨ v.22 and ■ v.98). *A total of 25 food items of the BDA v.22 were not considered in this analysis because their data are still in phase 2 of the update standard protocol (i.e., not yet uploaded in the Microsoft Access database). The corresponding food items in the BDA v.98 were also excluded from the analysis, as applicable (N=10). Abbreviation: SFAs, saturated fatty acids.*

In conclusion, the composition and metadata of the 151 BDA v.98 pre-existent food items were updated among the cereals, sugars, and cereal-based BDA food groups. Moreover, the main features of the BDA v.22 were the inclusion of 51 (+128%) food components and 141 (+93%) new food items. Despite the great expansion of the database, no missing data were left among the final food list. This aspect remains the main aim of the BDA, as well as the traceability of the included data to facilitate their application in nutritional epidemiology (Gnagnarella et al., 2004). Data from the present update will be published online to be fully available to users by the end of 2022.

4.3. GLUTEN FREE FOOD COMPOSITION DATABASE

4.3.1 RESULTS FROM THE UPDATE

From an initial screening, we found that most products included in the first version of the database (Mazzeo et al., 2015) were outdated: some of them were no more available in the market, and others have a different formulation. A total of 630 branded products were included in the GF-FCDB, aggregated in 110 food items. Three old food items were deleted because no comparable product was found on the market, and 23 new food items were added. The full list of GF food items with their Italian names, their codes, number of included food products for each item, water source, and RF use are listed in **Table 22**.

Table 22. *Food item codes, names and additional information relative to the compiling procedures. Food names are listed in their Italian version.*

Food code	OLD Food name (Italian version)	NEW food name (Italian version)	FC code	N products	Water	RF
21	*Cous Cous SG*		8002	6	D	
22	*Salatini SG*		9001	2	D	yes
23	*Grissini SG*		9001	13	D	yes
24	*Crackers salati SG*		9001	9	D	yes
26	*Fette di cereali aggregati SG*		15002	9	D	yes
27	*Mini cracker SG*		9001	6	D	yes
28	*Bocconcini e crostini SG*		9001	7	D	yes
29	*Taralli SG*		9001	4	D	yes
36	*Pane grattugiato SG*		9002	9	D	yes
37	*Pan carré comune SG*		9002	3	A	yes
38	*Pane alle olive SG*		9002	2	D	yes
39	*Pane comune a fette SG*		9002	15	A	yes
40	*Farina SG*		8002	7	D	
41	*Farina per dolci, mix per dolci, SG*		8002	9	D	
42	*Farina, mix pane-pizza, SG*		8002	8	D	
43	*Pane integrale SG*		9002	4	A	yes
44	*Panino, rosetta, SG*	*Pane, tipo rosetta, tartaruga, roll*	9002	6	A	yes
45	*Panini all'olio SG*		9002	7	D	yes
46	*Ciabatte-baguette SG*	*Pane, tipo ciabatta, baguette, sfilatino*	9002	7	A	yes
47	*Piadina SG*		9002	6	A	yes
48	*Focaccia SG*		9002	4	A	yes
50	*Biscotti per la colazione SG*	*Biscotti secchi per la colazione*	15007	8	A	yes
52	*Farina per pasta, mix, SG*		8006	4	D	
55	*Pasta di mais SG*		8006	5	D	
56	*Pasta di riso SG*		8006	3	D	
57	*Pasta integrale SG*		8006	6	D	
58	*Pastina, da brodo, SG*		8006	6	D	
59	*Pasta all'uovo, fresca, SG*		8006	3	D	
60	*Pasta all'uovo, secca, SG*		8006	5	D	
61	*Tortellini alla carne SG*		8007	6	A	
62	*Ravioli di carne SG*		8007	4	A	
63	*Pizza con pomodoro e mozzarella SG*		9002	6	A	
64	*Calzone SG*		9002	2	D	
65	*Pasta, base per pizza SG*	*Pasta base per pizza, precotta*	9002	6	A	yes
66	*Ravioli ricotta e spinaci SG*		8007	10	A	
73	*Filetti di pesce panati SG*		11001	5	D	

81	*Lasagne alla bolognese SG*		8007	4	D	
83	*Wafer alla vaniglia SG*		15007	3	A	yes
84	*Wafer al cioccolato SG*		15007	5	A	yes
85	*Wafer alla nocciola SG*		15007	4	A	yes
86	*Wafer ricoperti al cioccolato SG*		15007	5	D	yes
87	*Cantucci SG*		15007	4	D	yes
90	*Biscotti al cioccolato SG*		15007	21	D	yes
91	*Biscotti ripieni SG*	*Biscotti farciti SG*	15007	14	D	yes
92	*Frollini semplici SG*	*Biscotti frollini semplici SG*	15007	21	A	yes
93	*Biscotti ricoperti di cioccolato SG*		15007	12	D	yes
94	*Savoiardi SG*		15007	5	A	yes
97	*Biscotti da te SG*		15007	6	D	yes
98	*Fette biscottate SG*		15002	7	D	yes
99	*Muesli SG*		8003	6	D	
102	*Petto di pollo impanato SG*		10050	4	D	
111	*Canestrelli G*		15007	4	D	yes
112	*Biscotti al cocco SG*		15007	4	D	yes
113	*Biscotti con marmellata SG*		15007	7	D	yes
114	*Biscotti integrali SG*		15007	6	A	yes
115	*Preparato per zuppe SG*		28002	7	D	
116	*Pasta, con condimento, SG*	*Pasta al pomodoro SG*	8007	3	D	
383	*Gnocchi SG*		1001	10	D	
2022	*Panettone, tradizionale SG*		15001	8	A	yes
2023	*Pandoro SG*		15001	8	A	yes
2024	*Pan brioche SG*		15001	2	D	yes
2025	*Brioche, vuote, SG*		15001	3	A	yes
2026	*Brioche, con marmellata, SG*		15008	4	A	yes
2028	*Merendine, al cioccolato, SG*		15008	14	A	yes
2029	*Merendine, con marmellata, SG*		15008	7	A	yes
2030	*Merendine, cannolo, SG*		15008	4	D	yes
2031	*Pan di Spagna SG*		15006	2	D	yes
2032	*Pasta sfoglia SG*		15006	2	D	yes
2033	*Torta Margherita SG*		15006	4	D	yes
2034	*Muffin SG*		15001	2	A	yes
2035	*Plum cake SG*		15001	7	A	yes
2036	*Plum cake, al cioccolato, SG*		15008	7	D	yes
2037	*Brioche, al cioccolato, SG*		15008	6	A	yes
2038	*Panettone, al cioccolato SG*		15001	5	A	yes
2039	*Merendine, pan di spagna SG*	*Merendine, non farcite SG*	15001	6	A	yes
2040	*Pane rustico, con semi SG*		9002	11	A	yes

2042	*Torta al cioccolato SG*		15006	2	D	yes
2044	*Crostata cioccolato e nocciole SG*		15001	5	A	yes
2045	*Muffin, al cioccolato SG*		15008	7	A	yes
2046	*Colomba SG*		15001	11	A	yes
2048	*Crostata marmellata SG*		15001	6	A	yes
9048	*Tiramisù SG*		15006	2	D	
19033	*Gelato, biscotto, SG*		14006	4	D	
19034	*Gelato, cono, SG*		14006	3	D	
65100	*Sofficini al formaggio, surgelati SG*		28009	2	D	
999058	*Barrette di cereali, al cioccolato SG*	*Barrette di cereali e cioccolato SG*	15009	5	D	
999059	*Barrette di cereali SG*	*Barrette di cereali e frutta secca SG*	15009	3	D	
n01		*Farina mix rustico SG*	8002	2	D	
n02		*Pasta di cereali misti e legumi SG*	8006	4	D	
n03		*Pasta di legumi SG*	8006	12	D	
n04		*Pasta multicereali SG*	8006	11	D	
n05		*Pasta di grano saraceno SG*	8006	2	D	
n08		*Pasta pronta al pesto SG*	8007	2	D	
n09		*Ravioli vegetariani, freschi SG*	8007	10	D	
n13		*Pane per hamburger e hot dog SG*	9002	5	A	yes
n14		*Grissini integrali SG*	9001	3	D	yes
n15		*Snack ai cereali e formaggio SG*	9001	5	D	yes
n16		*Friselle SG*	9001	2	A	yes
n17		*Crackers integrali SG*	9001	4	D	yes
n18		*Piadina integrale SG*	9002	2	A	yes
n19		*Tortilla wrap SG*	9002	4	A	yes
n20		*Pagnotta intera SG*	9002	1	D	yes
n21		*Cialda o cono per gelato SG*	15007	2	D	yes
n22		*Waffle SG*	15008	1	D	yes
n23		*Fette biscottate integrali SG*	15002	4	D	yes
n24		*Merendine, al latte SG*	15008	3	A	yes
n25		*Merendine, senza zuccheri aggiunti SG*	15008	6	A	yes
n26		*Cantucci al cioccolato SG*	15007	2	D	yes
n27		*Muffin, ripieno alla frutta SG*	15008	4	A	yes
n28		*Barrette ricoperte al cioccolato ripiene SG*	15009	3	D	

Abbreviations: D, calculated by difference; A, analytical; FC: food category.

Table 23 A, and **B** shows the mean macronutrient composition of GF products clustered in food groups. Due to the compiling methodology, the database does not present any missing data and presents values for 86 food components. Food groups with the greatest variability in their energy and nutrient content were "Miscellaneous ready-to eat-products", that include different product types, such as cereal soups, breaded fish, breaded cheese and breaded chicken, "Filled pasta, ready-to-eat pasta and gnocchi" which included both meat and vegetal fillings, and "Breakfast products" that include melba toast and muesli. Moreover, also more homogeneous food groups such as "Pasta" and "Bread and substitutes" present some variability. Particularly, pastas showed a high protein value with surprisingly high variability (9.3±4.7). Fat content were particularly high in "Savoury snacks" (12.5±5.6), and in all sweet products excluding breakfast products, ranging from 15.0±2.8 ("Ice-creams") to 19.0±7.4 ("Biscuits"). Sodium and fibre were very variable in each food group mostly depending on the brand and product type.

Table 23.A. *Macronutrient composition (per 100g of food) among the food groups: "Flour", "Pasta", "Filled pasta, ready-to-eat pasta, gnocchi", "Savoury snacks", "Bread and substitutes", "Pizza", and "Miscellaneous ready-to eat dishes".*

	Flour (N=6)	Pasta (N=10)	Filled pasta, ready-to-eat pasta, gnocchi (N=8)	Savoury snacks (N=10)	Bread and substitutes (N=15)	Pizza (N=2)	Miscellaneous ready-to-eat dishes (N=4)
Energy (kJ)	1411±40	1393±123	963±317	1797±144	1185±143	907±50	993±316
Energy /kcal)	331±9	328±28	228±75	427±35	281±33	215±11	235±74
Protein (g)	4.6±3.1	9.3±4.7	6.7±3.3	3.9±1.7	3.5±0.6	6.5±0.3	11.2±3.6
Fats (g)	1.1±0.7	2.6±1.0	6.5±4.6	12.5±5.6	6.5±1.4	8.2±0.0	8.0±4.0
Cholesterol (mg)	0.1±0.1	16.7±35.2	78.9±79.5	5.2±5.3	0.2±0.7	26.5±23.8	26.7±17.9
Available carbohydrates (g)	78.3±5.4	69.1±11.6	36.6±14.4	72.1±4.5	49.1±7.3	29.3±2.5	29.9±21.5
Starch (g)	74. 0±5.0	68.4±11.8	35.1±14.0	68.6±3.8	45.7±8.1	27.0±2.3	27.6±20.9
Soluble carbohydrates (g)	4.2±3.5	0.7±0.5	1.5±1.2	3.5±2.5	3.4±1.5	2.2±0.2	2.2±1.9
Fibre (g)	4.5±2.2	3.7±2.2	2.3±1.1	4.8±2.1	6.0±1.6	2.7±0.2	2.9±3.5
Water (g)	10.4±1.3	14. 3±8.0	46.1±17.7	4.0±2.3	32.8±7.7	51.6±3.4	46.0±24.1
Sodium (mg)	203±180	35±62	378±232	917±285	634±157	397±137	342±225
Saturated fatty acids (g)	0.3±0.1	0.6±0.3	2.4±1.8	2.7±1.4	1.3±0.5	3.9±0.2	1.5±0.8
Monounsaturated fatty acids (g)	0.2±0.1	0.8±0.5	2.3±1.8	5.2±2.6	3.0±1.1	3.4±0.4	2.9±1.8
Polyunsaturated fatty acids (g)	0.5±.3	0.8±0.2	1.1±0.7	3.8±2.5	2.0±0.6	1.4±0.4	3.0±2.2

Data are expressed as mean±SD.

Table 23.B. *Macronutrient composition (per 100g of food) among the food groups: "Ice-cream", "Cakes and desserts", "Breakfast products", "Biscuits", and "Sweet snack bars".*

	Ice cream (N=2)	Cakes and desserts (N=28)	Breakfast products (N=4)	Biscuits (N=18)	Sweet snack bars (N=3)
Energy (kJ)	1285±11	1557±244	1630±61	1915±184	1843±263
Energy (kcal)	306±3	371±58	386±15	456±45	439±64
Protein (g)	3.7±0.3	4.3±0.9	6.7±2.9	4.9±1.6	6.6±1.2
Fats (g)	15.0±2.8	17.3±5.4	8.0±4.9	19.0±7.4	18.5±9.3
Cholesterol (mg)	5.8±0.7	71.4±42.1	0.1±0.1	39.5±42.0	2.2±2.2
Available carbohydrates (g)	40.6±6.1	50.3±9.5	70.3±8.4	67.9±6.0	62.4±5.8
Starch (g)	17.1±5.8	27.4±8.0	62.2±16.5	40.8±10.5	26.6±8.0
Soluble carbohydrates (g)	23.5±0.2	22.8±9.4	8.1±8.1	27.0±6.4	35.7±5.7
Fibre (g)	1.9±1.2	3.1±1.4	6.5±1.0	3.1±1.3	5.4±3.7
Water (g)	38.0±2.4	23.7±10.1	6.5±3.8	3.9±3.5	5.9±3.5
Sodium (mg)	83±30.4	218±91.1	423±211.9	171±74.1	137±50.1
Saturated fatty acids (g)	10.5±1.3	6.2±3.4	1.9±1.6	9.7±6.3	7.9±5.0
Monounsaturated fatty acids (g)	3.3±0.4	5.6±2.0	2.9±2.0	5.7±2.6	7.2±3.7
Polyunsaturated fatty acids (g)	1.0±0.0	4.6±2.9	2.6±1.3	2.7±1.3	2.6±1.2

Data are expressed as mean±SD.

Since the GF market is fast changing, the composition of GF foods has to be updated periodically. Compared with data from the first version of the Italian GF-FCDB (Mazzeo et al., 2015), the greatest changes were found in the protein content of flours, pasta, biscuits, and breads and substitutes, and in the fibre content of pasta and savoury snacks, which were found to be higher in 2020-2021. That was probably due to the increased presence in the GF market of products labelled as "wholegrain". However, as observed by other authors, most of GF foods showed high energy density, and content of fats, SFAs, sugar and salt (Fajardo et al., 2020).

The present comprehensive FCD may be very useful to allow an assessment of the dietary habits of coeliac patients. The evaluation of the nutritional adequacy of those patients is, in fact, very challenging. To date, several studies have been carried out on the macronutrient adequacy of coeliac patients' diet, reporting energy and fat intakes comparable or lower than those of the general population and lower fibre intake (Mazzeo et al., 2015). A recent review on GF diets showed key inadequacies: several mineral and vitamin deficiencies were commonly found in coeliac patients before, but also during adherence to the GF diet treatment (Melini and Melini, 2019). Population studies highlighted that GF diets were generally found to be ineffective in resolving the mineral and vitamin deficiencies observed at diagnosis (Shepherd and Gibson, 2013; Sue et al., 2018). During adherence to GF diets, several patients were found to have an inadequate intake of fat, sodium and vitamins, while protein intake is controversial, but a trend towards some improvement in GF diets has emerged with more adequate levels of fibre and sugars that in the past (Melini and Melini, 2019). Another Italian study on coeliac disease children underlined that they may be at risk of consuming too much fat and insufficient fibre, iron, vitamin D, and calcium. Moreover, their intake of folate, magnesium, zinc, and foods with a high glycaemic index has been found to be significantly altered (Di Nardo et al., 2019). However, results are overall inconsistent,

and the evaluation of micronutrient adequacy was rarely comprehensive. Micronutrient intakes in previous research was found to be very variable. The discrepancies among different studies may be partially due to missing data and inadequate FCDBs.

On the contrary the approach proposed in the present work and the use of standardised and well-documented procedures allowed us to obtain a reliable database representative of GF food items available on the Italian market for its use in nutritional research. Even if some limitations have to be stated, such as the lack of chemical analysis on macro- and micronutrient —which are the gold standard to estimate the nutrient composition of foods—, and the impossibility to assess accuracy and transparency of the nutritional declaration provided by the manufacturers (Traka et al., 2020), the present database included a large sample of branded products from multiple brands and provide a comprehensive nutrient composition derived from standardised calculation procedures. Furthermore, in the literature there is a limited knowledge on the micronutrient composition of GF foods (Rybicka, 2018) and the present database is the only one representing the composition of GF products available in Italy. Indeed, GF -FCDBs generally collect only NL information, without imputing micronutrient composition (Babio et al., 2020; Fajardo et al., 2020; Lasa et al., 2019). Only Missabach and colleagues (Missbach et al., 2015) imputed micronutrient values using the recipe approach, and few FCDBs included analytical values for a limited list (mainly bread) of GF products (ANSES, 2020; Norwegian Food Safety Authority, 2020; RIVM, 2021; USDA, 2018).

In conclusion, despite its limitations, the present database provides a comprehensive overview of the macro- and micronutrient composition of a set of 110 GF manufactured products present in the Italian market. The updated GF-FCDB may be extremely useful to assess the micronutrient adequacy of the diet of Italian coeliac disease population in further dietary assessment studies.

4.3.2. WATER CONTENT OF MANUFACTURED PRODUCTS

Moisture determination on 93 GC products and 88 corresponding GF foods showed a general incoherence with data calculated by difference from NLs ($p<0.001$; **Table 24**). This may be due to the methodologies used by manufacturer to derive the nutritional declaration. Indeed, manufacturer may choose to declare nutrient values derived from calculation procedures from national or international databases, without documenting any choice and calculation method (Pennington, 2008; Traka et al., 2020). The foods analysed in this work were mainly from large companies/brand leaders, and the reliability of smaller companies' NLs remains unknown. However, the amount of the observed difference was found to be minimal (-2.2±3.3g/100g), slightly affecting the overall macronutrient accuracy of the NL. This is reassuring, since the considerable increase in the past decades in manufactured complex foods available on the market, and the great use of food label data in nutritional research (Carter et al., 2016; Fajardo et al., 2020; Lasa et al., 2019).

Table 24. *Comparison between analytical and calculated water content in a sample of 181 gluten free and gluten containing products.*

	Mean±SD	**Median (25^{th}–75^{th})**	**Min**	**Max**	**p-value**
Moisture determination (g/100g)	22.5±12.6	22.1(14.7–31.6)	0.5	47.4	
Water calculated from NL (g/100g)	24.6±13.2	23.7(17.3–34.5)	1.0	49.1	
Difference (MD-WCNL) (g/100g)	-2.2±3.3	-1.8(-3.4 – -0.7)	-17.5	6.1	<0.001

Abbreviations: MD, moisture determination; WCNL: water calculated from NL.

We also observed a general higher water content in water calculations from NL then the analytical value, meaning that macronutrient composition may be underestimated in the label. The maximum overestimation of water was observed in a GC white sliced pre-packaged bread (-17.5 g/100g) and in 2 GF pre-packaged chocolate cakes from minor brands (-17.1 g/100g and -12.8 g/100g). An underestimation of water greater than 2 g/100g was observed only in GF products: 4 GF breads and croissants showed a difference greater than 5 g/100g. Water content is very important in FCD because variation in water content is the main determinant of the content of other components. Data on water content makes it possible to compare nutrient values of different foods. Water content information is also essential when comparing data from different sources, or when different analytical methods are applied (Greenfield and Southgate, 2003).

When comparing the differences in calculated and analysed water values, no significant differences were observed between GF and GC products, meaning that the lack of accuracy in NLs is similarly observed in GF and GC manufactured foods (data not shown). Furthermore, the analyses showed that GF products had generally a greater water content than the corresponding GC products (**Table 25**). The only GF food items with suggestively higher water values that their GC counterparts were muffins (-13 g/100g to -15 g/100g). On the other hand, GF and GC biscuits and cakes had comparable mean water contents. Water content of GF products may be different from traditional GC foods due to their specific formulation. GF foods may be richer of additives, water and hydrocolloids to compensate the lack of the gluten structure (Cappa et al., 2013;

Mancebo et al., 2015). Thus, borrowing water values from their GC counterparts may be risky when compiling a FCDB.

The current analysis showed that the difference between GF and GC "Filled-pasta", "Biscuits", and "Cakes and desserts" food groups were not statistically significant. However, GF and GC products present significantly different water content in the "Bread and substitutes" food group ($p=0.0156$; see **chapter 4.3.3**). Indeed, the presence of gluten is considered fundamental for successful breadmaking, and innovative technologies have been recently studied. On the other hand, it is easier to produce GF biscuits than GF bread. Compared to bread, gluten plays a minor role in biscuits so a wider variety of flours might be employed without particular concerns (Di Cairano et al., 2018). In breads, the use of GF ingredients markedly changes the rheological behaviour of the dough, which may result in different processing performance and post-baking quality. Generally, GF bread tends to have poor texture, low nutritional value, reduced mouthfeel and flavour, as well as a shorter shelf-life (Conte et al., 2019). Moreover, water required to breadmaking may vary accordingly to the type of flour used; corn flour required the largest amount of water (120%), while, rice flour required the lowest (80%). Hydration levels of gluten-free dough/batter are important determinants of the viscoelastic behaviour and rheological characteristics of the resulting bread (Morreale et al., 2018). Bread is in fact one of the most challenging manufactured product to formulate without the support of the gluten structure.

Table 25. *Comparison between analytical water content of gluten free (GF) and gluten containing (GC) food types.*

	GF water Mean±SD	GC water Mean±SD	GF-GC
BREAD AND SUBSTITUTES			
Sandwich bread (g/100g)	40.1±4.4	33.7±3.5	6.5
Sliced bread (g/100g)	37.2±5.1	30.9±1.1	6.3
Bread, wholegrain (g/100g)	37.2±4.1	32.1±1.2	5.1
Bread, *"rosetta"* (g/100g)	36.8±2.3	36.2±1.1	0.6
Bread, buns (g/100g)	33.5±1.5	31.5±3.9	2.0
Bread, hamburger (g/100g)	39.6±4.2	29.8±1.6	9.8
Pizza base, dry (g/100g)	27.3±3.6	31.5±0.5	-4.2
"Focaccia" (g/100g)	31.0±3.4	26.7±0.8	4.2
Pizza, tomato and mozzarella (g/100g)	49.2±1.5	47.4±2.1	2.2
"*Piadina"* (g/100g)	29.5±5.6	25.7±2.0	3.8
FILLED PASTA			
Filled pasta, meat filling, dry (g/100g)	28.7±0.2	8.3±0.1	20.4
Filled pasta, meat filling, refrigerated (g/100g)	33.7±0.6	31.9±0.8	1.8
Filled pasta, spinach &ricotta, dry (g/100g)	31.3±2.4		
Filled pasta, spinach &ricotta, refrigerated (g/100g)	50.4±0.9	39.3±1.2	11.1
BISCUITS			
Breakfast biscuits (g/100g)	0.9±0.1	1.5±0.2	-0.6
Biscuits, *"frollini"* (g/100g)	2.3±1.5	1.6±0.4	0.7
Wholegrain biscuits (g/100g)	2.0±1.2	2.1±0.6	-0.1
Vanilla Wafer (g/100g)	0.7±0.0	0.9±0.3	-0.2
Hazelnut Wafer (g/100g)	1.9±0.6	0.9±0.5	1.0
"Savoiardi" (g/100g)	4.8±1.0	7.2±0.6	-2.4
CAKES AND DESSERTS			
Croissant, plain (g/100g)	27.9±3.2	19.7±1.6	8.2
Croissant, chocolate (g/100g)	31.1±0.9	21.7±0.5	9.4
Croissant, fruit jam (g/100g)	33.4±6.9	21.7±3.0	11.7
Croissant, cream (g/100g)		21.8±0.5	
Muffin, cocoa (g/100g)	14.6±5.2	18.5±0.5	-13.3
Muffin, fruit jam (g/100g)	17.5±4.2	20.9±0.3	-16.7
Muffin, plain (g/100g)	19.1±0.9		
Muffin, chocolate crumbs (g/100g)	15.9±1.8	17.1±0.3	-15.3
Chocolate cakes (g/100g)	21.2±6.8	18.6±5.4	2.6
Milk cakes (g/100g)	20.3±0.3	19.5±0.4	0.8
Jam cakes (g/100g)	25.3±8.6	22.5±2.3	2.8
Plum cake (g/100g)	17.9±0.1	19.6±1.1	-1.7
Sponge cake (g/100g)	16.6±21	18.2±1.7	-1.7
Sugar-free cakes (g/100g)	19.8±2.6		
Tart, fruit jam (g/100g)	12.5±0.5	11.7±0.5	0.8
Tart, chocolate (g/100g)	5.1±0.8	5.3±0.0	-0.2
"Panettone" (g/100g)	29.6±1.8	21.9±0.5	7.7
"Pandoro" (g/100g)	26.5±1.8	19.1±0.6	7.4
"Colomba" (g/100g)	28.2±9.3	18.8±0.5	9.5

4.3.3. GLUTEN FREE *VS.* GLUTEN CONTAINING PRODUCTS

Table 26 A and **B** shows the mean nutrient composition of 174 GF and GC foods grouped in 4 food groups: "Bread and substitutes" (**A**), "Filled pasta" (**A**), "Biscuits" (**B**), and "Cakes and desserts" (**B**). Energy content was found to be significantly different only for GF and GC cakes and desserts ($p=0.0059$), with greater mean and median values observed for GC foods. As expected, protein content was found to be significantly different ($p<0.01$) in all the selected food groups. GC breads (8.4±1.6g/100g), filled pasta (12.4±2.1g/100g), biscuits (7.3±1.6g/100g), and cakes (6.6±1.2g/100g) contain more protein than their GF counterparts (5.9±3.0 g/100g, 7.7±1.7g/100g, 4.6±2.6g/100g, 4.2±1.3g/100g, respectively). Sugar content was significantly higher in GC filled pasta than in GF (3.1±1.5 g/100g *vs.* 0.6±0.6g/100g, respectively, $p=0.0220$). Fibre (available in 87.8% of the collected NLs) and water content (analytically determined) were significantly higher in GF breads than in GC breads ($p<0.0156$). et al.,

Several studies have been recently conducted to analyse the different nutritional composition of GF foods in respect to their GC counterparts in Italy (Angelino et al., 2020; Cornicelli et al., 2018), and in Europe (Allen and Orfila, 2018; Babio et al., 2020; Fajardo et al., 2020; Fry et al., 2018; Missbach et al., 2015; Myhrstad et al., 2021), collecting data on 110 (Missbach et al., 2015) to 2247 (Babio et al., 2020) food products. A complete agreement was found for protein intake, which was found to be lower in GF than in GC products, as in the current analysis. The lower protein content in GF products indicate that the gluten protein markedly impacts the overall protein content in the GC foods (Myhrstad et al., 2021). However, in an overall diet-perspective, it has to be underlined that, despite GF cereal-based products contain low amounts of proteins, other contributors of dietary protein intake are naturally free of gluten (pulses, fish, dairy products, eggs, and meat). Indeed, despite protein intake was found to decrease in a previous study after 1 year of GF diet treatment than at coeliac disease diagnosis, protein intake was found to be generally adequate meeting the DRVs (Shepherd and Gibson, 2013). For other macronutrients, results are mixed probably due to differences in country-specific food market and in the selection and aggregation of products included in the analysis. Moreover, previous authors from several countries reported a significantly higher price of GF than GC products (Babio et al., 2020; Missbach et al., 2015; Myhrstad et al., 2021).

In accordance to previous Italian data (Cornicelli et al., 2018), energy, sugar, and total fat content was overall comparable among GF and GC food products with few exceptions: in the present work we observed a lower energy content in GF cakes than in GC cakes which was not detected by Cornicelli and colleagues, while they found a lower energy intake in GF breads than in GC breads. Moreover, higher SFA content was reported by Cornicelli and colleagues in GF biscuits than in their GC counterparts (Cornicelli et al., 2018). We found higher fibre content in GF than in GC bread similarly to previous Italian results (Cornicelli et al., 2018). Contrarily, in the present paper no differences were found regarding fibre and salt content of biscuits, and total carbohydrate content of biscuits and bread and substitutes.

Conversely, many more differences between GF and GC food bread and substitutes were found by other Italian authors (Angelino et al., 2020) than those reported in the present work: a lower energy, lower carbohydrates, lower sugars, higher total fats and higher SFA content in GF breads

than in GC breads; and a lower energy and macronutrient content (except from carbohydrates, which were higher) in GF bread substitutes compared to their GC counterparts. However, Angelino and colleagues (Angelino et al., 2020) included in their analysis only GF breads and substitutes available in the e-commerce section of the major Italian retailers present on the Italian market, excluding GF-specific retailers and pharmacies, which may still be common food supply options for coeliac disease patients since GF products availability in supermarkets is generally limited (Gorgitano et al., 2019).

In conclusion, our analysis confirmed that GF foods tent to be less nutritious than their GC counterparts particularly regarding protein content, while the differences between GF and GC foods in the content of other NL-derived nutrients remain variable and mainly reliant on the product-specific formulation. However, some limitations should be considered in the present analysis. Firstly, nutrient composition was derived from NLs except for water, which was the only food component directly analysed. Another limitation is the small sample size compared to other literature studies. However, GF manufactured foods included in the present analysis were carefully selected from specific food groups and rigorously matched to their GC corresponding products, assuring that each GC product type (i.e., GC-FDCB food item) was matched with at least one corresponding GF product.

Table 26.A. *Comparison between nutrient values from nutritional label and analytical water (per 100g) of gluten containing and the corresponding gluten free foods from the "Bread and substitutes" and "Filled pasta" food groups.*

		Bread and substitutes (GC=29; GF=29)			**Filled pasta (GC=8; GF=3)**		
		Mean±SD	Median (25^{th}–75^{th})	p-value	Mean±SD	Median (25^{th}–75^{th})	p-value
Energy (kcal)	**GC**	271±34	296(251–287)	0.2464	307±59	293(271–328)	0.2099
	GF	264±30	261(241–275)		255±48	278(239–283)	
Water (analytic)(g)	**GC**	32.2±5.4	31.2(28.3–35.1)	**0.0156**	28.8±13.2	32.7(25.2–38.6)	0.5403
	GF	35.9±6.6	35.1(31.6–39.8)		37.6±11.3	33.7(31.2–42.0)	
Protein (g)	**GC**	8.4±1.6	8.5(7.6–9.0)	**<0.001**	12.4±2.1	13.0(10.8–13.3)	**0.0088**
	GF	5.9±3.0	3.0(2.3–4.1)		7.7±1.7	8.6(7.2–8.7)	
Fats (g)	**GC**	5.0±3.7	3.9(2.8–5.7)	0.0641	8.1±2.8	8.2(6.4–9.3)	0.9289
	GF	6.0±2.5	5.5(3.7–7.9)		8.2±1.6	7.7(7.4–8.9)	
SFAs (g)	**GC**	1.0±0.9	0.6(0.4–1.2)	0.0902	3.0±1.3	2.9(2.0–3.3)	0.8112
	GF	1.4±1.2	1.0(0.5–1.5)		3.2±0.3	3.3(3.1–3.4)	
Carbohydrates (g)	**GC**	46.5±5.6	48.4(45.0–50.0)	0.9009	44.7±9.6	41.0(38.8–46.7)	0.3561
	GF	46.7±7.3	46.0(43.1–52)		36.8±8.3	39.0(33.0–41.2)	
Sugars (g)	**GC**	4.2±2.4	4.4(2.1–6.0)	0.7974	3.1±1.5	3.2(1.8–4.0)	**0.0220**
	GF	4.5±2.9	3.4(2.7–6.5)		0.6±0.6	0.8(0.4–1.0)	
Fibre (g)	**GC**	3.4±1.8	2.9(2.1–4.1)	**0.0017**	2.4±0.7	2.3(12.0–2.8)	**0.0393**
	GF	5.0±1.9	5.0(3.9–6.4)		0.8±0.4	0.8(0.7–1.0)	
Salt (g)	**GC**	1.3±0.4	1.3(1.2–1.5)	0.3871	1.1±0.4	1.1(0.9–1.4)	0.6824
	GF	1.6±0.6	1.4(1.2–1.6)		1.7±1.5	0.8(0.9–1.4)	

P values <0.05 were highlighted in bold typeface. Abbreviations: GF, gluten free; GC, gluten containing; SFAs, saturated fatty acids.

Table 26.B. *Comparison between nutrient values from nutritional label and analytical water (per 100g) of GC and the corresponding GF foods from the "Biscuits" and "Cakes and dessert" food groups.*

		Biscuits (GC=14; GF=14)			Cakes and dessert (GC=38; GF=37)		
		Mean±SD	Median (25^{th}–75^{th})	p-value	Mean±SD	Median (25^{th}–75^{th})	p-value
Energy (kcal)	**GC**	458±44	460(438–480)	0.6790	404±33	410(381–421)	**0.0059**
	GF	465±38	464(447–483)		375±54	380(339–408)	
Water (analytic)(g)	**GC**	2.3±2.2	1.5(1.3–2.0)	0.8542	18.7±4.0	19.1(18.1–20.9)	0.2613
	GF	2.1±1.6	1.5(0.8–3.3)		21.6±8.4	20.2(16.1–28.0)	
Protein (g)	**GC**	7.3±1.6	7.9(6.7–8.5)	**0.0130**	6.6±1.2	6.5(5.8–7.2)	**<0.001**
	GF	4.6±2.6	4.1(2.8–5)		4.2±1.3	4(3.4–5.1)	
Fats (g)	**GC**	17.4±9.0	16.7(11.3–25.3)	0.8457	18.1±4.5	18.0(15.0–22.0)	0.0763
	GF	16.9±6.4	15.8(12.3–21.5)		15.9±6.0	16.0(12.0–20.0)	
SFAs (g)	**GC**	7.6±9.7	1.8(1.3–16.0)	0.2063	7.6±3.5	7.6(4.7–11.0)	0.0530
	GF	7.8±7.0	5.3(2.8–9.4)		6.0±3.7	5.1(2.4–9.5)	
Carbohydrates (g)	**GC**	67.7±7.4	67.4(61.1–74.9)	0.2112	52.3±6.0	52.0(49.0–55.0)	0.8651
	GF	71.4±7.9	71.0(67.7–77.9)		52.6±7.7	51.0(47.4–58.0)	
Sugars (g)	**GC**	22.3±11.5	20.3(19.3–25.3)	0.9572	26.7±8.7	27.5(24.0–30.9)	0.9525
	GF	22.1±8.5	21.8(19.0–26.7)		26.6±8.1	27.0(20.0–33.0)	
Fibre (g)	**GC**	4.5±3.2	3.0(2.8–5.9)	0.0842	2.7±1.6	1.9(1.7–3.5)	0.7773
	GF	3.2±3.1	1.9(1.0–3.8)		3.0±2.2	2.6(1.4–3.9)	
Salt (g)	**GC**	0.5±0.3	0.8(0.8–2.1)	0.9059	0.5±0.2	0.5(1.4–0.6)	0.7950
	GF	0.6±0.4	0.5(0.3–0.8)		0.5±0.2	0.5(0.4–0.6)	

P values <0.05 were highlighted in bold typeface. Abbreviations: GF, gluten free; GC, gluten containing; SFAs, saturated fatty acids.

4.4. RESULTS FROM A PILOT STUDY ON BABYFOODS

Abstract presented at the Italian Society of Human Nutrition (SINU) National Congress
F. Fiori, F. Concina, P. Gnagnarella, G. Carioni, M. Parpinel (2020). Update of "babyfoods" and "snacks" categories from the food composition database used for the analysis of infants diet at 18 months of age in PHIME study. NMCD. NUTRITION METABOLISM AND CARDIOVASCULAR DISEASES, vol. 30, p. 539.
Genova, November 2019

From a total sample of 389 7-dDR collected as part of the PHIME study a study-specific FCDB containing 754 food items was created (Concina et al., 2021, 2016). The total database included mostly BDA foods. However, 1.3% were from other FCDBs or literature data (breast milk), and 36.3% of the foods recorded in the diary were manufactured foods (babyfoods, snacks, and cakes) uploaded in the Microdiet software with only NL nutrient data.

Our work concerned 191 foods recorded in 10 7-dDR, 10% of which were manufactured foods. In the original database, vitamin, mineral, and fatty acid profiles present meanly 9%, 7% and 12% of missing data, respectively. In the updated database all missing data were filled, except those of breast milk (0.5% of total foods, accounting for <1% of nutrient missing data).

The comparison between the intakes estimated from the application of the original and those estimated from the updated FCDBs highlighted a general underestimation using the original database particularly for fatty acids (ranging from 18±22% for PUFAs and linoleic acid, to 27.9±20.7% for linolenic acid), cholesterol (18.3±32.5%), vitamin E (32.5±21.2%), vitamin D (11.2±20.9%), and retinol (19.2±24.0%) (**Table 27**). Mineral intakes were underestimated in a minor extent, with the greatest underestimation found for iron (6.5±3.7%). The maximum underestimation was observed in one food record where 11 out of 55 entries (20%) were represented by manufactured foods. In this record, the underestimation of cholesterol intake observed using the original database *vs.* the updated one was greater than 100%, while the underestimation of vitamin D, E, retinol, MUFAs, PUFAs, oleic, linoleic and linolenic acid intake was greater than 50% (data not shown).

Contrarily, macronutrient, sugar, fibre, sodium, and SFA intakes were comparable, as expected. Thus, these nutrient values were provided for most of the manufactured products included in the database. However, also the intakes of calcium, vitamin B2, B12, and C were found to be comparable. The analysis of the present NLs, which were collected prior than 2014, showed that, contrarily to the labels currently available on the market which have to comply with the European regulation on food labelling (1169/2011 Reg UE, 2011) —fully implemented stating from December 2014—, reported frequently micronutrient data also if in minor amounts. The current regulation would not allow the nutritional declaration of micronutrient values if those are present in the food in amounts lower than 15% or 7.5% of the reference daily allowance (RDA) for foods and beverages, respectively.

Table 27. *Percentage underestimation of nutrient intakes (mean, SD) observed when comparing data derived from the original and the updated FCDB.*

	Mean	**SD**
Protein	0.00%	0.00%
Fats	0.00%	0.01%
Available carbohydrates	0.00%	0.00%
Soluble carbohydrates	3.70%	7.50%
Fibre	1.94%	1.36%
Starch	9.36%	11.29%
Saturated fatty acids	3.41%	8.92%
Monounsaturated fatty acids	19.58%	18.43%
Polyunsaturated fatty acids	18.30%	22.44%
Cholesterol	18.29%	32.53%
Oleic acid	21.52%	18.35%
Linoleic acid	18.22%	22.46%
Linolenic acid	27.94%	20.69%
Sodium	1.84%	4.55%
Potassium	5.66%	2.80%
Calcium	1.07%	1.58%
Iron	6.46%	3.70%
Zinc	5.82%	4.59%
Vitamin B1	4.30%	3.55%
Vitamin B2	1.55%	1.66%
Vitamin B6	3.12%	2.33%
Vitamin B12	2.10%	4.59%
Vitamin C	1.20%	1.92%
Vitamin D	11.18%	20.92%
Vitamin E	32.50%	21.17%
Retinol	19.19%	23.98%
Vitamin A	7.73%	11.64%
Niacin	3.63%	6.26%
Folates	5.30%	5.10%

Despite macronutrient intakes have been found to be generally comparable when using different databases (Iguacel et al., 2022), nutrient intake underestimations are common biases derived from unsuitable, incomplete, or out-of-date FCDB use. As an example, NLs are typically used in nutritional epidemiology and in customer-targeted mobile apps to monitor food and nutrient intakes without considering the vast amount of micronutrient missing data (Ocké et al., 2021; Tosi et al., 2021; Westenbrink et al., 2012). Indeed, imputation and/or calculation procedures are very time-consuming, and given the extremely fast change in formulations of manufactured foods over the years, those procedures would need to be implemented continuously.

The present method allowed to estimate missing nutrient values starting from easily accessible food label mandatory information (i.e., ingredient list and NL). Despite the calculation process followed international standards and was likely to produce reliable data for nutritional epidemiology use, it was extremely time-consuming, based on a manual trial and error approach, and thus user-dependent. Other approaches may be more easily implemented on big data, such as the mapping approach proposed by Carter (Carter et al., 2016), that consisted in the matching of

branded food data to pre-existing data from similar products in the former FCDB. However, this approach may lead to several errors, in particular when the formulation of the product is peculiar. Regarding babyfoods, several recent studies underlined that products specifically targeted for children have a different nutritional composition with generally more free sugars that food products targeted for the general population (Gilbert-Moreau et al., 2021; Hutchinson et al., 2021). Thus, the mapping approach may be less suitable for particularly targeted foods. Furthermore, this approach was seen to be time and cost-consuming, as well as ours (Carter et al., 2016; Ocké et al., 2021).

In conclusion, the present attempt to compile missing nutrient values in manufactured products consumed by 18-month-old infants from the NAC-II cohort led to a complete FCDB. Our analysis highlighted a noteworthy underestimation of fatty acid intakes while the underestimation of most vitamins and minerals was negligible. However, despite the label-based recipe calculations provided a complete database, the approach may be of difficult application when required for great amount of data.

4.5. A NOVEL TOOL TO IMPUTE INGREDIENTS' WEIGHT

The integration of the genetic algorithm in a java user interface resulted in a novel tool that allows the compiler to insert parameters, refine them, and visualise results in order to guide decision-making within the label-based recipe calculation standard procedures.

The user interface is in Italian, and it is divided in: the control section, on the left, and the solution section (*"soluzioni"*), on the right (**Figure 16**). The control section is further divided in 4 subsections (algorithm, *"algoritmo"*; ingredients, *"ingredienti"*; nutrients *"nutrienti"*; FCD, *"composizione nutrizionale"*). The variability value (*"variabilità"*), on the top of the control section indicates the maximum total error acceptable, in grams, summing errors from the whole NL nutrient composition plus water. In the ingredient section, single ingredients can be added, moved among the ingredient list and/or doubled. When adding an ingredient, its minimum and maximum weight can be specified (if not, 0.1 g and 199.9 g are pre-set as minimum and maximum values, respectively). Moreover, if in the label a percentage contribution of an ingredient is stated, the percentage value can be uploaded in the form of a range *(%min*; *%max*) in the dedicated box. The value "-3" denotes missing data, as in the standard BDA coding system (Gnagnarella et al., 2015).

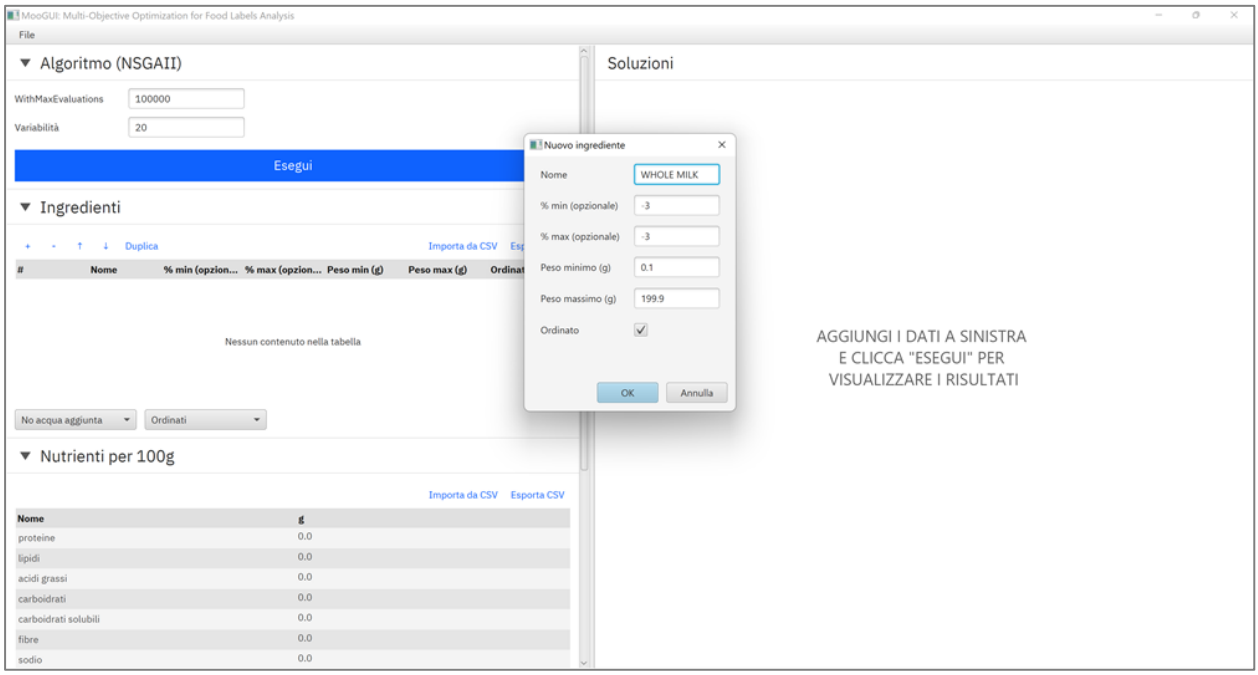


Figure 16. User interface starting view and the new ingredient upload window (Italian version).

The first line of the ingredients section is always dedicated to evaporated water in the user interface, which is not considered in the ranked order of weights of the ingredient list stated in the food label. If water is present in the ingredient list, an additional enter should be placed in the ingredient list to be considered in the ranked order. On the other hand, if water is not reported in the ingredient list of the food label but it is likely to be added as an ingredient in that given food, it may still be uploaded, in this case in the last position of the list. Indeed, water may be added as

an ingredient even if it is not reported in the ingredient list when in the final product added water content is less than 5% (1169/2011 Reg UE, 2011). To sum up, the first position of the ingredient list is always dedicated to evaporated water, while the last position may be dedicated to added water not mentioned in the food label. In the latter case, "*acqua fuori lista*" (i.e., water not reported in the ingredient list) has to be selected from the top down menu of the dedicated box, in order to exclude the last position to the ranking order. Another selection box allows to apply the ranking order constraint, based on the checklist which appears on the right of each ingredient (**Figure 17**). Typically, the first ingredients on the list comply with the ranking, while the last ingredients may be not ordered if they are present in amounts <2% of the final product (1169/2011 Reg UE, 2011).

