Mediterranean diet and beyond

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MEDITERRANEAN DIET AND BEYOND: AN ITALIAN PERSPECTIVE

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MEDITERRANEAN DIET AND BEYOND: AN ITALIAN PERSPECTIVE

DISSERTATION

to obtain the degree of Doctor at Maastricht University

on the authority of the Rector Magnificus,

Prof. Dr. Rianne M. Letschert

in accordance with the decision of the Board of Deans,

to be defended in public

on Monday 1 March 2021, at 13:00 hours

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To my family

CONTENTS

Chapter 1	pag. 9
General Introduction	
Chapter 2	21
Food group consumption in an Italian population using the updated food classification system FoodEx2: Results from the Italian Nutrition & HEalth Survey (INHES) study	
Chapter 3	45
Consumption of whole grain food and its determinants in a general Italian population: Results from the INHES study	
Chapter 4	57
Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population	
Chapter 5	67
Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study	
Chapter 6	79
Egg consumption and risk of total and cause-specific mortality in a healthy Mediterranean population	
Chapter 7	113
Chili Pepper Consumption and Mortality in Italian Adults	
Chapter 8	127
Daily coffee drinking is associated with lower risks of cardiovascular and total mortality in a general Italian population: results from the Moli-sani Study	
Chapter 9	139
General Discussion	
Chapter 10	151
Valorization	
Chapter 11	157
General Conclusions	

Chapter 12	159
Summary	
Chapter 13	161
Candidate's Curriculum vitae	
Chapter 14	163
Candidate's Publications	
Chapter 15	165
Acknowledgements	

CHAPTER 1. GENERAL INTRODUCTION

In the Fifties, the American scientist Ancel Keys from the University of Minnesota, described the eating habits of countrymen in Southern Italy in this way: “... *homemade minestrone, pasta of all varieties, with tomato sauce and a sprinkling of Parmesan, only occasionally enriched with a few pieces of meat or served with a small fish of the place beans and macaroni ..., so much bread, never removed from the oven more than a few hours before being eaten, and nothing with which spread it, lots of fresh vegetables sprinkled with olive oil, a small portion of meat or fish maybe a couple of times a week and always fresh fruit for dessert* ”. This was the first description of the Mediterranean way of eating (1), later on referred to as the Mediterranean Diet (2).

Even today, the Mediterranean diet is considered the golden standard of healthy nutrition and is described as the traditional dietary pattern of the olive tree-growing areas of the Mediterranean basin, characterized by high intake of plant foods (cereals, fruits, vegetables, legumes, tree nuts, seeds and olives), olive oil as the principal source of added fat, high to moderate intakes of fish and seafood, moderate consumption of eggs, poultry and dairy products (cheese and yoghurt), low consumption of red meat and moderate intake of alcohol (mainly wine) during meals (2).

On 16 November 2010, UNESCO approved the inscription of the Mediterranean Diet in the Representative List of the Intangible Cultural Heritage of Humanity summarizing it as follows: “*The Mediterranean Diet constitutes a set of skills, knowledge, practices and traditions ranging from the landscape to the table, including the crops, harvesting, fishing, conservation, processing, preparation and, particularly, consumption of food. The Mediterranean Diet is characterized by a nutritional model that has remained constant over time and space, consisting mainly of olive oil, cereals, fresh or dried fruit and vegetables, a moderate amount of fish, dairy and meat, and many condiments and spices, all accompanied by wine or infusions, always respecting the beliefs of each community*” (3).

The healthy traditional Mediterranean dietary pattern has been popularized through a pyramid representation at the base of which are placed foods that should sustain the diet, while at the upper levels, foods to be eaten in moderate/low amounts.

The health benefits associated with the Mediterranean diet have been well established by several epidemiological studies consistently showing that adherence to this dietary pattern is

associated with a significant reduction in the risk of developing cardiovascular disease, cancer and neurodegenerative diseases as well as all-cause mortality (4).

Nevertheless, late evidence has been suggesting a global shifting in Mediterranean societies that are rapidly withdrawing from this eating pattern rather orienting their food choices towards products typical of the Western diet pattern, an unhealthy eating behavior characterized by high intake of nutrient-poor foods, refined grains, animal fats, sugars, processed meat but poor in legumes, cereals, fruits and vegetables (5).