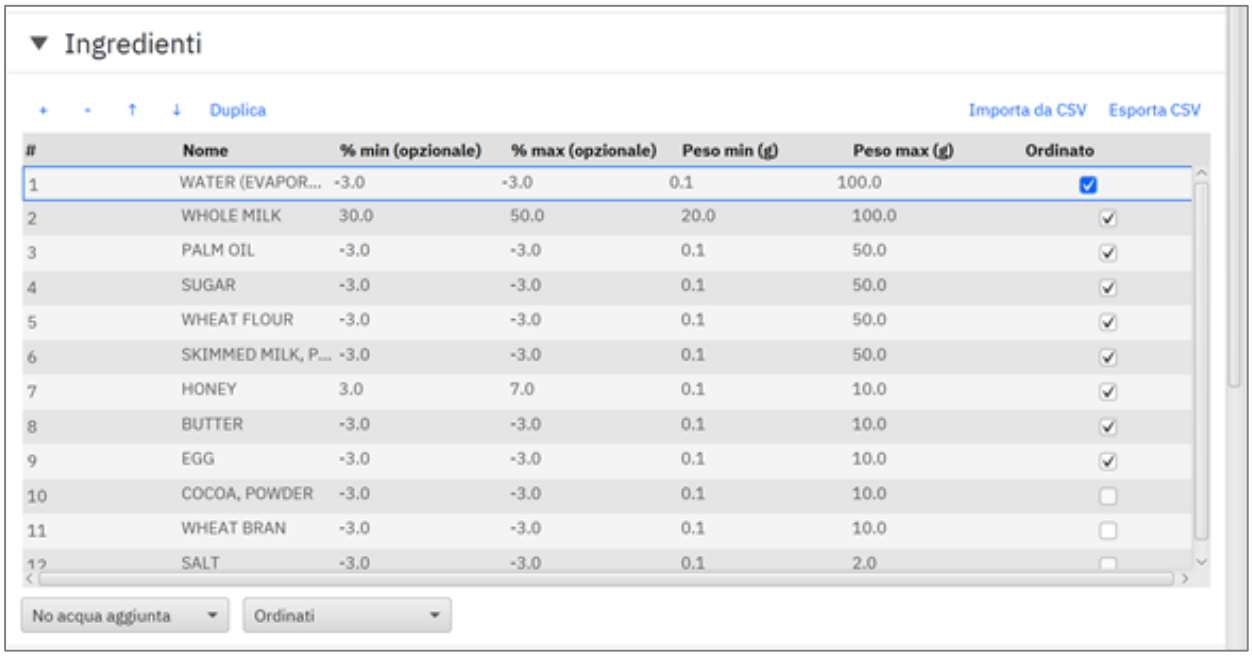
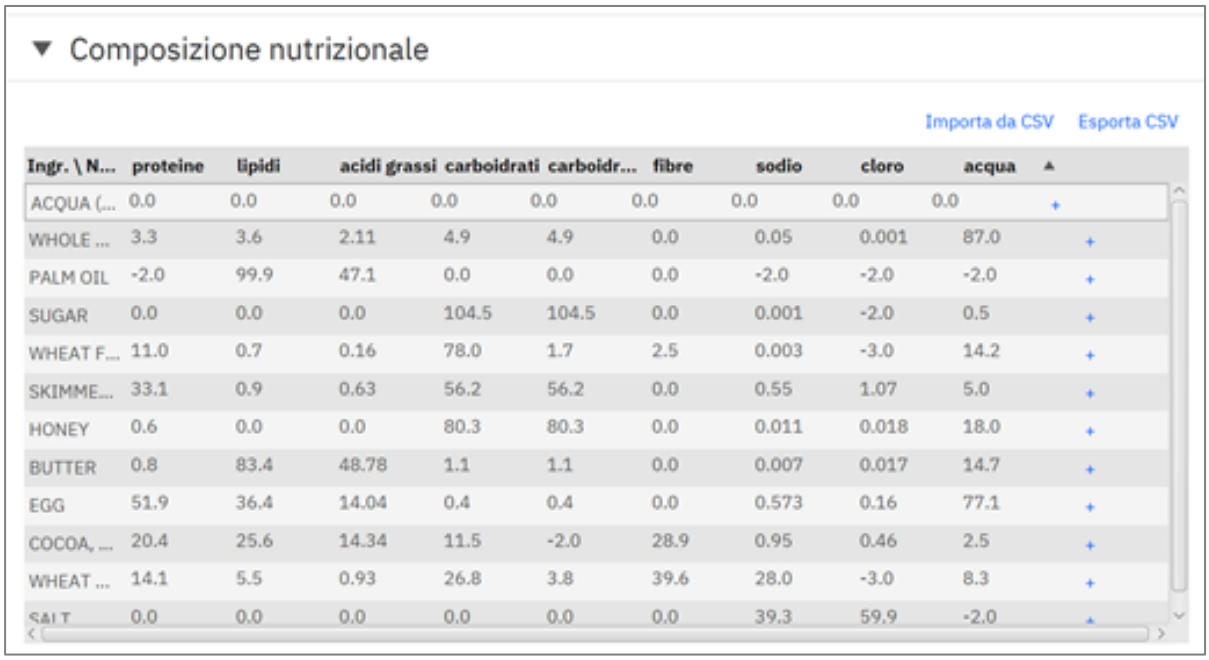


Figure 17. Example of an uploaded ingredient list, from the food label of a popular manufactured cake snack. Last column indicates the order rule checking list (Italian version).

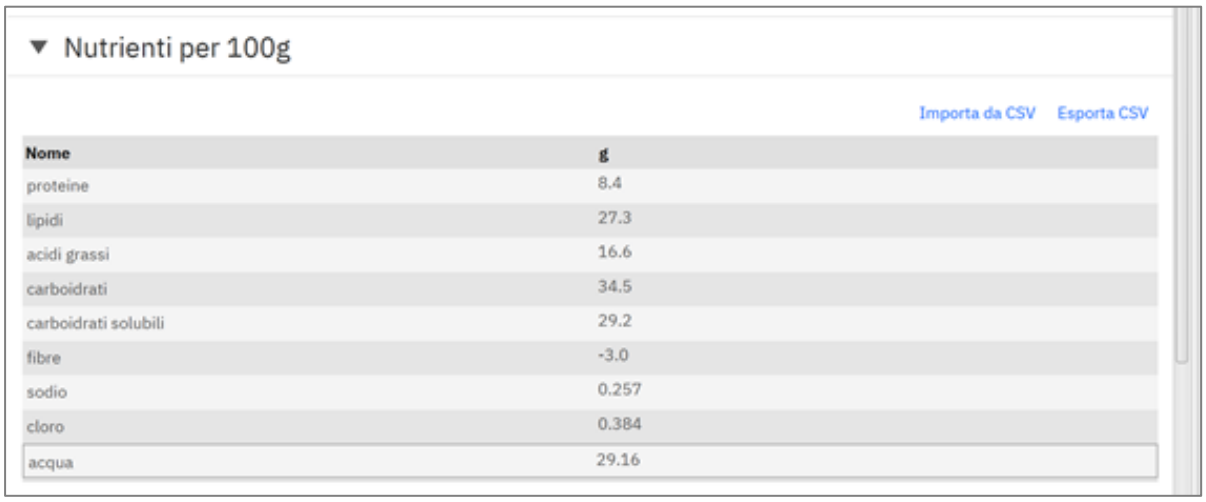
After one ingredient is added in the ingredient section, that ingredient name pops up in the FCD section. In this section the food composition of single ingredients can be easily uploaded clicking on the "+" button from a "BDA.csv" linked file. As in the BDA coding system, "-3" indicates missing, and "-2" indicates trace values (**Figure 18**).



Ingr. \ N...	proteine	lipidi	acidi grassi	carboidrati	carboidr...	fibre	sodio	cloro	acqua
ACQUA (...	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WHOLE ...	3.3	3.6	2.11	4.9	4.9	0.0	0.05	0.001	87.0
PALM OIL	-2.0	99.9	47.1	0.0	0.0	0.0	-2.0	-2.0	-2.0
SUGAR	0.0	0.0	0.0	104.5	104.5	0.0	0.001	-2.0	0.5
WHEAT F...	11.0	0.7	0.16	78.0	1.7	2.5	0.003	-3.0	14.2
SKIMME...	33.1	0.9	0.63	56.2	56.2	0.0	0.55	1.07	5.0
HONEY	0.6	0.0	0.0	80.3	80.3	0.0	0.011	0.018	18.0
BUTTER	0.8	83.4	48.78	1.1	1.1	0.0	0.007	0.017	14.7
EGG	51.9	36.4	14.04	0.4	0.4	0.0	0.573	0.16	77.1
COCOA, ...	20.4	25.6	14.34	11.5	-2.0	28.9	0.95	0.46	2.5
WHEAT ...	14.1	5.5	0.93	26.8	3.8	39.6	28.0	-3.0	8.3
SALT	0.0	0.0	0.0	0.0	0.0	0.0	39.3	59.9	-2.0

Figure 18. Example of the nutritional composition imported from the BDA for the selected ingredients (Italian version).

In the nutrient section, the NL nutritional data must be uploaded after the appropriate conversions: salt needs to be converted in sodium and chloride and expressed in grams **Figure 19**). Water content may be calculated by difference or uploaded as an analytically determined value (.

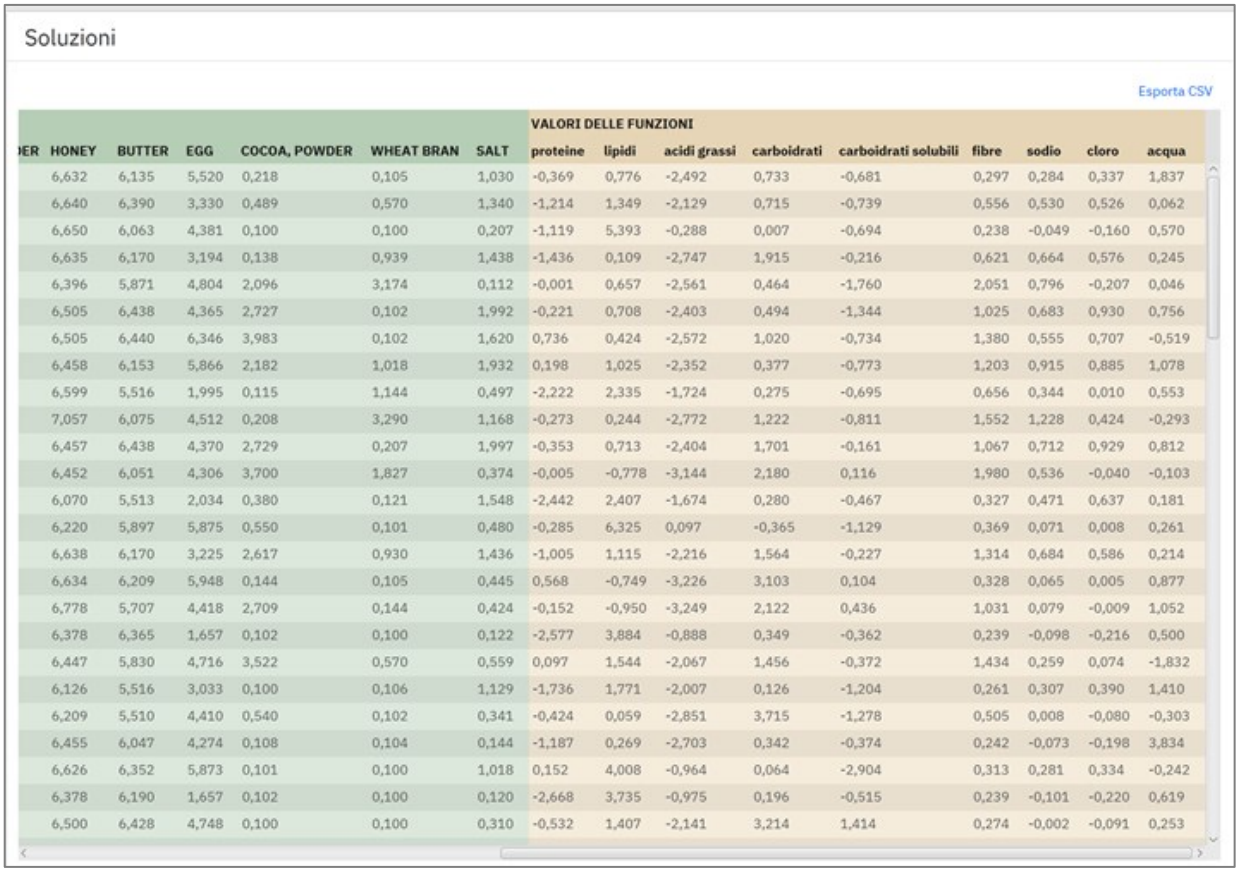


Nome	g
proteine	8.4
lipidi	27.3
acidi grassi	16.6
carboidrati	34.5
carboidrati solubili	29.2
fibre	-3.0
sodio	0.257
cloro	0.384
acqua	29.16

Figure 19. Example of the uploaded NL nutrient composition (Italian version).

Finally, the search for the optimal solutions is launched by clicking the run button (i.e., *"esegui"*) in the algorithm section. At this point, the section on the right will show all possible "good solutions" (i.e., meeting the constraints), or "bad solutions" (i.e., one or more constraints are not satisfied). As displayed in **Figure 20**, columns with a green background show calculated ingredients' weight, while columns with a yellow background (on the right) show the error of that given solution, for each nutrient (i.e., amount of the difference between NL declared nutrient and calculated nutrient). Solutions are presented in ascending order of total error. Thus, the first "good

solution" displayed is the most optimised one. However, solutions can be ordered by nutrient-specific errors by clicking on the column title.



Soluzioni

Esporta CSV

							VALORI DELLE FUNZIONI								
ER	HONEY	BUTTER	EGG	COCOA, POWDER	WHEAT BRAN	SALT	proteine	lipidi	acidi grassi	carboidrati	carboidrati solubili	fibre	sodio	cloro	acqua
	6,632	6,135	5,520	0,218	0,105	1,030	-0,369	0,776	-2,492	0,733	-0,681	0,297	0,284	0,337	1,837
	6,640	6,390	3,330	0,489	0,570	1,340	-1,214	1,349	-2,129	0,715	-0,739	0,556	0,530	0,526	0,062
	6,650	6,063	4,381	0,100	0,100	0,207	-1,119	5,393	-0,288	0,007	-0,694	0,238	-0,049	-0,160	0,570
	6,635	6,170	3,194	0,138	0,939	1,438	-1,436	0,109	-2,747	1,915	-0,216	0,621	0,664	0,576	0,245
	6,396	5,871	4,804	2,096	3,174	0,112	-0,001	0,657	-2,561	0,464	-1,760	2,051	0,796	-0,207	0,046
	6,505	6,438	4,365	2,727	0,102	1,992	-0,221	0,708	-2,403	0,494	-1,344	1,025	0,683	0,930	0,756
	6,505	6,440	6,346	3,983	0,102	1,620	0,736	0,424	-2,572	1,020	-0,734	1,380	0,555	0,707	-0,519
	6,458	6,153	5,866	2,182	1,018	1,932	0,198	1,025	-2,352	0,377	-0,773	1,203	0,915	0,885	1,078
	6,599	5,516	1,995	0,115	1,144	0,497	-2,222	2,335	-1,724	0,275	-0,695	0,656	0,344	0,010	0,553
	7,057	6,075	4,512	0,208	3,290	1,168	-0,273	0,244	-2,772	1,222	-0,811	1,552	1,228	0,424	-0,293
	6,457	6,438	4,370	2,729	0,207	1,997	-0,353	0,713	-2,404	1,701	-0,161	1,067	0,712	0,929	0,812
	6,452	6,051	4,306	3,700	1,827	0,374	-0,005	-0,778	-3,144	2,180	0,116	1,980	0,536	-0,040	-0,103
	6,070	5,513	2,034	0,380	0,121	1,548	-2,442	2,407	-1,674	0,280	-0,467	0,327	0,471	0,637	0,181
	6,220	5,897	5,875	0,550	0,101	0,480	-0,285	6,325	0,097	-0,365	-1,129	0,369	0,071	0,008	0,261
	6,638	6,170	3,225	2,617	0,930	1,436	-1,005	1,115	-2,216	1,564	-0,227	1,314	0,684	0,586	0,214
	6,634	6,209	5,948	0,144	0,105	0,445	0,568	-0,749	-3,226	3,103	0,104	0,328	0,065	0,005	0,877
	6,778	5,707	4,418	2,709	0,144	0,424	-0,152	-0,950	-3,249	2,122	0,436	1,031	0,079	-0,009	1,052
	6,378	6,365	1,657	0,102	0,100	0,122	-2,577	3,884	-0,888	0,349	-0,362	0,239	-0,098	-0,216	0,500
	6,447	5,830	4,716	3,522	0,570	0,559	0,097	1,544	-2,067	1,456	-0,372	1,434	0,259	0,074	-1,832
	6,126	5,516	3,033	0,100	0,106	1,129	-1,736	1,771	-2,007	0,126	-1,204	0,261	0,307	0,390	1,410
	6,209	5,510	4,410	0,540	0,102	0,341	-0,424	0,059	-2,851	3,715	-1,278	0,505	0,008	-0,080	-0,303
	6,455	6,047	4,274	0,108	0,104	0,144	-1,187	0,269	-2,703	0,342	-0,374	0,242	-0,073	-0,198	3,834
	6,626	6,352	5,873	0,101	0,100	1,018	0,152	4,008	-0,964	0,064	-2,904	0,313	0,281	0,334	-0,242
	6,378	6,190	1,657	0,102	0,100	0,120	-2,668	3,735	-0,975	0,196	-0,515	0,239	-0,101	-0,220	0,619
	6,500	6,428	4,748	0,100	0,100	0,310	-0,532	1,407	-2,141	3,214	1,414	0,274	-0,002	-0,091	0,253

Figure 20. Example of the list of good solutions provided by the algorithm. In green ingredients' weight, and in yellow error values for each NL nutrient and water (Italian version).

As an example, **Figure 21** shows the solutions generated setting to 10 the variability and considering 2 ingredient percentages and the ranked weight order for the first 8 ingredients (excluding evaporated water).

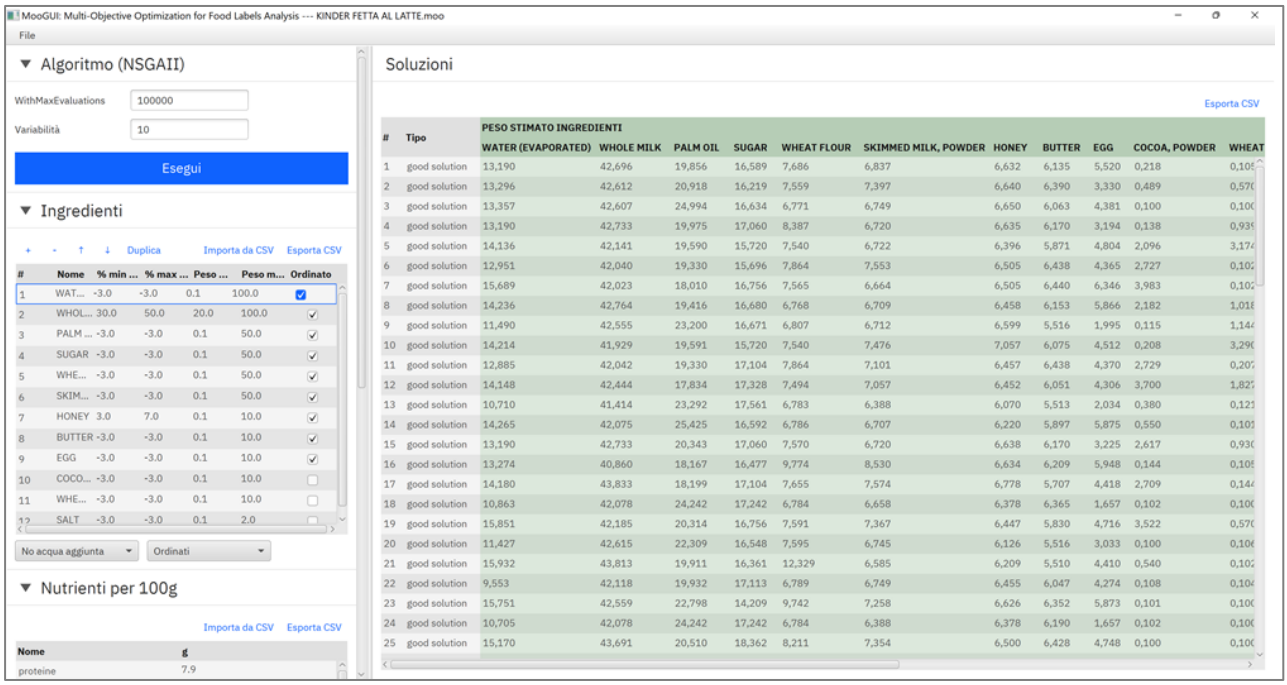


Figure 21. Example of solution generated setting to 10 the variability, considering ingredient percentages and ranked weight order of the first 8 ingredients, excluding evaporated water (Italian version).

Decision on which solution to use for micronutrient value calculations is then a compiler's responsibility. To refine solutions and minimise the overall error, 2 approaches are presented.

- The reduction of the variability parameter in the algorithm section.
- The reduction of the ingredient's domain (i.e., the min-max range for weights).

In both cases, if the reduction is excessive, it is common to obtain "bad solution" only. In the second approach the strategy may be the following:

- Choose a nutrient whose value is mainly dependent on one ingredient, as applicable.
- Check/order the solutions to identify those that minimise the error for that nutrient.
- Check the ingredients' weight range for that solutions.
- Reduce the ingredient's domain accordingly
- Run the algorithm.
- Repeat the procedure for other nutrients.

In the present work, the multi-objective optimization algorithms have been seen to be useful in the identification of the optimal ingredients' weight for label-based recipe calculations because they allowed the concurrent optimization of several criteria (also referred as "objectives"). Given that there is no single optimal solution when considering several criteria, the algorithm is likely to find a multitude of potential solutions with varying degrees of tradeoff between the objectives. Decision-makers are responsible for exploring this set of potential solutions and identifying the solution(s) to be implemented. While eventually the responsibility for the identification of the optimal solution is of the decisionmaker, optimization tools are intended to assist the process.

4.5.1. STRENGHTS AND LIMITATIONS

Retrocalculation of the quantity of ingredients used to produce a manufactured product is typically done by a trial-and-error manual technique, where the best solution is searched until a calculated value is found to be sufficiently close to the known variation in values (Machackova et al., 2018; Schakel et al., 1997). Recently, advances in information technologies, the increasing use of big data, and the development of mobile applications is fostering the exploration of FCD in new ways (Carter et al., 2016; Traka et al., 2020).

The implementation of the NSGAII algorithm allowed to standardise the decision-making process and to minimise errors. Moreover, the algorithm considers water loss due to cooking method. Comparing preliminary results obtained from the application of the multi-objective optimization algorithm, and those obtained from the manual compiling of a manufactured food trough the label-based recipe, the final level of accordance to the NL has been found to be similar. However, the present approach remains user-dependent. Indeed, the compiler's knowledge of nutrient composition of foods is essential for the use of the tool. Furthermore, as other approaches (Carter et al., 2016), also the present one is overly time-consuming, thus data are going to be challenging to maintain up-to-date.

In conclusion, the tool needs to be further optimised for the compiler's use. At present, the application presents some bugs, and the management of composite ingredients (i.e., ingredients for which an additional ingredient list is provided in brackets) is still missing. A dedicated software including the implementation of the algorithm as well as the automatization of micronutrient data production using the chosen solution (i.e., the ingredients' weight) would be extremely useful to fasten the overall process.

4.6. RESULTS FROM THE UPDATE OF THE CASE-CONTROL FOOD COMPOSITION DATABASE ON VARIABLES OF INTEREST

4.6.1. CHOLINE AND SPHINGOMYELINS

Information from tables, databases, and scientific literature have been searched and critically evaluated for their inclusion in the FCDB. Several published papers (Ahn and Schroeder, 2002; Hellgren, 2001; Koc et al., 2002; Lewis et al., 2014; Li et al., 2020; Liu et al., 2020; Norris and Blesso, 2017; Panel and Products, 2016; Richard et al., 2016; Rombaut et al., 2007; Takama et al., 1999; Vesper et al., 2000; Wang et al., 2021), and USDA FCDBs and tables (Patterson et al., 2008; USDA, 2018, 2020b, 2020a) have been found to report data on choline and/or SMs composition of foods. However, most of the data published in the literature were found to be unsuitable for their use in FCDBs, where it is essential to identify correctly dietary components and their metadata. Indeed, different analytical methodologies, different data expression and units of measure, undetailed food and component description, and lack of supplementary compositional data of the analysed foods, were some of the main issues encountered. FCD may change substantially due to the use of different analytical methods (Phillips et al., 2019; Westenbrink et al., 2012), thus it is important to check the homogeneity of compound definition, methodologies and data expression in the source tables or databases. Moreover, information on the water content and macronutrient composition may be extremely important during matching procedures to evaluate the similarity of the foods being matched. Eventually, water or macronutrient composition of the source food may be used to perform a value reproportioning based on the water or macronutrient composition of the given FCDB food item.

To give an example, to our knowledge Li and colleagues (Li et al., 2020) and Takama and colleagues (Takama et al., 1999) were the only authors who have analysed shellfish and squids for their SMs composition. However, SMs composition data were mainly obtained from shellfish and squids fished in the Pacific Ocean, rarely available in the European market, and SMs data where expressed as nmol/g or percentage of polar lipids, respectively. Another potential important source of data was published by Rombaut and colleagues (Rombaut et al., 2007) on the SMs content of a large sample of cheese products. However, data were presented as percentage of polar lipids, and, when converted in mg/100g of total food, results appeared to be not comparable with the USDA data. Indeed, converting USDA SMs data, originally expressed as choline moiety/100g (ChM/100g), in mg/100g is challenging. SMs are in fact a complex group of compounds with different molecular weights. To perform the conversion, a mean value of 751 g/mol was applied (Wang et al., 2021). However, this conversion would inevitably lead to inaccurate estimates. As a result, because of the fragmentation and lack of harmonization of the SMs composition data among the literature, in the present update it was decided to use only SMs data expressed in choline

moiety. Indeed, at present, the largest collection of SMs data was published (together with the corresponding choline data) by USDA (Patterson et al., 2008).

The final case-control FCDB was then compiled using 7 and 5 publicly available data sources for choline and SMs, respectively (Lewis et al., 2014; Patterson et al., 2008; Richard et al., 2016; USDA, 2020b, 2020a, 2018), and SMs content in foods was expressed as choline moiety, in accordance to the USDA methodology. Thus, the USDA Database for Choline Content of Common Foods (Patterson et al., 2008) and the USDA Standard Reference Database (USDA, 2018) were the primary sources for obtaining data on SMs and choline, respectively. The priority order of the selected literature sources, as well as the type, number (% of the total food items) and quality of the performed matches is presented in **Table 28**.

Table 28. *Matching of choline and sphingomyelins content from literature sources to the case-control food composition database' food items for the analysis of the food frequency questionnaire.*

					Items [N (%)]	
	Source	**Last updated**	**Matching**	**Quality code**	**Choline**	**SMs**
1	USDA Foundation	2020	Matched to exact food item	A	9 (3.0)	9 (3.0)
			Matched to similar food item	B	6 (2.0)	19 (6.3)
2	Richard et al.	2016	Matched to exact food item	A	6 (2.0)	6 (2.0)
			Matched to similar food item	B	3 (1.0)	3 (1.0)
3	Lewis et al.	2014	Matched to exact food item	A	7 (2.3)	7 (2.3)
			Matched to similar food item	B	5 (1.7)	5 (1.7)
4	USDA Choline Content of Common Foods	2008	Matched to exact food item	A	86 (28.5)	86 (28.5)
			Matched to similar food item	B	29 (9.6)	75 (24.8)
			Closest match	C	-	9 (3.0)
5	USDA Standard Reference	2018	*Matched to exact food item or imputed reproportioning based on SR calculation method	B	116 (38.4)	-
6			Imputed by ingredient calculations (mixed foods, recipes)	B	12 (4.0)	13 (4.3)
7			Imputed from same food category	C	15 (5.0)	61 (20.2)
8	USDA Survey	2018	*Matched to exact food item, metadata unknown	C	3 (1.0)	-
9			Assumed zero		1 (0.3)	4 (1.3)
10			Cannot match or impute		4 (1.3)	5 (1.7)
Total					302	302

Sources are listed in priority order. *non-analytical imputed data in the original source. Quality code A: food and descriptors from the specific DB match with food and descriptors from the FCD source, analytical data in the original source. Quality code B: same food in different form (reproportioned if needed), similar botanical origin, mean value of multiple foods, exact match but non-analytical data in the FCD source. Quality code C: closest match possible. Abbreviation: SMs, sphingomyelins.

Overall, regarding SMs, 212 foods were matched to USDA exact or similar foods; 62 food items were matched to the mean SMs value of the food group or category (vegetables: 10, pulses: 1, fruit: 15, cheese: 8, cereals in grains: 2, red meat: 7, poultry: 1, fish: 6, oils: 3, sweeteners: 5, and spices: 4); 9 foods were matched to a similar food in a different form and thus the SMs values were recalculated based on dry matter; and 13 foods were complex foods, so their SMs content was calculated based on the ingredient contribution to the total recipe. Contrarily to SMs, choline

composition data were comprehensively reported in the USDA Standard Reference database comprising an outstanding food list (USDA, 2018). As a result, several direct matches were available from this source. However, choline content reported in the USDA Standard Reference Database was frequently derived from non-analytical imputations (USDA, 2018). At the end of the matching and imputation procedure, 4 and 5 foods present missing data for SMs and choline, respectively. However, these foods are likely to have a minimal impact on the 83 FFQ aggregated items.

CHOLINE AND SPHINGOMYELIN CONTENT IN FOODS

Choline was seen to be ubiquitarian (297 out of 302 foods; 92.4%), and abundant in many foods (eggs, meat, pulses, fish, cereal-based products, and vegetables of the family *Brassicaceae*). Eggs, offal, meat and fish were the major food sources, with choline contents ranging from 43 mg/100g ("*wurstel"*) to 680 mg/100g (egg yolk). Pulses mean choline content was equal to 52.0±33.9 mg/100g, while fresh fruits contain 6.5±2.8 mg/100g and vegetable oils presents negligible choline amounts (<0.3 mg/100g). The only foods not presenting choline were alcoholic and carbonated beverages, sweeteners, vinegar, and water. In fact, choline is a nutrient essential for the structural integrity and signalling functions of cell membranes; for cholinergic neurotransmission; for muscle function; for fat transport from liver; and it is the major methyl-group donor in the diet (Zeisel, 2006). Dietary choline intake may be important to assess, since it is still debated if endogenous synthesis is sufficient or not to cover choline requirements. Biosynthesis is in fact promoted by oestrogens, and it may be inadequate in certain life stages (Fischer et al., 2007).

On the other hand, SMs were found only in a limited set of foods. Particularly, high values were observed for eggs (whole egg: 25.1 mg ChM/100g; egg yolk: 45.0 mg ChM/100g), and offal (22.1±3.5 mg ChM/100g). In decreasing order of SMs content: meat, cheese, fish, milk and dairy products were also found to be SMs dietary sources. SMs are, in fact, a class of animal sphingolipids provided with a sphingosine base which represents only a minor component of the human diet. Thus, dietary SMs are mainly supplied from animal-based foods. However, according to some authors, small or trace amount of SMs are also present in soy and in some fruit and vegetables (Patterson et al., 2008; Vesper et al., 2000; Wang et al., 2021).

CONCLUSIONS

The update of the case -control FCDB for choline and SMs showed the importance of the punctual definition of foods, food components and units of measure, the detailed description of the methodology, sampling and extraction procedures, and the clear expression of the data. For SMs in fact, several composition data were available in the literature, but they were overall extremely heterogenous and thus, impossible to use in FCDBs.

In conclusion, despite the methodological difficulties and poor quality of some SMs data, the update of the case-control database for SM and choline produced a suitable FCDB to assess dietary choline and SM intakes in a large observational case-control study.

4.6.2. PREBIOTICS

PREBIOTIC COMPOSITION IN ITALIAN FOODS

A trending interest in the nutritional field is currently the study of prebiotics compounds that can be beneficial to the gut microbial composition. However, current epidemiological results are not yet significant (Delgado et al., 2021). This is mainly due to the lack of FCD regarding the content of prebiotic compounds in foods, and the complex and not yet clear biological mechanisms that intercede in the gut, reducing the scientific strength of possible epidemiological findings.

The content of GOSs and FOSs in the 78 analysed food sources and the content of ITFs in the 7 analysed food sources are reported in **Table 29**. The primary food sources of ITFs were represented by garlic (25.1 g/100g) and jerusalem artichoke (16.7 g/100g), as reported in previous studies (Judprasong et al., 2011; Van Loo et al., 1995), followed by banana (4.8 g/100g), shallot (4.5 g/100g), artichoke (4.3 g/100g), onion (1 g/100g) and leek (0.9 g/100g). However, garlic, onion and shallot, which are typical ingredients used in traditional dishes of the Mediterranean diet, are usually consumed in minimal amounts. As observed in the latest Italian food consumption survey, of a total of 222±112 g/day mean consumption of vegetables in the Italian population, 20±26 g/day (0.3% contribution to total energy intake, 2.4% contribution to total fibre intake) are attributable to the food group: "Bulb and root vegetables" (Piccinelli et al., 2011; Sette et al., 2013), which includes most of the sources of ITFs.

Jerusalem artichoke also represents the main source of total FOSs (4.45 g/100g), while in the other foods analysed, the content is less than 1 g/100 g. Shallot (0.90 g/100g), garlic (0.37 g/100g), wholegrain biscuits (0.34 g/100g) and other cereal-based products are also relevant sources of FOSs, particularly kestose. Moreover, wholegrain cereal-based products (N=9; 0.08±0.12 g/100g) generally contain a greater amount of FOSs compared to refined cereal-based products (N=13; 0.06±0.08 g/100g). Vegetables contain extremely variable amount of FOSs (N=32; 0.19±0.80 g/100g) in different chemical forms. Fruits and pulses contain FOSs in detectable amounts only in the form of kestose, particularly banana (0.17 g/100g) and apricot (0.14 g/100g), with the other foods in the same food categories containing less than 0.03 g/100g.

Pulses, excluding green beans, were the primary source of GOSs, expressed as the sum of raffinose and stachyose (1.2±0.9 g/100g). Raffinose was particularly abundant in dried peas (0.50 g/100g) and chickpeas (0.46 g/100 g) and stachyose in dried beans (1.91 g/100g), peas (1.81 g/100g), and chickpeas (1.62 g/100 g) followed by canned beans (1.27 g/100 g), and canned and dried lentils (0.41 g/100g and 0.39 g/100g, respectively). With values slightly lower than those of pulses, cereals and cereal-based products also represent a significant source of GOSs, almost exclusively in the form of raffinose. Wholegrain products generally showed a higher content of raffinose than their refined counterparts. The raffinose-richest food products included whole wheat flour (0.30 g/100g), barley (0.22 g/100 g), and other wholegrain-based products (e.g., wholegrain biscuits, wholegrain pasta). Raffinose was detected also in white wheat pasta, biscuits, flour, rice, and in commercially prepared cakes, but in lower amounts (<0.06 g/100 g).

Table 29. *Inulin type fructans, fructo- and galacto-oligosaccharides composition of a selection of Italian foods.*

Common name	**ITFs**	**FOSs**			**GOSs**	
		Kestose	Nystose	FF-Nystose	Raffinose	Stachyose
FRUITS						
Apple		0.009	<LOQ*	<LOQ*	0.003	0.003
Apricot		0.137	<LOQ*	<LOQ*	<LOQ‡	<LOQ*
Banana	4.8	0.168	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Cherry		<LOQ*	<LOQ*	<LOQ*	0.040	<LOQ*
Grapefruit		0.015	<LOQ*	<LOQ*	0.012	<LOQ*
Grapes		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Kiwi fruit		0.006	<LOQ†	<LOQ†	0.006	<LOQ‡
Melon, cantaloupe		0.028	<LOQ*	<LOQ*	0.005	0.004
Melon, honeydew		0.007	<LOQ*	<LOQ*	0.003	<LOQ*
Orange		0.012	<LOQ*	<LOQ*	0.011	0.005
Peach		0.015	<LOQ‡	<LOQ*	0.005	<LOQ*
Pear		<LOQ‡	<LOQ‡	<LOQ*	<LOQ‡	<LOQ‡
Plum		0.003	<LOQ*	<LOQ*	0.003	<LOQ*
Strawberries		0.007	<LOQ†	<LOQ†	0.005	<LOQ*
Watermelon		0.004	<LOQ*	<LOQ*	<LOQ*	0.003
VEGETABLES						
Artichoke	4.3	0.024	0.005	0.003	<LOQ*	<LOQ*
Asparagus		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Aubergine/ eggplant		0.005	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Cabbage, green		<LOQ*	<LOQ*	<LOQ*	0.024	<LOQ*
Carrot		0.022	<LOQ*	<LOQ*	<LOQ‡	<LOQ‡
Cauliflower		0.004	<LOQ*	<LOQ*	0.012	<LOQ*
Celeriac		0.022	<LOQ*	<LOQ*	0.005	<LOQ*
Celery		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Chard		0.003	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Chicory		0.004	<LOQ*	<LOQ*	0.003	<LOQ*
Chicory, radicchio, green		<LOQ*	<LOQ*	<LOQ*	0.007	<LOQ*
Chicory, radicchio, red		0.004	<LOQ*	<LOQ*	0.063	<LOQ*
Courgettes/ zucchini		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Cucumber		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Endive		0.006	0.002	<LOQ*	0.003	<LOQ*
Endive, curly		0.006	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Endive, Scarola		0.004	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Fennel		0.003	<LOQ*	<LOQ*	<LOQ*	0.003
Garlic	25.1	0.31	0.045	0.016	<LOQ‡	<LOQ*
Jerusalem artichoke	16.7	1.521	1.597	1.336	<LOQ*	<LOQ*
Leek	0.9	<LOQ*	<LOQ*	<LOQ*	<LOQ‡	<LOQ*
Lettuce		0.004	<LOQ*	<LOQ*	0.003	<LOQ*
Onion	1.0	0.097	0.008	<LOQ†	<LOQ‡	<LOQ‡
Peppers/ *Capsicum* sweet type		0.009	<LOQ*	<LOQ*	<LOQ*	0.003
Potato		<LOQ†	<LOQ*	<LOQ*	<LOQ*	<LOQ*

Pumpkin/ squash		0.072	<LOQ†	<LOQ†	0.010	0.012
Red Radish		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Shallot	4.5	0.544	0.219	0.141	<LOQ*	<LOQ*
Spinach		<LOQ*	0.002	<LOQ*	0.003	<LOQ*
Sweet potatoes		0.029	<LOQ*	<LOQ*	0.010	<LOQ*
Tomatoes		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
Tomatoes, peeled, canned		<LOQ*	<LOQ*	<LOQ*	<LOQ*	<LOQ*
PULSES						
Beans, common, canned		0.002	<LOQ*	<LOQ*	0.141	1.270
Beans, common, dried		<LOQ†	<LOQ†	<LOQ†	0.133	1.905
Chickpeas, canned		<LOQ*	<LOQ*	<LOQ*	0.089	0.277
Chickpeas, dried		<LOQ†	<LOQ†	<LOQ†	0.463	1.619
Green beans		0.022	<LOQ*	<LOQ*	0.004	0.006
Lentils, canned		<LOQ*	<LOQ*	<LOQ*	0.032	0.410
Lentils, dried		<LOQ*	<LOQ*	<LOQ*	0.033	0.387
Peas, canned		0.010	<LOQ*	<LOQ*	0.052	0.267
Peas, dried, “BIO”		0.004	<LOQ*	<LOQ*	0.498	1.814
CEREAL AND CEREAL PRODUCTS						
Barley, pearl		0.151	0.094	0.024	0.223	0.011
Biscuits		0.052	0.003	<LOQ*	0.044	<LOQ⁑
Biscuits, wholegrain		0.175	0.083	0.081	0.220	0.003
Bread, white		0.008	<LOQ*	<LOQ†	<LOQ*	<LOQ*
Bread, whole wheat		0.017	<LOQ*	<LOQ*	0.004	<LOQ*
Breadsticks		<LOQ‡	<LOQ*	<LOQ†	<LOQ⁑	<LOQ*
Breadsticks, wholegrain		<LOQ†	<LOQ‡	<LOQ†	<LOQ†	<LOQ†
Brioche		<LOQ‡	<LOQ*	<LOQ*	0.005	<LOQ⁑
Pastry roll, chocolate		0.166	<LOQ†	<LOQ†	0.017	<LOQ*
Crackers		0.014	<LOQ*	<LOQ*	0.009	<LOQ⁑
Crackers, wholegrain		<LOQ†	<LOQ†	<LOQ†	0.015	<LOQ†
Cream puff (“*bignè*” pastry)		0.027	<LOQ*	<LOQ*	0.034	<LOQ*
Italian traditional Easter cake (“*colomba*”)		0.082	<LOQ*	<LOQ*	0.014	0.006
Melba toast		<LOQ‡	<LOQ†	<LOQ†	<LOQ*	<LOQ⁑
Melba toast, wholegrain		<LOQ‡	<LOQ*	<LOQ†	<LOQ⁑	<LOQ*
Pasta		0.106	0.021	<LOQ†	0.064	<LOQ⁑
Pasta, whole wheat		0.184	0.019	<LOQ‡	0.169	<LOQ⁑
Rice, brown		0.008	<LOQ*	<LOQ*	0.026	<LOQ⁑
Rice, grains, white, Arborio		0.002	<LOQ*	<LOQ*	0.010	<LOQ⁑
Sponge cake		0.022	<LOQ*	<LOQ*	0.035	<LOQ†
Wheat flour, white, type 00		0.042	0.0033	<LOQ*	0.044	<LOQ*
Whole-wheat flour, “BIO”		0.136	0.0041	<LOQ*	0.299	0.018

Limit of quantification: *<0.002; ⁑<0.005; †<0.010; ‡<0.020. Abbreviations: LOQ, limit of quantification, FF-Nystose, 1F-β-fructofuranosylnystose; ITFs, inulin-type fructans; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides.

COMPARISON WITH LITERATURE DATA

A comparison between FOSs and GOSs content in the analysed foods sources *vs.* data from literature was performed (**Table 30.A, B, C, and D)**. Excluding historical data from the USDA's tables (Matthews et al., 1987), which do not reflect the current scientific knowledge, other authors have published data on the content of FOSs (Biesiekierski et al., 2011; Campbell et al., 1997; Hogarth et al., 2000; Judprasong et al., 2011; Muir et al., 2009) and GOSs (Biesiekierski et al., 2011; Kotha et al., 2020; Muir et al., 2009) in plant-based products (fruits, pulses and vegetables) and cereal-based products available in the geographical region of interest.

In general, the comparison highlighted a certain variability, probably due to the geographical origin of the product, the cultivar, the physical form or preparation of the food, or the method of analysis which carries dissimilar limits of quantification. The bigger differences among literature data have been found in the total FOSs content of jerusalem artichoke, onion and shallot. Differences greater than 0.15 g/100g have also been found for the kestose content of banana and artichoke; nystose content of asparagus, cabbage, fennel and garlic; 1F-β-fructofuranosil-nystose content of garlic. The major differences regarding GOSs content have been found particularly on the raffinose content of pulses, onion, bread and wheat flour. Khota and colleagues (Kotha et al., 2020) reported the most different values of GOSs in dry pulses, which may be due to methodological differences since the authors developed and validated the analytical method they used to quantify soluble oligosaccharides.