In light of this, it is crucial to provide an estimation of the actual adherence to the Mediterranean diet among Mediterranean populations, also by offering insights on the main determinants of dietary habits.

As discussed in a recent commentary by Jeremiah Stamler, one of the pioneer researchers of the Mediterranean diet and trusty collaborator of Ancel Keys, several things have changed since the original definition of the Mediterranean diet and many problematic nutritional aspects emerged over the last decades (6). The high total energy and salt intake, greater red meat consumption are just some examples of how modern dietary habits shifted from the traditional dietary pattern.

Thus, it is particularly important to re-organize the major food groups presently included in the Italian diet in order to evaluate which are the most consumed foods and their determinants. Among them, a crucial role is played by grain products, that nowadays are almost exclusively from white flour (not whole grain flour) and whose high intake has been associated with increased risk of diabetes (7) and metabolic syndrome (8).

The Italian Nutrition & Health Survey (INHES) has offered the most up-to-date evidence on the consumption trends of major food groups consumed in Italy between 2010-2013, and also provided detailed information on major determinants associated with dietary habits, meal patterns and other factors possibly influencing diet.

In epidemiological studies, adherence to a given diet is commonly measured by *a-priori* dietary scores including a variable number of food groups or nutrients.

The theoretical advantage of studying a dietary pattern characterized by fewer food groups is that it more readily lends itself to application across populations.

The 'a priori-defined' approach is based on predefined diet quality indices using current nutrition knowledge about the relationships between food and disease (like the food pyramids). This approach is not necessarily wrong (on the contrary in most of the cases

reflects accurate information from series of valid studies), but shares the limitations of “static” evaluation that cannot incorporate actual particularities of individuals’ choices in relation to the development of a disease. Also, the *a-priori* approaches quantitatively measure the features of dietary habits that already have been recommended as ‘healthy’.

Along, with *a-priori* dietary scores, nutritional epidemiology also relies on the use of *a-posteriori* approaches also known as “empirically, or data-driven, derived dietary patterns” (9).

One key advantage of the latter approach is that it takes into account many aspects of the diet rather than focusing on a few hypothesized key food groups. Furthermore, food groups used in most *a-priori* scores are usually broadly defined and do not take into account subtle differences in the nutrients and phytochemicals in individual food items.

Since the *a posteriori* approaches (e.g. factor analysis) produce dietary patterns on the basis of available empirical data without any *a-priori* hypothesis, they might not necessarily represent optimal dietary patterns (10).

Several scores are used to measure the adherence to the Mediterranean diet. The most widely used is the one conceived by Trichopoulou and colleagues (11). The Mediterranean Diet Score is the most extensively used index due to its ease of application, and many variants have been created for the evaluation of multiple diet–health relationships.

The traditional Greek Mediterranean Diet (MDS-1) (12) originally comprised 8 food groups: 1) High ratio of monounsaturated: saturated fat; 2) Moderate alcohol intake; 3) High legume intake; 4) High intake of grains including bread and potatoes; 5) High fruit intake; 6) High vegetable intake; 7) Low intake of meat and meat products and 8) Moderate intake of milk and dairy products. The MDS-1 was based on assigning a score from 0 to 1 according to the daily intake of the eight components. In general, the medians of the sample, specific for sex, were used as cut-off points and grams per day were used as the intake measurement. A subject received a point if his intake was over the sample median for a protective component (vegetables, fruits, etc.) and below the median for non-protective components (dairy products, meat, etc.). In the case of alcohol (except when specified) 1 point was scored for males if their consumption was within 10 and 50 g/day, and within 5 and 25 g/day for women. If all the characteristics of the diet were incorporated, the highest score was obtained and reflected a greater adherence to the MD. Therefore, the MDS-1 usually ranged from 0 (minimal adherence) to 8 (maximum adherence) if the index had eight components.

Generally, a score of 4 or more was associated with satisfactory MD adherence and better health implications.

In 2003, another prospective investigation by the same group of Authors analyzed a population of 22,043 Greek adults, 20-86 years old, during a median of 44 months of follow-up (16). In this case, adherence to the traditional Mediterranean diet was assessed by the same scale developed by Trichopoulou et al. (1995) (12), but revised to include fish intake.

The addition to the Mediterranean diet score of a ninth component incorporating fish intake was deemed necessary not only because fish is an important part of the Mediterranean diet, but also because of strong evidence of an inverse correlation between fish consumption and the risk of death from coronary heart disease (13).

Due to the central importance of fish intake in the diet, and the paucity of data from Mediterranean populations, one of the papers included in this thesis (**Chapter 5**) addressed the role of this food towards the risk of developing CVD.

The Mediterranean Diet Score, or its modified versions, has been proven to be predictive of disease/mortality risk also in non-Mediterranean populations (14-16), and sometimes resulted as the best performing dietary model as compared to other healthy dietary patterns (17-19).

Along with the Greek Mediterranean Diet Score, a number of other indices were developed and many of them were conceptualized in Mediterranean countries: 1) In Greece the Mediterranean diet score proposed by Panagiotakos and colleagues (20); 2) In Italy Mediterranean Adequacy Index (MAI) by Alberti-Fidanza and colleagues (21); 3) Sofi and colleagues proposed a literature-based adherence score (22); 4) in EPICOR a new Italian Mediterranean Index developed by the authors (23) by adapting the Greek Mediterranean Index to the Italian eating behavior, with emphasis on EVOO and pasta intake.

Although including a large number of foods typical of the Mediterranean diet, the most common dietary indices measuring the Mediterranean diet do not provide any indication for some other widely consumed foods (e.g. eggs, potatoes) or beverages, such as coffee, nor quantify the use of spices which are an integral part of the Mediterranean diet.

In light of the above, it will be of interest to analyze whether foods and beverages, some already well-known for their health advantages (e.g. coffee), not included in the Mediterranean Diet Score, nor in the majority of its modified versions, are associated with health outcomes in a population with typical Mediterranean dietary habits, and also to test

whether the inclusion of these foods and beverages might improve risk prediction beyond a traditional Mediterranean Diet Score.

In order to fulfill the different purposes of this thesis, two large datasets were used; the Italian Nutrition & Health Survey (INHES) offered the opportunity to determine the actual dietary trends of consumption of major food groups and identify the main dietary patterns in Italy along with their demographic and socioeconomic determinants.

The second source of data is represented by the large population-based cohort of the Moli-sani Study, which allowed to address longitudinally the relation between diet and food items with risk of chronic disease or mortality.

The use of integrated data sources at national level (INHES Study), and from a representative Italian Mediterranean region (Moli-sani Study) offers a unique opportunity to obtain a complete and informative Italian perspective on diet and surroundings from which to confirm or to plan novel public health actions.

Therefore, the aims of this thesis are the following:

- 1) to re-organize the major food groups presently included in the Italian diet;**
- 2) to evaluate the adherence to the Mediterranean Diet in Italy and its major socioeconomic and demographic determinants;**
- 3) to address the health effects possibly associated with fish intake;**
- 4) to evaluate the association with mortality of largely consumed foods (chili pepper and eggs) and beverages (coffee) that are not included in the traditional Mediterranean Diet Score, in a large sample of the Italian population recruited in the Moli-sani Study cohort;**
- 5) to verify whether the inclusion of some of these foods/beverages into the Mediterranean Diet Score will improve its risk prediction.**

1. Assessment of food groups included in the Italian diet

The first paper here presented (**Chapter 2**) contains updated information on food group consumption in Italy by using data from the Italian Nutrition & Health Survey (INHES). The last suitable Italian public health surveillance program on nutrition has been held by the INRAN (National Institute for Research on Foods and Nutrition) covering the years 2005-06 (24). Since then, no further information has been gathered at the national level for food intake all over Italy.

The INHES study is a 3-year telephone-based survey on nutrition and health specifically designed to collect information on the dietary habits (quality, quantity and patterns), food choice determinants and food health awareness of the Italian population according to different geographical distribution (Northern, Central and Southern Italy), age, gender and socioeconomic profile. Between November 2010 and November 2013, 9,319 women and men aged ≥ 5 years from all over Italy were enrolled.