Table 30.A. *Comparison of fructo- and galacto-oligosaccharides content in Italian fruits with literature data (Campbell et al., 1997; Hogarth et al., 2000; Muir et al., 2009).*

	Kestose	Nystose	FF-Nystose	Raffinose	Stachyose	Type/original name, if different
APPLE						
Present	0.009	<0.002	<0.002	0.003	0.003	
Muir et al, 2009	nd	nd		nd	nd	apple, Granny Smith and Pink Lady, unpeeled and peeled
Campbell et al, 1997	0.010	0	0			apple, Red Delicious, Granny Smith, Jonagold
Campbell et al, 1997	0	0	0			apple, Golden Delicious, Rome
Hogarth et al, 2000	<0.02	<0.02	<0.02			
BANANA						
Present	0.168	<0.002	<0.002	<0.002	<0.002	
Muir et al, 2009	nd	nd		nd	nd	banana, common, firm
Muir et al, 2009	tr	nd		nd	nd	banana, common, medium ripeness
Campbell et al, 1997	0.140	0	0			banana
Campbell et al, 1997	0.160	0	0.040			banana, ripe
Hogarth et al, 2000	0.02-0.11	<0.02	0.020			Bananas, stage 1
Judprasong et al, 2011	0.090	0	0			Banana, common, ripe
Judprasong et al, 2011	0.020	0	0			Banana, common, unripe
GRAPEFRUIT						
Present	0.015	<0.002	<0.002	0.012	<0.002	
Muir et al, 2009	nd	nd		nd	nd	
GRAPES						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	
Muir et al, 2009	Nd	nd		nd	nd	grapes, black muscatel, Ralli seedless, Thompson, Red Globe, Red
Campbell et al, 1997	0.010	0	0.010			grapes, black
Campbell et al, 1997	0	0	0			grapes, Thompson
Hogarth et al, 2000	<0.02	<0.02	<0.02			grapes, seedless
KIWI						
Present	0.006	<0.010	<0.010	0.006	<0.005	
Muir et al, 2009	nd	nd		nd	nd	
Campbell et al, 1997	0	0	0			
MELON						
Present	0.028	<0.002	<0.002	0.005	0.004	Cantaloupe
Present	0.007	<0.002	<0.002	0.003	<0.002	Honeydew
Muir et al, 2009	nd	nd		nd	nd	melon, honeydew and cantaloupe
Campbell et al, 1997	0	0	0			cantaloupe
ORANGE						

Present	0.012	<0.002	<0.002	0.011	0.005	
Muir et al, 2009	nd	nd		nd	nd	orange, navel
Campbell et al, 1997	0.020	0	0.010			orange, navel
PEACH						
Present	0.015	<0.005	<0.002	0.0050	<0.002	
Muir et al, 2009	0.080	0.510		nd	nd	nectarine
Muir et al, 2009	tr	nd		nd	nd	peach, clingstone and white
Campbell et al, 1997	0.040	0	0			
PEAR						
Present	<0.005	<0.005	<0.002	<0.005	<0.005	
Muir et al, 2009	nd	nd		nd	nd	pear, Packham, peeled, firm and ripe
Campbell et al, 1997	0.010	0	0			pear, bosc
Campbell et al, 1997	0	0	0.020			pear, d'Anjou
PLUM						
Present	0.003	<0.002	<0.002	0.003	<0.002	
Campbell et al, 1997	0.020	0	0			plum, red
STRAWBERRY						
Present	0.007	<0.010	<0.010	0.005	<0.002	
Campbell et al, 1997	tr	0	0			
WATERMELON						
Present	0.004	<0.002	<0.002	<0.002	0.003	
Muir et al, 2009	0	0.200		0	0	watermelon, seedless

Table 30.B. *Comparison of fructo- and galacto-oligosaccharides content in Italian vegetables with literature data (Biesiekierski et al., 2011; Campbell et al., 1997; Hogarth et al., 2000; Judprasong et al., 2011; Matthews et al., 1987; Muir et al., 2009).*

	Kestose	**Nystose**	**FF-Nystose**	**Raffinose**	**Stachyose**	**Type/original name, if different**
ARTICHOKE						
Present	0.024	0.005	0.003	<0.002	<0.002	
Muir et al, 2009	tr	tr		nd	nd	
Campbell et al, 1997	0.150	0.060	0.0300			
Hogarth et al, 2000	<0.02-0.04	<0.02-0.05	<0.02-0.04			artichoke, hearts
ASPARAGUS						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	
Judprasong et al, 2011	0	0	0			
Muir et al, 2009	0.090	0.340		nd	nd	
Campbell et al, 1997	0	0	0			
Hogarth et al, 2000	<0.02	<0.02	<0.02			
CABBAGE						

Present	<0.002	<0.002	<0.002	0.024	<0.002	
Muir et al, 2009	nd	0.460		nd	nd	
Matthews et al, 1987				0.100	0.100	
CARROT						
Present	0.022	<0.002	<0.002	<0.005	<0.005	
Judprasong et al, 2011	0.040	0	0			
Muir et al, 2009	nd	nd		nd	nd	
Campbell et al, 1997	0.030	0	0			carrot, Bunny Luv
Campbell et al, 1997	0.020	0	0			carrot, Dole
Matthews et al, 1987				0.100	0.100	
CAULIFLOWER						
Present	0.004	<0.002	<0.002	0.012	<0.002	
Muir et al, 2009	nd	nd		nd	nd	
Matthews et al, 1987					0.100	
CELERY						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	
Campbell et al, 1997	0	0	0			
CHICORY						
Present	0.004	<0.002	<0.002	0.003	<0.002	chicory
Present	0.004	<0.002	<0.002	0.063	<0.002	chicory, radicchio, red
Present	<0.002	<0.002	<0.002	0.007	<0.002	chicory, radicchio, green
Muir et al, 2009	0.0500	0.120		nd	0.080	chicory leaves
Matthews et al, 1987				1.200	0.300	chicory, raw
COURGETTES/ ZUCCHINI						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	
Muir et al, 2009	nd	nd		nd	nd	
Campbell et al, 1997	0	0	0			
CUCUMBER						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	
Muir et al, 2009	nd	nd		nd	nd	cucumber, peeled/ cucumber, unpeeled
EGGPLANT / AUBERGINE						
Present	0.005	<0.002	<0.002	<0.002	<0.002	
Judprasong et al, 2011	0	0	0			
Campbell et al, 1997	0	0	0			
ENDIVE						
Present	0.006	0.002	<0.002	0.003	<0.002	endive
Present	0.004	<0.002	<0.002	<0.002	<0.002	endive, scarola
Present	0.006	<0.002	<0.002	<0.002	<0.002	endive, curly
Muir et al, 2009	nd	nd		nd	nd	lettuce, Red Coral
Campbell et al, 1997	0	0	0			endive

FENNEL						
Present	0.003	<0.002	<0.002	<0.002	0.003	
Muir et al, 2009	0.150	0.16		nd	0.100	fennel, bulb
GARLIC						
Present	0.306	0.045	0.016	<0.005	<0.002	
Judprasong et al, 2011	0.340	0.320	0.300			
Muir et al, 2009	0.210	0.710				
Campbell et al, 1997	0.330	0.040	0.020			
JERUSALEM ARTICHOKE						
Present	1.521	1.597	1.336	<0.002	<0.002	
Judprasong et al, 2011	2.020	1.750	1.390			
Muir et al, 2009	tr	nd		nd	nd	
Campbell et al, 1997	1.920	1.920	2.000			
LEEK						
Present	<0.002	<0.002	<0.002	<0.005	<0.002	
Judprasong et al, 2011	0.110	0.060	0.060			
Campbell et al, 1997	0.07	0.01	0.01			
Matthews et al, 1987				0.100	0.600	
LETTUCE						
Present	0.004	<0.002	<0.002	0.003	<0.002	
Muir et al, 2009	nd	tr		nd	nd	lettuce, butter
Campbell et al, 1997	0.030	0.010	0.010			lettuce
Matthews et al, 1987				0.100	-	lettuce, cos, raw
ONION						
Present	0.097	0.008	<0.010	<0.005	<0.005	
Judprasong et al, 2011	0.140	0.030	0.020			onion
Judprasong et al, 2011	0.970	0.800	0.700			onion, red
Muir et al, 2009	0.130	0.260		0.190	nd	onion, white
Campbell et al, 1997	0.110	0.020	0.010			onion, red
Campbell et al, 1997	0.170	0.090	0.060			onion, white
Campbell et al, 1997	0.150	0.060	0.040			onion yellow
Hogarth et al, 2000	0.020	0.020	<0.02			
Matthews et al, 1987				1.400	0.700	onions, mature, raw
PEPPER/ CAPISCUM, SWEET						
Present	0.009	<0.002	<0.002	<0.002	0.003	
Muir et al, 2009	nd	nd		nd	nd	Capsicum, green / capsicum, red
Matthews et al, 1987				0.100	-	pepper, sweet, green, raw
POTATOES						

Present	<0.010	<0.002	<0.002	<0.002	<0.002	
Judprasong et al, 2011	0	0	0			
Muir et al, 2009	nd	nd		nd	nd	Potato, unpeeled
Campbell et al, 1997	0	0	0			Potato, Idaho
PUMPKIN/ CUSHAW SQUASH						
Present	0.072	<0.010	<0.010	0.010	0.012	
Judprasong et al, 2011	0	0	0			
Muir et al, 2009	nd	nd		nd	nd	
Hogarth et al, 2000	<0.02	<0.02	<0.02			
Matthews et al, 1987				0.100	0.100	
RED RADISH						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	
Judprasong et al, 2011	0	0	0			
Campbell et al, 1997	0	0	0.010			
SHALLOT						
Present	0.544	0.219	0.141	<0.002	<0.002	
Judprasong et al, 2011	1.630	1.810	1.900			
Campbell et al, 1997	0.450	0.230	0.170			
SPINACH						
Present	<0.002	0.0023	<0.002	0.0025	<0.002	
Muir et al, 2009	nd	nd		nd	nd	spinach, baby
SWEET POTATOES						
Present	0.029	<0.002	<0.002	0.010	<0.002	
Judprasong et al, 2011	0.140	0	0			
Muir et al, 2009	nd	nd		nd	nd	
Campbell et al, 1997	0.020	0	0			
Hogarth et al, 2000	<0.02	<0.02	0.030			
TOMATO						
Present	<0.002	<0.002	<0.002	<0.002	<0.002	raw
Present	<0.002	<0.002	<0.002	<0.002	<0.002	tomato, peeled, canned
Muir et al, 2009	0.09	nd		nd	nd	tomato, common
Muir et al, 2009	0.07	0.01		nd	nd	tomato, roma
Muir et al, 2009	nd	nd		nd	nd	tomato, cherry
Campbell et al, 1997	0	0	0			tomato; tomato, cherry; tomato, Roma
Hogarth et al, 2000	0.030	<0.02	0.220			tomato paste
Hogarth et al, 2000	<0.02	<0.02	<0.02			tomato puree
Matthews et al, 1987				0	-	tomato paste

Table 30.C. *Comparison of fructo- and galacto-oligosaccharides content in Italian pulses with literature data (Biesiekierski et al., 2011; Campbell et al., 1997; Judprasong et al., 2011; Kotha et al., 2020; Matthews et al., 1987; Muir et al., 2009).*

	Kestose	Nystose	FF-Nystose	Raffinose	Stachyose	Type/original name, if different
BEANS, COMMON						
Present	0.002	<0.002	<0.002	0.141	1.270	canned
Present	<0.010	<0.010	<0.010	0.133	1.905	dry
Biesiekierski et al, 2011	nd	nd		0.100	0.510	beans, mixed, canned
Biesiekierski et al, 2011	0.100	nd		0.480	0.520	borlotti beans, canned
Biesiekierski et al, 2011	0.080	0.140		0.050	0.370	butter beans, canned
Biesiekierski et al, 2011	nd	nd		0.250	0.840	haricot beans, boiled
Biesiekierski et al, 2011	0.510	nd		0.800	1.160	red kidney beans, boiled
Campbell et al, 1997	0	0.010	tr			bean, kidney
Campbell et al, 1997	0	0.010	tr			bean, kidney, dry matter
Matthews et al, 1987				0.300	1.500	raw
Matthews et al, 1987				0.200	0.700	cooked
Kotha et al, 2020				0.342	3.847	white kidney, dry
CHICKPEAS						
Present	<0.002	<0.002	<0.002	0.089	0.277	canned
Present	<0.010	<0.010	<0.010	0.463	1.619	dry
Muir et al, 2009	tr	nd		0.680	0.570	soaked and boiled
Biesiekierski et al, 2011	nd	0.070		0.110	0.080	canned
Matthews et al, 1987				0.700	2.400	raw
Matthews et al, 1987				0.400	0.500	cooked
GREEN BEANS						
Present	0.022	<0.002	<0.002	0.004	0.006	
Muir et al, 2009	nd	nd		nd	nd	
Campbell et al, 1997	0	0	0			
LENTILS						
Present	<0.002	<0.002	<0.002	0.032	0.410	canned
Present	<0.002	<0.002	<0.002	0.033	0.387	dry
Biesiekierski et al, 2011	nd	nd		0.050	0.410	lentils green, boiled
Biesiekierski et al, 2011	nd	0.170		0.060	0.400	lentils, red, boiled
Biesiekierski et al, 2011	nd	nd		0.030	0.190	lentils, canned
Matthews et al, 1987				0.300	1.900	raw
Kotha et al, 2020				0.257	2.738	lentils, dry
PEAS						
Present	0.010	<0.002	<0.002	0.052	0.267	canned
Present	0.004	<0.002	<0.002	0.498	1.814	dried, BIO

Judprasong et al, 2011	0.090	0	0			garden peas
Biesiekierski et al, 2011	nd	nd		0.330	1.550	split peas, boiled
Campbell et al, 1997	0	0	0.010			peas
Matthews et al, 1987				0.700	2.100	peas, split, raw
Kotha et al, 2020				0.883	3.267	green split peas, dry

Table 30.D. *Comparison of fructo- and galacto-oligosaccharides content in Italian cereal products* with literature *data (Biesiekierski et al., 2011; Hogarth et al., 2000; Matthews et al., 1987).*

	Kestose	**Nystose**	**FF-Nystose**	**Raffinose**	**Stachyose**	**Type/original name, if different**
BARLEY, GRAINS						
Present	0.151	0.094	0.024	0.223	0.011	
Hogarth et al, 2000	0.140	0.050	<0.02			barley, quick cook
BISCUITS						
Present	0.052	0.003	<0.002	0.044	<0.005	
Biesiekierski et al, 2011	nd	nd		nd	nd	biscuit, sweet, plain and shortbread
BREAD, WHEAT						
Present	0.008	<0.002	<0.010	<0.002	<0.002	white
Present	0.017	<0.002	<0.002	0.004	<0.002	wholegrain
Biesiekierski et al, 2011	nd	0.110		0.200	nd	white
Biesiekierski et al, 2011	nd	nd		0.230	0.360	wholegrain
CRAKERS						
Present	0.014	<0.002	<0.002	0.009	<0.005	
Present	<0.010	<0.010	<0.010	0.015	<0.010	wholegrain
Biesiekierski et al, 2011	nd	nd		nd	nd	biscuit savoury, plain and wholemeal
PASTA, WHEAT						
Present	0.106	0.021	<0.010	0.064	<0.005	
Biesiekierski et al, 2011	nd	nd		nd	nd	
RICE, GRAINS						
Present	0.0022	<0.002	<0.002	0.010	0	white, Arborio
Present	0.0076	<0.002	<0.002	0.026	<0.005	brown
Hogarth et al, 2000	<0.02	<0.02	<0.02			rice cereal
Biesiekierski et al, 2011	nd	nd		nd	nd	white and brown
WHEAT FLOUR						
Present	0.042	0.003	<0.002	0.044	<0.002	white, type 00
Present	0.136	0.004	<0.002	0.299	0.018	whole wheat, BIO
Hogarth et al, 2000	0.150	<0.02	<0.02			
Matthews et al, 1987				0.200		wheat and whole wheat flour

Abbreviations: FF-Nystose, 1F-β-fructofuranosylnystose; ITFs, inulin-type fructans; FOS, fructo-oligosaccharides; GOS, galacto-oligosaccharides.

INULIN DEFINITION AND METHODOLOGICAL ISSUES

ITFs are a group of water soluble, non-digestible and fermentable carbohydrates having a prebiotic effect. The main, if not exclusive, presence of β (2→1) fructosyl-fructose glycosidic bonds endow ITFs with unique physicochemical and physiological properties, rendering them resistance to hydrolysis by digestive enzyme of the human upper gastrointestinal tract (Roberfroid et al., 2010). ITFs include inulin and FOSs, depending on their degree of polymerization (DP). All these compounds exert strong bifidogenic effects, although the length of polymerization may influence these effects. However, while the definition of FOSs is clear (DP of 2–10), the definition of inulin is not fully agreed. Some authors describe inulin as the product of physical separation techniques to remove all but high DP (>10) ITFs (Kelly, 2008). Others (Roberfroid, 2007; Shoaib et al., 2016; Van Loo et al., 1995) referred to inulin as the generic extract of chicory root plant fructans wherein the majority of fructosyl units are linked by means of β (2→1)-bonds; thus, including in this definition total ITFs, and possibly other type of fructans, depending on the analytical method and enzyme used. It is not clear whether dissimilar methods are capable of accurately discriminating ITFs to other types of fructans without interference. This may be the case of cereal and pulse products, which contains significant amounts of fructans (Biesiekierski et al., 2011). Indeed, cereals, which are an important fructan source in the western diet, mainly contain graminian-type fructans (Haskå et al., 2008), which, contrarily to the linear-chained ITFs, present both β 2→1 and β 2→6 bonds (Verspreet et al., 2015). For example, Van Loo and colleagues (Van Loo et al., 1995) reported a total inulin content of wheat ranging from 1 to 4%, mainly with low DP, while Biesiekierski 2011 reported a total fructan value of 0.68 g/100g of which only 0.11 g/100g from nystose.

The comparison between the analysed food sources and data from literature regarding ITFs content are shown in **Table 31,** together with the corresponding analytical method used. As noted, there was an overall heterogeneity in methodologies. Therefore, the comparison of the results showed significantly different data. Indirect enzymatic methods based on hydrolysis with inulinase (Van Loo et al., 1995) or inulinase plus amyloglucosidase (Judprasong et al., 2011) combined with gas-chromatography and flame ionization detector (GC-FID), are typically used to analyse (most exclusively) ITFs and FOSs. However, these methods do not give indication on the degree of polymerization (DP).

Table 31. *Comparison between methods and resulted composition of banana, artichoke, garlic, and jerusalem artichoke, according to 4 previous studies (Judprasong et al., 2011; Muir et al., 2007; Van Loo et al., 1995).*

METHODS	***Present***	***Judprasong et al, 2011***	***Van Loo et al, 1995***	***Muir et al, 2007***
Based on:	AOAC 997.08	AOAC 997.08	-	AOAC 999.03
Enzymes:	inulinase + amyloglucosidase	inulinase + amyloglucosidase	inulinase	fructanase
Determination:	HPAEC-PAD	GC-FID	GC-FID	-
Compound detected:	ITFs (most exclusively)	ITFs (most exclusively)	ITFs	fructans (inulin and graminian-type)
FOODS	***Present***	***Judprasong et al, 2011***	***Van Loo et al, 1995***	***Muir et al, 2007***
Banana	4.8	0.06; 0.4	0.3-0.7	
Artichoke	4.3		2.0-6.8	1.2
Garlic	25.1	22.4±2.86	9.8-16.0	17.4
Jerusalem artichoke	16.7	19.4±1.04	16.0-20.0	12.20
Leek	0.9	0.48	3.00	0.50
Onion	1.0	0.44; 3.56±0.95	1.1-7.5	1.8; 2.1
Shallot	4.5	8.86±0.75		8.9

Data are expressed as punctual value, range (-), distinct values (;), or mean±SD, as reported in the original source. Abbreviations: GC-FID, gas chromatography paired with flame ionization detector; HPAE-PAD: high-performance anion-exchange chromatography coupled with pulsed amperometric detection; ITFs: inulin-type fructans.

CONCLUSIONS

Despite ITFs, GOSs, and FOSs distribute widely in a large variety of plant products, there is lack of comprehensive FCD regarding naturally occurring prebiotics in commonly consumed foods in Italy. Moreover, there is no harmonization in definitions, analytical methods, and form in which data are presented in the literature, particularly regarding ITFs. Due to methodological and terminological heterogenicity it may be challenging to use literature data in FCDBs. Large amount of missing data, especially in the most consumed foods in the country, as well as errors in the FCDB compilation process may lead to possible under- or over-estimation of prebiotic dietary intake. Accordingly, the present work produced quantitative data on some of the major short chain carbohydrates with prebiotic effects in a wide range of commonly consumed raw and processed foods in Italy.

4.7. APPLICATION OF THE PREBIOTICS DATA IN NUTRITIONAL EPIDEMIOLOGY

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Authors: Turati F., Concina F., Rossi M., Fiori F., Parpinel M., Taborelli M., Giacosa A., Crispo A., Pagan E., Rosato V., Garavello W., Negri E., La Vecchia C.
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PREBIOTIC INTAKE

Within the "PrebiotiCa" project, we determined the daily intake of six fibre fractions with prebiotic activity (ITFs, nystose, kestose, 1F-β-fructofuranosylnystose, raffinose, and stachyose) in either cases and controls, using novel analytical FCD (**Chapter 4.6.2**). For the purpose of our study, the study-specific FCDB was compiled with the prebiotic content of simple foods (e.g., flour, specific legumes, fruits and vegetables) and complex recipes (e.g., bread, pasta, sweets) and applied to the self-reported weekly frequency of consumption of foods, groups of foods, or recipes obtained from the FFQ.

Of the food products investigated in the FFQ, legumes, whole-wheat flour, whole-grain based products, and barley represented the principal sources of GOSs. Raffinose was detected in certain amounts also in wheat products, while stachyose was detected almost exclusively in pulses. Accounting for consumption of food, the largest contributors of raffinose and stachyose in our population were cereal-based products and legumes, while other food groups, including vegetables and fruit, provided limited contributions (**Table 32**).

Table 32. *Percentage contribution of prebiotic intakes from different food groups.*

	Cereals and CBPs	*Pulses*	*Soups (vegetables, cereals, and pulses included)*	*Fruit*	*Vegetables*
Raffinose	**42%**	11%	16%	15%	
Stachyose		34%	**62%**		
ITFs	12.%*			**57%**	21%
Kestose	**37%**			35%	
Nystose	**64%**				

Only percentage contributions >10% were displayed; * mainly derived from garlic in cereal-based recipes. The main food group contribution for each prebiotic compound is highlighted in bold typeface. Abbreviations: CBPs, cereal based products; ITFs: inulin-type fructans.

ITFs were very abundant in garlic, but when consumption of food was accounted for, banana was the most important source, accounting alone for more than 50% of ITF intake in our population. As for dietary FOSs, the largest contributors of kestose in our population were cereals and fruit, in a similar proportion. Nystose and 1F-β-fructofuranosylnystose were detected in very small concentrations in a limited range of foods, consumed in small amounts by our population. As such, a low daily intake of nystose and 1F-β-fructofuranosylnystose were estimated in our

population, with limited variation across subjects. The quintiles upper cut points of daily prebiotic fibre intakes in our population are reported in **Table 33**.

Table 33. *Quintiles of daily prebiotic fibre intakes.*

	Upper cut point				
	Q1	Q2	Q3	Q4	Q5
Inulin-type fructans (mg/d)	377	642	978	1705	---
Kestose (FOS) (mg/d)	120	152	183	230	---
Nystose (FOS) (mg/d)	11	14	17	21	---
1F-β-fructofuranosylnystose (FOS) (mg/d)	0.8	1.9	3.1	8.0	---
Total FOSs (mg/d)	0.13	0.17	0.21	0.26	---
Raffinose (GOS) (mg/d)	68	85	102	125	---
Stachyose (GOS) (mg/d)	93	163	224	341	---

Abbreviations: d, day; Q, quintile; FOS, fructo-oligosaccharides; GOS: galacto-oligosaccharides.

Moreover, within our population, the intake of prebiotic fibre was positively correlated with total fibre intake: Pearson correlation coefficients were 0.31 (ITFs), 0.49 (kestose), 0.36 (nystose), 0.70 (1F-β-fructofuranosylnystose), 0.70 (total FOSs), 0.72 (raffinose), and 0.45 (stachyose). While a wealth of data is available on the role of fibre on colorectal cancer risk (Reynolds et al., 2019), no epidemiological study appears to have evaluated the association of this neoplasms with naturally occurring dietary prebiotics. The hypothesis of the role of dietary prebiotics in the prevention of colon cancer originate from the findings that prebiotics selectively stimulate the growth and the activity of some beneficial colonic bacteria, particularly *Bifidobacetria*. Such changes in the microbiota's composition have been recognised to be a marker of intestinal health. There is also tentative evidence in animal studies that a prebiotic rich diet may decrease the incidence of colon cancer, obesity, metabolic syndrome and type 2 diabetes (Roberfroid et al., 2010).

COLON RECTAL CANCER RISK

In the present analysis, both colon and rectal cancer cases were older and reported more frequently family history of intestinal cancer than controls. Colon, but not rectal, cancer cases were more educated than controls and reported more frequently a low level of physical activity. Participants with higher prebiotic intake were less frequently women, had higher total energy intake, reported less frequently history of diabetes, and, with the exception of stachyose, tended to be younger. Subjects with higher intake of ITFs tended to have a lower level of physical activity and to be more frequently current smokers; those with higher intakes of raffinose and total FOSs tended to be more frequently alcohol drinkers. Women with higher ITF, raffinose, or total FOS intakes were less frequently in menopause (data not shown).

Total ITFs, kestose, nystose and 1F-β-fructofuranosylnystose, and consequently total FOSs, were not associated with colorectal cancer risk.

On the other hand, a significant inverse association was observed with the intake of GOSs. The OR for an increase of intake equal to the difference between the 80° and 20° percentiles were 0.85 (95% CI, 0.76-0.96) for raffinose and 0.81 (95% CI, 0.74-0.89) with stachyose. In the analysis of quintiles of intake, the OR for the highest versus the lowest category were 0.73 (95% CI, 0.58-0.92) for raffinose and 0.64 (95% CI, 0.53-0.77) for stachyose, with significant trends of decreasing risk across quintiles. When further adjustment for total fibre intake was performed, the

association with raffinose intake attenuated (OR for Q5 versus Q1: 0.80, 95% CI, 0.62-1.04) while that with stachyose intake remained unchanged (OR for Q5 versus Q1: 0.66, 95% CI, 0.54-0.80, p for trend across quintiles: <0.001). Although the association with raffinose intake was attenuated when additional adjustment for total fibre intake was made, the association with stachyose intake remained unchanged, suggesting that part of the protection afforded by fibre consumption on colorectal cancer development may be through prebiotic effects (data not shown).

Supporting our results, the intakes of wholegrain products and fibre from grains have been favourably related to the risk of colorectal cancer in various studies (Larsson et al., 2005; Oh et al., 2019; Schwingshackl et al., 2018). Interesting, an association with the intake of grain fibre was observed even in studies in which no association with total fibre was detected (Bradbury et al., 2020; He et al., 2019; Hullings et al., 2020). Evidence on the favourable role of legume intake on colorectal cancer is less clear. According to a meta-analysis published in 2015 and based on 14 cohort studies, higher legumes consumption was associated with an approximately significant 10% reduced risk of colorectal cancer (Zhu et al., 2015). A 2018 meta-analysis based on a partially overlapping set of studies found, however, no association with legume intake based on a high versus low meta-analysis as well as on a dose-response meta-analysis (Schwingshackl et al., 2018). Furthermore, the intake of fibre from legumes was associated with a non-significant 16% decreased risk of colorectal cancer in a meta-analysis of 6 studies published in 2019 (Oh et al., 2019). On the other hand, the intakes of fruit and fruit fibre were not appreciably associated with colorectal cancer risk (Bradbury et al., 2020; Oh et al., 2019; Vieira et al., 2017).

STRENGHTS AND LIMITATIONS

Despite being the first large epidemiological study evaluating prebiotic dietary exposure and colorectal cancer risk, the present study presents some limitations to consider. The FFQ used for the dietary assessment was not specifically designed to measure the intake of prebiotic fibres and, although the main sources of prebiotics were addressed; the FFQ did not include items on certain foods rich in prebiotics such as rye products, spelt, jerusalem artichoke, oats, soya beans, and chicory (Biesiekierski et al., 2011; Campbell et al., 1997; Hogarth et al., 2000; Judprasong et al., 2011; Moshfegh et al., 1999; Muir et al., 2007); the FFQ did not distinguish between whole-grain and non-wholegrain items, apart from bread. Thus, individual estimates of prebiotic fibre intakes may be misclassified. However, possible exposure misclassification should not be unbalanced between cases and controls. Regarding novel prebiotic FCD, it has to be noted that ITFs was quantified only in 6 foods included in our FFQ.

Furthermore, owing to the limitations of the case-control design, we cannot exclude the possibility of selection and information biases. However, we considered a number of possible confounding factors in the analysis, we applied strictly exclusion criteria, the participation rate was high, and the FFQ was found to be valid and reproducible.

In conclusion, our results suggest an inverse association between dietary GOS intake and colorectal cancer risk and no association with total ITFs and FOSs. However, possible limitations in the estimation of dietary prebiotic intake due to the FFQ and ITFs methodological issues suggest caution in the interpretation of our results.

5. CONCLUSIONS

Nutrition is one of the major modifiable determinants of chronic diseases. Therefore, the availability of high quality and comprehensive FCD is essential for public health surveillance and nutritional epidemiology. FCDBs should be continuously updated to allow reliable assessment of nutrient exposure in a given population by limiting missing data on foods and food components. Indeed, the use of the BDA in its 2015 version and the lack of branded food complete nutritional composition has been found to potentially underestimate the dietary intakes of some nutrients of interest in different populations (children, obese adults, and centenarians). Food labels have been proven to have great potential in the food composition field; their improvement (in terms of nutrient coverage and accuracy) would not only ensure better consumer information but also provide very useful data for nutritional research and epidemiology.

As a result, the present work has highlighted the need for accessible, reliable, quality-documented, and complete FCD and produced novel data using standard and innovative approaches:

- The application of the EuroFIR standard compiling procedures resulted in the update of the cereal, sugars and cereal-based food groups that will be published in the BDA v.22 at: www.bda.ieo.it. In addition, the emerging need for more comprehensive FCBDs addressing the composition of an outstanding list of manufactured food items (GF, babyfoods) as well as new bioactive compound of epidemiological interest (choline, SMs, ITFs, FOSs, and GOSs), resulted in the update of an Italian GF-FCDB and other BDA-based FCDBs for their epidemiological use.
- Moreover, value can be added in the field of food composition by developing tools to compile missing nutrient values of branded foods. In the present work, a novel technological tool was investigated to replace the manual trial-and-error method used to quantify the weight of ingredients in the label-based recipe approach (based on EuroFIR standards). The ultimate goal was to automatically calculate missing nutrient values from food label information (NL and ingredient list). The resulting javaFX application —implementing a genetic algorithm— was able to reliably impute ingredients' weight, but it was not able to overcome the main criticalities encountered with the manual trial-and-error approach, such as dependency on the user (i.e., the compiler) and time-cost. Additional work may be needed to further optimise the tool and fasten the whole process.

The approaches used in this thesis provided useful guidance for further development of electronic platforms for the management of food composition data within local and international projects, such as METROFOOD-RI.

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Adherence to Dietary Recommendations of 7-Year-Old Children from a Birth Cohort in Friuli Venezia Giulia, Italy

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Abstract: Few Italian and European studies have assessed adherence to dietary recommendations in primary school children using dietary records. No Italian studies have provided an index-based nutritional adequacy assessment. We provided a comprehensive overview of dietary intake in 381 7-year-old children from NAC-II cohort study, Friuli Venezia Giulia (Italy). Energy, macro-, and micronutrient intakes were derived from 3-day dietary records. Standard (median and percentage) and index-based (Nutrient Adequacy Ratio (NAR) and Mean Adequacy Ratio (MAR)) approaches were used to evaluate adequacy to Italian dietary reference values at nutrient- and overall-diet-level. Percentage contribution of macronutrients to energy intake (%En) was unbalanced towards total fats and protein. In 25% of children, total fats intake exceeded the reference intake upper limit. In ~63% of children, protein intake was at least doubled in their child-specific population reference intake. Median intakes of sodium (1.7 g/day), saturated fatty acids (12.2 %En), and soluble carbohydrates (19.4 %En) exceeded the suggested dietary target in most (65–84%) children. Inadequacy was also observed for micronutrients, with median NARs ranging from 0.11 (vitamin D) to 0.90 (zinc). The median MAR was 0.75 (0.69–0.79), with 1 indicating optimal overall dietary intake. In conclusion, the enrolled children showed suboptimal intakes of several macro- and micronutrients, in line with Italian and European studies on primary school children. Based on the current findings, public health interventions may be targeted to specific nutrients or subpopulations.

Keywords: dietary habits; dietary intake; energy and nutrient intake; nutritional adequacy; primary school children; nutrient adequacy ratio; mean adequacy ratio; dietary reference values; food groups; dietary record

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1. Introduction

Over recent decades, environmental conditions, including nutrition, have been increasingly recognized to provide short- and long-term effects on health [1,2]. A balanced diet during pregnancy, infancy, and childhood is likely to provide a healthier development over the lifespan [3] and to prevent from the onset of the most common noncommunicable diseases, including obesity [1], type 2 diabetes [4], cardiovascular disease [5], and cancer [6], with childhood obesity itself being one of the major risk factors in the development of other noncommunicable diseases [7–9]. In Europe, the prevalence of overweight and obesity

in primary school children is still a considerable public health issue. Italy is one of the most affected countries [10], reaching 20.4% and 9.4% of prevalence, respectively [11]. In addition, in children, type 2 diabetes is becoming more common [12], even if it is still an occasional event [13]. A balanced nutritional intake including some key nutrients in childhood, such as polyunsaturated fatty acids (PUFAs), iron, iodine, and vitamin B12, is also important in children's neurodevelopment early in life, as well as in its short-term maintenance [14–18].

Childhood is a crucial period of growth and development [19]. Entering primary school, children markedly change their lifestyle. European primary school children spend 65% of school time in sedentary activities [20]. Together with modest physical activity levels outside school time [21,22], this suggests monitoring of their dietary patterns may improve our understanding of nutritional status, and the potential associations between diet and diseases [23]. Information on nutrient intakes at the population level may also provide support to organize targeted nutritional programs [24]. To address this goal, good-quality data on the macro- and micronutrient profile of children's diets are a key element [25].

Different dietary assessment tools have been traditionally used for investigating nutritional habits in children. Among them, food frequency questionnaires (FFQ) are useful to assess the habitual food consumption over long periods in large samples but may introduce errors at individual level for the assessment of energy, macro-, and micronutrient intake. Thus, dietary records (food diaries) or 24-h recalls are usually preferred when the aim is to compare nutrient intakes with country- and age-specific dietary recommendations or estimate mean energy and nutrient intakes [26].

A few studies so far have described nutrient intakes, food sources, and/or adherence to national and international dietary recommendations in primary school children. In Europe, their dietary habits were assessed using 24-h recalls or dietary records obtained from one [27,28] or multiple waves [29–31] of existing cohort studies. In Italy, a cohort was established in 2007 to investigate energy and nutrient intakes from a FFQ in 2–10-year-old children from the Lombardy region, in the north of Italy [32]; two time points were available for the description of dietary habits of primary school children (i.e., 8 and 10 years). Other dietary assessments have been carried out in Italian primary school children using 24-h recalls or dietary records too [24,33–37]. Among all the Italian studies, four [32,34–36] compared nutrient intakes with the corresponding Italian Dietary Reference Values (DRVs) [38]. The standard approach followed for each nutrient included: 1. comparison of observed mean/median intakes and DRVs; 2. calculation of the percentage of subjects meeting the DRV requirement. To our knowledge, no Italian studies so far have provided an index-based assessment of the nutrient-specific or overall-diet-specific adequacy in primary school children. Firstly introduced in Madden et al. [39], the Nutrient Adequacy Ratio (NAR) expresses an individual's intake of a nutrient as a percentage (capped at 100%) of the corresponding recommended allowance for that nutrient, given the respondent's age and sex. Later applied to the pediatric population by Hatløy et al. [40] and more recently by Eldridge [41], NARs provided the basis for the mean adequacy ratio (MAR) index. The MAR quantifies the overall nutritional adequacy of a population based on an individual's diet using the current recommended allowance for a group of nutrients of interest [40,41].

The aim of the current paper is to provide a comprehensive overview of dietary intake in 7-year-old Italian children from the Northern Adriatic Cohort (NAC-II), Friuli Venezia Giulia, in the northeast of Italy, by following different approaches:

1. Standard evaluation of adequacy to the DRVs [38];
2. Index-based evaluation of adequacy to DRVs at the following levels:
    a. Nutrient-level adequacy, through the NAR index;
    b. Overall-diet-level adequacy, through the MAR index;
3. Percentage contribution of different food sources to macro- and micronutrient intakes.

2. Materials and Methods

2.1. Study Population

Between 2007 and 2009, 900 pregnant women were enrolled in the prospective Italian NAC-II study [42], within the framework of the 'Public health impact of long-term, low-level, mixed element exposure in susceptible population strata' (PHIME) European Union project [43]. The project included a Mediterranean cohort involving 4 birth cohorts from Italy (NAC-II), Slovenia, Greece, and Croatia, with the aim of investigating the association between low-level mercury exposure from food consumption in pregnancy and child neurodevelopment at 18 months. Overall diet of mother–infant pairs was originally assessed (during pregnancy, and at 18 months of the child) to provide adjustments for potential confounding factors [43]. Within NAC-II, child's dietary habits were further assessed at 7 years (2014–2016), following an additional extended protocol [44]. Briefly, at the 7-year follow-up, parents of those children tested for the neurodevelopment outcomes at 18 months (N = 632) were contacted for further dietary and neurodevelopment evaluation. The current paper considered dietary intakes for the 381 children whose parents filled in the corresponding dietary record at 7 years of age. A comprehensive description of dietary intake at 18 months has been recently published [45].

The study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Institute for Maternal and Child Health IRCCS Burlo Garofolo (CE/V-109-12/04/2010). All participating families were informed and consented to participate to the study.

2.2. Parental and Children's Characteristics

Parents filled in a questionnaire assessing lifestyle of the enrolled 7-year-old children. Socio-demographic characteristics of both parents, including education level, marital status, and citizenship, were obtained from a questionnaire administered at delivery [43].

Children's height and weight were measured from healthcare staff during the neuropsychological assessment at 7 years [44]. Body mass index (BMI; kg/m^2) was calculated as: (weight (kg)/height^2 (m^2)). Children were categorized as normal weight, underweight, overweight, or obese according to the World Obesity Federation [46] based on International Obesity Task Force (IOTF) BMI cut-offs for thinness, overweight, and obesity [47].

2.3. Dietary Assessment

Dietary data were collected using a 3-day dietary record (3-dDR) filled in at home by one parent instructed on how to record type and portion size of the foods consumed by the child. Common kitchen utensils were suggested as an alternative to traditional kitchen scales to measure solids and fluids (e.g., teaspoon, glass); in this case, estimated equivalents in grams were also indicated to the parents. Food fortification or supplement use was not captured within the 3-dDR as the instructions did not suggest collection of this information. Three children filled in the diary for less than 3 days and 4 children filled in the diary for more than 3 days. A researcher's telephone contact was provided whenever parents need clarification while filling in the 3-dDR.

Intakes of 39 selected macro- and micronutrients were derived after uploading individual food information from the 3-dDRs in the Microdiet V4.4.1 software (Microdiet software–Downlee Systems Ltd., High, Peak, UK), which contains the Italian "Food Composition Database for Epidemiological Studies in Italy" [48], integrated with information from nutritional labels when needed. Full details of the methodology were published elsewhere [49].

For each nutrient, the Microdiet software provided total intake over the observation period; we calculated mean daily intakes by dividing total intake by the number of collection days. Total energy intake was estimated by summing the mean daily intake of the single macronutrients each multiplied by the corresponding energy conversion factor.

Single food items from the 3-dDRs were classified into 18 food groups obtained after modification of food grouping schemes provided in previous publications of our

group [45,50]. To disentangle the main food sources, percentage contribution of the 18 food groups on energy and nutrients was calculated for each child.

All procedures were conducted by a trained food technologist and three nutritionists, who were fully familiar with the management of food composition data, nutritional assessment, food preparation methods, and nutritional labels.

2.4. Nutritional Adequacy

Individual nutrient intakes were compared with the DRVs proposed by the Italian Society for Human Nutrition [38], when available. The DRVs include adequate intake (AI), reference intake (RI) range for macronutrients, average requirement (AR), population reference intake (PRI), and suggested dietary target (SDT) for the corresponding nutrient.

For protein intake, the Italian Society for Human Nutrition provided 3 DRVs including RI range, AR, and PRI. Given the preeminent role of AR and PRI for protein and availability of anthropometric information for most of the children (N = 350; ~92%), we calculated the AR and PRI child-specific cut-offs using the individual weights and the age-specific DRVs for 7-year-old children, as: AR(protein) (g/day)= 0.8 g/kg weight per day $\times$·kg of weight; PRI(protein) (g/day)= 0.98 g/kg weight per day $\times$ kg of weight. This integrates information on RI range, which was calculated by difference, as: RI(protein) = 100% − RI(total fats) − RI(available carbohydrates) [38].

We evaluated the adequacy of individual diets at the nutrient- as well as at the overall-diet-level using the NAR and the MAR, respectively [40]. In detail, the NAR is defined as the ratio of each child's intake to the national DRV for the appropriate age category. The MAR is the sum of all (nutrient-specific) NARs divided by the total number of NARs. As any ratio, a NAR equal to 1 indicates that the corresponding subject meets the requirement fixed for that nutrient. A MAR equal to 1 indicates that the subjects meet the requirements for all the selected nutrients.

To take into account inadequacy due to excess intake, we extended the approach proposed by Atløy in children [40] to those macro- and micronutrients for which a maximum desirable intake is available. In detail:

- For all micronutrients with one DRV indicating the minimum desirable intake (i.e., AI or AR), we truncated all NARs greater than 1 to 1 so that these nutrients could not compensate those with a NAR lower than 1 in the MAR calculation;
- For the remaining macro- and micronutrients indicating a maximum desirable intake (i.e., RI: protein, available carbohydrates, total fats, monounsaturated fatty acids (MUFAs), total PUFAs, PUFAs ω-3 and ω-6; SDT: soluble carbohydrates, saturated fatty acids (SFAs), sodium, and chloride), we followed suggestions by Hilbig [51] and redefined NARs greater than 1 (inadequate intake by excess) to be equal to: 1 minus the exceeding amount. For example, when the original NAR was equal to 1.15, our modified NAR value is equal to 0.85.

To assess the importance of the individual nutrients in the MAR calculation, we also carried out an influence analysis where the single components were removed one at a time from the MAR definition.

We additionally evaluated nutrient-specific adequacy of protein intake using child-specific AR and PRI.

2.5. Statistical Analysis

General characteristics of parents and children were presented as frequency and percentage distribution for categorical variables, and as median, 25th, and 75th centile for continuous variables with a non-normal distribution. Normality assumption was tested for each continuous variable using the Shapiro–Wilk test.

Standard evaluation of nutritional adequacy was carried out using median, 25th and 75th centile and percentage of children meeting the DRV requirements. Sex-specific median, 25th, and 75th centile were also provided and the presence of potential sex differences was investigated using the two-sample Wilcoxon rank-sum (Mann–Whitney) test. Furthermore,

we investigated the presence of potential inadequacy in individual protein intakes by comparing the observed intakes (g/day) with the corresponding AR and PRI (g/day) with the two-sample Wilcoxon rank-sum (Mann–Whitney) test. Index-based evaluation of adequacy was based on median, 25th, and 75th centile of NAR and MAR.

Statistical significance for all tests was set at 0.05. Stata (StataCorp. 2013. Stata Statistical Software: Release 13. StataCorp LP, College Station, TX, USA) was used for all statistical analysis.

3. Results

3.1. Lifestyle and Anthropometric Characteristics of the Study Population

In total, 381 children (females: 48.3%; males: 51.7%) whose parents filled in 3-dDR were included in the present study. Mother and father's socio-demographic characteristics at enrollment are reported in Supplementary Table S1. Only 6.3% of the mothers were foreign citizens. Almost 82% of the mothers and ~70% of the fathers had a high school diploma (45.1% and 47.0%, respectively) or a higher educational level (38.6% and 22.0%, respectively).

Children's lifestyle and anthropometric characteristics at 7 years of age are presented in Table 1.

Table 1. Children's lifestyle and anthropometric characteristics at 7 years of age. Northern Adriatic Cohort II (NAC-II), 2014–2016 (*N* = 381).

	Median	**25th–75th Centile**
Child weight at 7 years (kg) [1]	25.5	22.8–29.2
Child height at 7 years (cm) [1]	124.3	121.0–128.5
	N	%
Sex		
Male	197	51.7
Female	184	48.3
Weight status [1]		
Underweight	9	2.6
Normal weight	255	72.9
Overweight	67	19.1
Obese	19	5.4
Extra-curricular sport or play activities		
Never	15	3.9
1–3 days/week	303	79.5
>4 days/week	58	15.2
Not reported	5	1.3
Videogames activity		
Never	106	27.8
<1 h/day	172	45.1
1–2 h/day	64	17.6
3 h/day	5	1.3
Not reported	34	8.9
Television use		
Never	15	3.9
<1 h/day	105	27.6
1–2 h/day	233	61.2
3–4 h/day	22	5.8
Not reported	6	1.6

Table 1. *Cont.*

	N	%
Food consumption while screen-time activities		
Yes	103	27.0
No	274	71.9
Not reported	4	1.0
Caregiver (weekdays) [2]		
Mother	326	86.2
Father	146	38.7
Grandparents	135	35.8
School	164	43.4
Baby-sitter	13	3.4
Others	19	5.1

[1] Anthropometric information was available for 350 children only. [2] More than a category was available for responders; therefore, the total does not sum up to 381. In detail, 376 parents selected the "Others" option, 377 parents selected the "Father", "Grandparents", and "Baby-sitter" option, 378 parents selected the "Mother" or "School" option.

Children's median age was 7.1 (7.1–7.2) years. Approximately 72.9% of the children were normal weight, whereas 19.1% and 5.4% were overweight and obese, respectively. Most of the children (79.5%) practiced extra-curricular sport activities from 1 to 3 days per week, with a 15.2% practiced sport more than 4 days per week. Approximately half of the children (45.1%) played videogames less than 1 h per day or never (27.8%), whereas 66.9% watched TV more than 1 h per day (Table 1). During the weekend, the percentage of children playing videogames and watching TV increased in all categories (data not shown).

Mothers spent more time with children during weekdays, but a prevailing caregiver role was also identified for school (43.4%), fathers (38.7%), and grandparents (35.8%); during the weekend fathers spent more time together with the child (data not shown).

3.2. Description of Daily Dietary Nutrient Intake and Standard Comparison with the Italian Dietary Reference Values

3.2.1. Energy and Macronutrients

Overall and sex-specific descriptive statistics (median, 25th–75th centile) of the observed intakes of energy and macronutrients per day are reported in Table 2, together with the corresponding Italian DRVs. The Italian DRVs include RI range, SDT, or AI, as applicable [38].

Table 2. Distribution of energy and macronutrient intakes of 7-year-old children in the overall sample and stratified by sex. Northern Adriatic Cohort II (NAC-II), 2014–2016 (*N* = 381).

					Females (*N* = 184)			**Males (*N* = 197)**			
	Median	**25th**	**75th**	**DRVs**	**Median**	**25th**	**75th**	**Median**	**25th**	**75th**	***p*-Value**
Energy (kJ/d)	6291.8	5593.2	6982.2		6139.2	5261	6862.2	6404.4	5773.6	7113.2	0.002 *
Energy (kcal/d)	1503.0	1336.2	1668.0		1466.6	1256.8	1639.3	1530.0	1379.3	1699.3	0.002 *
Protein (g/d)	55.6	47.2	64.3		54.0	46.3	63.4	56.8	48.4	64.5	0.077
Protein (%En)	14.8	13.2	16.5	12–18 %En (RI) [1]	14.9	13.3	16.9	14.8	13.0	16.3	0.165
Total fats (g/d)	52.2	42.6	61.7		51.1	42.0	60.4	53.3	43.4	63.7	0.084
Total fats (%En)	31.3	27.4	35.1	20–35 %En (RI)	32.0	27.7	35.1	30.8	27.2	35.1	0.309
Saturated fatty acids (g/d)	20.5	16.5	24.5		20.2	14.9	24.0	20.9	17.0	25.0	0.055

Table 2. *Cont.*

					Females (*N* = 184)			Males (*N* = 197)			
	Median	**25th**	**75th**	**DRVs**	**Median**	**25th**	**75th**	**Median**	**25th**	**75th**	***p*-Value**
Saturated fatty acids (%En)	12.2	10.6	14.0	<10 %En (SDT)	12.2	10.8	14.0	12.2	10.4	14.1	0.865
Monounsaturated fatty acids (g/d)	18.0	14.5	22.1		18.0	14.0	21.7	18.1	14.8	22.1	0.307
Monounsaturated fatty acids (%En)	10.8	9.3	12.6	10–15 %En (RI) [2]	11.1	9.4	12.7	10.7	9.3	12.4	0.137
Oleic acid (g/d)	16.5	13.4	20.1		16.6	13.2	20.0	16.5	13.8	20.1	0.405
Polyunsaturated fatty acids (g/d)	5.2	4.1	6.6		5.3	4.1	6.7	5.1	4.1	6.5	0.792
Polyunsaturated fatty acids (%En)	3.1	2.5	3.9	5–10 %En (RI)	3.3	2.6	4.0	3.0	2.4	3.7	0.021 *
Arachidonic acid (mg/d)	146.3	95.6	219.7		143.2	96.4	206.5	154.2	94.5	226.9	0.503
Linoleic acid (g/d)	3.9	3.1	5.2		3.9	3.1	5.3	3.8	3.0	5.2	0.509
PUFAs ω-6 (%En)	2.4	2.0	3.2	4–8 %En (RI)	2.6	2.1	3.3	2.3	1.9	3.0	0.007 *
Alpha-linolenic acid (g/d)	0.6	0.4	0.7		0.5	0.4	0.7	0.6	0.5	0.7	0.101
EPA + DHA (mg/d)	61.0	23.7	208.3	250 mg/d (AI)	53.9	24.0	207.3	67.3	23.7	210.0	0.620
PUFAs ω-3 (%En)	0.4	0.3	0.5	0.5–2.0 %En (RI)	0.4	0.3	0.5	0.4	0.3	0.5	0.572
Cholesterol (mg/d)	185.3	143.0	224.8		186	141.9	225.5	183.0	143.3	224.4	0.852
Available carbohydrates (g/d)	197.6	163.7	223.9		189.3	153.0	215.8	204.1	172.0	230.4	<0.001 *
Available carbohydrates (%En)	51.8	48.3	56.6	45–60 %En (RI)	51.3	47.7	55.6	52.5	48.7	57.3	0.095
Soluble carbohydrates (g/d)	72.5	59.0	87.5		71.2	58.6	85.8	74.7	60.1	91.3	0.057
Soluble carbohydrates (%En)	19.4	16.4	23.0	<15 %En (SDT)	19.4	16.3	23.3	19.4	16.4	22.7	0.948
Fiber (g/1000 kcal/d)	7.0	5.7	8.7	8.4 g/1000 kcal (AI)	7.2	5.8	8.9	7.0	5.4	8.5	0.139

[1] Reference Intake calculated by difference: RI(protein) = 100%- RI(total fats)- RI(available carbohydrates); [2] Reference Intake calculated by difference: RI(MUFAs) = RI(total fats)- RI(PUFAs)- SDT(SFAs). Abbreviations: d, day; DRVs, Dietary Reference Values; RI, Reference Intake; SDT, Suggested Dietary Target; AI, Adequate Intake; %En, percentage of daily energy intake; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. The two-sample Wilcoxon rank-sum (Mann–Whitney) test was applied to detect any statistical differences between females and males; * $p < 0.05$.

The median daily energy intake of the overall sample was 1503.0 kcal (1336.2–1668.0), with a statistically significant difference by sex ($p < 0.05$): females tended to have a lower intake with a median of 1466.6 kcal (1256.8–1639.3) compared to 1530.0 kcal (1379.3–1699.3) for males.

Overall, the percentage contribution of protein, total fats, and available carbohydrates to daily energy intake (%En) was found to be in line with the recommendations. The median %En from total fats (31.3 %En; 27.4–35.1) was close to the upper limit of the RI range (20–35 %En). Conversely, the median %En from available carbohydrates (51.8 %En; 48.3–56.6) was closer to the lower limit of the recommendations (45–60%). A statistically significant difference was observed for available carbohydrates (g/day) between females and males ($p < 0.05$) (females: 189.3; 153.0–215.8 vs. males: 204.1; 172.0–230.4). Based on the %En of total fats and available carbohydrates, the median %En of protein (14.8 %En; 13.2–16.5) was at the middle point of the RI range (12–18 %En). Furthermore, the median %En from total PUFAs, PUFAs ω-6, and PUFAs ω-3 were below the lower limit of the RI

range. Within the PUFAs dietary profile, median intake of the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (mg/day) was below the AI. Higher %En from total PUFAs and PUFAs ω-6 were observed in females than in males ($p < 0.05$).

In median, soluble carbohydrates and SFAs intakes exceeded the SDT (<15 %En and <10 %En, respectively) in the overall sample: 19.4 %En (16.4–23.0) for soluble carbohydrates and 12.2 %En (10.6–14.0) for SFAs.

The median fiber intake (7.0 g/1000 kcal per day; 5.7–8.7) did not reach the AI (8.4 g/1000 kcal per day).

3.2.2. Protein Intake: Comparison with Average Requirement and Population Reference Intake

Providing a focus on protein intake, 100% of the enrolled children reached their AR; 99.7% of the children reached their PRI too, with only one child having an intake between AR and PRI. However, 63% of the children showed an observed protein intake 2–4 times higher than their PRI.

Figure 1 shows a comparison between individual-level distributions of observed (i.e., from the 3-dDR) and required (i.e., expressed as their age-specific AR and PRI) intakes for our 7-year-old children. In the absence of sex-specific differences in observed intakes, this analysis was carried out on the overall sample of children. The distribution of the observed protein intake modestly overlapped with that of the corresponding required intakes (p-values from the two-sample Wilcoxon rank-sum (Mann–Whitney) test for observed vs. AR intake and observed vs. PRI intake <0.0001). Corresponding summary statistics expressed as median (25th–75th centile) were equal to: observed intake: 56.2 g/day (47.7–64.3) vs. AR intake: 20.4 g/day (18.3–23.3), and PRI intake: 25.0 g/day (22.4–28.6).

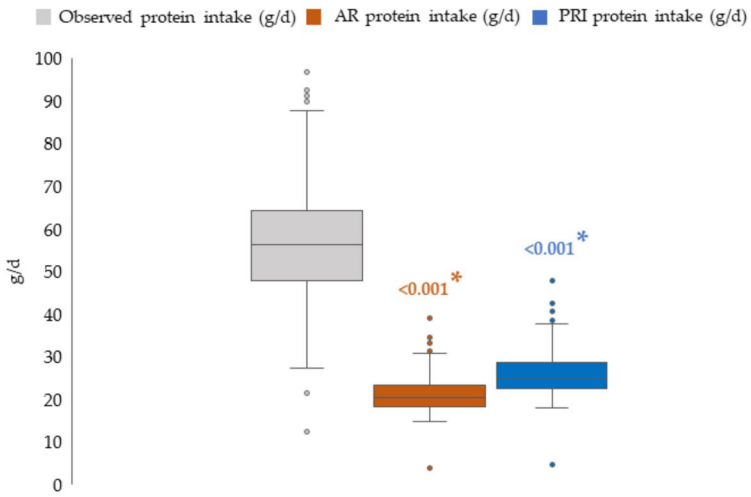


Figure 1. Box-and-whiskers plots comparing the observed protein intake of children and their protein dietary reference values. NAC-II, 2014-2016 ($N = 381$). Each child's ($N = 350$) protein requirement was estimated using Average Requirement (AR = 0.8 g/kg of weight per day) and Population Reference Intake (PRI = 0.98 g/kg of weight per day) for 7-year-old children. The bottom and top edge of each box represent the 25th and 75th centile (interquartile range); the line within each box represents the median; the ends of the bottom and top whiskers represent the minimum and maximum values and the circles represent outliers. Abbreviation: d, day. The two-sample Wilcoxon rank-sum (Mann–Whitney) test was applied to detect any statistical differences between observed and estimated intakes (AR and PRI). * $p < 0.05$.

3.2.3. Micronutrients

Overall and sex-specific descriptive statistics (median, 25th–75th centile) of the observed intakes of micronutrients per day are reported in Table 3, together with the corresponding Italian DRVs. The Italian DRVs include AI, SDT, AR, or PRI, as applicable [38].

Table 3. Distribution of micronutrient intakes of 7-year-old children in the overall sample and stratified by sex. Northern Adriatic Cohort II (NAC-II), 2014–2016 (*N* = 381).

					Females (*N* = 184) (*N* = 197)			Males (*N* = 197)			
	Median	**25th**	**75th**	**DRVs**	**Median**	**25th**	**75th**	**Median**	**25th**	**75th**	***p*-Value**
Sodium (g/d)	1.7	1.3	2.1	1.1 g/d (AI); 1.5 g/d (SDT)	1.7	1.3	2.1	1.8	1.4	2.1	0.207
Potassium (g/d)	1.8	1.4	2.1	3 g/d (AI)	1.7	1.4	2.1	1.8	1.5	2.1	0.719
Calcium (mg/d)	537.8	409.6	706.3	900 mg/d (AR); 1100 mg/d (PRI)	515.9	392.4	657.0	567.7	420.1	721.7	0.034 *
Magnesium (mg/d)	83.5	65.5	105.8	130 mg/d (AR); 150 mg/d (PRI)	81.8	61.7	104.2	85.3	67.0	106.0	0.193
Phosphorus (mg/d)	819.0	693.8	966.9	730 mg/d (AR); 875 mg/d (PRI)	778.7	668.5	941.4	846.2	715.7	975.8	0.020 *
Iron (mg/d)	5.9	4.8	7.2	5 mg/d (AR); 13 mg/d (PRI)	5.6	4.6	7.1	6.1	5.0	7.5	0.066
Zinc (mg/d)	6.3	5.3	7.4	7 mg/d (AR); 8 mg/d (PRI)	6.0	5.1	7.3	6.6	5.6	7.4	0.004 *
Selenium (µg/d)	18.5	13.0	28.2	30 µg/d (AR); 34 µg/d (PRI)	17.4	13.2	27.4	19.1	12.9	28.7	0.336
Copper (mg/d)	0.4	0.2	0.5	0.4 mg/d (AR); 0.6 mg/d (PRI)	0.4	0.2	0.5	0.4	0.3	0.5	0.288
Chloride (g/d)	1.3	1.0	1.7	1.7 g/d (AI); 2.3 g/d (SDT)	1.2	1.0	1.7	1.3	1.0	1.7	0.331
Manganese (mg/d)	0.4	0.2	0.6	1.2 mg/d (AI)	0.4	0.2	0.6	0.4	0.2	0.6	0.650
Iodine (µg/d)	75.0	51.8	104.6	100 µg/d (AI)	69.3	47.2	104.8	80.0	55.1	104.6	0.077
Vitamin B1 (mg/d)	0.7	0.6	0.9	0.6 mg/d (AR); 0.8 mg/d (PRI)	0.7	0.6	0.8	0.7	0.6	0.9	0.127
Vitamin B2 (mg/d)	1.1	0.8	1.3	0.7 mg/d (AR); 0.8 mg/d (PRI)	1.0	0.8	1.3	1.1	0.9	1.3	0.014 *
Niacin (mg/d)	9.6	7.7	12.2	9 mg/d (AR); 12 mg/d (PRI)	9.6	7.6	12.1	9.5	7.7	12.3	0.890
Pantothenic acid (mg/d)	2.1	1.6	2.6	3.5 mg/d (AI)	2.2	1.7	2.7	2.1	1.6	2.6	0.979
Vitamin B6 (mg/d)	1.3	1.0	1.5	0.7 mg/d (AR); 0.9 mg/d (PRI)	1.2	1.0	1.5	1.3	1.1	1.5	0.394
Biotin (µg/d)	11.5	8.6	14.7	20 µg/d (AI)	11.5	8.4	14.8	11.5	8.7	14.6	0.929
Folate (µg/d)	160.6	127.5	199.7	210 µg/d (AR); 250 µg/d (PRI)	155.1	125.5	194.1	165.7	135.0	204.2	0.072
Vitamin B12 (µg/d)	2.4	1.8	3.3	1.3 µg/d (AR); 1.6 µg/d (PRI)	2.4	1.7	3.1	2.5	1.9	3.4	0.133
Vitamin A (µg/d) [1]	603.7	438.8	853.4	350 µg/d (AR); 500 µg/d (PRI)	589.6	421.8	856.2	617.4	450.1	853.1	0.419
Vitamin C (mg/d)	63.2	40.7	98.4	45 mg/d (AR); 60 mg/d (PRI)	61.6	41.0	97.5	63.7	40.4	98.4	0.984

Table 3. *Cont.*

					Females (N = 184) (N = 197)			Males (N = 197)			
	Median	**25th**	**75th**	**DRVs**	**Median**	**25th**	**75th**	**Median**	**25th**	**75th**	***p*-Value**
Vitamin D (µg/d)	1.1	0.7	1.5	10 µg/d (AR); 15 µg/d (PRI)	1.0	0.7	1.3	1.1	0.8	1.6	0.142
Vitamin E (mg/d) [2]	5.1	4.0	6.6	8 mg/d (AI)	5.2	4.1	6.6	5.1	4.0	6.6	0.496

[1] Expressed as retinol equivalents; [2] Expressed as alpha-tocopherol equivalents. The two-sample Wilcoxon rank-sum (Mann–Whitney) test was applied to detect any statistical differences between females and males; * $p < 0.05$. Abbreviation: d, day.

Median intakes of potassium, magnesium, manganese, iodine, pantothenic acid, biotin, and vitamin E were below the corresponding AI. Median sodium intake (1.7 g/day; 1.3–2.1) reached the AI (1.1 g/day), but it exceeded the SDT (1.5 g/day).

Moreover, median intakes of calcium, chloride, magnesium, zinc, selenium, folate, and vitamin D were below the AR and consequently the PRI. Median intakes of phosphorus, iron, copper, vitamin B1, and niacin reached the corresponding AR, but not the PRI Finally, median intakes of vitamin B2, vitamin B6, vitamin B12, vitamin A, and vitamin C reached both their AR and PRI.

Significantly higher intakes were observed in males as compared to females for calcium, phosphorus, zinc, and vitamin B2 ($p < 0.05$, Table 3).

3.3. Index-Based Evaluation of Diet Adequacy to Dietary Reference Values

3.3.1. Nutrient-Level Adequacy

The proportion of children with a nutrient intake above, below, or within the recommendations, together with the corresponding median NARs, were presented in Figures 2–5.

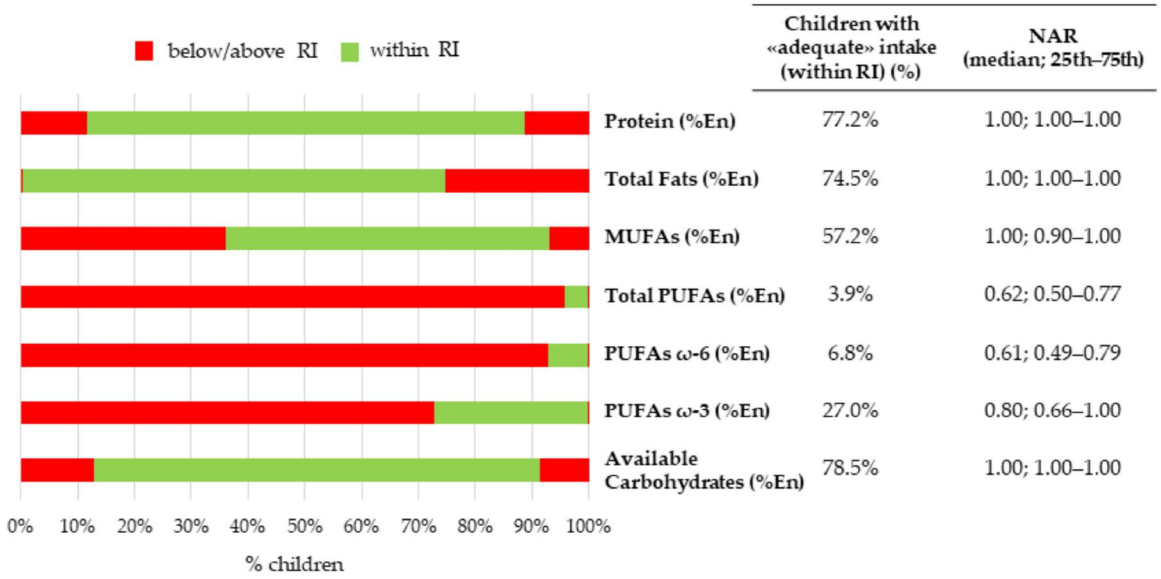
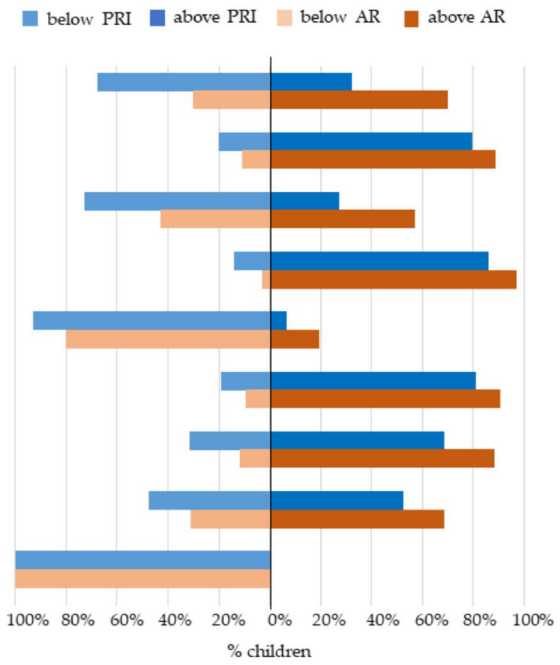
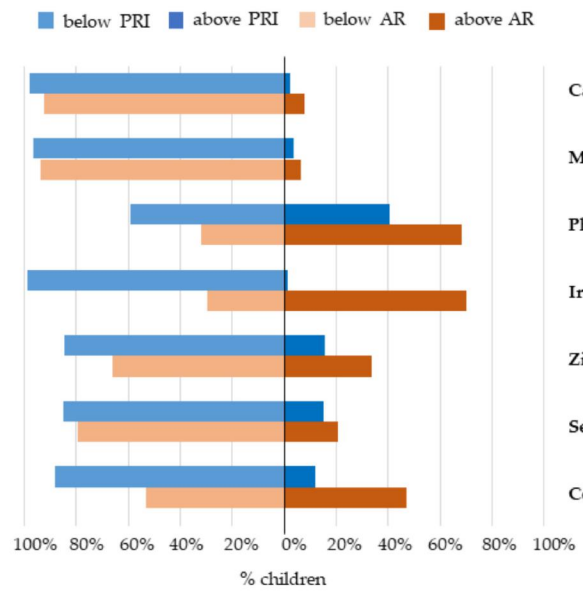


Figure 2. Nutritional adequacy of macronutrients relative to the reference intake. NAC-II, 2014–2016 (N = 381). NAR was based on the RI range. Children having intakes equal to the cut-off values were considered to be adequate for that specific nutrient.



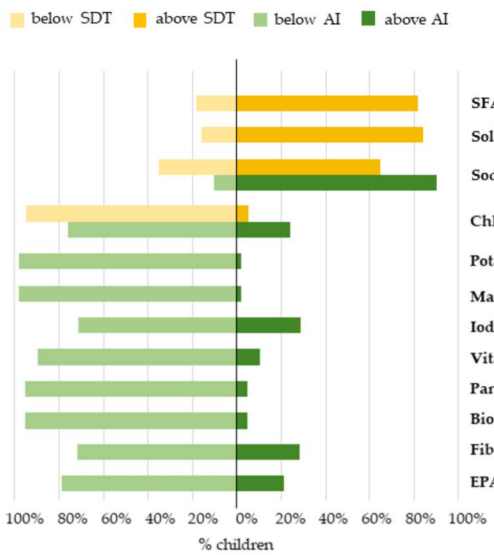
	Children with «adequate» intake (above PRI) (%)	Children with «adequate» intake (above AR) (%)	NAR (median; 25th–75th)
Vitamin B1 (mg/d)	32.3%	69.8%	1.00; 0.95–1.00
Vitamin B2 (mg/d)	79.8%	89.0%	1.00; 1.00–1.00
Niacin (mg/d)	27.3%	57.2%	1.00; 0.85–1.00
Vitamin B6 (mg/d)	86.1%	97.1%	1.00; 1.00–1.00
Folate (µg/d)	6.8%	19.7%	0.76; 0.61–0.95
Vitamin B12 (µg/d)	81.1%	90.6%	1.00; 1.00–1.00
Vitamin A (µg/d)	68.5%	88.2%	1.00; 1.00–1.00
Vitamin C (mg/d)	52.5%	68.8%	1.00; 0.90–1.00
Vitamin D (µg/d)	0.0%	0.0%	0.11; 0.07–0.15

Figure 3. Nutritional adequacy of micronutrients (i.e., vitamins) relative to the average requirement and population reference intake. NAC-II, 2014–2016 (*N* = 381). NAR was based on the AR. Children having intakes equal to the cut-off value were considered to be adequate for that specific nutrient.



	Children with «adequate» intake (above PRI) (%)	Children with «adequate» intake (above AR) (%)	NAR (median; 25th–75th)
Calcium (mg/d)	2.1%	7.6%	0.60; 0.46–0.78
Magnesium (mg/d)	3.4%	6.3%	0.64; 0.50–0.81
Phosphorus (mg/d)	40.7%	68.2%	1.00; 0.95–1.00
Iron (mg/d)	1.3%	70.3%	1.00; 0.97–1.00
Zinc (mg/d)	15.5%	33.9%	0.90; 0.75–1.00
Selenium (µg/d)	15.0%	20.7%	0.62; 0.43–0.94
Copper (mg/d)	11.8%	47.0%	0.96; 0.60–1.00

Figure 4. Nutritional adequacy of micronutrients (i.e., minerals) relative to the average requirement and population reference intake. NAC-II, 2014–2016 (*N* = 381). NAR was based on the AR. Children having intakes equal to the cut-off value were considered to be adequate for that specific nutrient.



	Children with «adequate» intake (below SDT) (%)	Children with «adequate» intake (above AI) (%)	NAR (median; 25th–75th)
SFAs (%En)	18.4%	-	0.78; 0.60–0.94
Soluble Carbohydrates (%En)	15.8%	-	0.71; 0.47–0.91
Sodium (g/d)	35.0%	89.8%	0.80; 0.58–1.00
Chloride (g/d)	94.8%	23.9%	0.75; 0.58–0.95
Potassium (g/d)	-	2.1%	0.59; 0.48–0.70
Manganese (mg/d)	-	2.1%	0.33; 0.17–0.47
Iodine (µg/d)	-	28.9%	0.75; 0.52–1.00
Vitamin E (mg/d)	-	10.2%	0.64; 0.50–0.83
Pantothenic acid (mg/d)	-	4.7%	0.61; 0.47–0.75
Biotin (µg/d)	-	5.0%	0.58; 0.43–0.73
Fiber (g/1000kcal/d)	-	28.1%	0.84; 0.68–1.00
EPA+DHA (mg/d)	-	21.3%	0.24; 0.09–0.83

Figure 5. Nutritional adequacy of macro- and micronutrients, relative to the adequate intake and suggested dietary target. NAC-II, 2014–2016 (*N* = 381). NAR was based on the SDT and AI Children having intakes equal to the AI cut-off value were considered to be adequate for that specific nutrient, while children having intake equal to the SDT cut-off value were considered to be inadequate.

The RI lower limits for total fats (%En), protein (%En), and available carbohydrates (%En) were reached by 74.5%, 77.2%, and 78.5% of children, respectively. This was confirmed by median NARs being all equal to 1.00 (1.00–1.00)—i.e., the ideal cut-off for nutrient adequacy—for all the previous nutrients (Figure 2).

Approximately 96%, 93%, and 73% of children did not reach the RI lower limit for total PUFAs (%En), PUFA ω-6 (%En), and PUFA ω-3 (%En), respectively. The corresponding median NARs were far from 1 and equal to 0.62 (0.50–0.77), 0.61 (0.49–0.79), and 0.80 (0.66–1.00), respectively, thus suggesting a substantial inadequacy (Figure 2).

In addition, ~80% of children had intakes of vitamin B2, vitamin B6, vitamin B12, and vitamin A above the AR; the corresponding median NAR was equal to 1.00, with the 25th centile already reaching 1. Almost 70% of children had intakes of vitamin B1 and vitamin C above the AR, with median NARs of 1.00, but the 25th centile reached 0.95 and 0.90, respectively. Less than 20% of children had an intake above the AR for folate, with a median NAR of 0.76 (0.61–0.95). No child had an intake above the AR for vitamin D, with a median NAR as low as 0.11 (0.07–0.15) (Figure 3).

Furthermore, almost 70% of children had an intake of iron and phosphorus above the AR, with median NARs of 1.00 (0.97–1.00) and 1.00 (0.95–1.00), respectively. However, only 1% of children had an iron intake above the PRI cut-off. Regarding selenium, zinc, and copper, 20.7%, 33.9%, and 47.0% of children had an intake above the corresponding nutrient-specific AR, respectively; the median NARs were 0.62 (0.43–0.94), 0.90 (0.75–1.00), and 0.96 (0.60–1.00), respectively, thus indicating that the observed intakes for zinc and copper were closer to the AR than selenium. The AR for calcium and magnesium was reached by less than 10% of the children (NARs equal to 0.60 and 0.64, respectively) (Figure 4).

Finally, for fiber, iodine, chloride, and EPA + DHA intake the AI was similarly reached by 20–30% of the children, but the median NARs varied from 0.24 (0.09–0.83, EPA + DHA) to 0.84 (0.68–1.00, fiber). Less than 10% of children had an intake of potassium, manganese, pantothenic acid, and biotin above the AI; the corresponding median NARs varied from 0.33 (0.50–0.81, manganese) to 0.64 (0.50–0.83, vitamin E) (Figure 5).

Overall, 90% of the children had a sodium intake above the AI; however, only 35% had an intake not exceeding the SDT cut-off value, as reflected by a median NAR smaller than 1 (NAR: 0.80, 0.58–1.00). Even if most children exceeded the SDT for SFAs and soluble

carbohydrates (81.6% and 84.2%, respectively), the corresponding median NARs were 0.78 (0.60–0.94) and 0.71 (0.47–0.91), thus witnessing a modest distance of observed intakes from the SDT (Figure 5). However, when analyzing individual intakes with more stringent cut-offs, no child from our sample had a soluble carbohydrates intake <5 %En, 4 children only were <10 %En, vs. 60 (15.8%) who were <15 %En, which corresponds to the Italian SDT. Finally, 57 children (15.0%) had a soluble carbohydrates intake exceeding the recommended 25% of energy intake [38] (data not shown).

3.3.2. Overall-Diet-Level Adequacy

In our population, no children reached the optimal MAR value of 1.00, targeting adequacy on all the available nutrients. Overall, the median MAR was 0.75 (0.69–0.79). In the influence analysis, median values of the MAR ranged from 0.74 to 0.76, after removal of one component at a time. No statistically significant differences were found in median MAR values between females and males.

3.4. Sources of Nutrient Intakes: Food Groups

Figure 6 shows the percentage contribution of food groups to energy, protein, total fats, available carbohydrates, soluble carbohydrates, and fiber. The full list of food groups and their contribution to intake of fatty acids, cholesterol, and micronutrients are presented in Supplementary Tables S2 and S3.

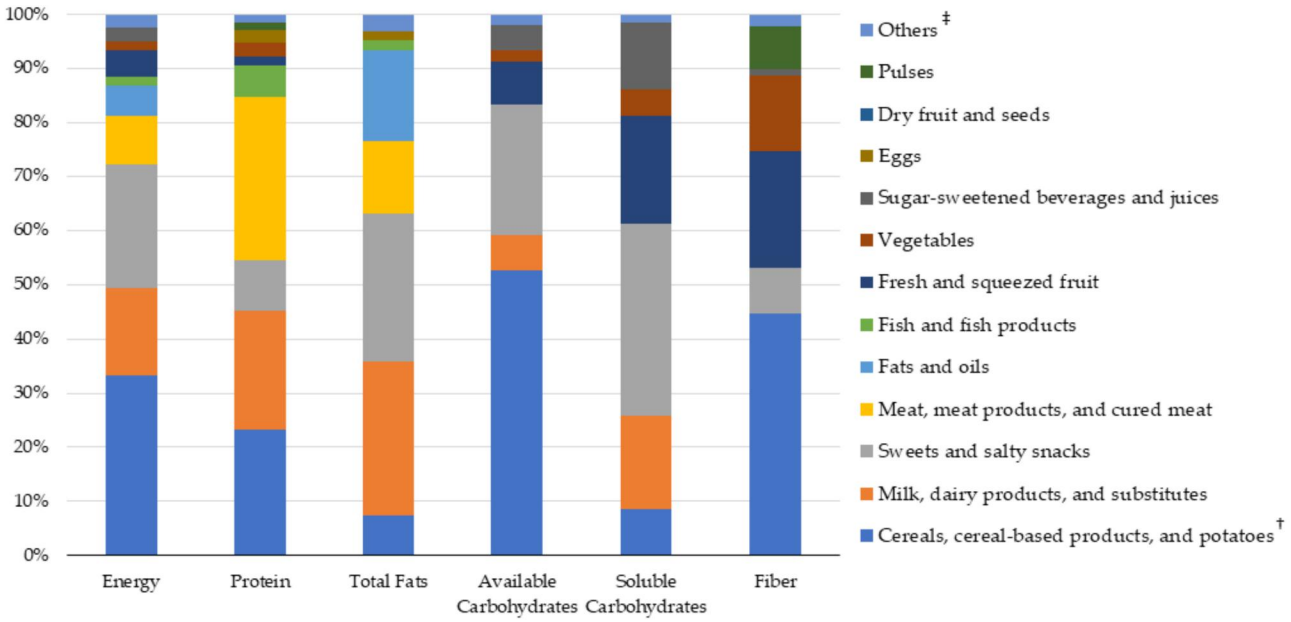


Figure 6. Percentage contribution of the different food groups to total intake of energy and macronutrients. NAC-II, 2014–2016 (*N* = 381). ‡ For each variable, the category "Others" included all the remaining food groups. † "Cereals and cereal-based products" and "Potatoes" were clustered together, as well as "Meat and meat products" and "Cured meat". The full list of food groups and their contribution to intake of fatty acids, cholesterol, and micronutrients are presented in Supplementary Tables S2 and S3.

The main sources of energy intake were "Cereals, cereal-based products and potatoes" (33.2%), "Sweet and salty snacks" (22.6%), and "Milk, dairy products, and substitutes" (16.1%). "Sweet and salty snacks" were the main sources of soluble carbohydrates (35.4%), followed by "Fresh and squeezed fruit" (20.1%), "Milk, dairy products, and substitutes" (17.2%), and "Sugar-sweetened beverages and juices" (12.4%). The major contributors of protein intake were "Meat, meat products, and cured meat" (30.3%), "Cereals, cereal-based products and potatoes" (23.2%), and "Milk, dairy products, and substitutes" (22.0%). "Fish

and fish products" contributed to protein intake only for ~6.0%. "Milk, dairy products, and substitutes" (28.5%) and "Sweet and salty snacks" (27.1%) were the main sources of total fats, followed by "Fats and oils" (16.7%) and "Meat, meat products, and cured meat" (13.6%). Finally, the major sources of available carbohydrates and fiber were similar and included "Cereals, cereal-based products and potatoes" (52.6% and 44.7%), "Sweet and salty snacks" (24.2% and 8.4%), "Fresh and squeezed fruit" (8.0% and 21.6%), and "Vegetables" (2.0% and 13.9%). "Pulses" contributed to fiber intake only for the 7.8%.

4. Discussion

The current study evaluated nutritional adequacy in 381 7-year-old children from Friuli Venezia Giulia, Italy, who were enrolled within a cohort study aimed at evaluating the effects of mercury on infant neurodevelopment [42]. Results revealed an inadequate intake of key nutrients, as highlighted by standard analyses and the NAR indexes, and suboptimal adequacy of the overall dietary profile, as expressed by the MAR index. In the standard comparison with DRVs, distribution of macronutrient intakes in percentage of energy was unbalanced in favor of protein and fats, with protein intake exceeding the recommendation from 2 to 4 times. Similarly, inadequacy by excess intake was found for most (range: 65.0–84.2%) of the children for soluble carbohydrates, SFAs, and sodium. Within a range of median values between 0.11 and 0.90, the NAR-based analysis further confirmed and allowed to quantify inadequacy by defect for some micronutrients, including vitamin D and folate; it also downgraded evidence on zinc inadequacy, previously emerged in standard DRV-based analysis. A median MAR value of 0.75, with no child reaching the optimal adequacy value of 1, suggested a suboptimal adequacy of the overall diet in the study population.

Considering available carbohydrates, total fats, and protein, most of the children from our sample met the Italian DRVs and showed a NAR index equal to 1. Although apparently reassuring, this hides a substantial unbalance of the overall diet towards total fats and protein. Indeed, in our sample, no child was below the RI lower limit for total fats, and 1 out of 4 children (25.2%) exceeded the upper RI limit. In addition, by calculating child-specific PRI cut-offs for protein, ~63% of the children at least doubled their recommended PRI and ~11% at least tripled it. From a different perspective, the median protein intake of our sample is 55.6 g/d, which is comparable to the daily protein requirement of an adult woman of 60 kg of weight (54 g/day) [38]. The described unbalance towards protein and total fats has been already documented in most of the other Italian [34,35] and European [27,29–31] studies on primary school children, except for one older Italian study [32] where available carbohydrates of 8-year-old children reached 60% of total energy intake. In addition, our analysis on food groups suggested that at least 60% of protein daily intake was from animal sources, indicating a low plant-based protein intake, as previously observed in children from the same age in Italy, Spain, and Belgium [37,52,53]. Western dietary pattern, which is high in animal sources, has been previously associated with an increased risk of metabolic syndrome [54].

Still in line with the Italian and European data [24,34,35,55], we observed: 1. an excess intake of SFAs, with 82% of children being above the SDT; and 2. intakes of total PUFAs below the RI lower limit in 96% of the children.

We similarly observed an excess contribution to energy intake from soluble carbohydrates, when using the SDT as the reference cut-off [24,34,35,55]. In addition, 15% of the enrolled children derived at least 25% of their total energy intake from soluble carbohydrates, against recommendations of the Italian Society of Human Nutrition [38], who considered intakes >25% to be at risk for adverse effects on health. Furthermore, no child from our sample had a soluble carbohydrates intake <5 %En, 4 children were < 10 %En vs. 60 (15.8%) < 15 %En, which corresponds to the Italian SDT. This is far from the World Health Organization recommendation to reduce the intake of free sugars to <5 %En, due to their effects on body fat deposition, overweight and obesity, cardiovascular risk, and dental caries [56].

Even if underestimation of sodium is likely to occur in dietary records, our median sodium intake (1.7 g/day) was in line with the one reported by Rosi et al. [35] (1.8 g/day), whereas Verduci et al. [34] reported a lower median intake (1.2 g/day); in the UK-based Avon Longitudinal Study of Parents and Children (ALSPAC) study sex-specific medians were higher (2.1 and 2.3 g/day) and sodium was estimated without considering added salt [30]. In addtion, 65% of our enrolled children had a sodium intake above the SDT [38], without any other possible comparisons except for Verduci et al. [34], where 9% of children were above the SDT, in line with their lower median intake. Major food sources included "Cereal and cereal-based products" (34.4%), followed by "Herbs, spices and added salt" (14.1%), probably due to an increased frequency of consumption of bread substitutes [57] and ready-to-eat products [58,59], which are rich in salt [48]. The "Sweets and salty snacks" food group provided a nonnegligible contribution (8.9%) to sodium intake, with sweets accounting for 92.9% of the food group contribution. This suggests sodium is present in sweets too. Although in "Milk, dairy products, and substitutes" and "Cured meat" salt is traditionally used as a preservative [60], in sweets it is commonly used as a flavor enhancer [60].

Except for iron, copper, and phosphorus, intake of other minerals was generally inadequate (i.e., median intake lower than the corresponding AR) in our sample. Similar conclusions were reached for iron, phosphorus, sodium, calcium, potassium, and zinc, in one or more of the available Italian studies [34,35]. However, generally, higher mean/median intakes were shown in the comparison with previous European studies [30,31,61], as well as with the very detailed but older Italian INRAN-SCAI study [24]. Downgrading evidence on zinc inadequacy from standard DRV-based analysis, the NAR-based approach revealed a modest deviation of zinc intake from the DRVs in most children (median NAR = 0.90). This indicates that dietary inadequacy was not severe in our sample, as also hypothesized for zinc deficiency in serum of European children [62].

Seven out of 12 available vitamins showed a median adequate intake, with five of them (vitamin B2, vitamin B6, vitamin B12, vitamin A, and vitamin C) even reaching the PRI. However, in our analysis we detected a major contribution of food groups of animal origin to vitamin B2 (>60%), vitamin B6 (>43%), and vitamin B12 (100%), as well as to energy (>30%). Among others, the worst degree of inadequacy was observed for folate and vitamin D. Folate median intake was below the AR and 80% of children did not reach it. In Italy and Europe, mean/median intake of folate in primary school children was similarly low [30,31,35,61]. In our sample, the main source of the folate was "cereals and cereal-based products", where folate intakes may be underestimated due to cooking losses. An inadequate intake of vitamin D was also observed in our sample: no child met the AR, and the median intake of children was 11%, as compared to the AR cut-off value (median NAR = 0.11). This is alarming, but in line with dietary data of other Italian [24,34,35] and European studies [30,31]. Evidence of serum deficiency of vitamin D was also reported in a pediatric population in Italy [63,64], suggesting an increased sun exposure and dietary intake has to be reached.

We did not observe substantial variation in nutrient intake between males and females. Most of the significant differences were found for macronutrients, with the higher available carbohydrates intake in males likely reflected in their higher energy intake, as also found in Verduci et al. [32]. This is in line with previous results from the ALSPAC [30] and from our NAC-II cohort in children at 18 months [45], where, however, soluble carbohydrates were also significantly different between males and females. Only four micronutrients showed a significantly different intake in males and females, but those differences were small and likely to be not nutritionally meaningful.

The major strength of the present work stands in its comprehensive description of dietary intake following different approaches. To our knowledge, we were the first group in Italy to propose the use of NAR and MAR indexes for a quantitative evaluation of dietary adequacy at the nutrient- and overall-diet-levels. We extended the MAR index in two directions: 1. including additional macro- and micronutrients; 2. considering

nutrient inadequacy by excess intake together with deficiency in the calculation of the corresponding NARs. We also carried out an influence analysis to assess the importance of single nutrients in the calculation of the MAR index, with reassuring results. We finally summarized information on percentage contribution of selected food groups to macro- and micronutrients, to provide an updated benchmark information for future Italian studies on primary school children. This comprehensive approach has been possible because we collected information based a 3-dDR, which provided a precise quantification of daily food intake [26]. In addition, we referred to the "Food Composition Database for Epidemiological Studies in Italy" [48] to derive intakes of a complete list of 86 macro- and micronutrients. Among the 37 nutrients compared with DRVs, 24 did not show missing values in the BDA. We were only unable to compare four nutrients with the available Italian DRVs [38]: chrome, fluorine, and molybdenum were not provided by the BDA and vitamin K was fraught with so many missing values (86% of the total BDA items) to likely not providing a reliable estimate of its intake.

The current study also has limitations. The cohort was enrolled in the Friuli Venezia Giulia region with a different aim, so generalizability of results to the Italian population of 7-year-old children of the same or next time span is questionable. However, percentages of males and females, prevalence of overweight, as well as percentages of mothers and fathers with a high school diploma were similar to those reported in the national survey "OKkio alla SALUTE" on 8–9-year-old children in 2016, for the Friuli Venezia Giulia region [65] and at the national level [66]. Prevalence of obesity in our sample was in line with data from Friuli Venezia Giulia (5.0%, [65]), but lower than the national-level data (9.3%, [66]). This likely reflects the high frequency of practicing sports and the modest screen time detected in our sample; however, a proper comparison with "OKkio alla SALUTE" was not possible due to differences in questionnaires. Although a dietary record is the gold standard dietary assessment method [26], its use may still lead to biases. Dietary records were filled in by caregivers, who may not be fully aware of child food consumption, especially when the child has lunch at school and/or more than one caregiver is in charge of him/her. Dietary records may be incomplete or inaccurately completed. In case of missing information, standard recipes and standard portion sizes were used. In our 3-dDRs, added salt was not accurately reported by all subjects, and no information on its iodization has been provided. Similarly, water consumption was sporadically reported, leading to possible underestimation of minerals, especially of calcium. An additional source of underestimation of nutrients—common to other studies—included missing data in the food composition tables. Moreover, the use of nutritional labels for the conversion of complex commercial products (~4% of the total food items) may have led to inaccurate estimates of a few macronutrients and/or underestimation of micronutrient intakes. We were also unable to properly estimate child-specific energy requirement due to lack of a dedicated tool to assess physical activity level. In our application, we have to acknowledge that the lack of standardized cut-offs for NAR and MAR have limited our ability to distinguish between modest and severe nutritional inadequacy. Finally, we cannot exclude that inadequacy observed in the present study simply reflected a limited dietary variety [67] or a low adherence to the Mediterranean diet [68,69]. Dietary pattern analysis may provide additional insight into children's overall dietary behavior and its potential relation with nutritional adequacy.

5. Conclusions

In line with previous Italian and European studies on primary school children, the nutritional assessment of a sample of 7-year-old children enrolled in the NAC-II cohort from Friuli Venezia Giulia, Italy, has revealed an unbalanced macronutrient profile towards protein and fats and a suboptimal intake of several macro- and micronutrients. For the first time in an Italian study, our paper has explored the use of two indexes integrating the standard evaluation of nutritional adherence to DRVs. These indexes may provide the basis for targeted public health interventions. They indeed may allow to identify critical

nutrients whose intakes have to be modified or subsets of subjects to be targeted within the general population.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu14030515/s1, Table S1: Parents general information at delivery. Northern Adriatic Cohort II (NAC-II), 2014–2016 (N = 381); Table S2: Percentage contribution of food groups to total intake of fatty acids and cholesterol. NAC-II, 2014–2016 (N = 381); Table S3: Percentage contribution of food groups to total intake of micronutrients. NAC-II, 2014–2016 (N = 381).

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ORIGINAL ARTICLE
EPIDEMIOLOGY AND CLINICAL MEDICINE

A 3-year school-based intervention improved physical fitness and reduced the prevalence of overweight and obesity in Italian prepubertal children

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ABSTRACT

BACKGROUND: Schools constitute the ideal setting in which children's physical activity, physical fitness, and health status can improve. However, intervention protocols and their effectiveness vary considerably. The purpose of the study was to investigate the differences in physical fitness and overweight and obesity prevalence between children attending structured physical education classes held by a specialized teacher (EXP) or traditional classes of equal duration held by an ordinary teacher (TRAD).

METHODS: Anthropometric and fitness parameters were assessed in a convenience sample of 12,519 1st grade schoolchildren over 3 subsequent school years. Six field-based tests were used to assess physical fitness.

RESULTS: Physical fitness improved more in the EXP group than in the TRAD group, except for flexibility (sit and reach). At the end of the 3rd year, the EXP children performed better than did the TRAD children (P<0.001) in Léger (girls: +34%, boys: +30%), agility shuttle (girls: -10%, boys: -9%), long jump (girls: +9%, boys: +8%), frontal basketball throw (girls: +11%, boys: +10%), and standing balance (girls: +18%, boys: +28%). The prevalence of obesity and overweight was 5% lower in the EXP than in the TRAD group at the 3-year follow-up.

CONCLUSIONS: The proposed teacher-driven intervention, which was focused on the quality rather than the duration of time spent in the gym during school hours, was effective in improving children's physical fitness. Furthermore, the decrease in the prevalence of obesity and overweight suggests the intervention can improve heavier children's weight status.

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KEY WORDS: Exercise; Physical education and training; Body Mass Index; Physical fitness; Pediatric obesity; Child.

Physical activity (PA) is one of the major determinants of physical fitness (PF), which is defined as the set of attributes related to a person's ability to perform physical activities. Both PF and PA have been associated with cardiovascular health,[1, 2] weight status,[3, 4] and physical and mental well-being in children.[5-8] Although the importance of regular PA has been shown, some authors have reported that children have an inactive lifestyle[9, 10] as well as an inadequate level of physical fitness,[11] leading to an elevated prevalence of overweight and obesity,[9, 12] especially in southern Europe. In fact, most European children do not adhere to national and international guidelines, recommending at least 60 minutes of daily moderate-to-vigorous physical activity (MVPA), to achieve health benefits.[10] Moreover, it has been reported that currently, less than half of the time allotted for physical education (PE) classes in schools are devoted to MVPA and that children's active participation in PE at school can be low.[13] Therefore, simple and efficient interventions need to be developed and implemented to counteract this trend.

Because many children can be affected and there are infrastructures to promote PA, the school environment is commonly viewed as a favorable setting in which children's PA can be increased.[14] Although results are inconsistent and the types of interventions vary considerably, several authors have reported that school-based PA interventions can improve physical fitness,[15-18] the percent of time children spend performing MVPA during PE,[19] health-related quality of life,[18] and body mass (BM) or Body Mass Index (BMI).[17, 19-21] The interventions have ranged from minimal changes in PE sessions to modifications in the content, duration, and type of activities performed during the entire school day. In this context, the role of PE teachers' specific training may be crucial in enhancing children's motivation and improving their physical literacy.[22]

Although some fewer intensive interventions not specifically designed to target PF have been shown to have no effect on PF,[19, 23] even modest interventions can have health benefits, especially in high-risk young people such as obese children.[21] Consequently, since Italy has one of the highest prevalence of childhood obesity in Europe[12] and the public cost of obesity is high,[24] it is important to determine whether a simple school-based PE intervention can reduce the prevalence of obesity in schoolchildren, as well as improve their physical fitness.

The primary purpose of the present study was to analyze, over a 3-year follow-up period, the differences in physical fitness between a large sample of 1st grade schoolchildren taking structured PE classes held by a specialized teacher (EXP) and a control group taking traditional classes of equal duration held by their ordinary classroom teacher (TRAD). The secondary purpose was to investigate changes in children's weight status and in the prevalence of overweight and obesity after a mild teacher-driven PE intervention in Italian primary schools.

Materials and methods

A convenience sample of 12,519 Italian 1st grade schoolchildren (6 years old) who attended different public schools in the Friuli Venezia-Giulia region (Italy) from 2016 to 2019 and participated in the project "MOVIMENTO in 3S: promozione della salute nelle scuole attraverso lo sport" (MOVIMENTO in 3S project: promoting health in schools through sport) was enrolled. Schoolchildren were assigned either to an experimental (EXP: 3542 girls and 3848 boys) or to a traditional (TRAD: 2498 girls and 2631 boys) physical education program and were followed up for 3 years.

The study was approved by the University of Udine Ethics Committee on Human Research for Medical Science. The following criteria were adopted to identify eligible children: elementary school attendance and the absence of any disease or disability that could make the child unable to participate in the scheduled school physical education program. Before the study began, the purpose and objectives were carefully explained to each child and his or her parents. The children gave their verbal consent, and written informed consent was obtained from their parents.

Experimental protocol

For each class included in the experimental physical education (EXP) program, both the ordinary teacher and an external specialized teacher held the physical education classes for the whole 3-year period. Each specialized teacher was specifically trained in physical education (degree in Exercise and Sport Sciences) and was responsible for implementing a class-specific physical activity plan. The type of physical activity (exercises, games, circuits, etc.) was coordinated through monthly meetings, according to a previously standardized plan. A broad range of physical activities were offered to the children assigned to the EXP group, including traditional games and exercises of increasing intensity and duration throughout the study. The program was designed to promote the joy of movement, body awareness, spatial perception, motor skills, coordination, physical literacy, team spirit and compliance with the rules to achieve long-term modifications in behavioral patterns and to minimize children's level of inactivity. All the sessions had the same two goals: they were designed to be playful and enjoyable and to encourage the children to perform 40 minutes of MVPA within a 50-minute PE class. The classes were scheduled twice a week according to the standard Italian primary school curriculum. Hence, the intervention was focused on the quality rather than the duration of time spent in the gym during school hours. Active participation during PE class was continuously monitored to minimize the children's inactivity time.

In contrast, the traditional physical education group (TRAD) followed the standard physical education program during the 50-minute classes scheduled twice a week. The classes were held by ordinary classroom teachers.

Anthropometric measurements and physical fitness parameters were taken at the beginning of the project and at the end of each school year during school hours. The measurements were taken in both the EXP and TRAD groups by Sport Sciences students who were previously trained to correctly collect the data for each test.

Anthropometric characteristics

Each child's stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board, and body mass (BM) was measured to the nearest 0.1 kg with a calibrated manual weighing scale (Seca 709, Seca GmbH & Co. KG., Hamburg, Germany). Body Mass Index (BMI) was calculated as BM (kg) · stature^{-2} (m). The children were considered OW or OB based on BMI/age-specific curves when their BMI was higher or equal to the international cut-off point, corresponding to the centile curve that passes through either the BMI curve for 25 or 30 kg · m^{-2}, respectively, at 18 years of age.[25]

Physical fitness

To obtain a representative status of the children's physical fitness, 6 of the several physical fitness tests suitable for the selected age group[26, 27] were considered. Priority was given to the accuracy of the measurements, which were taken by a group of trained sports scientists within a short period of time in all the schools involved in the study. The 6 tests selected were administered to children on 6 different weekdays during their physical education classes so that each test was not influenced by the results of the previous test. The following tests, which were comprehensively described elsewhere,[3] were chosen to assess aerobic capacity, whole body agility, lower limb explosive power, upper body power, hip and low back flexibility, and balance capacity: the Léger, shuttle run, long jump, frontal throw of the basketball, sit and reach, and standing balance tests, respectively.

Statistical analysis

For the analysis, the collected data were first screened for incorrect inclusions. When data were not plausible, the records were excluded from the database. Anthropometric characteristics and physical fitness are expressed as means and standard deviations (SDs), and they were stratified by sex and the number of years of follow-up. To identify the distributions of the variables, the Kolmogorov-Smirnov Normality Test was used. A generalized linear repeated mixed model of the main effects of sex (girls *vs.* boys), age and sex × age interaction was used to compare the EXP and TRAD groups in terms of anthropometric characteristics and physical fitness variables at each follow-up. All statistical analyses were performed by SAS, version 9.4 (SAS Institute, Cary, NC, USA), with $P<0.05$ indicating statistical significance.

Results

No significant differences were found between the EXP and TRAD groups regarding the anthropometric characteristics at the beginning of the study and at the end of each academic year. Table I, II show the anthropometric characteristics of the 12,519 1st grade schoolchildren followed for 3 years (6, 7 and 8 years old). A total of 3700 children (1175 girls and 1925 boys) from the EXP group and 3530 (1757 girls and 1773 boys) from the TRAD group completed all the measurements and fitness tests throughout the 3-year follow-up period. Stature and body mass (BM) differed significantly between sexes ($P<0.001$) and

TABLE I.—*Anthropometric characteristics of the experimental (EXP) physical education groups, stratified by gender and evaluated at the end of the 1st (6 years old), 2nd (7 years old) and 3rd (8 years old) year (yr) of follow-up.*

EXP	Girls			Boys			P		
	1st yr (N.=3542)	2nd yr (N.=2807)	3rd yr (N.=1775)	1st yr (N.=3848)	2nd yr (N.=3070)	3rd yr (N.=1925)	Sex	Age	S×A
Stature (m)	1.21±0.06	1.24±0.06	1.31±0.06	1.21±0.06	1.25±0.06	1.32±0.06	0.001	0.001	0.045
BM (kg)	23.8±4.3	26.2±5.1	29.8±6.3	24.4±4.5	26.5±5.0	30.2±6.1	0.001	0.001	0.845
BMI (kg·m^{-2})	16.3±2.2	16.8±2.4	17.4±2.9	16.5±2.2	16.7±2.2	17.3±2.6	0.127	0.001	0.572

All values are mean±SD. Significance by general linear mixed model of the main effects of sex (girls *vs.* boys), age and sex × age interaction (S×A).

TABLE II.—*Anthropometric characteristics of the traditional (TRAD) physical education groups, stratified by gender and evaluated at the end of the 1st (6 years old), 2nd (7 years old) and 3rd (8 years old) year (yr) of follow-up.*

TRAD	Girls			Boys			P		
	1st yr (N.=2498)	2nd yr (N.=2440)	3rd yr (N.=1757)	1st yr (N.=2631)	2nd yr (N.=2589)	3rd yr (N.=1773)	Sex	Age	S x A
Stature (m)	1.20±0.05	1.22±0.06	1.29±0.05	1.19±0.05	1.23±0.06	1.29±0.06	0.001	0.001	0.037
BM (kg)	22.9±4.1	25.0±4.7	28.2±5.7	23.0±3.6	25.2±4.7	28.6±5.5	0.001	0.001	0.248
BMI (kg·m^{-2})	16.0±2.1	16.7±2.4	17.0±2.7	16.3±1.9	16.7±2.2	17.2±2.5	0.078	0.001	0.487

All values are mean±SD. Significance by general linear mixed model of the main effects of sex (girls *vs.* boys), age and sex × age interaction (S×A).