This paper identified over 70 food groups that were analyzed by gender, age group, geographical area, educational level or type of meal (e.g. breakfast, lunch).

2. Whole grain as a key component of the Mediterranean diet pyramid

Chapter 3 reports data on whole grain food intake and also addresses its major demographic, socioeconomic, psychosocial and behavioral determinants in the general Italian population of adolescents and adults recruited in the INHES Survey.

The Mediterranean diet pyramid recommends the consumption of one or two servings of cereals at each main meal (in the form of bread, pasta, rice, couscous and others), preferably whole grain (25).

The latest updated data on whole grain consumption in Italy derive from the INRAN-SCAI 2005-06, a cross-sectional survey conducted on a representative sample of 1300 households randomly selected and stratified into the four main geographical areas of Italy (North-West, North-East, Centre, South and Islands). This study showed that whole grain products, over the 3-day survey period, were consumed in only a quarter of Italian children and adults, with mean intakes being substantially lower than quantitative whole grain recommendations (26). Countries including the USA, Canada, Denmark and Australia have specific dietary guidelines on whole grain intake but others, as Italy, do not explicitly indicate the amount of whole grain to be consumed, rather they only suggest to regularly consume cereals preferably as whole grain.

3. Determinants of adherence to the Mediterranean diet

The third paper of the thesis (**Chapter 4**) investigated the actual adherence to the Mediterranean diet in the adult population recruited from the INHES study and discusses its major demographic, socioeconomic, psychosocial and behavioral factors.

More recently, a global shifting from the Mediterranean diet was documented especially in the Mediterranean regions that experienced the greatest loss of adherence as opposed to non-

Mediterranean Countries (27), to such an extent that a nutrition transition issue emerged also for Mediterranean populations (28).

In light of the above, improving the understanding of main determinants of the Mediterranean diet adherence during the 21st century is of crucial importance in order to develop effective public health strategies for prevention of major chronic diseases, especially at a time of financial crisis during which efforts and resources should be strategically delivered.

An additional key component of the bundle of foods included in the Mediterranean diet pyramid is fish, that was only added to the final version of the Mediterranean Diet Score, in 2003. However, little is known on the association of fish and cardiovascular risk within Mediterranean population settings, and available evidence dates back to men recruited in the Italian segment of the Seven Countries Study (29).

The fourth paper of this thesis (**Chapter 5**) reports the association between fish intake and risk of composite coronary heart disease (CHD) and cerebrovascular disease in the Moli-sani Study, a large population-based cohort of 24,325 men and women aged ≥ 35 years living in a Southern Italian region. In this paper a first attempt has been made to evaluate the healthy benefit of processed fish.

4. Beyond the traditional Mediterranean diet: foods, beverages and spices not included in the Mediterranean Diet Score

Generally, the following foods and beverages are not considered when assessing the adherence to the Mediterranean diet in epidemiological studies.

Eggs are a very common food in our diet, they contain are an inexpensive source of high quality protein, all B vitamins, folate, fat-soluble vitamins, as well as, several essential minerals that play a fundamental role in basic nutrition. On the other hand, eggs are a main source of cholesterol and relevant of saturated fat that are potentially associated with an increased risk of cardiovascular diseases and type 2 diabetes (30).

Spices, included chili pepper, have long been an essential part of the traditional Mediterranean diet, and are placed, along with herbs, at the base of the Mediterranean diet pyramid both for their nutritional properties and as a valuable substitute for salt (31). Health benefits of chili peppers have been ascribed to capsaicin, its major pungent compound, which has been observed to favorably improve cardiovascular function and metabolic regulation in experimental and population studies (33).

Coffee is one of the most commonly consumed non-alcoholic beverages worldwide. In general, the association between coffee consumption and a variety of conditions and diseases has been examined extensively, often with contrasting results. Although some studies reported that coffee consumption was associated with higher mortality in general population (35), most published studies have shown that regular coffee consumption is associated with a lower incidence of diabetes (34) and certain types of cancers (35), and with a lower total and CVD mortality (36, 37).