TABLE III.—*Underweight (UW), normal-weight (NW), overweight (OW) and obese (OB) prevalence in the experimental (EXP) physical education groups, stratified by gender and evaluated at the end of the 1st (6 years old), 2nd (7 years old) and 3rd (8 years old) year (yr) of follow-up.*

EXP	Girls			Boys		
	1st yr (N.=3542)	2nd yr (N.=2807)	3rd yr (N.=1775)	1st yr (N.=3848)	2nd yr (N.=3070)	3rd yr (N.=1925)
UW (%)	9	7	8	8	7	7
NW (%)	66	70	70	68	71	73
OW (%)	18	16	16	18	16	15
OB (%)	7	7	6	6	6	5

TABLE IV.—*Underweight (UW), normal-weight (NW), overweight (OW) and obese (OB) prevalence in the traditional (TRAD) physical education groups, stratified by gender and evaluated at the end of the 1st (6 years old), 2nd (7 years old) and 3rd (8 years old) year (yr) of follow-up.*

TRAD	Girls			Boys		
	1st yr (N.=2498)	2nd yr (N.=2440)	3rd yr (N.=1757)	1st yr (N.=2631)	2nd yr (N.=2589)	3rd yr (N.=1773)
UW (%)	7	6	6	7	5	5
NW (%)	68	69	67	69	72	70
OW (%)	20	18	18	19	17	18
OB (%)	6	7	8	5	6	7

increased significantly from the 1st year to the 3rd year of follow-up in both the EXP and TRAD groups (P<0.001) (by a mean of +0.06 m and +3 kg per year in both the girls and boys in each group, except for the TRAD boys, whose height increased by a mean of +0.05 m per year). Accordingly, BMI increased significantly by year (by a mean of +0.5 and +0.4 $kg \cdot m^{-2}$ per year in the EXP girls and boys, respectively, and a mean of +0.5 $kg \cdot m^{-2}$ per year in the TRAD girls and boys).

Table III, IV show the prevalence of underweight, normal weight, overweight and obesity in the EXP and TRAD girls and boys at the end of each year of follow-up. In the EXP group, the prevalence of OB and OW decreased from the 1st year to the end of the 3rd year of follow-up by -1% and -2%, respectively, in the girls and by -1% and -3%, respectively, in the boys. In contrast, in the TRAD group, OB prevalence increased by +2% from the 1st year to the end of the 3rd year of follow-up in the girls and boys, and OW prevalence decreased by -2% in the girls and -1% in the boys.

Therefore, the prevalence of OB and OW at the 3rd year of follow-up was lower in the EXP group than in the TRAD group by -2% and -3%, respectively.

Children's physical fitness

No significant differences were found between the EXP and TRAD groups in any of the PF results at the beginning of the project and at the end of the first academic year. However, significant differences in PF scores were found between the girls and boys for all tests (P<0.001); hence, the results were presented separately for the girls and boys in both the EXP and TRAD groups.

In the Léger test (Figure 1A, B), performance improved more in the EXP group (by a mean of +37% per year in both the girls and boys) than in the TRAD group (by a mean of +30% per year in both the girls and boys) over the follow-up period (P<0.05). Accordingly, the children assigned to the EXP group completed significantly more levels than did those assigned to the TRAD group at the end of the 2nd and 3rd years of follow-up (+30 and +34%, respectively, in the girls; and +31 and +30%, respectively, in the boys, P<0.001).

Figure 1C, D show that the children in the EXP group required less time to complete the agility shuttle test each year (by a mean of -5% per year in both sexes) than did the children in the TRAD group (by a mean of -3% per year in both sexes; P<0.05). The girls and boys who followed the EXP protocol completed the agility shuttle test in significantly less time than did those who followed the TRAD protocol at the end of the 3rd year of follow-up (-10% in the girls and -9% in the boys, P<0.001).

For the long jump test (Figure 1E, F), the children in the EXP group increased the distance of their jump each year (by a mean of +8% per year in both sexes) more than those in the TRAD group did (by a mean of +5% per year in both sexes; P<0.05). Consequently, the girls in the EXP group jumped significantly farther than the girls in the TRAD group did at the end of the 2nd and 3rd years of follow-up

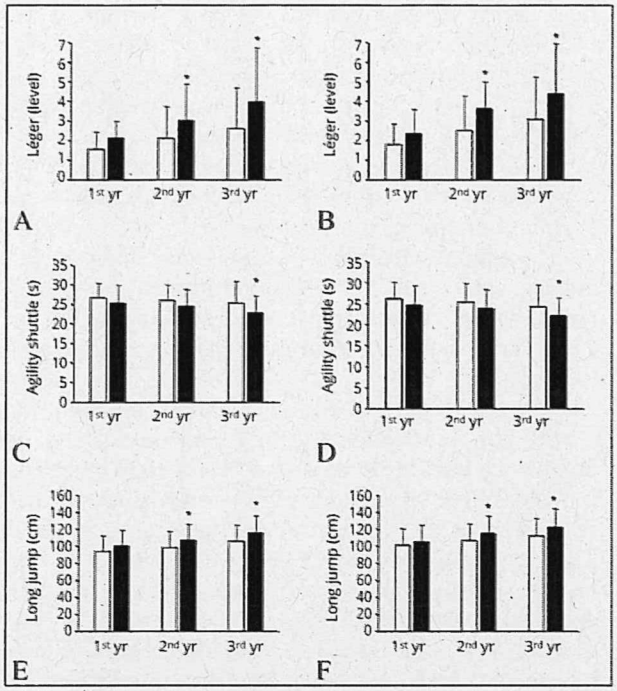

Figure 1.Physical fitness: A, B) Léger test; C, D) agility shuttle test; E, F) long jump test in the experimental (EXP, ■) and traditional (TRAD, □) physical education groups, stratified by gender (girls [A, C, E]; boys [B, D, F]) and evaluated at the end of the 1st (6 years old), 2nd (7 years old) and 3rd (8 years old) year (yr) of follow-up.
All values are mean±SD.
*Significantly different relative to TRAD (P<0.001).

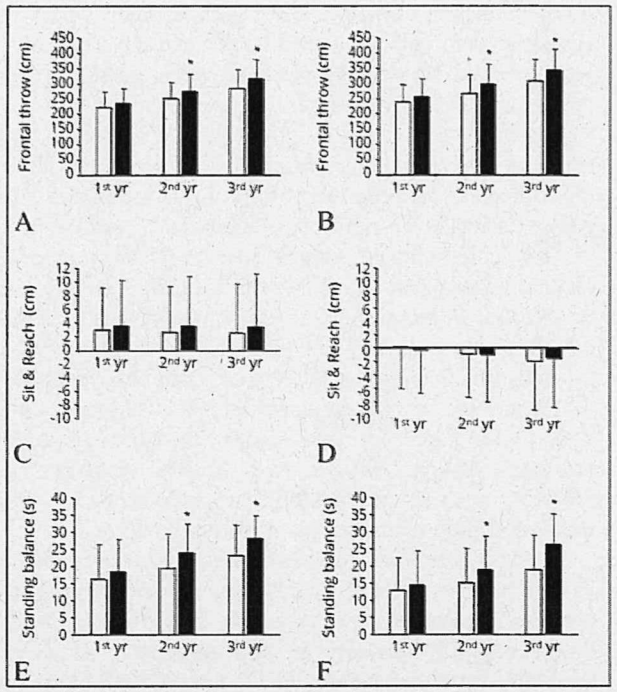

Figure 2.Physical fitness: A, B) frontal throw test; C, D) sit and reach test; E, F) standing balance test in the experimental (EXP, ■) and traditional (TRAD, □) physical education groups, stratified by gender (girls [A, C, E]; boys [B, D, F]) and evaluated at the end of the 1st (6 years old), 2nd (7 years old) and 3rd (8 years old) year (yr) of follow-up.
All values are mean±SD.
*Significantly different relative to TRAD (P<0.001).

(+8 and +9%, respectively), and the boys in the EXP group jumped significantly farther than boys in the TRAD group did (+8% at the end of the 2nd year and +8% at the end of the 3rd year, P<0.001).

Figure 2A, B show the results of the frontal throw test. The children in both groups threw the ball farther each year, but those in the EXP group increased their throwing distance more than those in the TRAD group did (by a mean of +16% per year in both the girls and boys in the EXP group and +13% per year in both the girls and boys in the TRAD group; P<0.05). Accordingly, at the end of the 2nd and 3rd years of follow-up, the children in the EXP group threw the ball significantly farther than those in the TRAD group did (+9% in the girls and +11% in the boys at the end of the 2nd year, and +11% in the girls and +10% in the boys at the end of the 3rd year, P<0.001).

Regarding the sit and reach test (Figure 2C, D), no differences were found between the EXP and TRAD groups at the end of each year. The girls' changes in flexibility by year were not significant in either the EXP or the TRAD group. On the other hand, the flexibility capacity of the boys decreased less each year in the EXP group than in the TRAD group (by a mean of -137% per year in the EXP group and -279% per year in the TRAD group; P<0.05).

Finally, Figure 2E, F show larger improvements in the standing balance test scores over the follow-up period in the EXP group (by a mean of +24 and +35% per year in the girls and boys, respectively) than in the TRAD group (by a mean of +19 and +21% per year in the girls and boys, respectively; P<0.05). Accordingly, the children in the EXP group maintained their balance significantly longer than those in the TRAD group did at the end of the 2nd and 3rd years (+19 and +18%, respectively, in the girls; and +20 and +28%, respectively, in the boys, P<0.001).

Discussion

The main results showed that, in the present sample of Italian 1st grade children, the higher competences in planning and delivering PE lessons by specialized teachers: 1) posi-

tively influenced children's PF at the end of the 2nd and 3rd academic year, with the exception of flexibility; and 2) the prevalence of OB and OW was 5% lower in the EXP group than in the TRAD group after the 3-year intervention.

Teachers are key figures in increasing PE participation and enjoinment among schoolchildren, and specialized PE teachers have been found to be more successful than general teachers in improving children's physical fitness in a similar learning environment.[28] A previous study demonstrated that minimal teacher-directed interventions can be effective in increasing children's MVPA during school days and improving BM.[29] In particular, intervention effectiveness may be enhanced by the program being modified for the specific school setting in which it is delivered[22] and by its ability to promote participants' enjoyment during PA sessions.[20] Although PA outcomes were not assessed in the present study, participation in PE activities was constantly monitored and maintained at a high level.

We show that the intervention was effective in increasing PF in both sexes. The girls and boys in the EXP group improved their aerobic capacity, agility, and lower and upper limb strength at similar rates. Balance capacity improved more by year in the boys than in the girls in the EXP group. Moreover, starting from the second year of follow-up, the children assigned to the EXP group increased their aerobic capacity, balance, and muscular strength in the lower and upper limb more than those assigned to the TRAD group did, while the magnitude of increase in the agility scores did not differ between the two groups throughout the 3-year follow-up period. Accordingly, in previous studies on PA-focused interventions implemented in schools, significantly better results were found in the intervention group than in the control group in shuttle runs,[23, 30-33] long jump tests,[15, 28, 30] and coordination tests.[34] However, contrary to the present study, most of the previous interventions included a longer PE class or additional PA allocated time and had varied follow-up durations, ranging from 5 months[34] to 5 years.[30] Conversely, the results of a similar school-based intervention showed non-significant differences between the intervention and control groups in either PF test performed,[17] and a recent review[19] showed inconsistences regarding the effects of PE-based interventions on PF in schoolchildren, concluding that only those specifically designed to influence fitness outcomes were found to be effective. Regarding flexibility scores, even though no significant differences were found between the EXP and TRAD groups in either the girls or boys in the present study during the whole follow-up period, the intervention seemed to reduce the magnitude of decrease in flexibility observed in the boys at the end of the 3rd year of follow-up compared to that in the TRAD group. This general decreasing trend has also been found in a previous study in schoolboys living in the same geographic region.[3] The non-significant differences in flexibility scores could be due to the activities performed during PE, which were more focused on dynamic and play exercises than on specific fitness training components.

In summary, although some previous studies implementing similar PA interventions during school hours reported no significant improvements in PF in the children assigned to the experimental group compared to those assigned to the control group,[17, 34] the present evidence showed that even a minor intervention, modifying PE quality but not duration (PE or PA allocated time), can improve children's PF more than the standard curriculum is. Accordingly, some authors[28] have found that improving the quality of the standard PE curriculum, by recruiting a specialized teacher, could improve children's PF more than the standard curriculum implemented by a general classroom teacher. Furthermore, not only the intervention design but also the follow-up duration has been found to be crucial.[23] Previous comparable interventions that were inefficient in improving children's PF were implemented for a shorter period than was the intervention in the present study.[17, 34] As a confirmation, in the present study, a one-year intervention was not enough to yield significant differences between the EXP and TRAD groups in any PF test, while better results were obtained at the end of the 2nd and 3rd years.

Regarding the BM outcomes of school-based PA interventions aimed at preventing childhood obesity, in the literature, the results are generally inconsistent; effectiveness seems mainly driven by the type, intensity, and duration of the intervention, with better results obtained by interventions with an increased time allocated to structured PA.[19, 20, 35, 36] Although the intervention in the present study focused on increasing MVPA during PE, the differences in BM and BMI between the EXP and TRAD groups at the end of the study period were not significant, which is consistent with the results of a comparable study implementing a teacher-based intervention to enhance PE quality but not duration.[28] Hence, we can assume that 100 minutes of high-quality PE weekly during school hours, that is, the amount of time proposed by the standard Italian Curriculum, is not enough to slow the increase in BM and BMI in growing children. In accordance, some authors[29] have found that weight status improves after a 3-year intervention only when the total

weekly time allocated to structured PA at school is greater than 135 minutes.

Furthermore, school-based interventions targeting PA seem to be more effective in reducing BM in overweight and obese children than in normal-weight children.[21, 37] Sacchetti *et al.*,[17] despite reporting non-significant BMI differences between the groups, observed a larger reduction in the prevalence of obese and overweight children in the intervention group than in the control group after a 2-year intervention modifying PE quality and quantity. The extent of this reduction is comparable to that reported in the present study, where the prevalence of OB and OW in the EXP group was 5% lower than that in the TRAD group after a 3-year intervention in which PE quality but not duration was modified. Although modest, the extent of this reduction may be of great relevance in public health, considering the entire national schoolchildren population. In fact, the economic consequences of childhood obesity become relevant when obese children become obese adults. Approximately one-third of obese preschool children and approximately half of obese school-age children become obese adults.[38, 39] The estimated annual costs of treating obesity-related illnesses in Italian overweight, obese and severely obese individuals are higher than that of treating the normal weight counterparts by means of 10, 21 and 42%, respectively (which correspond on average to 530, 580 and 680 euro/year per capita for overweight, obese and severely obese subjects, respectively).[24, 40] Therefore, reducing the prevalence of overweight and obese children could also lead to a significant reduction in national healthcare costs.

Conclusions

In conclusion, the present teacher-driven intervention (EXP), which was focused on the quality rather than the duration of time spent in the gym during school hours, was effective in improving children's physical fitness. In addition, at the end of the three-year follow-up period, the prevalence of obesity and overweight was 5% lower in the EXP group than in the TRAD group, suggesting that the intervention was effective in improving heavier children's weight status. By reducing the prevalence of overweight and obese children, effective school-based interventions could also lead to a relevant reduction in the healthcare costs associated with obesity. Therefore, the present findings reveal that qualified physical education teachers in primary schools could play an important role in physical fitness and health promotion.

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Applied nutritional investigation

Accuracy of applications to monitor food intake: Evaluation by comparison with 3-d food diary

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ABSTRACT

Objective: The availability of nutrition applications (apps) has increased in recent years. The aim of this study was to assess the accuracy of nutrient intake calculations from some of the leading apps.
Methods: We identified five apps according to some selection criteria: >4-star ratings, >1 million downloads, including a food composition database, and in Italian language. Apps were used for 2 wk each. Using a 3-d food diary, the nutritional values obtained from each app were compared to a reference method including the Food Composition Database for Epidemiologic Studies in Italy. Energy intake differences were calculated for single nutrient and 3-d food diary between single app and reference method after food-item matching. Bland–Altman plots were used to assess agreement of the methods.
Results: Apps identified were FatSecret, Lifesum, MyFitnessPal, Yazio, and Melarossa. Apps tended to underestimate total energy intake compared with the reference method, from a minimum of −2 kcal for Lifesum, to a maximum of −5.4 kcal for Yazio (average per item). Apps tended to underestimate lipids, and to a lesser extent carbohydrate and fiber intake, except for Yazio and Lifesum, which overestimated the intake of protein. These discrepancies appear to be due to the use of no country-specific food composition databases and to user customization of the food list.
Conclusions: The present findings suggest that the leading nutrition apps present critical issues in assessing the intake of energy and nutrients. Implementation of a framework for quality assessment is necessary to drive the design and development of higher-quality apps. Further research on efficacy and use of apps to monitor food intake is also warranted and some recommendations are provided.

Introduction

Recent advancements in technology and communication have permitted the development of new branches of medicine and the birth of a new field of e-health: mobile health (mHealth). The Global Observatory for eHealth defined *mHealth* as a medical and public health practice supported by mobile devices, such as mobile phones, patient monitoring devices, personal digital assistants, and other wireless devices [1]. These means can provide remote clinical health care, improving the therapeutic process. With >3 billion smartphones worldwide [2] and with the majority of the population using the internet, the availability of mobile applications (apps) has increased in the past few years. In particular, use of health- and fitness-related mobile apps is widespread in all age groups, with a prospective of a positive effect on daily behavior and weight management [3,4]. By using nutrition apps, users can track their dietary consumption with web-based food diaries, manage their weight, and receive health tips based on their daily behavior [5]. The advantages of these new technological tools are related to the direct and daily involvement of users. Indeed, users can set goals to enhance their motivation and can benefit from real-time feedback to correct wrong behaviors. Nevertheless, scientific research is still limited regarding the evaluation of data reliability and the effective use of apps in clinical practice: there is an

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obvious need to investigate the potential of these apps as well as their limitations [6]. As these apps allow the user to self-monitor and evaluate food consumption, they can present disadvantages: improper use can cause health problems as well as potentially trigger or maintain eating disorder symptomatology [7,8].

We have planned to develop a new app for smartphones that includes the Food Composition Database for Epidemiologic Studies in Italy (BDA) [9]. It is a tool that provides the composition in terms of energy and food components for the most important nutrients in the main food items consumed in Italy [10,11]. The BDA is a widely used food composition database designed for nutritional research. It is compiled according to well-defined methodology, starting from food composition data of Italian and foreign sources and from scientific papers. The BDA has been certified according to the European Food Information Resource system and all data are documented accordingly [12].

Food composition databases are of great importance in different fields, from clinical practice to nutritional research, for educational purposes, and also in the food industry [13,14]. The nutrient content of a given diet is generally calculated with programs that are based on available country-specific food composition databases. Dietary intake is difficult to measure and no available method can perfectly assess dietary exposure: 24-h recall, food diaries, and food frequency questionnaires present some limitations [15,16]. Considerable progress has been made in the accuracy of dietary intake assessment methods, but new methods such as web-based tools, augment reality apps using wearable devices or cameras and personal digital assistants incorporating images or voice recording options need higher costs and they do not solve all problems related to self-reporting [6,17].

Given these considerations, the aim of this study was to evaluate the characteristics of popular available nutrition apps for smartphones and their accuracy in the nutrient intake calculations by comparison with a reference method (RM). All information collected will be used to develop a new app for smartphone to monitor food intake.

Materials and methods

Identification and selection of apps

Applications were located on Google Play Store for Android devices available in Italy. We identified 450 ranked apps in the Top Selling and Top Download sections of the categories "Food and Drink," "Health and Fitness," "Medical," and "Books and References" between December 2018 and January 2019. After an initial screening based on the descriptions provided by the store, we excluded some apps belonging to the "Medical" and "Books and References" categories and those that were fitness-related because they were not relevant to the study. Inclusion criteria were set to reduce the number of apps to evaluate. We finally identified 10 apps with >4 star-rating, >1 million downloads, with Italian language availability, and the presence of a food composition database. The first five apps ranked by popularity were selected and included in the analysis.

A researcher downloaded the identified apps on a smartphone to be used for about 2 wk each. The researcher uploaded in each app some information to standardize the evaluation (i.e., age, height, weight, and circumferences of a healthy normal-weight woman, and 3-d diary of physical activities). All feedback and information supplied from each app were collected in a dedicated form in Excel, regarding body mass index, ideal weight, basal metabolism, or energy requirement. After an initial free period, all apps included a paid version to use all testable features. We paid particular attention to evaluate the accuracy of contents, checking the presence or absence of summary graphs, recipes, barcode scanner, reminders, and so on. The scientific quality was also evaluated in terms of presence or absence of bibliographic references, scientific supervision, source of food composition data, and information about authors and developers.

Evaluation of energy and nutrient intake

To assess the accuracy of the identified apps in terms of estimation of energy and nutrients, a 3-d food diary (3-DFD) containing fresh food, packaged food, recipes, and drinks was uploaded and divided into meals (breakfast, lunch, dinner, and snacks). For each food included in the 3-DFD, a corresponding match item was identified in each app. Numerous foods with similar denominations have been found in all apps. Items offering the most similar description were chosen. If a corresponding item was not available or in the case of a recipe (cappuccino, spinach omelet, fruit salad, and beef ragout recipe), a list of ingredients with their respective quantity was uploaded.

The nutritional values calculated from each app were manually downloaded to be compared with those obtained by using the BDA [11], by means of a software (Microdiet, Downlee Systems Ltd., UK), defined as RM. BDA provides the composition for 978 Italian food items in terms of energy and food components (macronutrients, minerals and trace elements, water- and fat-soluble vitamins, fatty acids, amino acids, and sugars). To standardize the comparison in all apps, we flagged missing values and components recorded as "trace" (defined as very low concentration of nutrient not nutritionally significant) and transformed them into zero [18]. Nutrient values indicated as undetectable below a given threshold were replaced by the mean between zero and the threshold value for data included in the BDA. We calculated total daily content of protein, fat, carbohydrates, and fiber in grams for each app. Energy expressed in kcal was obtained using international conversion factors: 3.75 kcal/g of carbohydrate (expressed as monosaccharide), 4 kcal/g of protein, 9 kcal/g of fat, and 2 kcal/g of fiber, in addition to energy reported by a single app. We were not able to include alcohol in the energy calculation because the apps did not account for this value.

Statistical analysis

Summary statistics (mean, median, interquartile range [IQR], 5th and 95th percentiles) for 3-DFD total energy and single nutrient intake (carbohydrates, fiber, lipids, and proteins) were calculated for each app and for the RM (Microdiet). The differences between the total energy intake of the two measurement tools for each app by a single nutrient were also calculated after food-item matching. Total energy intake distribution was visually inspected by using jittered scatterplots. The differences between the two measurement tools for each app were tested for zero location by the signed rank sum test. Spearman's correlation coefficient for energy and nutrient intake between the two measurement tools was calculated. Additionally, the agreement between the two tools was estimated by the Bland–Altman plots, using 5th and 95th percentiles of the differences as the lower and upper limits of agreement respectively. Normality assumption was checked graphically by a QQ plot and by using the Shapiro–Wilk test. All tests were two-tailed and considered significant at the 5% level. All statistical analyses were carried out using SAS version 9.4 (SAS, Cary, NC, USA).

Results

From an initial group of 450 apps available on Google Play Store, five apps meeting the inclusion criteria (Fig. 1) were identified and downloaded to perform the analysis. The five apps identified were FatSecret, Lifesum, MyFitnessPal, Yazio, and Melarossa. They were used for an average of 20 d each. The principal characteristics of the apps are reported in Table 1. Except for Melarossa, which included Italian food composition data (CREA, Rome, Italy) and was supervised by the Italian Society of Nutritional Science, the other apps were based on non-Italian food composition databases and were developed in other countries. Assistance and scientific coverage were adequate only in Melarossa and Lifesum. Total number of nutrients available in each app exhibited great variability ranging from 1 to 32 (Table 1 and Supplementary Table 1).

Considering the mean intake of carbohydrates, fiber, lipids, and proteins in g/3-DFD reported by single app (Supplementary Table 2), we observed an overall tendency of underestimation for fiber and lipids. Lifesum tended to overestimate the intake of carbohydrates and proteins and Yazio only the protein intake compared with the RM. Considering mean energy intake, FatSecret tended to underestimate (−355 kcal) and Lifesum to overestimate (+165 kcal). Melarossa was the only app to not report a total daily energy and nutrient intake, but did provide energy (kcal) for single items selected. Calculating the mean energy intake, Melarossa showed the worst energy intake compared with the RM (−390 kcal), mainly due to the elevated number of food items not available in the app (Supplementary Table 2). To better analyze these discrepancies, excluding Melarossa, we evaluated the accuracy of energy calculation item matching of proteins, carbohydrates, lipids, and fiber from FatSecret, Lifesum, MyFitnessPal, and Yazio with the RM. Apps tended to underestimate the mean energy

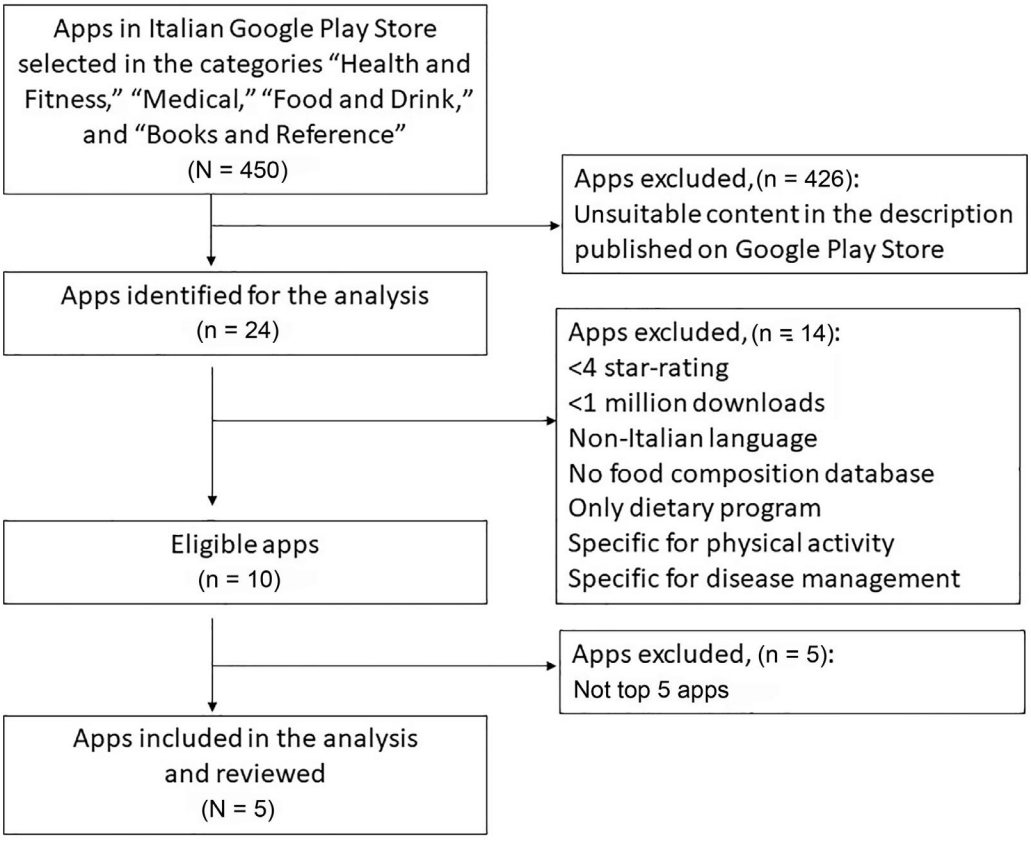

Fig. 1. Study flowchart for the process of selecting the identified apps.

intake compared with the RM, from a minimum of −2 kcal (IQR, 13.3) for Lifesum, to a maximum of −5.4 kcal (IQR, 12.7) for Yazio (Table 2) per item. There was a comparable degree of variability, from a minimum of 10.8 kcal to a maximum of 12.7 kcal IQR for MyFitnessPal and Yazio, respectively, although the positive skewness distributions showed long tails (Fig. 2), resulting in quite different values for the 5th and 95th percentiles and biased means (Table 2).

Yazio tended to underestimate energy intake more compared with other apps over 3 d (−329 kcal). Comparisons between the two measurement tools were not significant, except for MyFitnessPal underestimating total energy intake of −4.4 kcal per item (P = 0.03). In Table 3, differences are reported between the two measurement tools by nutrients. Overall, three of the five apps tended to underestimate the nutrient intake; Yazio and Lifesum overestimated protein intake by +1.5 kcal and +2 kcal per item, respectively, although the IQRs were low, with both at 1.1. Over 3-DFD, Yazio underestimated energy intake from lipids of −238 kcal and Lifesum overestimated energy from protein of +114 kcal. Comparisons between the two measurement tools were not statistically significant for nutrients, except for MyFitnessPal for lipids (−2.9 kcal, P = 0.001). Carbohydrates and lipids presented the highest IQRs (between 4.8 and 7, 2 and 5.7, respectively), indicating a higher degree of variability (Table 3). Overall, correlation coefficients between the two measurement tools were high (FatSecret: r = 0.96; MyFitnessPal and Lifesum: r = 0.93; and Yazio: r = 0.92; Supplementary Table 4). The lowest correlation coefficients were observed for fiber in MyFitnessPal, Lifesum, and Yazio at r = 0.68, r = 0.82, and r = 0.85, respectively and for lipids in Yazio at r = 0.85. None of the apps showed any evident systematic difference or proportional bias compared with the RM, although different counts of outliers for each app was observed on Bland–Altman plots (Fig. 3). Outliers ranged from a minimum of five foods for FatSecret and

Table 1
Characteristics of the five apps evaluated

App	Country	Assistance	Scientific supervision or references	Source of FCDB*,£	Food diary	Total components/ nutrients, n
FatSecret	Australia	No	No	App and users	Yes	12
Lifesum	Sweden	Yes†	Yes	United States, Sweden, United Kingdom, Germany, and users	Yes	11
MyFitnessPal	United States	Yes†	No	App and users	Yes	15
Yazio	Germany	No	No	App and users	Yes	32
Melarossa	Italy	Yes	Yes‡	Italy§	No	1

FCDB, food composition database.
*No references were available, except for Italy.
†In English.
‡Supervised by the Italian Society of Nutritional Science.
§Italian food composition database (CREA, Rome).

Table 2
Mean energy intake (kcals) per food item and total energy intake per 3-DFD by single app and difference compared with the RM

Measurement tool	N*	Mean (median)	IQR	(5th percentile, 95th percentile)	3-DFD total
FatSecret (A)	60	109 (88)	105	(1.5, 268)	6540
RM (B)	61	112 (71.7)	101	(1, 391)	6832
Difference (A-B)	60	−4.9 (0)	11.9	(−69.4, 37.3)	−294
Lifesum (A)	57	112 (83.4)	105	(0.4, 399)	6384
RM (B)	57	114 (76.5)	111	(0.6, 391)	6498
Difference (A-B)	57	−2.0 (0.3)	13.3	(−51.3, 35.2)	−114
MyFitnessPal (A)	61	108 (83.8)	93.4	(0, 317)	6588
RM (B)	61	112 (71.7)	101	(1, 391)	6832
Difference (A-B)	61	−4.4 (−1.5)†	10.8	(−40.2, 32.3)	−268
Yazio (A)	61	107 (76.8)	101	(2, 295)	6527
RM (B)	61	112 (71.7)	101	(1, 391)	6832
Difference (A-B)	61	−5.4 (0.7)‡	12.7	(−121, 37.8)	−329

IQR, interquartile range; RM, reference method; 3-DFD, 3-d food diary.
*Number of items matched between single app and RM.
†Statistical significance corresponding to $P = 0.03$, all other differences are not significant.
‡Lowest non-significant $P = 0.61$.

Lifesum to a maximum of seven for MyFitnessPal and Yazio (Supplementary Table 5). Tiramisu was the common outlier for all apps.

Discussion

This study indicated that, overall, the most popular nutrition apps available on the market tended to underestimate energy intake compared with the RM. Lifesum underestimated energy intake less than the other apps. Nevertheless, the evaluation of nutrient intake seems to be more challenging and not following a specific scheme. In general, all the apps tend to underestimate lipids and to a less extent carbohydrate and fiber intake, whereas proteins were overestimated by Lifesum and Yazio.

The under- and overestimation over 3 d seems small, but if applied long-term and on a greater number of items and days, it may increase the inaccuracy of energy and nutrient intake. Yazio and FatSecret could underestimate energy intake by −768 and −686 kcal over 7 d, respectively, comparable to energy restrictions proposed to produce a weight loss in overweight/obesity management (deficit of 300−500 kcal/d will produce a weight loss of 300−500 g/wk) [19].

In some conditions (i.e., diabetes, hypertension, and dyslipidemia) app use can affect nutritional status if it is not adequately monitored by a specialist. Recent studies document the potential for these apps to trigger, maintain, or exacerbate eating disorder symptomatology [8,20].

Simpson and Mazzeo [7] explored associations between the use of calorie-counting and fitness-tracking devices in students. Individuals who reported use of calorie trackers manifested higher levels of eating concern and dietary restraint, controlling for body mass index. The author suggested encouraging individuals presenting with eating disorder-related impairment to use tracking technology in accordance with professional recommendations because it might be advantageous to target their use in treatment [7].

The cause of under- or overestimation may be due to various reasons. Crowd-sourced food options in some apps can be overwhelming and can compromise the accurate selection of the correct food item, as reported by Chen et al. [21]. The use of food

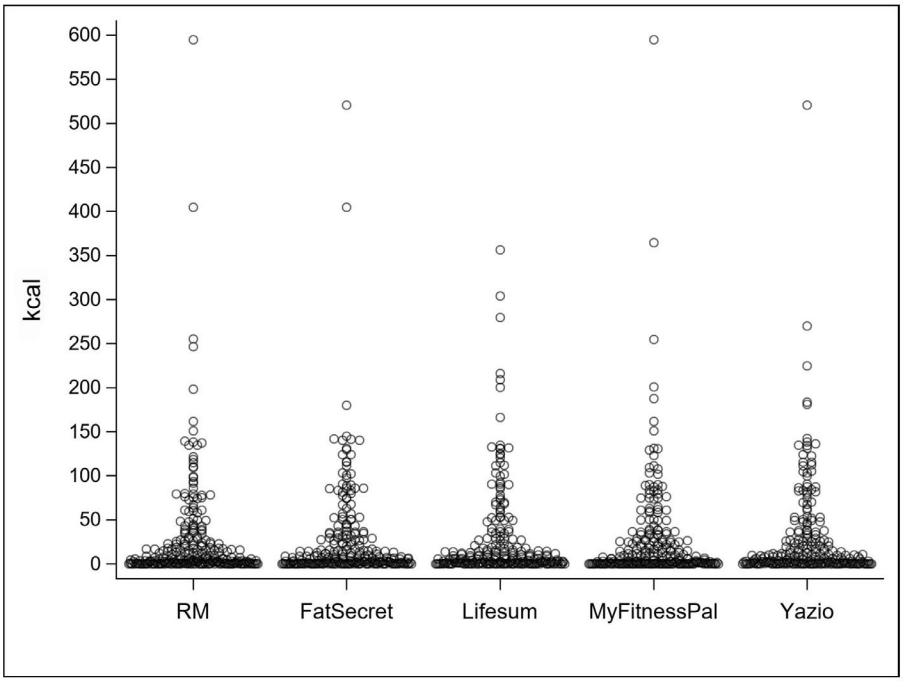

Fig. 2. Distribution of 3-d food diary energy intake by single app and the reference method (Microdiet) (kcal/item). RM, reference method.

Table 3
Difference in the mean energy intake (kcals) per single nutrient and per 3-DFD by app compared withRM

Nutrient	App	N*	Mean (median)	IQR	(5th percentile, 95th percentile)	3-DFD total
Carbohydrates						
	FatSecret	60	−1.9 (1)	7	(−47.3, 33.2)	−114
	Lifesum	57	−1.7 (0.2)	6.6	(−36.6, 36.3)	−96.9
	MyFitnessPal	61	−0.5 (0)	4.8	(−24.4, 31.5)	−30.5
	Yazio	61	−2.2 (0.4)	5.9	(−60.8, 30.2)	−134
Fiber						
	FatSecret	59	−0.3 (0)	0.6	(−10.8, 5.1)	−17.7
	Lifesum	57	−0.5 (0)	0.2	(−8.8, 3.9)	−28.5
	MyFitnessPal	57	−0.1 (0)	0.8	(−5.1, 3.2)	−5.7
	Yazio	46	0.1 (00)	0.5	(−2.5,3.7)	4.6
Lipids						
	FatSecret	60	−3 (0)	5.7	(−24.9, 18.4)	−180
	Lifesum	57	−1.8 (0)	2	(−28.4, 24.5)	−103
	MyFitnessPal	61	−2.9 (−0.3)†	2.2	(−16.2, 2.2)	−177
	Yazio	61	−3.9 (0)	4.1	(−24.3, 10.8)	−238
Proteins						
	FatSecret	60	0.2 (0)	1.4	(−9.8, 9.5)	12
	Lifesum	57	2 (0)	1.1	(−4, 24.5)	114
	MyFitnessPal	61	−0.6 (−0.1)‡	1	(−6.1, 5.4)	−36.6
	Yazio	61	1.5 (0)	1.1	(−4.8, 26.5)	91.5

IQR, interquartile range; RM, reference method; 3-DFD, 3-d food diary.
*Number of items matched between single app and RM.
†Statistical significance corresponding to $P = 0.001$, all other differences are not significant.
‡Lowest non-significant $P = 0.09$.

composition databases from different countries as reported by Lifesum (i.e., United States, Australia, United Kingdom, Sweden, Germany) may be particularly prone to error for nutrient intake estimation [22]. Foods are inherently variable in composition and limited comparability among countries is common. Regional differences arise, especially from the use of local varieties, different soil quality, or meteorologic aspects, and this variability is further increased in local foods, traditional recipes, manufacturer's formulations, and fortified foods defined by national laws [14,18].

An example is presented by outliers identified in the present analysis. Tiramisu is a typical Italian dessert whose nutritional composition included in the BDA is calculated from recipe (prepared with mascarpone cheese, Savoyard biscuits, eggs, sugar, and coffee). This food is energy-dense compared with items included in the other databases (probably manufacturer's formulations). This can explain differences found in other traditional Italian items such as bread prepared with olives, pizza, and tortellini, but it does not clarify differences found in other items (e.g., extra virgin olive oil, eggs, kiwi, and bananas). In fact, another cause of under- or overestimation may be due to the possibility that users might upload their own composition data as allowed in four of the five apps evaluated. The modifiability of food composition databases, although it offers the benefit of customization of foods consumed, presents shortcomings in the accuracy of nutrients estimation. When users are allowed to enter nutrient labels, they may insert errors (i.e., typos) leading to losses of the entire food database quality, as in the case of extra virgin olive oil (5 g = 20 kcal reported by Yazio compared with 45 kcal reported by RM). These issues may affect accuracy in tracking energy and nutrient intake, inserting additional sources of variability, therefore, a rigorous check of data uploaded by an expert is foreseen (i.e., dietitian/nutritionist).

Similar published studies have evaluated the comparability of nutrient intake estimates from nutrition-tracking apps to traditional food records. Ferrara et al. [23], as part of their testing, reported results mostly consistent with the present findings. Comparing five apps to the U.S. Department of Agriculture reference for a 3-d diet, they found that on average differences were 1.4% for calories, 1% for carbohydrates, 10.4% for proteins, and −6.5% for fats. If tracking of calories and carbohydrates closely matched estimates from the U.S. Department of Agriculture database, proteins and fats showed large deviations. Ferrara et al. suspected that the underestimation of fats was due to difficulties in estimating oils used in cooking, and in general inconsistencies due to food reformulations and the use of European database reported by some apps.

In 2018, Griffiths et al. [24] compared intake calculations from 30 24-h dietary recalls entered by a researcher into five nutrition apps, with nutrient intake estimated using Nutrition Data System for Research software. They found a pattern of underestimation of nutrient intake by the apps for most of the nutrients examined. The comparative validity of energy and macronutrients ranged from $r = 0.73$ to 0.96. For each app, one or more mean nutrient intake calculation was significantly lower than those from RM, with underestimation ranging from 7% to 41%. In Australia, Chen et al. [25] evaluated 23 of the most popular smartphone apps for weight loss. They found a mean absolute difference in energy intake estimates between the apps and the RM was of 127 kJ/d (corresponding to 30 kcal), but the differences ranged from 1001 to −700 kJ/d, indicating a great variability among apps.

Two recent studies compared recorded dietary intakes on MyFitnessPal to traditional methods [21,26] in two samples of populations. The first study recruited adults to use MyFitnessPal for 4 d consecutively and completed two 24-h recalls collected by phone. They found that daily energy intake was significantly underestimated by 1863 kJ (standard deviation = 2952 kJ; $P = 0.0002$) corresponding to 445 kcal [20]. They reported also that individuals tend to omit a mean of 18% (standard deviation = 15) of food items, particularly energy-dense and nutrient-poor foods from MyFitnessPal records. Moreover, Teixeira et al. [26] recruited students to compare MyFitnessPal with paper-based food records in Brazil. They found that although many nutrients were underestimated compared with paper records, there was moderate correlation between both methods. The Bland–Altman plots showed tendency to underestimate and relatively narrow limits of agreement. Carbohydrates and lipids showed trends of increasing the degree of overestimation with increased intake, even after data normalization.

In the present study, we found suboptimal information concerning scientific contents and accuracy of information linked to

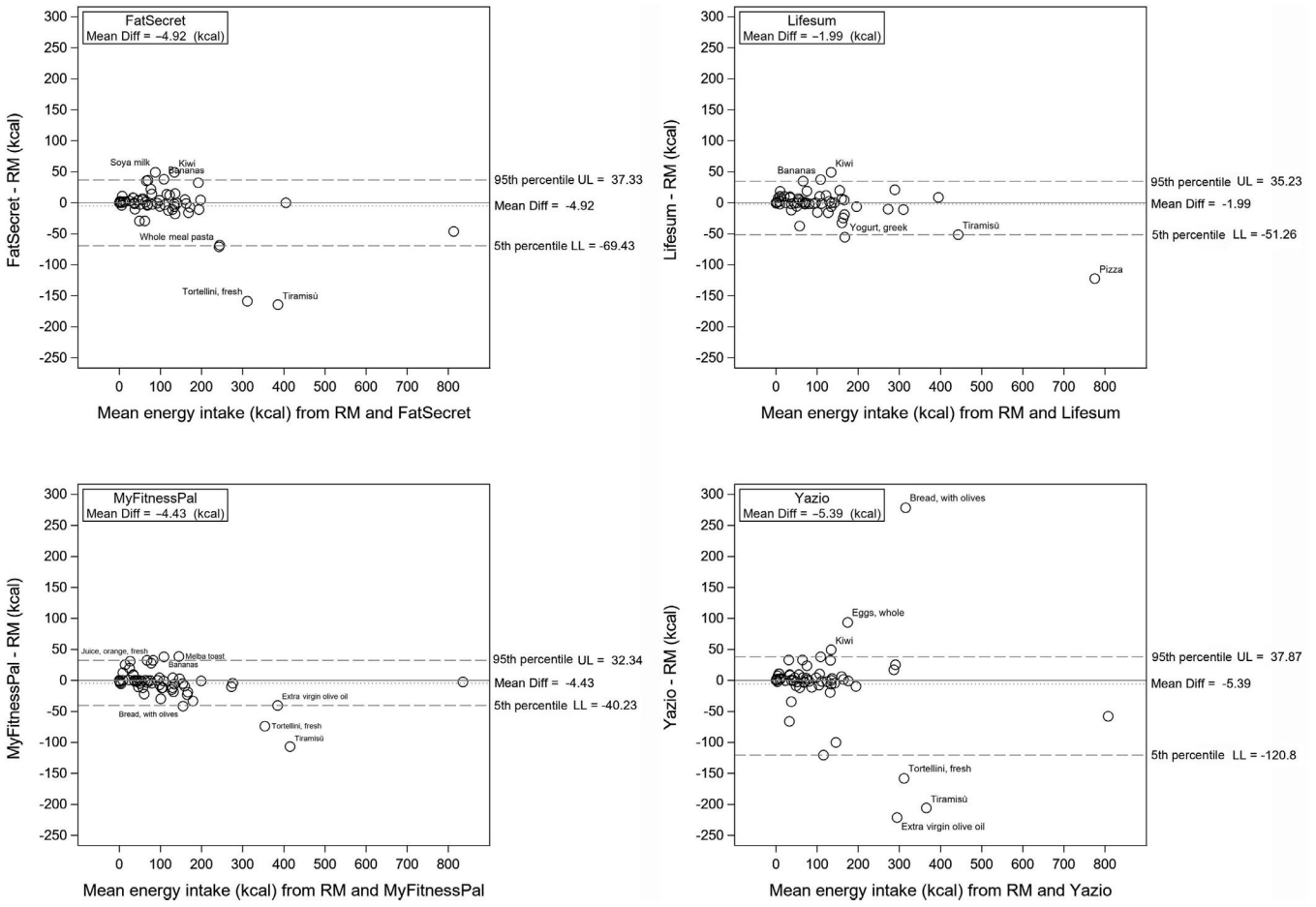

Fig. 3. Bland–Altman plots of the mean differences in energy intake (kcal) between the apps and the RM (Microdiet). RM, reference method.

recommendations from national guidelines (i.e., body mass index, estimated energy requirements), expressing alarms over general quality of these apps as reported by other authors [25,27] particularly for users with limited health literacy.

Nutritional research examining food consumption has always been limited due to a number of methodological challenges associated with dietary assessment [28]. This field has recently looked to technology to assist in advancing current methods. Electronic devices, such as apps, have the potential to overcome many of the limitations associated with traditional methods, reducing participant burden, and improving compliance associated with the more detailed measures of food intake [6]. Data collection and data quality could also be improved by reducing measurement errors and bias, giving the opportunity to prospectively and repeated collection of food consumption. They may also reduce researcher burden by decreasing costs and resources associated with data collection, coding, and reporting [29]. However, the inherent individual bias related to self-reported dietary intake will not be solved and more research is therefore crucial.

One of the main limitations of the present study was the inability to evaluate the large amount of nutrition apps available on the market, and for this reason some popular apps were missed. The energy and nutrient intake collected among all apps may have been influenced by the researcher dedicated to the study, trained in nutrition (i.e., a dietitian). Adults with a limited knowledge in nutrition would have found it more difficult to match foods or to choose serving size to enter. Moreover, in our experience, the popularity of apps seems to rely more on usability and features than on quality and scientific contents, and this can be monitored only with the supervision of health professionals who can ensure the correct use. Finally, to assess the accuracy of identified apps in terms of energy and nutrient estimation, a 3-DFD was identified. It contained mainly fresh food, packaged food, recipes, and drinks according, to a Mediterranean dietary pattern, not representing a standard diet.

One of the strengths of the present study was the thorough examination of the nutrient and energy intake of the apps evaluated, in addition to other characteristics assessed (scientific coverage, feature availability, and usability). This evaluation could train health professionals to analyze the specific features, as well as the strengths and weaknesses of the most popular, available apps, in order to give the correct recommendation or information to their patients.

Finally, this study offered insights into the limitations of the apps available and highlighted the need for a well-designed nutrition app for self-monitoring and dietary assessment. Many of the results of the analysis should be considered in the future to design a more suitable app to assess dietary intake in free-living adults. We outline recommendations accordingly reported in Table 4.

Conclusions

Leading nutrition apps present critical issues in assessing energy and nutrient intake and in providing adequate scientific

Table 4
Summary of recommendations to develop a high-quality app

Usability	Easy to use with smart menu. Graphic art for daily intake summary. Use of non-tiring eye colors. Possibility of rewarding frequent users with special lectures or educational material.
Scientific coverage of contents	App developed using scientific and sound contents, and obtaining scientific supervision by expert or by a scientific association. Provide links of certified nutrition website. List references of all contents available on the app. Update and choice of country-specific documents.
Food diary	Include a clear explanation on how to upload food consumed. Report some tips or indication for an easier choice of the correct item and to upload common portion size. Include the possibility of data export in Excel or pdf format.
Food composition data	Clear origin of food composition data included. Use only country-specific data. Should not allow user to add food composition data, unless a rigorous check of data uploaded by an expert (i.e., dietitian/nutritionist) to avoid typos or inconsistences or clear distinction between user's data and app data. Remove duplicate food items.
Technology-enhanced features	Optical barcode reader to load the data automatically from the nutritional label. Include data export, in Excel or pdf format. Provide short educational videos about specific topics.
Requirements	Ask user's age and permit the access only to adults. Keep record of diseases. Use national nutrient- recommended intake levels for the population.
Assistance	Feedback in national languages. Weekly answers from a nutritionist.

contents and accuracy of information. This evaluation offers clinicians and consumers an informed view of the apps available on the market. Implementation of a framework for quality assessment is necessary to drive the design and development of higher-quality apps. Further research on the efficacy and use of apps to monitor food intake is also warranted.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2020.111018.

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ORIGINAL ARTICLE

Effects of 3-month high-intensity interval training vs. moderate endurance training and 4-month follow-up on fat metabolism, cardiorespiratory function and mitochondrial respiration in obese adults

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Abstract

Purpose The purpose of this study was to investigate, in obese adults, changes in body composition, physical capacities, fat oxidation and ex vivo mitochondrial respiration induced by a 3-month either moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT); afterwards, the patients were followed for four months.

Methods Thirty-two patients (mean age 39 years; mean body mass index [BMI] 36 kg•m^{-2}) participated in this study attending ~ 34 sessions of training. At baseline (PRE), at the end of the program (POST) and after follow-up, body composition, peak O_2 uptake ($V'O_2$peak) and fat oxidation rate were measured. *Vastus lateralis* biopsies for the evaluation of mitochondrial respiration were performed only at PRE and POST.

Results At POST, body mass (BM) and fat mass (FM) decreased (− 6 and − 14%, respectively, $P < 0.05$) in MICT and HIIT; $V'O_2$peak increased in both groups (+ 6 and + 16%, respectively, $P < 0.05$). Maximal fat oxidation rate increased only after HIIT ($P < 0.001$). Maximal ADP-stimulated mitochondrial respiration normalized by citrate synthase increased ($P < 0.05$) by 67% and 36% in MICT and HIIT, respectively, without significant difference. After follow-up, BM and FM were still lower (− 4 and − 20%, respectively, $P < 0.050$) compared with baseline in both groups. Only after HIIT, $V'O_2$peak (+ 8%) and maximal fat oxidation rate were still higher ($P < 0.05$).

Conclusions HIIT was more effective in improving and maintaining $V'O_2$peak and fat oxidation. These results may be relevant for an appropriate prescription of training programs designed to optimize aerobic fitness in obese subjects.

Keywords Obesity · HIIT · Lipid oxidation · Mitochondrial respiration · Aerobic function

Abbreviations

a-$\bar{v}O_2$ diff	Arteriovenous oxygen difference
BM	Body mass
BMI	Body mass index
CO	Cardiac output
CI	Oxidative phosphorylation complex I
CII	Oxidative phosphorylation complex II
DAP	Diastolic arterial pressure
ETS	Mitochondrial electron transport system
FAT	Lipid oxidation rate during the incremental test
FM	Fat mass
FFM	Fat-free mass
HIIT	High-intensity interval training
HOMA-IR	Homeostasis Model Assessment

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HR	Heart rate
IPAQ-SF	International Physical Activity Questionnaire Short Form
MICT	Moderate-intensity continuous training
SAP	Systolic arterial pressure
SF12	Short-Form 12, questionnaire about health-related quality of life
SF12_MI	Short-Form 12, mental index
SF12_PI	Short-Form 12, physical index
SVC	Systemic vascular conductance
$V'CO_2$	Pulmonary CO_2 output
$V'O_2$	Pulmonary O_2 uptake

Introduction

Obesity is a common health problem that is widely spread worldwide and is a major risk factor for type 2 diabetes mellitus, cardiovascular and respiratory diseases, musculoskeletal disorders and some types of cancer (Williams et al. 2015). It is generally accepted that the primary cause of obesity is energy imbalance, i.e. the amount of calories which are introduced is greater than the amount of calories which are utilized. In this context, molecular processes and pathways that directly regulate energy metabolism or caloric intake appear to be feasible targets for therapy. Compared to lean subjects, obese people have an impaired capacity to oxidize lipids (Lanzi et al. 2014), associated with low insulin sensitivity (Kelley and Simoneau 1994) and ease in gaining weight (Zurlo et al. 1990). Further, obesity is a risk factor for cardiovascular and respiratory diseases, musculoskeletal disorders and some types of cancer (Thrush et al. 2013; Devarshi et al. 2017; de Mello et al. 2018).

Optimizing fat oxidation capacity is an important objective both for performance (Hetlelid et al. 2015) and health (Achten and Jeukendrup 2004). Whole-body fat oxidation does not seem to be related with intrinsic mitochondrial oxidative capacity (Nordby et al. 2006; Dandanell et al. 2018), but rather to the amount of lean body mass (Nordby et al. 2006), mitochondrial content (Dandanell et al. 2018) and maximal oxygen uptake ($V'O_2max$) (Nordby et al. 2006; Hetlelid et al. 2015; Dandanell et al. 2018).

Numerous studies have demonstrated that skeletal muscle metabolism and mitochondrial content/function are impaired in obesity, but there is no general agreement about the issue (Ara et al. 2011; Nair et al. 2011; Fisher-Wellman et al. 2014). Mitochondrial content (Ritov et al. 2005; Larsen et al. 2012), the levels of mitochondrial proteins (Wijngaarden et al. 2013) and their (predominantly nuclear) genes (Mootha et al. 2003; Patti et al. 2003; Ritov et al. 2009; Hwang et al. 2010) have been shown to be reduced in the skeletal muscle of obese individuals, as well as in type 2 diabetes individuals, compared to lean controls. Mitochondrial dysfunction and impaired enzymatic activity of oxidative phosphorylation complexes have been confirmed more recently in obese skeletal muscle (Formentini et al. 2017; Devarshi et al. 2017; de Mello et al. 2018).

Compared to other methods, high-resolution respirometry in permeabilized muscle fibres, the approach which was utilized in the present study allows a more 'physiological' evaluation of mitochondrial function ex vivo. In studies carried out by this method in obese subjects, an impaired muscle mitochondrial metabolism was reported, in terms of mitochondrial respiratory capacity (state 3 respiration) (Vijgen et al. 2013). In obese sedentary adults, moderate-intensity physical activity combined with weight loss was reported to increase the enzymatic activities of the electron transport chain, which occurred without a significant increase in mitochondrial DNA, and was ascribed to an increase in mitochondrial cristae (Menshikova et al. 2007). However, mitochondrial adaptations resulting from different training modalities have been slightly investigated in obese subjects.

Thus, the aim of the present study was to determine the effects of 3 months of either high-intensity interval training (HIIT) or moderate-intensity continuous training (MICT) on body composition, $V'O_2peak$, fat oxidation, and cardiovascular and mitochondrial functions in healthy obese volunteers.

Greater responses were hypothesized for all variables following HIIT vs. MICT. An evaluation was also performed 4 months after the termination of the interventions, in order to evaluate the maintenance of the observed changes, considering that the weight maintenance after its reduction is a major issue (Donnelly et al. 2009). During the experimental period and follow-up, identical nutritional advices have been provided for both groups in order to avoid confounding nutritional variables on the outcomes.

Subjects and methods

Subjects

Thirty-two obese volunteers (17 males and 15 females) were recruited from the Exercise Physiology Laboratory of the University of Udine, where they underwent a medical and dietetic evaluation. The inclusion criteria were age between 18 and 50 years and body mass index (BMI) ≥ 30 kg m^{-2}. Subjects who had previously participated in weight management programs had cardiovascular, respiratory, neurologic, muscular-skeletal, metabolic and/or endocrine diseases or those who were taking any drugs known to influence energy metabolism and cardiorespiratory adjustments to exercise were excluded. No subject was taking beta-blockers. The Ethics Committee of the Friuli-Venezia-Giulia Region approved this study (protocol number 1764). Before the study began, the purpose and objectives had been carefully

explained to each subject and written informed consent had been obtained. A physical activity questionnaire was administered to exclude potential volunteers who engaged in any continuous activity longer than 20 min more than once a week, indicative of a moderate physical activity level (IPAQ-SF) (Craig et al. 2003).

Study protocol

After the first inclusion visit, subjects were admitted to 3 months of multidisciplinary weight management program, including lifestyle education, physical activity and dietary follow-up.

Control tests, including assessment of body composition, physical capacities, fat oxidation rate, physical activities and dietary habits, were performed during two weeks before the beginning and immediately after completing the weight management program. At the same time, skeletal muscle biopsies of the *vastus lateralis* muscle were taken for measurement of ex vivo mitochondrial respiration by high-resolution respirometry. In addition, anthropometric indices and physical performance were monitored monthly during the program, in order to adjust food allowances and physical activities individually. Four months after the end of the weight management program, control tests, including assessment of body composition, physical capacities, fat oxidation rate, physical activities and dietary habits, were performed.

Physical activity

During the 3-month weight management period, subjects followed a physical training program, including three training sessions per week under supervision. The subjects were split randomly in two groups, one group following a moderate-intensity continuous training (MICT, $n=16$) and the second group following high-intensity interval training (HIIT, $n=16$).

All subjects completed 34 ± 0.14 sessions of physical training. The intensity of MICT on the treadmill was set at a heart rate (HR) corresponding to 60% of the initial V'O_2peak, the duration of the training session was 44 ± 8 min. HIIT consisted of 10 min of warm up (50% of V′O_2peak) followed by 3–7 repetitions of 3 min bouts of high-intensity walking (100% of V′O_2peak), interspersed by 1.5 min walking at low intensity (50% of V′O_2peak) and followed by 5 min of cool down (50% of V'O_2peak); the duration of the training session was 33 ± 4 min. Exercise intensity was set up by adjusting the slope of the treadmill and verifying that the HR values corresponded to the values of 50 or 100% of V′O_2peak. When subjects improved their performance capacity and their HR tended to decrease, the slope was increased to ensure that HR reached values previously selected. The amounts of energy expended during the training sessions were similar for both groups: 20 kJ per kg of fat-free mass (FFM), which corresponds to about 1.5 MJ per session.

Research assistants and physical trainers were responsible for verifying that each subject, participated to each training session, performed the exercises correctly and completed at least 90% of the exercise sessions. At the end of each month, aerobic tests were performed to assess physical capacities and to adjust physical training intensity individually. All subjects were also advised to practice leisure physical activities during the weekend and holidays.

During the 4-month follow-up, the same training suggestions were given to all subjects. The suggestions consisted of three training session per week covering the full intensities range: one high intensity (90% HRpeak and less than 30 min), one medium intensity (~ 70–80% HRpeak and 30–50 min) and one low intensity (< 70% HRpeak and more than 60 min). Although the subjects were invited to use a heart rate monitor, training during the follow-up period was not supervised and compliance was checked by a questionnaire (Craig et al. 2003).

Diet and nutritional education

During the intervention period, the patients followed personalized diets formulated according to the Italian recommended dietary allowances (SIO-ADI 2016). Energy supply was about 1.3 times the initial basal metabolic rate (BMR) estimated using the Harris Benedict equation (Harris and Benedict 1918), as suggested by the Italian guidelines for obesity treatment (SIO-ADI 2016). Carbohydrates, lipids and protein supplied were 56, 27 and 17% of energy intake, respectively. Six weeks after the beginning of the weight management program, food allowances were further reduced. The reduction ranged between 2610 and 3766 kJ as suggested by SIO-ADI (SIO-ADI 2016), based on their prior diet and personal feedbacks. During the weight management period, subjects had dietetics lessons, including choice of foods, and they were instructed to maintain their food habits after the end of the weight management period.

Measurements

Anthropometric characteristics and body composition

The medical history and a physical examination of subjects were taken at the time of admission to the weight management program. Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm

on a standardized wall-mounted height board. BMI was calculated as BM (kg) × stature^{-2} (m). Body composition was measured by bioelectrical impedance (BIA, Human IM Plus; DS Dietosystem, Milan, Italy) according to the method of Lukaski et al. (Lukaski et al. 1986). Fat mass (FM) and fat-free mass (FFM) were calculated with equations derived in obese people either of different ages or BMI (fat-specific formulae) by utilizing a two-compartment model (Gray et al. 1989).

Dietary and physical activity habits

Dietary data were collected using a 4-day dietary (4-DD) record (food diary) given to the subjects in 3 different occasions in order to analyse their eating behaviour before the beginning of the intervention, at the end of the intervention and after the follow-up period. The diaries were given together with instructions on how to record type, quantity and mode of consumption of foods over a 24-h period on four separate days, including one during the weekend. Data extracted from food diaries were analysed using the Microdiet software (V2.8.6, Downlee Systems Ltd., High, Peak, UK) containing the Italian food composition database for epidemiological studies (Gnagnarella et al. 2015), integrated with information from nutritional labels when data were missing, data and the brand were specified in the diary.

To assess physical activity levels, we used the validated International Physical Activity Questionnaire Short Form (IPAQ-SF) (Craig et al. 2003). The questionnaire records vigorous intensity, moderate intensity, walking activities and the sitting time spent during the previous 7 days. The IPAQ-SF scores were converted into Metabolic Equivalent minutes per week (MET-min week^{-1}) using the 'Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)'(International Physical Activity Questionnaire 2005).

Further, all the participants completed the Short-Form 12 (SF12) questionnaire to investigate the health-related quality of life. The questionnaire is composed by 12 items from which physical (SF12_PI) and mental health (SF12_MI) indices are obtained (Ware et al. 1996).

Physical capacities and maximal fat oxidation rate

Peak oxygen uptake ($V'O_2$peak) and maximal fat oxidation rate were determined by a graded exercise test on a motorized treadmill (H/P/Cosmos Sports and Medical Gmbh, Germany), under medical supervision. Subjects were asked to avoid strenuous exercise the day before the test and came to the laboratory after a 12-h fasting. Before the beginning of the study, subjects were familiarized with the equipment and the procedures. Each test was undertaken at the same time of the day in the different periods of the study and comprised a 5-min rest period followed by walking in stages of 4-min duration until voluntary exhaustion. The rates in m s^{-1} and incline in % followed a sequence: 1.11 (0%), 1.11 (3%), 1.39 (3%), 1.39 (6%), 1.53 (6%), 1.53 (9%), 1.53 (12%), 1.53 (13%), 1.53 (15%), 1.53 (18%), 1.53 (21%) and 1.53 (24%) (Lazzer et al. 2017). During the experiment, ventilatory and gas exchange responses were measured continuously by indirect calorimetry (CPET, Cosmed, Italy). The flowmeter and gas analysers of the system were calibrated using, respectively, a 3-L calibration syringe and calibration gas (16.00% O_2; 4.00% CO_2). During the exercise test, an electrocardiogram was recorded continuously and displayed online for visual monitoring, and heart rate (HR) was measured with a dedicated monitor device (Garmin, US). $V'O_2$peak was estimated for each subject considering the last 20 s of the graded exercise tests.

The substrate oxidation rate was calculated from $V'O_2$ and $V'CO_2$ values determined during the last minute of each workload level, according to the protocol of Achten (Achten et al. 2002) and using the following equations (Frayn 1983):

$$\text{Fat oxidation rate } (\text{g min}^{-1}) = 1.67 \cdot V'O_2 \text{l} \cdot \text{min}^{-1} - 1.67 \cdot V'CO_2 \text{l} \cdot \text{min}^{-1} - 0.307 \cdot P_{oxi},$$

$$\text{Carbohydrate oxidation rate } \left(\text{g min}^{-1}\right) = 4.55 \cdot V'CO_2 \text{l} \cdot \text{min}^{-1} - 3.21 V'O_2 \text{l} \cdot \text{min}^{-1} - 0.459 \cdot P_{oxi}.$$

where P_{oxi} is the protein oxidation rate. P_{oxi} was estimated by assuming that protein oxidation contributed approximately 12% of resting energy expenditure (Frayn 1983):

$$\text{Protein oxidation rate } \left(\text{g min}^{-1}\right) = \left[\text{energy expenditure } \left(\text{kJ min}^{-1}\right) \cdot 0.12\right] \cdot 16.74^{-1} \left(\text{kJ g}^{-1}\right).$$

For each subject, the results of the graded exercise test were used to compute the relationship between fat oxidation rate as a function of exercise intensity, expressed as $\%V'O_2$peak. The best fit was obtained with a polynomial relationship of the second order. The graded exercise test on the motorized treadmill was performed in the same conditions (speed and incline) in the three different periods.

Cardiovascular function

During the graded exercise test on the motorized treadmill, at rest and during the first step at 1.11 m s^{-1} and 0% slope, stroke volume (SV) and cardiac output (CO) were monitored continuously by bioimpedance method (PhysioFlow, Manatec, France), following the procedure described by Charloux et al. (Charloux et al. 2000). This method has been validated during maximal incremental exercises (Richard et al. 2001) and has been utilized also in overweight and obese subjects (Vella et al. 2011). PhysioFlow and

metabolimeter values were synchronized and mean values were calculated every 10 s. Data obtained during the last 20 s of rest and of the first step were taken in account for further analysis. Arteriovenous oxygen difference (a-$\bar{v}$ O_2 diff.) was estimated by the following equation (Bloos and Reinhart 2009):

$$a - \bar{v}O_2\text{diff.}\left(\text{ml}O_2 \cdot 100\ \text{ml}^{-1}\right) = V'O_2\left(\text{ml} \cdot \text{min}^{-1}\right) \cdot CO^{-1}\left(\text{ml} \cdot \text{min}^{-1}\right) \cdot 100.$$

Systolic (SAP) and diastolic (DAP) arterial blood pressures were measured twice using an inflatable cuff and mean values were calculated the last minute of rest and of the first step. Mean arterial pressure (MAP) was calculated as [2•diastolic blood pressure + systolic blood pressure]•3^{-1}. Measures of arterial pressure were synchronized with the CO signal to calculate systemic vascular conductance (SVC), as the ratio between CO and MAP.

Biopsies and mitochondrial respiration ex vivo

Biopsies were obtained from the *vastus lateralis* muscle by percutaneous excision after an overnight fasting. A microneedle (Tru-cut Histocore, 12 G, Biomed Instrument and product GmbH, Germany) was used to collect the specimens; after anaesthesia of the skin using lidocaine (2%), a small incision was made to penetrate skin and fascia. Specimens were put in preserving BIOPS solution (BIOPS: 10 mM EGTA calcium buffer [free Ca^{2+} concentration 100 nmol l^{-1}], 20 mM imidazole, 20 mM taurine, 50 mM K^+/4 morpholinoethanesulfonic acid, 0.5 mM dithiothreitol, 6.56 mM $MgCl_2$, 5.77 mM ATP and 15 mM phosphocreatine, pH 7.1), containing 10% (wt vol^{-1}) fatty acid-free BSA and 30% (vol vol^{-1}) DMSO at 4 °C, then immediately frozen in liquid nitrogen and stored at − 80 °C until the analysis (Salvadego et al. 2016).

To measure mitochondrial respiration ex vivo, fibre bundles in ice-cold BIOPS solution were quickly cleaned from the connective and fatty tissues, and separated under magnification (MC170 HD, Leica Microsystems, Switzerland, LTD) with a sharp-ended needle, leaving only small areas of contact, and then incubated with 20 µg ml^{-1} saponin for 30 min with a continuous gentle stirring to ensure complete permeabilization. After being rinsed twice in respiration medium MiR05 (0.5 mM EGTA, 60 mM potassium lactobionate, 3 mM $MgCl_2$, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose and 1 g l^{-1} BSA, pH 7.1), permeabilized fibre bundles were measured for wet weight and immediately transferred into the respirometer chambers for O_2 consumption measurements. Measurements were performed by high-resolution respirometry (Oxygraph-2 k; Oroboros Instruments, Innsbruck, Austria) at 37 °C (Pesta and Gnaiger 2012) in duplicate using two specimens for each subject (2–4 mg tissue wet weight in each 2 ml glass chamber). The oxygen levels in the chambers were maintained above air saturation in the range of 300–450 µM (average O_2 partial pressure 250 mmHg) to prevent oxygen limitation of respiration. To this aim, intermittent re-oxygenation steps were performed by adding 0.3 mM hydrogen peroxide (MiR05 was added with 280 U ml^{-1} catalase before the measurements). Measurements were run in the presence of 25 µM blebbistatin to prevent ADP-induced contraction (rigor), particularly evident in small length fibres, such as those obtained from biopsy by microneedles (Hughes et al. 2015). A sequential, multiple substrate-uncoupler-inhibitor titration (SUIT) protocol was applied (Pesta and Gnaiger 2012) to measure different respiratory states and substrate/coupling control ratios. Cytochrome C (10 µM) was added to test for mitochondrial outer membrane integrity during the measurements, and only samples demonstrating a negligible damage (i.e. < 10% increase in respiration after cytochrome C addition) were taken in consideration (i.e. MICT PRE $n = 6$ and MICT POST $n = 8$; HIIT PRE $n = 6$ and HIIT POST $n = 7$). The main mean characteristics of these subgroups were not significantly different from whole group of subjects.

Data were digitally recorded and analysed using DatLab4 software (Oroboros Instruments). Chemicals and reagents were from Sigma (St. Louis, MO, USA).

Citrate synthase activity (CS)

CS activity was assayed spectrophotometrically (Srere 1969) by an EnSpire 2300 Multilabel Reader (PerkinElmer). After completion of the respirometer measurements, muscle fibres were recovered and processed as in Spinazzi et al. (2012). In MICT and HIIT groups, 13 and 14 subjects were considered, respectively. Briefly, muscle fibre was suspended in1: 20 wt vol^{-1} in a homogenization buffer containing 250 mM sucrose, 20 mM Tris, 40 mM KCl and 2 mM EGTA with 1:50 vol vol^{-1} protease inhibitors (Sigma-Aldrich), and submitted to a motor driven homogenization in a pre-cooled 1 ml glass–glass potter (Wheaton, USA). The homogenate was centrifuged at 600×*g* for 10 min and the clarified homogenate was assayed for protein concentration (Lowry et al. 1951). For CS assay, 10–20 µg of protein was added to each well of a 96-well microplate along with 100 µl of 200 mM Tris-Triton X-100 (0.2% vol vol^{-1}), 20 µl of 1 mM 5,5′-dithiobis-2-nitrobenzoate freshly prepared, 6 µl of 10 mM acetyl-coenzyme A and mQ water to a final volume of 190 µl. Finally, 10 µl was added to 10 mM oxaloacetic acid that started the reaction. All assays were performed at

25 °C in triplicate. Activity is expressed as mU (nmole/min) per mg of protein.

Statistical analyses

Statistical analyses were performed using SPSS 20.0 software (IBM, Chicago, USA), with significance set at $p < 0.05$. All results are expressed as means and standard error (SE). Normal distribution of the data was tested using the Shapiro–Wilk test. Sphericity was verified by Mauchly's test. When the assumption of sphericity was not met, the significance of the F ratios was adjusted according to the Greenhouse–Geisser procedure.

The differences on the training adherence between the two groups were analysed by student's test for unpaired data. Anthropometric characteristics, body composition, $V'O_2$peak cardiovascular parameters, data derived from questionnaire and food diary, glycolipid metabolism, and CS and mitochondrial function parameters were analysed with a generalized linear mixed, multilevel, growth model, fit by maximal likelihood, which takes into account the subjects as random effects and group (MICT vs HIIT), and gender, time, time^2 and interaction group × time as fixed effects. For CS and mitochondrial function parameters, it was not necessary add 'time^2' since there has been only two time points. The same analyses were applied to the fat oxidation during the exercise, adding the % of $V'O_2$peak as fixed factor. As a logistic regression, the overall fit of the model was tested using a Chi-square likelihood test. The smaller its deviance [minus twice the log-likelihood (– 2LL)], the better is the prediction. The model was implemented by adding the random and fixed effects one by one, until the smallest – 2LL. If, by adding an effect, the -2LL did not become significantly smaller, the effect was not considered. In case of the interaction and the group effects were not significant, the model considered the two groups as one group (Twisk 2006; Woltman et al. 2012).

If a statistical difference was detected in any of the fixed effects, the multilevel models were performed with partial pooling (Gelman et al. 2012). For instance, to break down the interaction group × time, the analysis was rerun three times to exclude every time one of the three time points; in this way, it was possible to find in which time point the interaction was located. If the gender distribution of the subjects was balanced in the two groups and no gender differences and no interaction between groups were found in the parameters studied, then male and female subjects were considered together. Finally effect size (ES) has been calculated as proposed by Feingold (2009). ES < 0.20 was considered small, < 0.50 medium and > 0.50 large as proposed by Cohen (1988).

Results

Adherence to the training program

During the training period, subjects were involved in 34.4 ± 0.2 and 34.8 ± 0.3 training sessions for MICT and HIIT groups, respectively, ($P = 0.811$) without adverse events. The average of energy expended during the training sessions were 23.8 ± 4.1 and 22.4 ± 2.5 kJ kg^{-1} of FFM for MICT and HIIT groups, respectively, ($P = 0.256$). The duration of each session was greater for MICT (44.3 ± 7.6 min) than for HIIT (33.6 ± 3.6 min, $P < 0.001$, ES 1.78).

Anthropometric characteristics and body composition

At PRE, no significant differences were found between MICT and HIIT for age (37.3 ± 0. 6 and 40.1 ± 0.4 years, group effect $P = 0.334$) and stature (1.72 ± 0.11 and 1.71 ± 0.07 m, group effect $P = 0.895$) (Table 1).

At POST, mean weight loss was 5.84 ± 0.15 kg (time effect $P < 0.001$, ES 0.40), BMI decreased by 2.03 ± 0.05 kg m^{-2} (time effect $P < 0.001$, ES 0.46), waist circumference decreased by 4.51 ± 0.17 cm (time effect $P < 0.001$, ES 0.39), hip circumference decreased by 4.75 ± 0.16 cm (time effect $P < 0.001$ ES 0.51), FM decreased by 5.37 ± 0.16 kg (time effect $P < 0.001$, ES 0.56) and FFM did not change significantly in MICT and HIIT groups, without differences between groups (interaction G × T ranging from $0.331 < P < 0.767$).

After 4 months of follow-up (Table 1), body mass increased by 2.15 ± 0.16 kg (time effect $P < 0.015$, ES 0.12) and BMI increased by 0.71 ± 0.05 kg m^{-2} (time effect $P < 0.003$ ES 0.13), in MICT and HIIT without differences between groups (interaction $G \times T$ respectively $P = 0.331$ and $P = 0.587$); the values, however, were still significantly lower than at PRE (time effect $P < 0.001$, ES respectively 0.29 and 0.34). Waist and hip circumference and FM did not change significantly during the follow-up period remaining significantly lower than at PRE (time effect $P < 0.001$, ES 0.76). FFM increased significantly during the follow-up period, by 3.18 ± 0.16 kg (time effect $P < 0.001$ ES 0.26) in MICT and HIIT groups, and were not significantly different than at PRE (Table 1).

Dietary and physical activity habits

Dietary habits

At PRE, no significant differences were found between MICT and HIIT groups in energy intake (9509 ± 763 vs. 7720 ± 522 kJ, group effect $P = 0.061$), and carbohydrates

Table 1 Anthropometric characteristic, physical capacities and physical activity habits before (PRE) and after 3 months (POST) of weight management program, and after 4 months of follow-up in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups

	MICT			HIIT			*P*		
	PRE (*n*:16)	POST (*n*:16)	FOLLOW-UP (*n*:14)	PRE (*n*:16)	POST (*n*:16)	FOLLOW-UP (*n*:12)	Gr	*T*	Gr × *T*
Body mass (Kg)	107.1 ± 4.4	101.2 ± 4.5*	103.8 ± 5.4*†	103.5 ± 2.7	97.8 ± 2.5*	98.0 ± 3.0*†	0.790	0.001	0.331
BMI (kg m^{-2})	36.1 ± 1.3	33.9 ± 1.2*	35.2 ± 1.5*†	35.1 ± 0.9	33.2 ± 1.0*	32.9 ± 0.9*†	0.638	0.001	0.587
Waist (cm)	113.0 ± 3.5	109.4 ± 4.0*	110.5 ± 4.4*	114.1 ± 2.2	108.8 ± 2.1*	107.4 ± 2.2*	0.406	0.001	0.590
Hip (cm)	123.1 ± 2.8	118.0 ± 2.9*	119.9 ± 3.1*	120.5 ± 1.8	116.1 ± 2.2*	113.9 ± 1.5*	0.875	0.001	0.500
FFM (kg)	69.4 ± 3.9	68.6 ± 4.1	71.0 ± 5.0†	65.1 ± 2.9	64.7 ± 2.7	69.3 ± 3.9†	0.386	0.001	0.767
FM (Kg)	37.7 ± 2.7	32.4 ± 2.3*	32.5 ± 3.0*	38.4 ± 2.1	32.9 ± 2.5*	28.8 ± 2.0*	0.970	0.002	0.433
HRpeak (bpm)	180.1 ± 0.9	177.2 ± 1.1*	173.0 ± 1.3*	181.0 ± 0.9	176.0 ± 0.5*	178.2 ± 0.83*	0.141	0.001	0.149
V′O2peak (L min^{-1})	3.02 ± 0.05	3.19 ± 0.05* [I]	2.95 ± 0.06*†	2.88 ± 0.04	3.35 ± 0.05* [I]	3.32 ± 0.06*†	0.288	0.001	0.001
V′O2peak (mL min^{-1} Kg^{-1} FFM)	43.58 ± 0.39	46.80 ± 0.39* [I]	42.02 ± 0.51*†	44.26 ± 0.45	51.51 ± 0.40* [I]	48.04 ± 0.56*†	0.093	0.001	0.001
IPAQ_TOT (MET-min $week^{-1}$)	818 ± 220	1481 ± 292*	1965 ± 552*	766 ± 253	1257 ± 208*	2223 ± 507*	0.954	0.001	0.635
IPAQ_VIG (MET-min $week^{-1}$)	190 ± 135	547 ± 281*	928 ± 372*	45 ± 45	310 ± 138*	660.0 ± 285*	0.264	0.003	0.779
IPAQ_MOD (MET-min $week^{-1}$)	247 ± 102	596 ± 204	384 ± 136	435 ± 243	431 ± 94	1060 ± 423	0.259	0.090	0.275
IPAQ_WALK (MET-min $week^{-1}$)	380 ± 127	337 ± 94	653 ± 294	286 ± 99	517 ± 162	503 ± 121	0.948	0.080	0.936
SF12_PI (pt)	504 ± 1.9	51.4 ± 2.3	52.4 ± 1.8	50.0 ± 2.2	53.1 ± 1.7	52.6 ± 1.6	0.888	0.161	0.976
SF12_MI (pt)	46.3 ± 2.7	49.1 ± 2.2	45.2 ± 2.6	52.4 ± 1.8	50.8 ± 2.2	51.7 ± 2.4	0.282	0.611	0.984

All values are presented as mean ± standard error

BMI body mass index, *FM* Fat Mass, *FFM* Fat-free Mass, *IPAQ-TOT* International Physical Activity Questionnaire, *IPAQ-VIG* vigorous activity, *IPAQ-MOD* moderate-intensity activity, *IPAQ-WALK* physical activity derived from walking, *SF12_PI* Short-Form 12, questionnaire about health-related quality of life concerning physical index, *SF12_MI* Short-Form 12, questionnaire about health-related quality of life concerning mental index

Gr group effect, *T*: time effect; Gr × *T*: groups × time effect. For statistical significance, see paragraph results

Significance by generalized linear mixed model (*see statistical paragraph*): *Significantly different from PRE, $P < 0.05$

†Significantly different from POST, $P < 0.05$; [I]Interaction Groups × Time (pre—post) effect $P < 0.05$

(45 ± 2 vs. 45 ± 1%, group effect $P = 0.953$), lipids (37 ± 2 vs. 36 ± 2%, group effect $P = 0.383$) and proteins (16 ± 1 vs. 16 ± 1%, group effect $P = 0.663$) contributions to energy intake.

At POST, mean energy intake decreased significantly by − 1689 ± 84 kJ day^{-1} (time effect $P < 0.001$, ES 0.62) without difference between groups (interaction $G \times T$ $P = 0.244$). Carbohydrates contribution to energy intake did not change significantly, but lipids decreased by − 4.27 ± 0.29% (time effect $P < 0.05$ ES 0.57) and protein increased by + 3.46 ± 0.15% (time effect $P < 0.001$ ES 1.02) without differences between groups.

After the follow-up period, mean energy intake increased significantly by 600 ± 100 kJ day^{-1} (time effect $P < 0.05$ ES 0.51) without differences between groups ($P = 0.215$); the values returned similar to those described at PRE (time effect $P = 0.098$). Carbohydrates, lipids and proteins contributions to energy intake did not change significantly from POST to follow-up, but lipids remained lower than at PRE (– 4%, time effect $P = 0.028$, ES 0.47).

Physical activity habits

Physical activity habits, evaluated by the IPAQ questionnaire, were similar between the two groups (MICT and HIIT) at baseline (group effect P values ranging from 0.259 to 0.954; Table 1) and the interactions $G \times T$ were not significant (P values ranging from 0.275 to 0.936; Table 1).

However, after the training period (POST, Table 2), total (IPAQ_TOT) and vigorous (IPAQ_VIG) physical activities increased by 72 and 264% (time effect, respectively, $P = 0.011$, ES 0.62 and $P = 0.040$, ES 0.77), respectively, in both groups. But, moderate activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) did not change significantly (time effect, respectively, $P = 0.090$ and $P = 0.080$) in both groups.

After the follow-up period (Table 2), total (IPAQ_TOT) and vigorous (IPAQ_VIG) physical activities did not change significantly and remained higher than at PRE by 100 (time effect $P = 0.001$, ES 1.38) and 168% (time effect $P = 0.004$, ES 1.72) in MICT and HIIT groups, respectively. Also, moderate-intensity activity (IPAQ_MOD) and physical activity derived from walking (IPAQ_WALK) after the follow-up period did not change significantly (time effect, respectively, $P = 0.080$ and $P = 0.090$) in both groups.

The quality of life assessed by the SF12 questionnaire concerning physical and mental indices showed no differences over time in both groups (Table 2).

Physical capacities

Cardiovascular parameters

At rest, $V'O_2$, CO, SV, SAP, DAP, SVC and a-$\bar{v}$ O_2 diff. values were similar between the two groups (MICT and HIIT) at baseline (group effect P values ranging from 0.132 to 0.748; Table 2) and the interactions $G \times T$ were not significant (P values ranging from 0.318 to 0.866; Table 1).

After the training period (POST), SAP decreased significantly at rest by mean − 5% (time effect $P < 0.001$, ES 0.55) in MICT and HIIT groups, without significant changes for the other parameters (Table 2).

After the subsequent follow-up period, SAP did not change significantly and remained significantly lower than at PRE by mean − 5% (time effect $P = 0.003$, ES 0.66) in MICT and HIIT groups. Also, DAP decreased significantly compared to POST, by 4% (time effect $P = 0.033$, ES 0.50) in MICT and HIIT groups; finally, $V'O_2$ increased significantly compared to PRE, by 19% (time effect $P = 0.039$, ES 1.28) in MICT and HIIT groups (Table 2), while a-$\bar{v}$ O_2 diff increased almost significantly between PRE and POST (time effect $P = 0.093$) and between POST and follow-up (time effect $P = 0.055$).

During walking at 1.11 m s^{-1} (4 km h^{-1}) and 0% slope (~ 40% of $V'O_2$peak), $V'O_2$, CO, SV, SAP, DAP, SVC and a-$\bar{v}$ O_2 diff values were similar between the two groups (MICT and HIIT) at baseline (group effect P values ranging from 0.195 to 0.951; Table 2) and the interactions $G \times T$ were not significant (P values ranging from 0.354 to 0.781; Table 1). Physical training caused a significant decrease in $V'O_2$ by − 7% (time effect $P = 0.039$, ES 1.78) in MICT and HIIT groups, and in SAP by − 11% ($P < 0.001$, ES 0.65) in MICT and HIIT groups. After the follow-up period, $V'O_2$ did not change significantly and remained significantly lower than at PRE by 12% (time effect $P = 0.011$, ES 0.21) in MICT and HIIT groups. SAP increased significantly but remained significantly lower than at PRE by 6% (time effect $P = 0.007$, ES 0.95) in MICT and HIIT groups. Finally, both CO and SVC were significantly lower than at PRE by 19% ($P < 0.001$, ES, respectively, 0.46 and 0.13) in MICT and HIIT groups (Table 2).

Peak oxygen uptake

At PRE, no significant differences were found between MICT and HIIT for HRpeak and $V'O_2$peak (Table 1).

At POST, HRpeak decreased by 3.90 ± 0.20 bpm (time effect $P < 0.001$) in MICT and HIIT groups, without difference between groups (Table 1). Absolute $V'O_2$peak increased by 0.17 ± 0.01 and by 0.46 ± 0.20 L min^{-1} (time effect $P < 0.001$) in MICT and HIIT groups, respectively, with a significantly lower increase in MICT than HIIT (+ 6%

Table 2 Cardiovascular parameters before (PRE) and after 3 months (POST) of weight management program, and after 4 months of follow-up in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups

	MICT			HIIT			*P*		
	PRE (16)	POST (16)	FOLLOW-UP (14)	PRE (16)	POST (16)	FOLLOW-UP (12)	Gr	*T*	Gr × *T*
At rest									
$V'O_2$ (L min^{-1})	0.35 ± 0.02	0.37 ± 0.02	0.39 ± 0.03*	0.37 ± 0.01	0.37 ± 0.02	0.49 ± 0.08*	0.132	0.012	0.318
CO (L min^{-1})	6.71 ± 0.33	5.95 ± 0.31	5.97 ± 0.30	7.16 ± 0.30	7.15 ± 0.30	7.09 ± 0.51	0.345	0.113	0.595
SV (ml)	88.82 ± 4.86	84.10 ± 5.63	84.54 ± 4.35	92.98 ± 4.49	96.05 ± 5.76	88.64 ± 6.69	0.158	0.393	0.817
SAP (mmHg)	132.06 ± 2.66	126.13 ± 2.20*	126.15 ± 1.95*	139.56 ± 3.46	131.56 ± 2.08*	128.75 ± 2.47*	0.620	0.002	0.339
DAP (mmHg)	83.88 ± 2.10	82.69 ± 1.36	80.00 ± 2.21†	87.50 ± 2.42	90.38 ± 1.73	86.08 ± 2.03†	0.439	0.002	0.383
SVC (ml min^{-1} $mmHg^{-1}$)	67.08 ± 2.71	60.68 ± 3.06	62.76 ± 3.44	69.71 ± 3.34	69.28 ± 3.74	70.85 ± 5.26	0.410	0.118	0.463
a-$\bar{v}$ O2 diff. (mL 100 mL^{-1})	5.42 ± 0.32	6.05 ± 0.35	5.66 ± 0.59	5.28 ± 0.30	5.45 ± 0.30	6.52 ± 0.60	0.748	0.039	0.866
Walking at 1.11 m s^{-1}, 0% slope									
$V'O_2$ (L min^{-1})	1.27 ± 0.06	1.17 ± 0.06*	0.05*	1.23 ± 0.05	1.15 ± 0.06*	1.08 ± 0.06*	0.431	0.001	0.384
CO (L min^{-1})	13.77 ± 0.61	12.08 ± 0.74	10.85 ± 0.37*†	13.31 ± 0.83	14.23 ± 0.72	11.06 ± 0.44*†	0.951	0.001	0.717
SV (ml)	136.60 ± 5.73	134.50 ± 8.92	120.18 ± 7.81	135.11 ± 8.05	147.38 ± 7.73	123.75 ± 6.88	0.611	0.077	0.781
SAP (mmHg)	143.57 ± 3.76	130.00 ± 1.64*	137.86 ± 3.80*†	149.69 ± 4.24	132.50 ± 1.64*	138.75 ± 3.14*†	0.195	0.001	0.354
DAP (mmHg)	85.00 ± 2.01	1.65	81.57 ± 2.12	87.50 ± 2.54	88.44 ± 1.56	85.00 ± 1.51	0.426	0.443	0.777
SVC (ml min^{-1} $mmHg^{-1}$)	133.11 ± 5.09	122.66 ± 7.78	108.90 ± 4.24*†	126.83 ± 7.43	139.30 ± 7.44	109.47 ± 5.56*†	0.498	0.012	0.762
a-$\bar{v}$ O2 diff. (mL 100 $^{mL\text{-}1}$)	9.58 ± 0.65	10.17 ± 0.65	10.33 ± 0.51	9.57 ± 0.46	8.65 ± 0.73	10.47 ± 0.83	0.893	0.073	0.481

All values are presented as mean ± standard error

$V'O_2$ oxygen consumption, *CO* cardiac output, *SV* stroke volume, *SAP* systolic arterial pressure, *DAP* diastolic arterial pressure, *a-$\bar{v}$ O2 diff.* arteriovenous difference of O_2, *SVC* systemic vascular conductance

Gr: group effect; *T*: time effect; Gr × *T*: groups × time effect

Significance by generalized linear mixed model (*see statistical paragraph*): *Significantly different from PRE, $P < 0.05$

†Significantly different from POST, $P < 0.05$;

and 16%, interaction $G \times T$ $P < 0.001$, ES 0.44, Table 1; Fig. 1a). $V'O_2$peak normalized by FFM increased by 3.3 ± 0.2 and by 7.2 ± 0.3 mL min^{-1} kg^{-1} FFM (time effect $P < 0.001$) in MICT and HIIT groups, respectively, with a significantly lower increase in MICT than HIIT (+8%, and + 16%, interaction $G \times T$ effect $P < 0.001$ ES 0.80, Table 1 and Fig. 1b).

HRpeak did not change significantly during the follow-up period, and remained significantly lower than at PRE (Table 1, time effect $P < 0.001$ ES 0.42). Absolute $V'O_2$peak decreased by 0.19 ± 0.02 and by 0.21 ± 0.01 L min^{-1} (time effect $P < 0.001$ ES 0.19) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HIIT (+8%, time effect $P < 0.001$, Table 1 and Fig. 1a). $V'O_2$peak normalized by FFM decreased by 4.79 ± 0.14 mL min^{-1} kg^{-1}FFM (time effect $P < 0.001$) in MICT and HIIT groups, returning at PRE values in MICT but remaining higher in HI time effect IT (+5%, time effect $P < 0.001$, Table 1 and Fig. 1b).

Fat oxidation rate

Before the training period (PRE), fat oxidation rate during the incremental test was not significantly different between groups (group effect $P = 0.110$). Maximal fat oxidation rate was observed at 41 ± 1% of $V'O_2$peak in MICT (0.41 ± 0.01 g min^{-1}, Fig. 2a) and at 43 ± 1% of $V'O_2$peak in HIIT (0.43 ± 0.1 g min^{-1}, Fig. 2b) groups. At exercise intensities above 60 ± 1% of $V'O_2$peak, fat oxidation rate decreased markedly and the contribution of fat oxidation to energy supply became negligible above 80 ± 1% of $V'O_2$peak.

After the training period (POST), there was a significant interaction $G \times T$ ($P = 0.016$); in the MICT group, fat oxidation rates were not significantly different from those at PRE at all the exercise intensities (Fig. 2a). On the other hand, the HIIT group exhibited a greater absolute rate of fat oxidation at 60% (+45%, time effect $P = 0.019$; ES 0.99), 70% (+119%, time effect $P < 0.001$; ES 1.67) and almost significantly greater at 80% (time effect P 0.076) of $V'O_2$peak (Fig. 2b), whereas at 40 and 50% of $V'O_2$peak, the values were not significantly different from those at PRE.

After 4 months of follow-up, in the MICT group, fat oxidation rates were still not significantly different from those at PRE at all the exercise intensities (Fig. 2a). On the other hand, the HIIT group exhibited a greater absolute rate of fat oxidation at 60% (+32%, time effect $P = 0.031$; ES 0.69), 70% (+28%, time effect $P < 0.001$, ES 0.40) and 80% (+80%, time effect $P = 0.047$, ES 0.68) of $V'O_2$peak (Fig. 2b), whereas at 40 and 50% of $V'O_2$peak, the values remained not significantly different from those at PRE.

Mitochondrial respiration ex vivo and citrate synthase activity

Results of citrate synthase (CS) activity assays are reported in Table 3. No significant differences were observed after the weight management program compared to the baseline values in both MICT and HIIT groups. Thus, none of the two protocols of exercise training affected CS activity. Conversely, the weight management program with both protocols affected significantly the intrinsic mitochondrial respiration. A CI + CII SUIT protocol was applied in order to obtain conditions of electron transport convergent at the Q-junction corresponding to the operation of the TCA cycle and mitochondrial substrate supply in vivo, thereby measuring the maximal oxidative phosphorylation capacity. Specifically, non-phosphorylating resting mitochondrial respiration was measured in the presence of malate (4 mM) and glutamate (10 mM), and in the absence of adenylates so that O_2 consumption was mainly driven by the back leakage of protons through the inner mitochondrial membrane (complex I state 2 respiration or 'leak' respiration). Saturating ADP (5 mM) was then added followed by succinate (10 mM), thereby achieving the maximal ADP-stimulated mitochondrial respiration sustained by complex I and complex II (complex I + II state 3 respiration). Next, electron transport system capacity (complex I + II ETS) was evaluated by the uncoupler protonophore FCCP titration. Rotenone (1 µM) was then added to inhibit complex I and to evaluate ETS sustained by complex II (rotenone insensitive) and by complex I (rotenone inhibited). The degree of oxidative phosphorylation coupling for a specific substrate supply (glutamate/malate in this case), referred to as OXPHOS coupling, was determined by calculating the ratio between complex I + II state 3 respiration minus complex I leak respiration and complex I + II state 3 respiration [(state 3 – leak)/state 3] (Pesta and Gnaiger 2012; Salvadego et al. 2016). Data of different respiration states and substrate/coupling control ratios are reported in Table 3. In particular, maximal ADP-stimulated mitochondrial respiration (CI + II state 3 respiration) increased significantly (time effect $P = 0.042$ ES 0.86) with respect to the baseline values after the weight management program (POST) in both MICT and HIIT groups. The increase was + 51%, without significant difference between the two groups (interaction $G \times T$ was $P = 0.708$). The data dealing with coupling control ratio (OXPHOS coupling) show that they were not affected by the two training protocols. At baseline, the values of the ratio were within 0.69–0.78 and did not change significantly after the weight management program, for both MICT and HIIT. Finally, the capacity of the electron transport system (ETS) uncoupled from the phosphorylating system augmented after the weight management program. Specifically, maximal ETS sustained by substrates of complexes I and II, glutamate/malate and succinate (ETS_CI + CII) increased

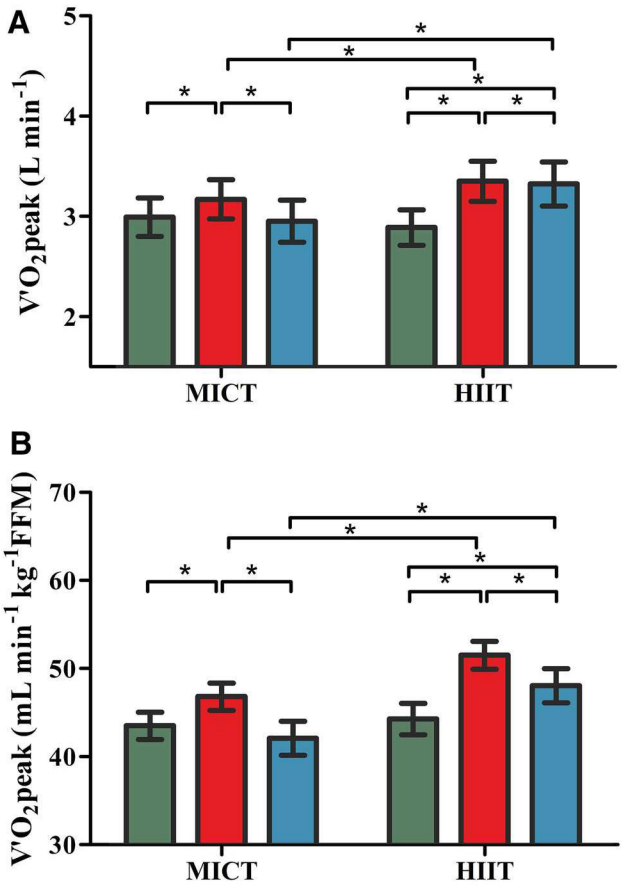

Fig. 1 Absolute peak oxygen uptake ($V'O_2$peak, panel **A**) and peak oxygen uptake normalized by fat-free mass ($V'O_2$peak FFM^{-1}, panel **B**) measured before (PRE, green square) and after 3 months (POST, red square) of weight management program, and after 4 months of follow-up (blue square), in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups. All values are presented as mean ± standard error. Significance by generalized linear mixed model (see statistical paragraph): *Significantly different, $P < 0.05$

significantly (time effect $P = 0.042$, ES 0.96) as compared with baseline, without significant difference between them (interaction $G \times T$ was $P = 0.953$). In addition, either rotenone-sensitive complex I ETS (ETS_CI) or -insensitive complex II ETS (ETS_CII) exhibited a pattern similar to that of ETS_CI + CII, indicating that both complex I and complex II were upregulated, although their increases vs. the baseline values did not reached the statistical significance.

Discussion

In obese patients, the 3-month weight management program entailing, in terms of exercise, MICT or HIIT, resulted in the following: (1) significant improvement of $V'O_2$peak in both groups, although more pronounced in HIIT; (2) significant increase in fat oxidation rate during submaximal exercise, only in the HIIT group; and (3) similar increases, in the two groups, of maximal ADP-stimulated mitochondrial respiration; (4) similar adherence (> 90%) without adverse events in the two groups; and (5) after 4 months of follow-up, only in the HIIT group $V'O_2$peak and fat oxidation rate were still greater compared to the baseline measurements.

HIIT program improving physical capacities and fat oxidation rate

Both MICT and HIIT increased the $V'O_2$peak, although a greater improvement was observed for HIIT, confirming previous research conducted in the general population and in obese people (Lazzer et al. 2017). HIIT would elicit $V'O_2$max improvements through both central and peripheral adaptations, whereas MICT would elicit $V'O_2$max improvements mainly through peripheral adaptation (Daussin et al. 2007). In this study, a $V'O_2$peak increment was measured in the MICT group and mitochondrial respiration (peripheral factor) was improved as well. It must be said also that despite mitochondrial respiration improved in MICT, fat oxidation (peripheral factor) did not. This might suggest that mitochondrial respiration improvement in obese subjects does not contribute to improve also fat oxidation and the $V'O_2$peak increment is occurred independently from fat oxidation following MICT. The intensity that elicits maximal whole-body fat oxidation is approximately 60% of $V'O_2$max in well-trained athletes (Achten et al. 2002), 50% in general population (Achten et al. 2002) and 40% in obese patients (Lazzer et al. 2017). During HIIT the most part of the energy comes from carbohydrates (Hetlelid et al. 2015), since above 80% of $V'O_2$max the contribution of the fat oxidation is almost negligible (Achten et al. 2002; Lazzer et al. 2017). Nevertheless, Hetlelid et al. (2015) found that, by comparing well-trained athletes and moderately active people, the higher performance by the former group during HIIT was mainly explained by their nearly threefold higher rates of fat oxidation capacity, that allows them to keep fat oxidation rate by far higher compared with moderately active people, both during high-intensity exercise and during recovery. In accordance with the Hetlelid results (Hetlelid et al. 2015), the fundamental role of fat oxidation during HIIT might explain why this type of training is more efficient than MICT in improving the fat oxidation rate. Further, previous studies showed that maximal fat oxidation rate was related to $V'O_2$max (Nordby et al. 2006; Hetlelid et al. 2015), suggesting that a training suited for improving $V'O_2$max might improve fat oxidation capacity as well. These data regarding HIIT group support this hypothesis but on the contrary the aerobic fitness improvement following MICT seems mainly due to central factors since mitochondrial respiration enhancements are not accompanied by an improvement

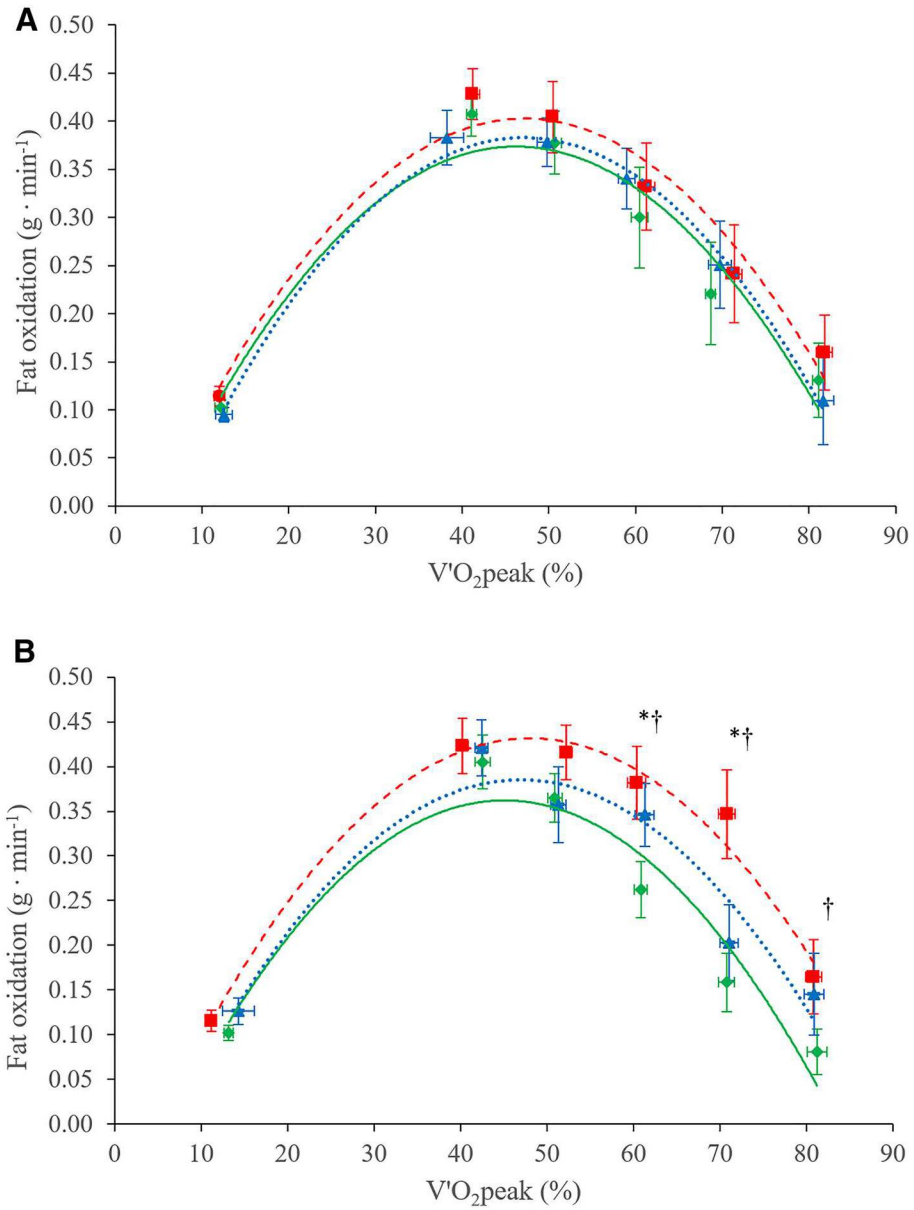

Fig. 2 Fat oxidation rate as a function of exercise intensity expressed as percent of peak oxygen uptake ($V'O_2$peak) before (PRE, green diamond continuous line) and after 3 months (POST, red square dashed line) of weight management program, and after 4 months of follow-up (blue triangle dotted line), in moderate-intensity continuous training (MICT, panel **a**) and high-intensity interval training (HIIT, panel **b**) groups. All values are presented as mean ± standard error. *Significantly different PRE *vs.* POST, $P < 0.05$. †Significantly different PRE *vs.* follow-up, $P < 0.05$

of the capacity to utilize fats during the exercise, maybe due to the more pronounced central limitation in obese (Vaccari et al. 2019). On the other hand, carbohydrate utilization during HIIT is not different between well-trained athletes and moderately active people (Hetlelid et al. 2015); moreover, it does not change following HIIT training (Lazzer et al. 2017); this further suggests that lipid oxidation capacity, rather than carbohydrate utilization, is important during HIIT in obese subjects.

$V'O_2$ and SAP, at rest and during walking at submaximal intensity, decreased in both training groups, with no differences. It is noteworthy that HIIT can improve a clinically relevant parameter, such as SAP, by an amount similar to that described following MICT, despite a lower training time requirement. A previous meta-analysis described a better ability by HIIT in reducing blood pressure in obese patients and in general, HIIT seems more effective in reducing markers correlated with cardiometabolic risk (García-Hermoso et al. 2016). According to the literature (Sawyer et al. 2016), HIIT might induce different vascular adaptations than MICT; given a similar $V'O_2$max increment, HIIT would increase arterial dilatation, whereas MICT would increase the resting arterial diameter (Sawyer et al. 2016). These data showed a slight increment on SVC and a-$\bar{v}$ O_2 diff during submaximal exercise, without differences between groups, suggesting an improvement of vascular function. According to previous research conducted on non-obese sedentary subjects (Daussin et al. 2007), HIIT improves the maximal performance of the cardiovascular system, whereas

Table 3 Mitochondrial parameters before (PRE) and after (POST) the weight management program in moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) groups

	MICT		HIIT		*P*		
	PRE ($n=6$)	POST ($n=7$)	PRE ($n=6$)	POST ($n=8$)	Gr	*T*	Gr × *T*
CS	307 ± 25	278 ± 18.10	308 ± 24	268 ± 21	0.996	0.142	0.479
Maximal ADP-stimulated respiration	1.88 ± 0.37	3.13 ± 0.48*	1.93 ± 0.59	2.63 ± 0.47*	0.403	0.042	0.708
ETS_CI + CII	1.97 ± 0.41	2.86 ± 0.48*	1.48 ± 0.37	2.38 ± 0.38*	0.274	0.042	0.953
OXPHOS coupling	0.78 ± 0.09	0.81 ± 0.05	0.69 ± 0.08	0.75 ± 0.08	0.309	0.520	0.835
ETS_CI	1.24 ± 0.23	1.63 ± 0.31	1.01 ± 0.14	1.61 ± 0.27	0.748	0.062	0.606
ETS_CII	0.73 ± 0.20	1.23 ± 0.23	0.48 ± 0.23	0.77 ± 0.17	0.083	0.058	0.610

All values are presented as mean ± standard error

CS: Citrate Synthase activity, expressed as mU/mg protein; the values of CS refer to n = 13 for MICT and n = 14 for HIIT groups unlike the above indicated. Maximal ADP-stimulated mitochondrial respiration: CI + II state 3 respiration measured in the presence of malate (4 mM), glutamate (10 mM), succinate (10 mM) and saturating ADP (5 mM), normalized by CS and expressed as pmol O_2 s^{-1} mU^{-1}. ETS_CI + CII: maximal Electron Transport System capacity sustained by CI + II, obtained by stepwise addition of the chemical uncoupler FCCP, normalized by CS and expressed as pmol O_2 s^{-1} mU^{-1}. OXPHOS coupling: [(state 3 – leak)/state 3], where leak is the non-phosphorylating resting respiration measured in the presence of malate (4 mM) and glutamate (10 mM), and in the absence of ADP (CI state 2/leak respiration); ETS_CI: ETS sustained by CI (rotenone-sensitive ETS), normalized by CS and expressed as pmol O_2 s^{-1} mU^{-1}. ETS_CII: ETS sustained by CII (rotenone-insensitive ETS), normalized by CS and expressed as pmol O_2 s^{-1} mU^{-1}

Significance by generalized linear mixed model (see statistical paragraph): *Significantly different from PRE, $P < 0.05$

improvements obtained by MICT would mainly manifest at submaximal intensities.

MICT and HIIT improving oxidative phosphorylation capacity but not efficiency

These results revealed an improvement of maximal ADP-stimulated respiration and maximal ETS capacity after both MICT and HIIT, without changes in oxidative phosphorylation coupling, suggesting that oxidative phosphorylation capacity but not efficiency was enhanced by exercise training. Furthermore, no improvement of mitochondrial content was observed, as estimated by CS activity, after both training. Thus, it may be inferred that the increase in mitochondrial function observed in the present study was due to changes in oxidative phosphorylation complexes activity/assembly, or remodelling of mitochondrial inner membrane. Menshikova et al. (2007) suggested a similar hypothesis in sedentary obese individuals undergoing moderate-intensity physical activity combined with weight loss. In particular, the authors observed an improved enzymatic capacity for oxidative phosphorylation without a significant change in mtDNA content, hypothesizing a mitochondrial cristae remodelling. These data showing an increase in maximal capacity of both complex I and complex II may be in accordance to such hypothesis. Intriguingly, studies on non-obese subjects showed that some markers of mitochondrial biogenesis increase in high-intensity interval running, more than in moderate-intensity continuous running (Wallman et al. 2009), and others showed that HIIT is more effective in improving markers associated with mitochondrial contents (MacInnis et al. 2016; MacInnis and Gibala 2017). However, the specific training's characteristics to increase mitochondrial content are not yet fully understood (Bishop et al. 2019). The key factor could be the volume, since Granata et al. (2016), did not find any changes in CS and other mitochondrial content markers following three training interventions at different intensities, but on the other hand, MacInnis et al. (2016) reported that sprint interval training increases mitochondrial content to a similar extent to MICT despite a reduced exercise volume. Nevertheless, it is important to consider that all the studies mentioned above refer to normal weight people, while Boyd et al. (2013) studied this topic in obese/overweight subjects and found no difference in the improvement of oxidative capacity of skeletal muscle and in the mitochondrial content following a low volume at low intensity compared to high-intensity high-volume training.

Overall, these results prompt to propose exercise training, irrespective of the differences between the two training interventions investigated in the present study, as a good strategy to counteract the alteration of the mitochondrial proteome recently observed in skeletal muscle of subjects with obesity (Kras et al. 2018). Indeed, such proteomic profile, with proteins forming the increased TCA cycle and those forming the decreased oxidative phosphorylation complexes, has increased capacity to produce reducing equivalents of NADH and FADH2 in an impaired electron transport chain, thereby generating oxidative stress (Kras et al. 2018).

In this study, despite both training modalities improved oxidative mitochondrial function, only after HIIT, the

capacity to oxidize lipids during exercise improved. It should be considered that mitochondrial oxidative capacity widely exceeds systemic O_2 delivery (Boushel et al. 2011) and does not seem to be related with total body fat oxidation (Nordby et al. 2006). Looking at these results, the improvement in fat oxidation in HIIT was not associated with changes in CS activity. This suggests that at least for 3 months of training, the improvement of fat oxidation is not due to mitochondrial adaptations, but due to other factors, like improvements in O_2 muscle supply, capillary density and O_2 diffusion. Indeed, endurance athletes, compared with untrained individuals, have higher whole-body maximal fat oxidation which, however, does not correlate with mitochondrial fat oxidation (Nordby et al. 2006); this further suggests that higher O_2 availability might be the main factor increasing the whole-body fat oxidation.

Training adherence

During the weight management programs, no differences in training adherence between MICT and HIIT and no adverse events were observed, in agreement with previous studies (Jung et al. 2015), but in contrast with Lunt et al. (2014). Lunt et al. (2014) showed that, despite the greater potential efficiency of HIIT in improving aerobic capacity compared to MICT, the effectiveness of HIIT could be reduced due to the lower adherence to training prescriptions. In the present study, a lower adherence to the HIIT program was not noticed, despite the training period was quite long (3 months): the number of training sessions (about 35) was the same in the two groups and the actual intensities were quite close to the programmed ones. HIIT was reported to be more enjoyable compared to MICT, at least inside a laboratory setting (Bartlett et al. 2011); this work showed that this is true even outside the laboratory setting and for a relatively long period of time. Given that 'lack of time' remains one of the most commonly cited barriers to regular exercise participation (Gillen and Gibala 2014), HIIT could be a time-efficient exercise strategy that warrants consideration for training prescription also in the obese population.

Follow-up

After 4 months of follow-up, $V'O_2$peak and fat oxidation rate decreased in both groups compared with POST. Only the HIIT group, however, maintained higher values of $V'O_2$peak and fat oxidation rate than at PRE, even though other cardiovascular and anthropometric characteristics were equally improved in both groups. This suggests a greater long-term efficiency of HIIT in maintaining the cardiovascular fitness and metabolic health, and so a greater ability to reduce cardiovascular risk and insulin sensitivity (Robinson et al. 2015).

Mean BM after follow-up was slightly increased in both groups compared with POST (although being still lower than at PRE). Interestingly, fat mass and waist and hip circumferences were unchanged between the end of the supervised training and the end of the follow-up period; FFM, on the other hand, increased. This suggests that physical activity, particularly vigorous physical activity, maintained after the training period, induced an increase in FFM.

Along with the increase in FFM, improvements were observed in arterial pressure, SVC and a-$\bar{v}$ O_2 diff, suggesting a positive effect of the lean mass increase on the cardiovascular system parameters (Pedersen and Febbraio 2012). Further, the hip and waist circumferences did not increase after the follow-up period despite the BM increment, suggesting a reduction in cardiovascular risk (O'Donovan et al. 2009).

CO decreased compared to POST and a-$\bar{v}$ O_2 diff tended to increase both at rest and during walking, suggesting an improved oxygen extraction of the peripheral tissues and an improvement in muscle oxidative function (Daussin et al. 2007), as confirmed by the mitochondrial data. On the other hand, in the follow-up, SAP during walking increased compared to POST and SVC decreased, suggesting an increased arterial stiffness (Saladini and Palatini 2017).

Limitations

In the present study, high-resolution respirometry measurements were performed on frozen muscle samples (see METHODS), which could reduce the magnitudes of the ADP-stimulated mitochondrial respiration, as observed previously (Larsen et al. 2012; Meyer et al. 2014). However, the cryopreservation procedure proposed by Kuznetsov et al. (Kuznetsov et al. 2003) and adopted by different laboratories (Wüst et al. 2011; Salvadego et al. 2016) was strictly followed for obtaining a reliable estimation of mitochondrial function. Furthermore, the intactness of outer mitochondrial membrane was verified by administering cytochrome *c* in the measurement chamber and the samples exhibiting a substantial increase in respiration (> 10%) were excluded from these analyses. This procedure allowed the authors to exclude significant damage to outer mitochondrial membrane in the samples considered in this analysis. In addition, the fibres fragility consequent to muscle microneedle biopsy and sample handling reduced the number of samples exploitable for the mitochondrial respiration analysis. Nevertheless, numerically homogeneous populations of samples ($n = 6$–8) were analysed successfully for both the ET and HIIT groups, and the main physiological mean characteristics in these subgroups were not significantly different from whole group of subjects.

Conclusions

In conclusion, MICT and HIIT improved the anthropometric characteristics, some cardiovascular markers and mitochondria intrinsic function at the same extent. However, HIIT was more efficient in improving $V'O_2$peak and fat oxidation capacity and in maintaining them after 4 months of follow-up. These results may be relevant for an appropriate prescription of training programs. Since improving aerobic fitness is very important in reducing the mortality risk in this population (Brown and Kuk 2015), HIIT should be designed not only to reduce energy imbalance but also to optimize and maintain aerobic fitness.

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Author contributions FV and SL conceived the overall study; all the authors contributed to design the research. FV, ADA, FDV, EC, BM and FF and conducted the experiments. FV, AD, JMS, BN, MC, FF, GB and AA analysed the data. FV wrote the manuscript with the help of AP, BM, MC, IM, FF, GB. SL, BG and IM who revised the manuscript. All the authors read and approved this manuscript.

Compliance with ethical standards

Conflict of interest No conflicts of interest, financial or otherwise, are declared by the author(s).

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ORIGINAL ARTICLE

Physical fitness reference standards in Italian children

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Abstract

Physical fitness in childhood is considered a marker of current and future health. For this reason, there is a need for a simple but reliable test to assess the different components of physical fitness even at school during physical education lessons. However, standard values are required to correctly interpret the results of such tests. Hence, this study aimed to generate sex- and age-specific normative percentile values for health-related physical fitness in Italian children. To this aim, 30,472 children aged 6–11 years from the Friuli Venezia-Giulia region (Italy) were examined. The fitness test battery included the Léger test (cardiorespiratory), the shuttle test (agility), standing long jumps, frontal throws of a basketball (lower and upper limb strength), the sit-and-reach test (flexibility), and the standing balance test. Sex- and age-percentile curves were determined using the General Additive Model for Location Scale and Shape (GAMLSS).

Conclusion: The reference standards are provided as 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th, and 99th percentiles in the form of both tables and charts and are roughly comparable with those of other European children.

What is Known:
- *Physical fitness in childhood is considered a marker of current and future health;*
- *Several tests have been developed to assess physical fitness in children;*
- *There are general European reference standards for a series of tests of the main fitness components for children.*

What is New:
- *The present study provides specific reference standards for a series of tests that are indicative of the main fitness components and easily applied in children, particularly those in the Italian population;*
- *Standing balance test and basketball frontal throw test references in a wide sample of children;*
- *The performance of children in the present study was roughly comparable to that of other European children.*

Keywords Reference values · Cardiorespiratory fitness · Muscular strength · Flexibility · Balance · Speed-agility

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Abbreviations

20-mSRT	Léger test, also called 20-m shuttle running test
Pn	*n* percentile
GAMLSS	General Additive Model for Location Scale and Shape method

Introduction

Physical fitness in childhood involves several health-related components, such as cardiorespiratory and muscular endurance, muscular strength, speed-agility, flexibility, and balance [1]. These components are considered markers of current and future health [2]. Some studies have shown that a low level of cardiorespiratory fitness is associated with cardiovascular diseases, some types of cancer, increased adiposity, and obesity [2]. Muscle strength is inversely related to cardiovascular diseases and metabolic risk factors [2] independent of cardiovascular fitness [3], and both upper and lower body muscle strength levels are considered important [4]. Additionally, muscle strength levels and speed-agility capacities are positively related to bone health and self-esteem [2]. Finally, even though there is only weak evidence indicating that flexibility and balance in childhood are correlated with health [5], the American College of Sports Medicine (ACSM) still includes exercise aimed at improving flexibility and balance in its guidelines for preventing worsening of physical fitness in adults [1] and elderly individuals. Since childhood physical fitness is important for public health [6], several tests have been developed to assess its components in a simple but reliable way. The Léger test is often used to evaluate cardiorespiratory fitness [7], and standing long jump and frontal throws of a basketball are considered reliable indices of lower [8] and upper [9] limb muscle fitness. In addition, the shuttle (10 × 5 m) [10], sit and reach [11], and standing balance [12] tests are considered reliable methods for evaluating speed-agility, flexibility, and balance, respectively. Sex- and age-specific reference values help identify children with a low level of fitness and promote healthy behaviours thereby preventing future health risks.

Although there are some reference values from different regions of the world [13], there is a scarcity of reference values for Italian children [13]. Even though reference from Europe already exist [14], there may be wide differences among countries or even within a single country [15]. Hence, this study aimed to generate sex- and age-specific normative percentile values for health-related physical fitness in Italian children aged between 6 and 11 years. Furthermore, this study aimed to provide a battery of tests by selecting the simplest tests to be performed.

Subjects and methods

A sample of 30,472 Italian schoolchildren (6–11 years old) involved in the "*MOVIMENTO in 3S: promozione della Salute nelle Scuole attraverso lo Sport*" (*MOVIMENTO in 3S* project: promoting Health in Schools through Sport) project was considered in the present study [6]. The project was a cross-sectional study; children were enrolled from different public schools in the Friuli Venezia-Giulia Region (Italy) between 2016 and 2018. One hundred thirty-seven of the 387 schools in the region joined the project and about 95% of the students of these schools participated in the measurements. The total number of primary schoolchildren in the Friuli Venezia Giulia region was about 60,000.

The experimental protocol was approved by the University of Udine Ethics Committee on Human Research for Medical Science. All the Children attending primary school were considered eligible for the study excluding those with any issues that prevented them from attending physical education classes. The procedures and purposes of the project were carefully explained to each child and his or her parents. Children gave verbal consent to participate and parents gave written informed consent. Thereafter, physical fitness tests were performed at the beginning and of each school year during school hours by a previously trained researcher to collect the data accurately and consistently.

Physical fitness

A battery of 6 tests was chosen to obtain a complete report of children's physical fitness [16]. Priority was given to the use of tests that were easy to perform without the use of any special tools. The 6 tests were administered to the children on 6 different days during their class hours.

Cardiorespiratory fitness

Cardiorespiratory fitness was evaluated by a single repetition of the Léger test [16], which has been also validated in children [17, 18]. For the test, children were required to run back and forth continuously between two cones placed 20 m apart. A pre-recorded audio signal started to beep at pre-set intervals. The children had to follow the rhythm imposed by the beeps and the starting speed was selected at 8.5 km h^{-1}. The children had to be in correspondence with the cone at each beep. Every minute the speed was increased by 0.5 km h^{-1} reducing the time between two consecutive beeps.

When the children being tested did not reach the final point in time or when the children stopped by fatigue, the last stage completed was recorded as their final score.

Speed-agility

Whole body speed-agility was evaluated by a shuttle test (10 × 5 m) [19–22], which has been validated in children [10]. Two lines have been depicted on the floor 5 m apart. At the "go!" signal, children had to run as fast as possible to the opposite line, cross it with both feet, run back across the starting line, and repeat the task for 10 shuttles (50 m), without brakes, in the shortest possible time. The best time of two trials was then taken into consideration.

Muscular power of the lower limbs

Lower limb explosive power was evaluated by means of a long jump test [12, 14, 19–26], which has been validated, by several authors, also in children [8, 16, 21]. Children were asked to jump for distance from a standing start. They were instructed to bend their knees with their arms in front of them, parallel to the ground, then to swing both arms and jump as far as possible, trying to land on their feet. The best trial between 3 tests was recorded.

Muscular power of the upper limbs

Upper limb power was evaluated by a frontal throw of a basketball (0.5 kg) [9]. The validity and reliability of this test has been previously confirmed [24]. Children were instructed to throw the basketball while sitting on the ground with their legs spread apart and with their back against the wall in order to use only upper limb's muscles. The ball was thrown with two hands maintaining back contact with the wall. The distance of the throws was considered between the wall and the first point of contact of the ball on the ground. The best of three throws was recorded in centimetres.

Flexibility

Hip and low back flexibility was evaluated by the sit-and-reach test [12, 19–22, 24–26], which has been previously validated [11]. Children were asked to sit on the floor with legs stretched forward and knee locked and pressed against the floor. Shoes were removed and soles of the feet were placed flat against a box. Children stretched as far as possible along the measuring line and held that position for 1 to 2 s while the distance was measured. The level of the feet was considered to be 15 cm [27] in order to make all the values positive.

Static balance

The standing balance test evaluates balance capacity in children [12, 27], and it is considered valid and reliable [17, 18]. Children were asked to remove their shoes and to keep their balance on their preferred leg. The free leg had to be flexed at the knee, and the foot had to be held by the hand against the buttock of the same side while the contralateral hand remained fixed at the level of the hip. The test started after 1 min of practice. Time in seconds was considered the score of the test. The test was considered concluded either after 30 s, when the children moved their supporting foot, or when they lost contact between the heel of the non-supporting leg and the buttocks.

Statistical analysis

Statistical analyses were performed using the GAMLSS (General Additive Model for Location Scale and Shape) package 5.1-4 [28] of the statistical software R version 3.6.3. All physical fitness results were expressed as the mean and standard deviation (SD). To perform the analysis, the collected data were first screened for incorrect inclusions. The percentile curves for the fitness variables were stratified by sex and calculated as a function of age using the GAMLSS method. The percentile curves for the 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th, and 99th percentiles were calculated based on the model that showed the best goodness of fit.

Results

The initial selected sample (n: 30472) was screened for any incorrect inclusions and 41 children were deleted from the original database due to temporary physical issues preventing them from performing the tests. Then, 30,431 children were considered in the present study. Tables 1 and 2 and Figs. 1, 2, and 3 display the age- and sex-specific percentiles (P_1, P_3, P_{10}, P_{25}, P_{50}, P_{75}, P_{90}, P_{97}, and P_{99}) for the different fitness tests in 30,431 Italian schoolchildren (6–11 years old). In the Léger test, boys performed better ($P < 0.05$) than girls, and older children performed better ($P < 0.05$) than younger children (Fig. 1a, b); in the speed-agility shuttle test, girls and boys had similar values, and older children performed worse ($P < 0.05$) than younger children (Fig. 1c, d). In the standing long jump test (Fig. 2a, b) and frontal throw test (Fig. 2 c, d), boys performed better ($P < 0.05$) than girls, and older children performed better ($P < 0.05$) than younger. In the sit-and-reach test, girls performed better ($P < 0.05$) than boys (Fig. 3a, b). In addition, girls' performance decreased with age from P_1 to P_{50}, while it increased with age from P_{75} to P_{99}; however, boys' performance decreased with age from P_1 to P_{99}. Finally, in the standing balance test, boys and girls had similar values (Fig. 3c, d); moreover, in both sexes, the increment with age was maximal around P_{50} and tended to be lower when approaching both P_{99} and P_1.

Table 1 Percentiles of the Léger test, speed-agility shuttle test, and long jump test, stratified by sex and class of age (6–11 years old)

	Percentile for girls										Percentile for boys									
	Age	1	3	10	25	50	75	90	97	99	Age	1	3	10	25	50	75	90	97	99
Léger test (*n*)	6–< 7	1.0	1.2	1.4	1.6	1.9	2.4	3.2	4.0	4.4	6–< 7	1.1	1.2	1.3	1.6	2.1	3.2	3.8	4.8	5.5
	7–< 8	1.3	1.5	1.7	1.8	2.2	2.6	3.6	4.2	5.3	7–< 8	1.1	1.3	1.4	1.8	2.3	3.6	5.2	6.1	6.7
	8–< 9	1.4	1.6	1.8	2.2	2.8	3.6	4.5	5.4	6.0	8–< 9	1.2	1.4	1.6	2.3	3.1	4.2	5.4	6.6	7.2
	9–< 10	1.5	1.8	2.0	2.8	3.4	4.3	5.1	6.6	7.3	9–< 10	1.3	1.5	1.6	2.4	3.6	4.8	5.8	7.0	8.2
	10–< 11	2.0	2.2	2.6	3.3	3.6	4.6	5.3	7.1	8.1	10–< 11	1.3	1.5	1.7	2.6	3.9	5.2	6.9	8.1	8.4
	11–< 12	2.1	2.4	2.8	3.4	3.9	4.8	5.5	7.4	8.4	11–< 12	1.4	1.6	1.9	2.8	4.2	5.6	7.3	8.4	9.2
Speed-agility shuttle test (s)	6–< 7	37.6	34.7	31.6	29.3	27.3	25.7	23.7	20.6	16.9	6–< 7	38.6	34.8	31.7	28.0	26.8	24.5	22.8	19.1	16.5
	7–< 8	37.1	34.1	31.0	28.1	26.7	24.0	22.3	18.8	16.5	7–< 8	35.8	34.5	30.7	27.4	25.3	23.1	21.3	18.8	15.9
	8–< 9	36.3	33.7	29.5	27.6	25.5	23.4	21.5	17.6	15.7	8–< 9	35.2	34.0	29.0	26.5	24.3	22.5	21.0	17.8	15.1
	9–< 10	35.0	32.6	28.7	26.5	24.4	22.3	20.8	16.9	14.9	9–< 10	34.1	32.2	28.2	25.4	23.5	21.8	19.8	16.7	14.7
	10–< 11	34.1	30.0	27.8	25.9	23.8	21.8	20.2	16.5	14.6	10–< 11	33.8	31.7	27.4	24.8	22.8	21.0	19.1	16.0	14.0
	11–< 12	33.3	29.4	27.1	25.1	23.2	21.3	19.6	16.2	14.3	11–< 12	33.0	29.6	26.5	24.2	22.1	20.4	18.2	15.5	12.9

The values correspond to the 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th, and 99th age- and sex-specific percentiles

Discussion

The present study provides age and sex reference standards for a series of tests indicative of the main fitness components that are easily applied in schoolchildren. The values for the percentiles presented are noteworthy because they come from a fairly wide sample of 30,431 children, and they could be very useful for educators. Moreover, they can be used to compare the fitness status of Italian children to that of European children and children worldwide. Finally, these reference standards can serve as a good reference for monitoring the decline in physical fitness state that is occurring in children around the world [29–32].

Because the relationship between cardiorespiratory fitness and health issues in children is well documented [2, 7, 33–35], the Léger test is often used to assess this important component at school [36]. The Léger test [17], also called the 20-m shuttle running test (20-mSRT), is one of the most widely used tests for evaluating cardiorespiratory fitness due to its validity and reliability and the ability to test a large number of children at the same time [37]. It can be scored by using the number of stages completed (as in the present study), the number of laps completed, or the speed reached at the last stage and allows us to estimate the maximal oxygen uptake [17, 38]. The values obtained in the present study were roughly comparable to those obtained previously in Spanish children [20, 39] and those in Tomkinson's study [22], particularly for girls. Even though the study by Tomkinson included children and adolescents aged 9–17 years old from 30 European countries, it is not possible to compare values for the younger children. Moreover, our data from children aged >9 years were comparable to those from Australian children [40]. The review by Olds et al. [13], which included 37 countries from around the world, showed that Italian children were among the worst performers on the Léger test, whereas northern European countries were the best performers. However, the reference standards provided by the present study appear to be roughly in line with those of other European countries. Hence, the situation of the Italian children considered in the present study does not seem to be so compromised when compared with the situation of those from other European countries.

Speed-agility is strongly related to health as well, in particular to bone mineral density and bone mass accumulation later in life [2, 3, 5, 35]. There are a wide variety of tests aimed at evaluating speed-agility, often based on running patterns and changes in direction. The shuttle test is one of them [19–22], and it has been previously used several times to assess speed-agility in children and adolescents [19–22, 34]. Overall, the studies confirm that girls tend to have higher values than boys and that younger children have better performance than older children, not only in children the same age as those in our study [20] but also in adolescents until 17 years old [21, 22, 34]. Furthermore, our values appear to be in line with or slightly higher than those observed in Spanish children [20].

Muscular strength/fitness is one of the most studied fitness components in relation to health [2–5, 35]. In particular, the standing long jump is considered a general index of muscular fitness [4]. Our results were similar to those found in Spanish

Table 2 Percentiles of the frontal throw test, sit and reach test, and standing balance test, stratified by sex and class of age (6–11 years old)

	Percentile for girls										Percentile for boys									
	Age	1	3	10	25	50	75	90	97	99	Age	1	3	10	25	50	75	90	97	99
Frontal throw test (cm)	6–< 7	90.5	124.0	158.7	190.6	220.0	251.4	285.7	320.2	370.2	6–< 7	110.5	135.2	170.8	200.7	240.0	280.3	310.1	350.6	380.6
	7–< 8	110.8	130.2	165.0	200.3	235.2	270.1	305.8	340.4	363.5	7–< 8	120.3	142.2	180.8	212.6	252.1	294.3	333.3	374.8	407.5
	8–< 9	145.4	170.7	210.7	240.4	280.5	321.2	360.5	392.5	425.0	8–< 9	155.1	180.0	225.5	266.6	310.0	354.4	392.4	440.2	465.5
	9–< 10	150.7	190.3	244.8	285.4	328.7	370.6	410.8	450.6	481.8	9–< 10	165.4	205.3	265.4	315.1	360.5	410.8	450.8	500.8	535.1
	10–< 11	160.4	221.2	285.3	325.8	365.2	410.6	454.3	500.8	540.1	10–< 11	180.6	250.4	315.1	360.2	410.1	455.1	500.4	545.4	580.2
	11–< 12	191.0	270.3	326.0	362.7	400.8	449.6	500.4	540.7	580.0	11–< 12	227.8	304.1	352.0	400.1	450.2	500.3	545.8	590.3	640.8
Sit and reach test (cm)	6–< 7	4.8	6.8	11.0	14.8	18.8	22.8	26.3	29.6	32.5	6–< 7	1.6	4.0	7.5	11.5	16.4	19.5	23.0	27.1	30.5
	7–< 8	2.4	4.8	9.8	14.5	18.6	22.6	26.4	30.7	33.5	7–< 8	1.4	2.8	6.0	10.8	16.1	18.6	22.8	25.9	29.6
	8–< 9	1.8	4.5	8.9	14.1	18.5	22.5	27.4	31.1	33.6	8–< 9	1.1	2.5	5.5	9.7	15.7	18.5	22.1	25.3	28.5
	9–< 10	1.6	4.0	8.5	13.6	17.5	23.1	27.5	32.5	34.5	9–< 10	0.8	2.1	4.6	9.2	14.8	18.2	21.8	25.0	28.1
	10–< 11	1.2	3.5	8.1	13.5	17.4	22.7	28.6	32.8	34.9	10–< 11	0.7	1.5	4.2	8.9	14.4	17.7	21.5	24.8	27.9
	11–< 12	1.1	3.2	7.8	13.1	17.0	23.1	29.5	33.2	35.4	11–< 12	0.4	1.3	3.9	8.1	14.3	17.5	21.1	24.3	27.4
Standing balance test (s)	6–< 7	0.8	2.1	3.7	7.7	13.8	25.3	26.5	27.1	27.9	6–< 7	0.9	1.4	3.8	5.5	11.2	19.0	23.5	26.7	29.4
	7–< 8	1.6	3.3	6.2	12.7	15.4	25.8	26.8	27.4	28.1	7–< 8	1.7	2.5	4.0	8.5	13.6	21.7	25.6	27.4	29.5
	8–< 9	3.6	6.1	10.5	14.1	21.7	27.6	27.9	28.3	28.7	8–< 9	2.3	3.4	6.2	12.1	17.4	23.4	26.1	27.8	29.7
	9–< 10	4.3	8.6	12.3	17.3	24.6	27.8	28.2	28.6	29.0	9–< 10	2.7	4.8	9.5	13.8	22.8	26.7	26.9	28.5	29.8
	10–< 11	4.8	9.6	14.4	21.4	26.3	28.4	28.6	29.1	29.6	10–< 11	3.0	5.7	12.1	17.5	25.1	26.3	27.6	28.8	29.8
	11–< 12	5.5	11.3	15.9	23.2	28.2	28.8	29.1	29.6	29.9	11–< 12	3.4	6.4	12.8	18.7	25.7	27.9	28.4	29.3	29.9

The values correspond to the 1st, 3rd, 10th, 25th, 50th, 75th, 90th, 97th, and 99th age- and sex-specific percentiles

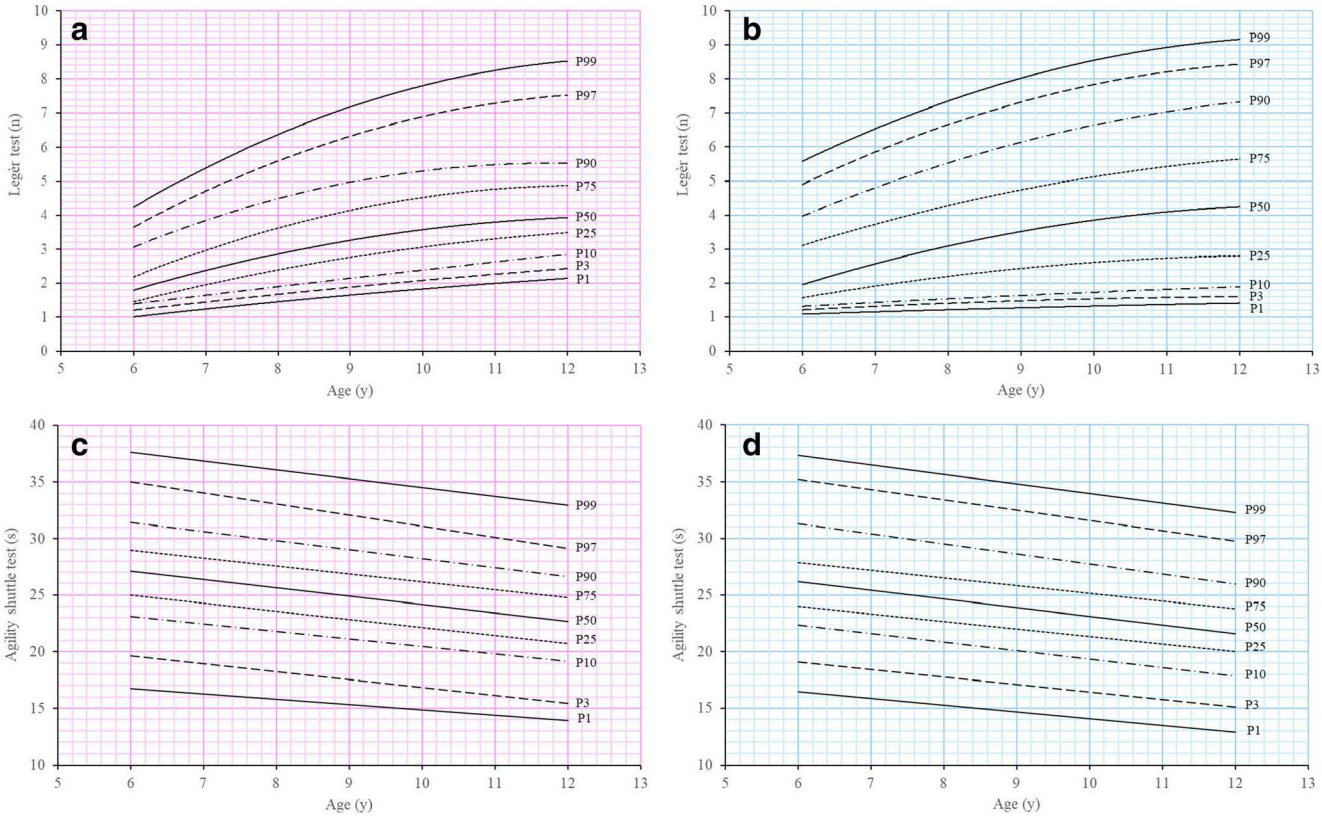

Fig. 1 Percentile curves of the physical fitness (Léger test: **a**, **b**; speed-agility shuttle test: **c**, **d**), stratified by sex (girls: **a**, **c**; boys: **b**, **d**) and class of age (6–11 years old)

children of the same age [20] and European children aged 6 to 9 years old [14] but lower than those found in Polish [21] and Australian children older than 9 years old. However, in all these studies, as in ours, boys and older children had higher outcomes than girls and younger children, respectively [14, 20, 21, 40]. In terms of muscle fitness, upper body muscle fitness is considered an important predictor of heath in children [4]. One of the most commonly used tests to assess this component was the handgrip test [4, 14, 20, 21, 41]. However, in our opinion, it is very important to provide an easy-to-perform test that does not require special tools to evaluate upper body muscle fitness. In light of this, the basketball frontal throw test was developed [42]. Our results were slightly higher than those observed in Australian children and adolescents older than 9 years old [40]. In addition, to the best of our knowledge, this is the first study that has provided reference values for the basketball frontal throw test in a wide sample of children.

The flexibility standard reference values provided by the present study are in line with those for Spanish children [20]; moreover, older boys and girls showed less flexibility levels than younger boys, while girls were more flexible than boys. The reference values from Dobosz [21] showed that Polish boys performed better than Italian boys. Finally, the review by Catley and Tomkinson [40] shows higher values in Australian children than in the children in our study, but it is not clear if the differences were due to different methods of assessment.

To our knowledge, the present study was the first to provide a standing balance test reference in children. This may help promote the use of this test in assessing balance, since it has been proven to be reliable to evaluate balance capacity in children [12, 18]. Nevertheless, although our results on balance were not directly comparable with others, the trends whereby older individuals performed better than younger individuals [14, 21, 34] and girls performed better than boys [14, 34] were confirmed by other studies.

For a practical application of these data, educators, coaches, and all other people involved in children physical activity may consider these reference values of fitness status: very poor ($X < P10$); poor ($P10 \leq X < P25$); medium ($P25 \leq X < P75$); good ($P75 \leq X < P90$); and very good ($X < P90$). The lowest percentiles, for example, 10th percentile, can represent a "warning signal" for further tests and investigations. Miguel-Etayo and colleagues [14] suggest that a fitness level below P5 is potentially pathological and cardiorespiratory and muscular fitness is particularly associated with health status [2]. Moreover, children with values below P25 may be introduced

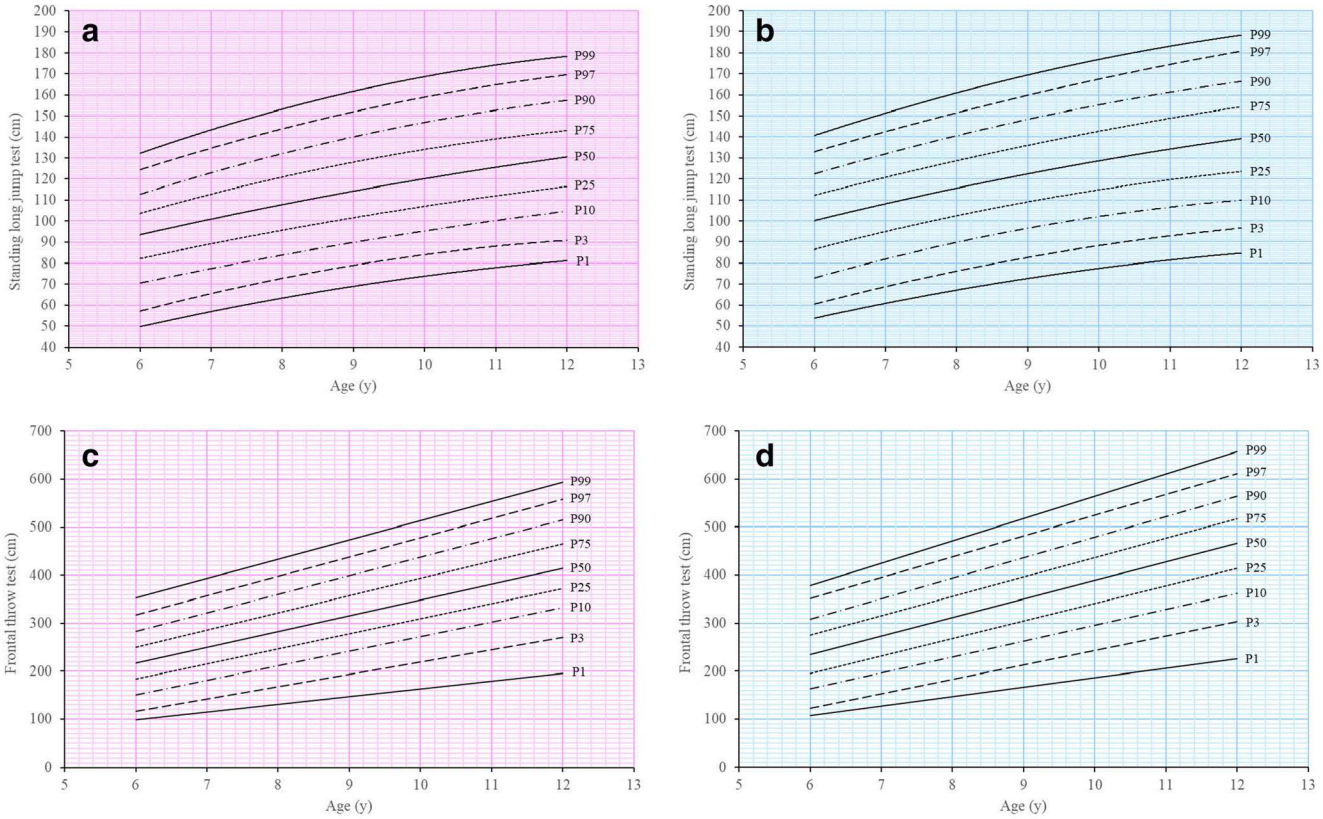

Fig. 2 Percentile curves of the physical fitness (standing long jump test: **a**, **b**; frontal throw test: **c**, **d**), stratified by sex (girls: **a**, **c**; boys: **b**, **d**) and class of age (6–11 years old)

to training programmes to improve their fitness. On the other hand, children with values above the highest percentiles may be advised for further athletic developments.

A further aspect to be considered is the secular trend in children's physical fitness; it seems that in many countries around the world, physical fitness has been worsening over the years [29–32, 38]. The study by Tomkinson et al. [38], which used the Léger test to assess cardiorespiratory fitness in 11 mainly developed countries around the world between 1980 and 2000, showed a marked decline in performance in both sexes, especially in older compared to younger individuals. In this light, the data provided by the present study may be useful for monitoring this phenomenon. Many factors can influence physical fitness, such as sex, age, children's body mass index, and the amount of physical activity, and these factors are independently related to each other [43]. Indeed, the study by Zaqout et al. [33], in accordance with our data, showed that boys had better cardiorespiratory fitness and upper- and lower-limb strength than girls, while girls had better balance and flexibility. Furthermore, increased body mass index (BMI) was related to lower cardiorespiratory fitness, flexibility and lower limb strength, balance, and increased upper limb strength as previously reported [6], while higher physical activity was connected to the improvement of all components of physical fitness except balance [43]. Children's BMI is also positively associated with their mother's obesity, but, luckily, CRF and muscular strength mitigate this relationship [44]. Therefore, physical fitness and physical activity are important, and the role of the school may be crucial, on the one hand, to identify children with low physical fitness levels and, on the other hand, to encourage children to be active. However, physical activity programmes should aim to improve not only cardiorespiratory fitness but also muscular fitness and speed-agility [2]. For this reason, physical activity is recommended in the form of both structured and unstructured activity in children; in particular, aerobic exercise is recommended daily, while activity focused on muscle and bone strengthening should be prescribed at least 3 days per week [45]. Recent findings [46] suggest that also active videogaming may be beneficial.

Two of the main strengths of the present study are the large number of children involved and the fact that all tests are easy to perform at school without specific tools. Furthermore, to the best of our knowledge, this is the first study to provide reference values of the standing balance test in children. On the other hand, the two main limitations are (1) the design of the study is cross-sectional while it would be preferable to obtain data from a longitudinal study on growing children and (2) the children included in the sample come from a single region of Italy.

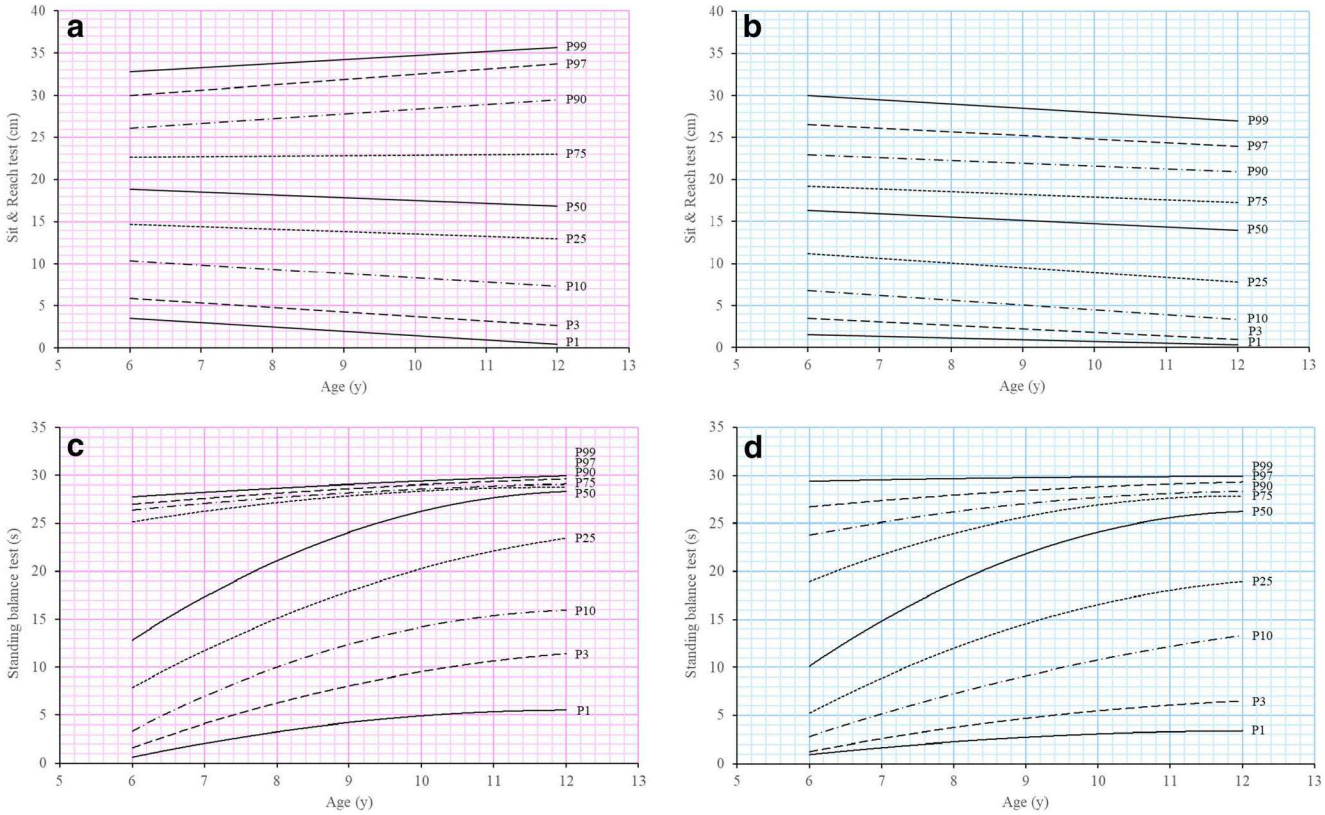

Fig. 3 Percentile curves of the physical fitness (sit and reach test: **a**, **b**; standing balance test: **c**, **d**), stratified by sex (girls: **a**, **c**; boys: **b**, **d**) and class of age (6–11 years old)

In conclusion, our results provide reference standards for a complete series of tests that are indicative of the main components of physical fitness and applicable to children aged between 6 and 11 years. It is important to note that the tests included in this work are easy to perform without any specific tools.

Furthermore, the performance of children in the present study was roughly comparable to that of other European children, and our data could be useful for monitoring the secular trend of physical fitness in children, which has been decreasing over the years.

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Authors' contributions SL conceived the overall study, all the authors contributed to design the research and to make the measurements. GB analysed the data. FV wrote the manuscript with the help of FF and GB. SL, MP, GM, RM revised the manuscript. All the authors read and approved the manuscript.

Data availability N/A

Declarations

Ethics approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors. The experimental protocol was approved by the University of Udine Ethics Committee on Human Research for Medical Science. Before the study began, the purpose and objectives were carefully explained to each child and his or her parents. Children gave their verbal consent, and written informed consent was obtained from their parents.

Consent for publication Patients signed informed consent regarding publishing their data

Conflict of interest The authors declare no conflict of interest.

Code availability N/A

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PLOS ONE

RESEARCH ARTICLE

Relationship between body mass index and physical fitness in Italian prepubertal schoolchildren

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Abstract

The objective of this study was to investigate the association between physical fitness and body mass index categories (obesity, OB; overweight, OW; normal-weight, NW; and underweight, UW) in prepubertal children. Anthropometric and physical fitness characteristics were collected from a convenience sample of 30472 Italian schoolchildren (6–11 years old). Six field-based tests were used: Léger, agility shuttle, long jump, frontal throw of the basketball, Sit & Reach and standing balance. Significant differences were found in the anthropometric characteristics, physical fitness and weight status prevalence between girls and boys ($p<0.05$) and, except for flexibility, by age class ($p<0.05$). Obese children performed worse than their NW counterparts in aerobic capacity ($p<0.001$), agility ($p<0.001$), muscular power of the lower limb ($p<0.001$) and balance ($p<0.001$). Conversely, children with obesity showed greater upper limb power than NW children ($p<0.001$). The discrepancy in physical fitness between OB and NW children increased in older girls (flexibility, $p = 0.002$; muscular power of the lower and upper limb, $p = 0.002$ and $p = 0.005$) and boys (aerobic capacity, $p = 0.009$; agility, $p = 0.006$; standing balance, $p = 0.019$; muscular power of the lower and upper limb, $p<0.001$ and $p = 0.011$) compared to their younger counterparts. On the other hand, UW children performed worse than NW children mainly in terms of muscular power of the arms ($p<0.001$). Additionally, there was an increasing disparity in the frontal throw test scores of UW and NW girls ($p = 0.003$) and boys ($p = 0.011$) in older children compared to younger children. In conclusion, the effect of body mass index on children's physical fitness intensifies with age. OB and OW negatively affect aerobic capacity, agility, lower limb power and balance but positively affect upper limb power. UW negatively affects upper limb power. This study underscores the importance of preventing childhood OW, OB, and UW in early life to promote children's health and proper fitness development.

Introduction

In the past four decades, the prevalence of childhood overweight and obesity has risen dramatically worldwide [1]. Italy has one of the highest prevalence of obese and overweight youth in

Europe although a trend towards a reduction was observed between 2008 and 2016 [2–4]. Paediatric obesity has a wide range of serious short and long-term health and social consequences. Because of their excess adiposity, obese children may exhibit early signs of multiple comorbidities such as cardiovascular dysfunction [5,6] and asthma [7]. Other short-term consequences of childhood obesity may be underachievement at school, lower self-esteem, psychological problems and decreased quality of life relative to healthy peers [8]. Furthermore, obesity tracks strongly from childhood into later life [9], causing an increased likelihood of morbidity in adulthood as well as all-cause mortality [5].

Another strong predictor of future health is physical fitness, which has been defined as a set of attributes related to a person's ability to perform physical activities [10]. In the literature, cardiorespiratory fitness and muscular strength have been associated in children with cardiometabolic risk factors [11,12] and recently, cardiovascular fitness has been positively associated with academic performance [13]. Therefore, physical fitness, and in particular cardiorespiratory fitness, could modify the impact that body mass index (BMI) has on the risk of cardiovascular disease and obesity-related comorbidities, especially in children and adolescents [14,15]. Moreover, research has found positive associations between children's adiposity and physical fitness, regardless of physical activity levels [10]. In particular, several authors have linked higher BMIs to poor performance in weight bearing activities, i.e., activities that require moving bodyweight through space [16–25], while inconsistences have been found in the association between children's weight status and muscular strength [18,20,21,23–25]. Given the connections that are present among fitness, weight and health status, monitoring physical fitness levels in prepubertal children may be essential to prevent fitness deficiencies, obesity and their possible health-related consequences [25]. Prepuberty can in fact be a critical time to promote healthy lifestyles in childhood and later life, and the school environment in particular may be suitable for intervention and monitoring programmes due to its capability to reach children across age groups.

After a preliminary evaluation of age- and sex-specific differences in prepubertal children's physical fitness, the primary purpose of the present study was to analyse the association between physical fitness (cardio respiratory fitness, speed, strength, balance and flexibility) and BMI categories (obesity, OB; overweight, OW; normal-weight, NW; and underweight, UW) in a large sample of Italian schoolchildren. The secondary purpose was to examine whether different physical fitness components improve as a function of age in groups of children of differing weight status. We hypothesized that an OW and OB status in children has a negative effect on physical performance and that such effects increase as a function of age class.

Subjects and methods

A convenience sample of 30472 Italian schoolchildren (6–11 years old) who participated in the project "*MOVIMENTO in 3S*: *promozione della Salute nelle Scuole attraverso lo Sport*" (*MOVIMENTO in 3S* project: promoting Health in Schools through Sport) was enrolled from different public schools in the Friuli Venezia-Giulia Region (Italy) during the period between 2016 and 2018. The experimental protocol was approved by the University of Udine Ethics Committee on Human Research for Medical Science. The following criteria were adopted to select eligible children: elementary school attendance and the absence of any disease or disability that could make a child unable to participate in the scheduled school physical education programme. Before the study began, the purpose and objectives were carefully explained to each child and his or her parents. Children gave their verbal consent, and written informed consent was obtained from their parents. Then, anthropometric measurements and physical fitness

parameters were recorded at the beginning of the school year during school hours. The measurements were taken by a group of Sports Sciences PhD students who were previously trained to correctly collect the data for each test.

Anthropometric characteristics

Children's stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board, and body mass (BM) was measured to the nearest 0.1 kg with a calibrated manual weighing scale (Seca 709, Germany). Body mass index (BMI) was calculated as BM (kg) · stature^{-2} (m). Children were considered OW or OB based on BMI/age-specific curves when their BMI was greater than or equal to the international cut-off point corresponding to the centile curve that passes through either the BMI curve for 25 or 30 kg · m^{-2}, respectively, at 18 years of age [26].

Physical fitness

To obtain a representative status of children's physical fitness, 6 of the several physical fitness tests suitable for the selected age group [27,28] were considered. Priority was given to the accuracy of measurements, which were taken by a group of trained sports scientists within a short period of time in all the schools involved in the study. The 6 tests selected were administered to children on 6 different weekdays, during their physical education classes, avoiding each test being influenced by the results of the previous test. The following tests, which were easy to perform in all the school environments, were chosen:

Aerobic capacity. Aerobic capacity was evaluated by the Léger test [29], which has confirmed validity and reliability in children [30–32]. The test involved running continuously between two points that were 20 metres apart. The runs were synchronized with a pre-recorded audio tape, which played beeps at set intervals. The interval of beeps was calculated to obtain a speed of 8.5 km· h^{-1} at the start, and to increase by 0.5 km· h^{-1} at each level thereafter. As the test proceeded, the interval between each successive beep decreased, forcing children to increase their speed over the course of the test until it was impossible to keep in sync with the recording. When the children being tested did not reach the final point in time, the last level completed was recorded as their final score.

Agility. Whole body agility was evaluated by a shuttle test (10 x 5 m) [17,18,33,34]. The reliability and validity of shuttle tests have been previously examined in children [35]. Two lines, 5 metres apart, were identified using cones. On the signal "ready", children were instructed to place their feet behind the starting line. Then, on the signal "go!", they sprinted to the opposite line, passed it with both feet, ran back to the starting point, and repeated the task, without a rest. Children were asked to repeat the track between the two lines 10 times, in order to run 50 metres in total. Two trials were performed, and the shortest time needed to complete the test was recorded in seconds.

Muscular power of the lower limb. Lower limb explosive power was evaluated by a long jump test [17,18,20,21,23,25,33,34,36,37]. The validity and reliability of jump tests have been previously evaluated in children by several authors [31,32,38]. Each child jumped for distance from a standstill. During the performance of the jumps, children were asked to bend their knees with their arms in front of them, parallel to the ground, then to swing both arms, push off vigorously and jump as far as possible, trying to land with their feet together and to stay upright. The test was performed three times and scored in centimetres. The longest jump length was recorded.

Muscular power of the arms. Upper body power was evaluated by a frontal throw of a basketball (0.5 kg) [39], which has been defined as being a valid and reliable test [40]. Children

were asked to sit on the ground with their legs apart and their back leaning against the wall, facing the direction where the ball was to be thrown. The ball was held with two hands and brought close to the body at the chest level, then vigorously thrown forward as far as possible, maintaining wall contact. The longest throw of three, as measured by the distance between the wall and the first contact point of the ball to the ground, was recorded in centimetres.

Flexibility. Hip and low back flexibility were evaluated by the Sit & Reach test [17,18,20,21,23,25,33,34], whose reliability and validity have been previously assessed in the literature [32,41]. In a seated position with their knees extended and their feet placed firmly against a vertical support, children reached forward along the measuring line as far as possible with their arms at the same level. The distance reached by their hands to the nearest centimetre was recorded as the score, using the level of the feet as zero, so that any measure that did not reach the toes was considered negative and any measure beyond the toes was considered positive.

Static balance. The standing balance test was used in previous studies to reliably [32] evaluate balance capacity in children [25,28]. Children removed their shoes and placed their hands on their hips. While balancing on the preferred leg, the free leg was flexed at the knee, and the foot was held close to the buttocks by the hand of the same side. Children had one minute to practice their balancing before starting the test. Then, children stood on their preferred leg for a maximum of 30 s. Time was recorded when children moved their supporting foot or when they lost contact between the heel of the non-supporting leg and their buttocks.

Statistical analysis

To perform the analysis, the collected data were first screened for incorrect inclusions. When they were not plausible, records were excluded from the database if the correct information was unavailable. Anthropometric characteristics and all physical fitness information were expressed as the mean and standard deviation (SD) or standard error (SE) in the graphs and stratified by sex and age and by sex, age and BMI categories, respectively. The effects of sex, age and BMI categories and the interaction among these variables on anthropometric characteristics and physical fitness were tested using two-way analysis of variance (ANOVA) after evaluating the homogeneity of variance with Levene's test. When significant differences were found, a Bonferroni post hoc test was evaluated implementing multiple comparisons to detect which variable means were significantly different from each other. Then, a simple linear regression equation was calculated for each sex and for each physical fitness parameter (including 6 to 11 age) to evaluate the difference between each BMI category. We compared the regression coefficients (β) of NW with those of other BMI categories (individually for males and females) to test the null hypothesis H_0: $\beta_{NW} = \beta_{OB/NW/UW}$ and to evaluate whether the dimension of a regression coefficient should be larger for one group than for another. All statistical analyses were performed by SAS, Release 9.4 (SAS Institute, Cary, NC, USA), with a significance set at $p<0.05$.

Results

The initial selected sample (n: 30472) was screened for any incorrect inclusions and 41 records were deleted from the original database. Table 1 shows the anthropometric characteristics of the 30431 children involved in the study. Stature and body mass (BM) increased significantly from age class 6 to age class 11 (by a mean of +0.05 m and +3.3 kg per year in both girls and boys) and differed significantly between girls and boys ($p<0.001$). In addition, BMI increased significantly with age (by a mean of +0.48 and +0.52 $kg \cdot m^{-2}$ per year in girls and boys,

Table 1. Anthropometric characteristics of children (n: 30431) stratified by sex (girls and boys) and age class (6–11 years old).

	Girls (n:14645)						Boys (n:15786)						P		
	6 years (n:845)	7 years (n:3161)	8 years (n:3176)	9 years (n:2914)	10 years (n:2690)	11 years (n:1859)	6 years (n:868)	7 years (n:3377)	8 years (n:3303)	9 years (n:3228)	10 years (n:2929)	11 years (n:2081)	Sex	Age	S X A
Stature (m)	1.19 ±0.05	1.21±0.06	1.28±0.06	1.33±0.06	1.39±0.07	1.44±0.07	1.17±0.06	1.21±0.06	1.26±0.06	1.32±0.07	1.38±0.07	1.44±0.07	<.001	<.001	<.001
BM (kg)	22.6±4.0	24.1±4.6	27.3±5.6	31.1±6.9	34.7±7.7	38.9±8.8	23.1±3.7	24.5±4.5	27.7±5.4	31.7±6.8	35.4±7.6	39.4±8.6	<.001	<.001	0.757
BMI ($kg \cdot m^{-2}$)	16.3±2.2	16.5±2.3	17.0±2.6	17.7±3.0	18.1±3.1	18.7±3.3	16.4±2.0	16.6±2.2	17.0±2.5	17.8±2.8	18.3±3.0	19.0±3.3	0.002	<.001	0.056

All values are means ± SD. Significant according to a generalized linear model of the main effects of Sex (Girls vs Boys), Age, and Sex × Age interaction (S × A).

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respectively) and differed significantly between girls and boys ($p<0.002$), except for age classes 6 and 7.

Table 2 shows the prevalence of underweight (UW; 7.5% *vs* 6.1%), normal-weight (NW; 67.4% *vs* 69.6%), overweight (OW; 19.2% *vs* 18.4%) and obesity (OB; 5.9% *vs* 5.8%) in girls and boys, respectively, grouped by age class. Significant differences ($p<0.05$) in weight status prevalence were found between girls and boys of all ages apart from those who were 6 years old ($p = 0.607$). Additionally, significant differences were found in UW, NW, OW and OB prevalence between age groups in girls and boys ($p = 0.001$, for both sexes).

Children's physical fitness

Aerobic capacity. Girls completed fewer levels than boys in the Léger test at each age class (-0.2, -0.4, -0.5, -0.9 and -0.4 levels for ages 6 to 11 years old, respectively, $p<0.011$), and older children completed more levels than younger children (by a mean of +0.3 levels each year, in both girls and boys). This performance improvement by age followed a diminishing pattern. Between class ages 6 and 7, the level reached by girls and boys increased by +0.5 and +0.7 levels, respectively, while between class ages 10 and 11, it increased by +0.1 and +0.4 levels, respectively (Table 3).

OB girls and OB boys performed worse on the Léger test than their NW counterparts (Fig 1a, 1aA and 1aB). In particular, significantly lower levels were reached by 8-, 9- and 10-year-old OB girls than by NW girls of the same ages (-50.0, -38.7 and -37.5%, respectively, $p<0.001$) and by 8-, 9-, 10- and 11-year-old OB boys than by NW boys (-36.4, -37.8, -55.8 and -43.6%, respectively, $p<0.001$). Additionally, OW girls completed lower levels than NW girls at 8, 9, 10 and 11 years old (-26.7, -25.8, -21.9 and -21.9%, respectively, $p<0.001$), and OW boys reached lower levels than NW boys at 9, 10 and 11 years old (-21.6, -34.9 and -28.2%, respectively, $p<0.001$).

The discrepancy in aerobic capacity among OB, OW and NW children increased at higher age classes relative to lower classes (Fig 2a, 2aA and 2aB). Nevertheless, the slopes of the linear

Table 2. Underweight (UW), normal-weight (NW), overweight (OW) and obesity (OB) prevalence in girls and boys of each age class (6–11 years old).

	Girls (n:14645)							Boys (n:15786)						
	6 years (n:845)	7 years (n:3161)	8 years (n:3176)	9 years (n:2914)	10 years (n:2690)	11 years (n:1859)	Total	6 years (n:868)	7 years (n:3377)	8 years (n:3303)	9 years (n:3228)	10 years (n:2929)	11 years (n:2081)	Total
UW (%)	7.3	6.5	7.2	7.3	8.1	9.2	7.5	6.9	6.3	7.2	5.4	5.5	6.1	6.1
NW (%)	66.8	69.4	67.3	65.7	67.6	67.0	67.4	69.7	72.9	70.6	67.9	69.0	66.6	9.7
OW (%)	19.8	17.6	19.2	20.7	19.3	19.4	19.2	18.1	15.5	16.6	19.7	19.9	22.0	18.4
OB (%)	6.2	6.5	6.3	6.4	5.1	4.5	5.9	5.3	5.4	5.7	7.0	5.5	5.4	5.8

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Table 3. Physical fitness scores of children (n: 30431) stratified by sex (girls and boys) and age class (6–11 years old).

	Girls (n:14645)						Boys (n:15786)						P		
	6 years (n:845)	7 years (n:3161)	8 years (n:3176)	9 years (n:2914)	10 years (n:2690)	11 years (n:1859)	6 years (n:868)	7 years (n:3377)	8 years (n:3303)	9 years (n:3228)	10 years (n:2929)	11 years (n:2081)	Sex	Age	S X A
Léger (n)	1.6±0.9	2.1±1.6	2.6±2.1	2.8±1.5	3.0±1.8	3.1±1.6	1.8±1.1	2.5±1.8	3.1±2.2	3.4±1.9	3.9±2.4	3.5±1.9	0.014	<.001	0.011
Agility Shuttle (s)	26.7±3.7	26.0±4.0	25.3±5.7	24.1±5.1	23.4±4.1	23.0±4.0	26.2±4.0	25.4±4.4	24.3±5.2	23.7±6.0	22.7±4.3	22.2±3.5	<.001	<.001	0.012
Long jump (cm)	94.4±17.4	97.0±18.4	106.6±18.8	115.3±19.3	122.8±20.6	128.8±20.6	101.0±19.2	103.6±19.3	113.8±20.1	122.9±20.5	130.7±21.5	137.7±22.3	<.001	<.001	0.006
Frontal throw (cm)	221.6±52.4	235.9±54.8	282.2±59.6	326.9±67.7	366.7±72.2	405.7±72.2	239.7±56.9	254.5±61.2	309.1±66.6	359.8±74.9	406.3±76.0	448.3±78.7	<.001	<.001	0.024
Sit & Reach (cm)	3.0±6.2	2.6±6.8	2.5±7.2	2.4±7.8	2.3±8.1	2.3±8.6	0.2±6.2	-0.9±6.5	-1.9±7.4	-2.9±7.5	-3.0±7.9	-3.6±8.4	<.001	<.001	0.027
Standing balance (s)	15.9±9.9	17.8±8.6	21.1±8.3	23.3±7.6	24.8±6.8	25.5±6.0	13.0±9.2	14.8±8.5	18.0±8.8	20.5±8.4	22.8±7.8	23.6±7.2	<.001	<.001	0.015

All values are means ± SD. Significant according to a generalized linear model of the main effects of Sex (Girls vs Boys), Age, and Sex × Age interaction (S × A).

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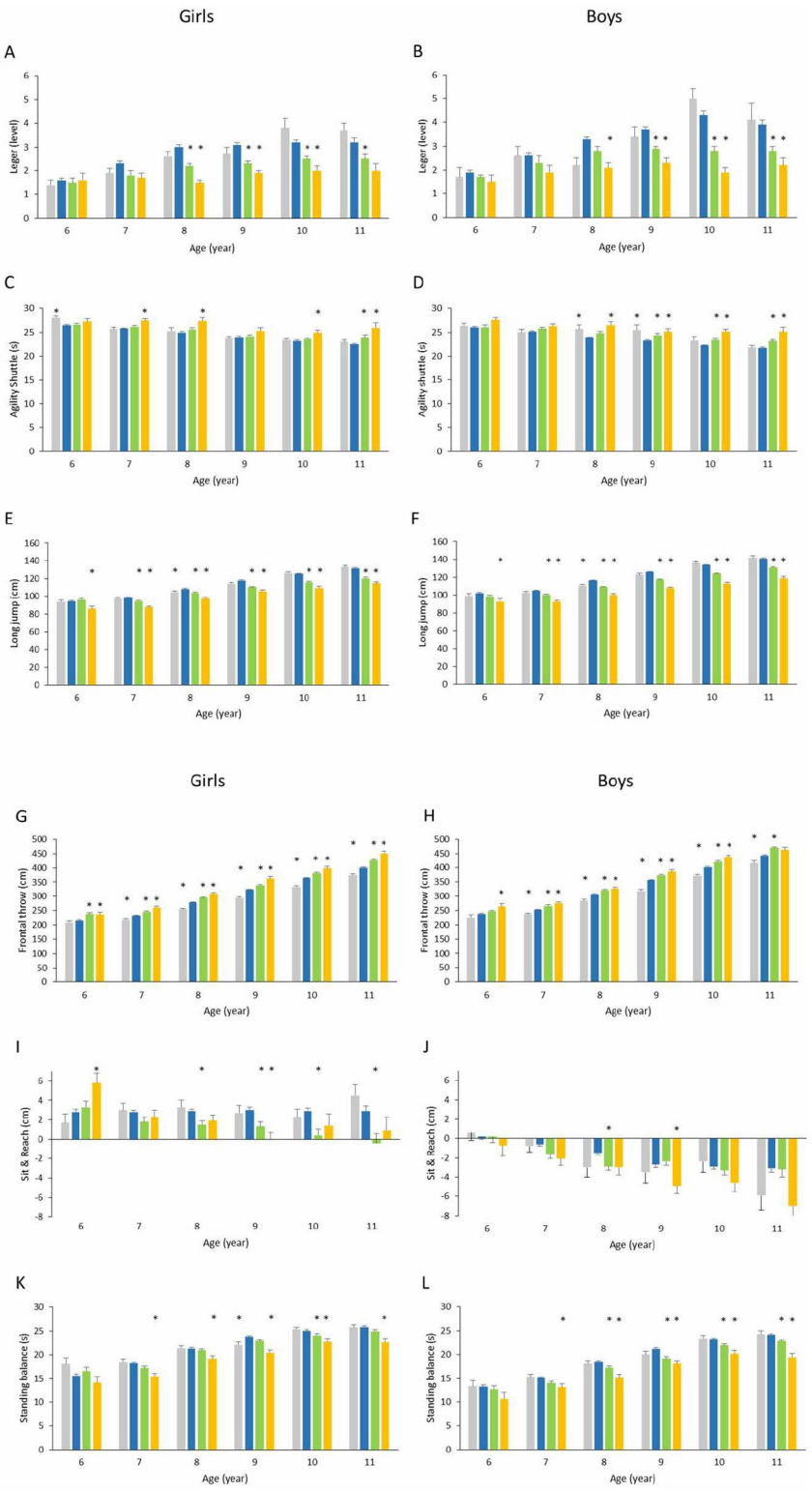

Fig 1. a. Children's physical fitness (Léger test: A, B; Agility shuttle test: C, D; Long jump test: E, F) reported as a function of BMI category and age class. Girls: A, C, E; Boys: B, D, F. Underweight, ; normal weight, ; overweight, ; and obese, . All values are means ± SE. *Significantly different relative to scores for normal weight children ($p<0.05$). b. Children's physical fitness (frontal throw test: G, H; Sit & Reach test: I, J; standing balance test: K, L) reported as a function of BMI category and age class. Girls: G, I, K; Boys: H, J, L. Underweight, ; normal weight, ; overweight, ; and obese, . All values are means ± SE. *Significantly different relative to scores for normal weight children ($p<0.05$).

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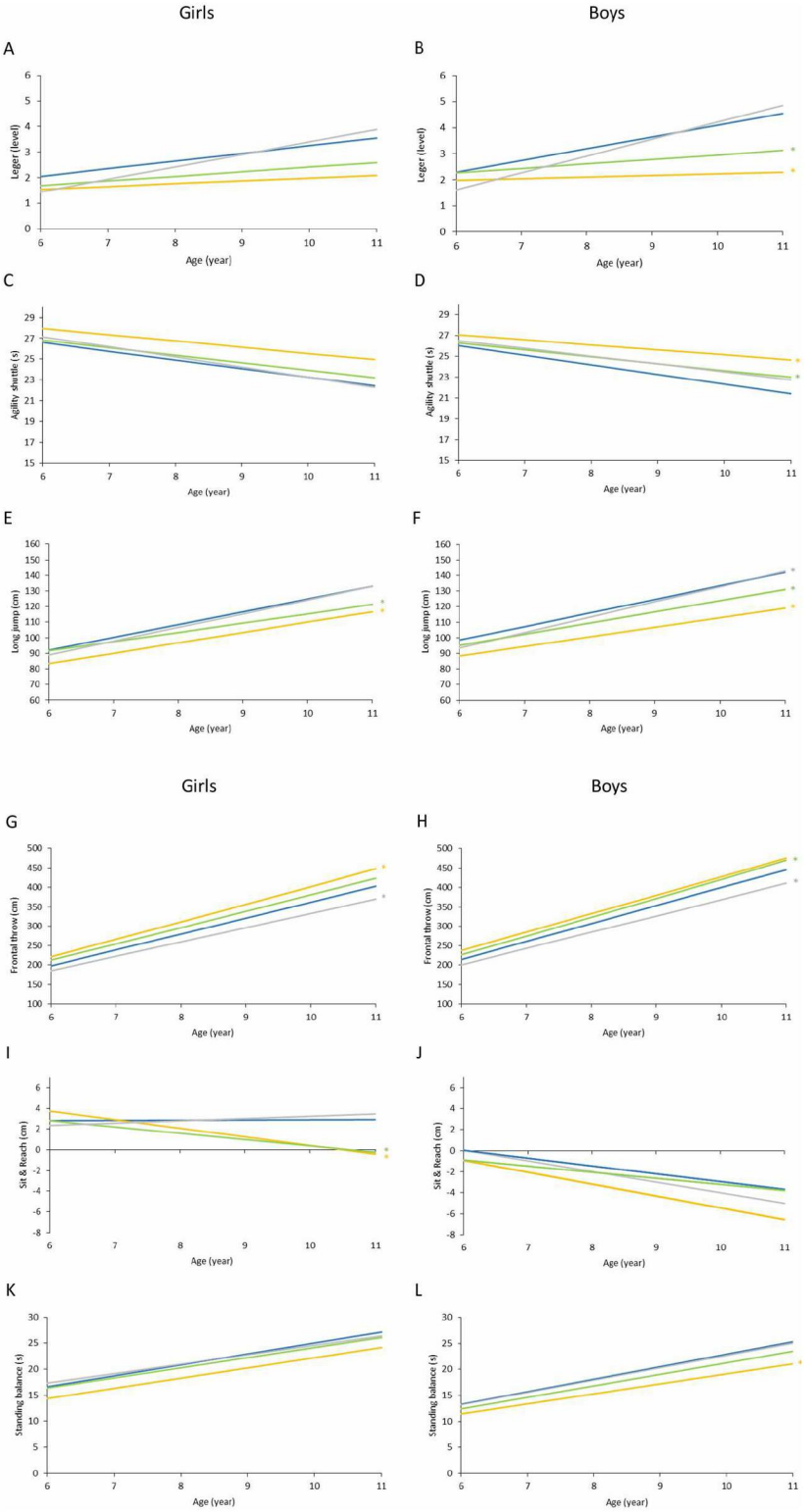

Fig 2. a. Regression models considering changes in children's physical fitness (Léger test: A, B; Agility shuttle test: C, D; Long jump test: E, F) reported as a function of BMI category and age class. Girls: A, C, E; Boys: B, D, F. Underweight, ----; normal weight, ----; overweight, ----; and obese, ----. *Significantly different slope relative to that for normal weight children ($p<0.05$). b. Regression models considering changes in children's physical fitness (frontal throw test: G, H; Sit & Reach test: I, J; standing balance test: K, L) reported as a function of BMI category and age class. Girls: G, I, K; Boys: H, J, L. Underweight, ----; normal weight, ----; overweight, ----; and obese, ----. *Significantly different slope relative to that for normal-weight children ($p<0.05$).

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regression models calculated for OB and OW girls were not significantly different from those for NW girls, whereas the slopes calculated for OB and OW boys were significantly lower than those for NW boys ($p = 0.009$ and $p<0.001$, respectively).

Agility shuttle. Girls needed more time than boys to complete the shuttle test at each age class (+0.5, +0.6, +1.0, +0.4, +0.7 and +0.8 s from 6 to 11 years, respectively, $p<0.012$). Moreover, agility improved progressively from age class 6 to age class 11 in children of either sex (by a mean of -0.7 s in girls and -0.8 s in boys each year).

The amount of time needed to complete the test was significantly longer in OB girls and boys than in NW girls and boys (Fig 1a, 1aC and 1aD). In particular, OB girls aged 7, 8, 10 and 11 needed more time than their NW peers (+6.6, +9.6, +7.8 and +15.6%, respectively, $p<0.001$), as did OB boys aged 8 to 11 (+10.9, +8.2, +13.5 and +15.7%, respectively, $p<0.001$). Similarly, 11-year-old OW girls took longer to complete the test than NW girls (+6.2%, $p = 0.0231$), as did 9-, 10- and 11-year-old OW boys compared to NW boys (+5.2, +5.4 and +6.5%, respectively, $p< 0.001$). On the other hand, UW girls showed worse agility capacity than NW girls at 6 years old (+6.2%, $p = 0.023$), and UW boys showed worse agility capacity than NW boys at 8 and 9 years old (+7.5% and +9.9%, respectively, $p<0.033$).

The agility gap between OB, OW and NW children increased in older children compared to younger children (Fig 2a, 2aC and 2aD). No significant slope differences were found between NW girls and other BMI categories. However, the slopes calculated for OB and OW boys were significantly greater than the slope calculated for NW boys ($p = 0.006$ and $p = 0.008$, respectively).

Muscular power of the lower limb. Girls jumps were shorter than those of boys for each age class (-6.6 cm at 6 and 7 years old, -7.2 cm at 8 years old, -7.6 cm at 9 years old, -7.9 cm at 10 years old and -8.9 cm at 11 years old, $p<0.006$). Muscular power of the lower limb was considerably greater in older children than in younger children (mean +6.9 cm in girls, and +7.3 cm in boys, per year). The peak improvement was observed between 7 and 8 years (+9.6 and +10.2 cm in girls and boys, respectively).

The recorded jump distance was shorter in OB and OW children than in NW and UW children for either sex (Fig 1a, 1aE and 1aF). In particular, OB girls jumped a significantly shorter distance than their NW counterparts at each age class (-9.3, -10.9, -10.0, -10.3, -12.7 and -13%, at 6, 7, 8, 9, 10 and 11 years old respectively, $p<0.001$), as did OB boys (-9.5, -11.6, -13.8, -14.4, -15.6 and -15.9%, respectively, $p<0.001$). OW girls jumped a shorter distance than NW girls at 7, 8, 9, 10 and 11 years old (-4.0, -4.1, -6.4, -7.5 and -8.6%, respectively, $p <0.001$), as did OW boys (-4.3, -6.2, -6.8, -7.3 and -7.6%, respectively, $p <0.001$). On the other hand, 8-year-old UW girls and boys jumped a significantly shorter distance than their NW counterparts (-3.3%, $p = 0.033$ and -4.9%, $p<0.001$, respectively).

Lower limb muscular power disparities among children of differing BMI categories were found to be wider in higher age classes than in lower age classes (Fig 2a, 2aE and 2aF). The slopes of the regression models calculated for OB and OW children were significantly lower than the slope calculated for NW children of either sex ($p<0.001$ and $p = 0.002$ for OB and OW girls, respectively, and $p<0.001$ for OB and OW boys). On the other hand, the regression slope for UW boys was significantly higher than that of NW boys ($p = 0.015$).

Muscular power of the arms. Girls scored lower than boys on the frontal throw test at each age class (-18.1, -18.6, -26.9, -32.9, -39.6, -42.6 cm from 6 to 11 years, respectively, $p<0.024$). Muscular power of the upper limb increased from age class 6 to age class 11 in both girls and boys (by a mean of +36.8 and +41.7 cm per year, respectively). A peak was observed between 7 and 8 years old (+46.3 and +54.6 cm in girls and boys, respectively).

The distance covered by the thrown ball increased gradually from lower to higher BMI categories (Fig 1b, 1bG and 1bH). In particular, OB girls threw the ball significantly farther than

NW girls at 6, 7, 8, 9, 10 and 11 years old (+9.3, +12.3, +10.5, +12.3, +9.3 and +12.3%, respectively, $p<0.001$), and OB boys threw the ball significantly farther than NW boys at 6, 7, 8, 9 and 10 (+10.9, +9.3, +6.3, +8.5 and +8.8%, $p<0.001$). Similarly, OW girls threw farther than NW girls at 6, 7, 8, 9, 10 and 11 years old (+9.9, +5.8, +5.7, +5.0, +4.8 and +6.2%, respectively, $p<0.001$), and OW boys threw farther than NW boys at 7, 8, 9, 10 and 11 (+5.9, +4.7, +5.1, +4.6 and +6.2%, respectively, $p <0.001$). Finally, UW girls scored significantly lower than NW girls at 7, 8, 9, 10 and 11 years old (-6.4, -8.9, -8.8, -8.5 and -6.6%, respectively, $p <0.001$), as did UW boys (-5.7, -7.5, -10.6, -7.5 and -5.7%, respectively, $p <0.001$).

The gap in muscular power of the arms among children belonging to different weight status categories was greater in older children than in younger children (Fig 2b, 2bG and 2bH). OB girls and OW boys showed a significantly greater linear regression slope than NW girls and boys, respectively ($p = 0.005$ and $p = 0.011$). In contrast, UW girls and boys showed significantly smaller slopes than NW girls and boys ($p = 0.003$ and $p = 0.011$, respectively).

Flexibility. Girls obtained higher Sit & Reach scores than boys at each age class (+2.8, +3.5, +4.4, +5.3, +5.3, +5.9 cm from 6 to 11 years, respectively, $p<0.027$). Moreover, there was no significant difference in flexibility scores obtained by girls of differing age groups; on the other hand, scores were found to be significantly lower for older boys than for younger boys (by a mean of -0.8 cm each year).

Flexibility was partially influenced by BMI status (Fig 1b, 1bI and 1bJ). OB girls showed greater flexibility capability at 6 years old and lower capability at 9 years old (+107.1 and -100.0%, $p<0.004$) than NW girls, while OW girls were significantly less flexible than NW girls at 8, 9, 10 and 11 years old (-48.3, -56.7, -86.2 and -113.8%, respectively, $p<0.005$). On the other hand, BMI was found to have less impact on the flexibility of boys: only 9-year-old OB boys and 8-year-old OW boys performed significantly worse than their NW peers (-81.5%, $p = 0.021$ and -93.3%, $p = 0.045$, respectively).

Additionally, it was observed that the flexibility gap between children of differing BMI categories was greater in older children than in younger children, especially in girls (Fig 2b, 2bI and 2bJ). Indeed, only the slopes calculated for OB and OW girls were significantly different from those of their NW peers ($p = 0.002$ and $p<0.001$, respectively).

Static balance. Balance capacity was found to be better in girls than in boys at each age class (+2.9, +3.0, +3.1, +2.8, +2.0, +1.9 s from 6 to 11 years old, respectively, $p<0.015$) and to increase by age in both sexes (by a mean of +1.9 s in girls and + 2.1 s in boys each year). A peak was observed between 7 and 8 years old (+3.3 and +3.2 s in girls and boys, respectively).

Significant differences in standing balance scores were observed among BMI categories at most age classes, except for age class 6 (Fig 1b, 1bK and 1bM). With respect to NW girls, worse balance capacity was shown at 7, 8, 9, 10 and 11 years old by OB girls (-14.8, -10.3, -14.3, -9.6 and -12.7%, respectively, $p<0.001$), at 10 years old by OW girls (-4.0%, $p = 0.034$), and at 9 years old by UW girls (-7.1%, $p = 0.008$). Similarly, at 7, 8, 9, 10 and 11 years old, OB boys maintained their balance for a significantly shorter time than NW boys (-13.2, -18.4, -14.6, -13.3 and -19.8%, respectively, $p<0.001$), as did OW boys at 8, 9, 10, and 11 years old (-7.0, -9.9, -6.0 and -5.8%, respectively, $p<0.001$).

Fig 2b, 2bK and 2bL show the regression models considering changes in the balance scores of girls and boys as a function of BMI category and age class. The only statistically significant slope difference was found between OB and NW boys ($p = 0.019$).

Discussion

The main results showed that in the present sample of Italian prepubertal children, 1) anthropometric characteristics and physical fitness differed significantly between girls and boys; 2)

OB and OW status negatively affected aerobic capacity, agility, lower limb power and balance but 3) positively affected upper limb power; 4) underweight negatively affected upper limb power; and finally, 5) BMI effect on physical fitness increased over the years.

Both the anthropometric characteristics and physical fitness of prepubertal children are affected by sex and age. As has already been observed in the literature, sex-related differences in anthropometric characteristics and physical fitness increase in older children, particularly after 12 years of age [34,42,43]. Actually, according to some authors [24,33,44], female improvement in physical fitness (particularly in strength) plateaus at approximately 12 years old, marking the emergence of the gender gap. However, as confirmed by the present study, sex-related differences could also be detected prior to the pubertal stage [18,21,37,45,46]. In agreement with several previous studies assessing children's physical fitness through field-based tests similar to those used in the present study, it was observed that boys perform better than girls in terms of cardiovascular fitness [18,33,34,37,44,46,47], muscular strength of the upper and lower limb [18,21,24,33,34,37,44,46,47] and speed-agility [18,21,33,34,37,46,47]. Conversely, girls perform better in terms of flexibility [18,21,33,34,37,44,47] and balance [33,37,46]. The observed sex-related differences might be due to both environmental and biological factors. It is known that children who play sports have better physical fitness than those who do not [21]. Moreover, physical activity attendance and type of sport practised could be different in girls and boys due to motivation, social interest or peer influence, resulting in girls being generally less active than boys [48]. Regarding biological factors, although we did not collect data on body composition to confirm these assumptions, previous studies showed that girls have a significantly greater percentage of fat mass and less fat free mass than boys [49] and that, during growth, the fat free mass of males increases at a faster rate than that of females, especially during puberty [50]. Consequently, these environmental and biological differences might have led to better physical fitness and muscular strength performances in boys than in girls, especially at older ages.

Additionally, consistent with previous studies regarding European prepubertal children [18,24,25,33,34,37,45,46], a general performance improvement by age was detected in each fitness parameter, except for flexibility. In this regard, it is known that gross motor coordination improves from childhood to puberty [44] even if inter-individual variation is still a major feature among typically developing young children [51]. Concerning flexibility, the present study revealed a significant decrement in boys' performance from younger to older age, as was reported by Gonzales et al. [18]. However, some authors [37] have found a non-significant variation in the flexibility scores of both sexes by age, while others have found an improvement with age in girls and an improvement after puberty in boys [34]. Such inconsistencies might be due to individual variation or the type of physical activity practised by the specific sample of children considered.

Not only sex and age can affect children's physical fitness. Body mass and body composition account for a substantial portion of the variation in performance during childhood [21,22]. Consistent with previous literature findings, the present study detected statistically significant differences between various physical fitness scores achieved by OB, OW, NW and UW children. Specifically, it is known that OW and OB children perform worse than NW children in weight bearing activities, as was the case in the Leger, shuttle and jump tests [16–25]. In the present study, this trend was found to be similar between sexes and more prominent in older children than in younger children, emphasizing the need to promote healthier lifestyles from an early age. Moreover, OW *vs* NW differences were less frequently significant than OB *vs* NW differences, and OW children performed better than OB children in weight bearing activities, as has been reported in the literature [17]. This could be due to the quantitative role of fat mass, which behaves as an inert load limiting physical movement, physical activity attendance

[52] and proper motor skills development in OB children more than in OW children. On the other hand, the increased mechanical work needed to lift the body off the ground in everyday-life activities could have a positive influence on absolute muscle strength, increasing fat free mass (FFM) more in OB children than in their NW counterparts [53,54]. A higher FFM, which is known to correlate with strength parameters in obese subjects [55], might be the reason why better throwing scores were obtained by the OB and OW children in the present and in previous studies [18,20,21,24] than by NW children. In contrast, some authors [23,25] have found comparable strength of the upper limb in OB, OW and NW children. These conflicting results might be explained by different throwing protocols, body composition or physical activity practised by children. Additionally, our results confirmed that being OB or OW has a negative effect on static balance capability [25] and, in older girls in particular, on flexibility [19–21,23,25]. In fact, the flexibility scores achieved by OW and OB girls worsened dramatically with age but remained stable in NW and UW girls. Finally, although the relationships between UW children and their physical fitness have been less studied in the literature, some authors noticed that UW children perform similarly to NW children apart from in upper [16,18,23,24] and lower limb strength [16,23], in which they obtained lower results. In the present study, UW children performed considerably worse than NW children in upper limb strength, while, regarding the long jump test, significantly lower scores were observed for the UW children than for the NW children exclusively at 8 years old. Other significant worse results were obtained by UW children than by NW children on the agility shuttle test. These findings suggest that not only obesity but also leanness could have an impact on children's physical fitness. Indeed, although being UW at 6 years old might not have major fitness consequences, it could lead to more consistent impairments at an older age, if not corrected.

Among participants taking part in the present study, we noticed that the prevalence of OW and OB (24.6%) was lower than in previous Italian studies [2,21,56,57], which is in line with the prevalence observed in northern Italy in 2016 [3]. Comparing our results with data regarding children of the same age in the same area, the general trend of OB, OW and UW appears to be slightly reducing [25]. This trend was also found in another Italian region in 2015 [56]. Nevertheless, the OB and OW prevalence in children still remains higher than the mean value observed in Europe between 2007 and 2010 [4] and needs to be reduced promptly.

Because of the alarming prevalence of OW and OB children and the low cardiorespiratory fitness levels in southern European youth [34], it is important to identify, at an early age, children who are likely to develop low levels of physical fitness to adopt appropriate measures to counter these deficiencies. Our results showed that BMI has a significant impact on physical fitness capacity and development in prepubertal children. Therefore, interventions should be primarily addressed to children with non-optimal weight status. Although some limitations must be considered when interpreting the findings of this study, such as the cross-sectional design and the limited geographical region considered, the large sample size allowed us to obtain informative data on Italian prepubertal children's physical fitness and weight status. In conclusion, the study provided evidence that sex, age, and BMI-related differences in physical fitness could be detected before puberty, and that preventing childhood overweight, obesity, and underweight in early life is extremely important to promote children's health and optimal physical fitness development, suggesting that the earlier the intervention is implemented, the more effective it will be.

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