Chapters 6 and 7 investigated the association of eggs and chilies with mortality risk in a large Mediterranean population of adult men and women, and assessed whether inclusion of chili pepper is able to improve risk prediction associated with a traditional Mediterranean Diet Score.

Finally, **Chapter 8** analyzed the association of coffee, and also tested whether inclusion of coffee intake would improve risk prediction beyond the traditional Mediterranean Diet Score. These last studies help clarify the health contribution of foods and beverages largely consumed by the Italian population that are not included in a traditional Mediterranean diet score.

LIST OF PAPERS INCLUDED IN THIS THESIS

1. Pounis G, Bonanni A, **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, Bonaccio M, Cerletti C, Riccardi G, Donati MB, de Gaetano G, Iacoviello L; INHES Investigators. Food group consumption in an Italian population using the updated food classification system FoodEx2: Results from the Italian Nutrition & HEalth Survey (INHES) study. *Nutr Metab Cardiovasc Dis.* 2017 Apr; 27(4):307-328.
2. **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, Bracone F, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Bonaccio M; INHES Study Investigators. Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population. *Eur J Public Health.* 2018 Jul 17.
3. Bonaccio M, **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A, Cerletti C, Donati MB, de Gaetano G, Iacoviello L. on behalf of the Moli-sani study Investigators. Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study.

Nutr Metab Cardiovasc Dis. 2017 Oct;27(10):865-873.

4. **Ruggiero E**, Bonaccio M, Di Castelnuovo A, Bonanni A, Costanzo S, Persichillo M, Bracone F, Cerletti C, Donati MB, de Gaetano G, Iacoviello L; INHES Study Investigators. Consumption of whole grain food and its determinants in a general Italian population: Results from the INHES study. *Nutr Metab Cardiovasc Dis.* 2019 Jun;29(6):611-620.
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7. **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Bonaccio M, on behalf of the Moli-sani Study Investigators. Daily coffee drinking is associated with lower risks of cardiovascular and total mortality in a general Italian population: results from the Moli-sani Study. *J Nutr.* 2020 Dec 31; nxaa365.

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CHAPTER 2.

Food group consumption in an Italian population using the updated food classification system FoodEx2: Results from the Italian Nutrition & HEalth Survey (INHES) study

Pounis G, Bonanni A, **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, Bonaccio M, Cerletti C, Riccardi G, Donati MB, de Gaetano G, Iacoviello L; INHES Investigators

Nutr Metab Cardiovasc Dis. 2017 Apr; 27(4):307-328.

Food group consumption in an Italian population using the updated food classification system FoodEx2: Results from the Italian Nutrition & HEalth Survey (INHES) study

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Abstract *Background and Aim:* Dietary habits evolve over time, being influenced by many factors and complex interactions. This work aimed at evaluating the updated information on food group consumption in Italy.

Methods and Results: A total of 8944 (4768 women and 4176 men) participants aged >18 years from all over Italy recruited in 2010–13 (Italian Nutrition & HEalth Survey, INHES) was analyzed. The recruitment was performed using computer-assisted-telephone-interviewing and one-day 24-h dietary recall retrieved from all participants. The updated, second version, of FoodEx2 food classification system was applied to extract data on food group consumption. The participation rate was 53%; 6.2% of the participants declared to follow a special diet, the most prevalent being hypo-caloric diets (55.7% of special diets). Men compared to women presented significantly higher intakes of “grains and grain-based products”, “meat and meat products”, “animal and vegetable fats and oils and primary derivatives” and “alcoholic beverages” (P for all < 0.001); moreover, men had lower intakes of “milk and dairy products”, “water and water-based beverages” and “products for non-standard diets, food imitates and food supplements” (P for all < 0.001). Differences in food group intake among age groups, geographical regions and educational level groups were also identified (P for all < 0.05).

Conclusions: Data on the consumption of more than 70 food groups and sub-groups were illustrated in different strata. The present analysis could be considered as an updated source of information for future nutrition research in Italy and in the EU.

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Introduction

Dietary habits evolve over time, being influenced by many factors and complex interactions. Income, prices, individual preferences and beliefs, cultural traditions, as well as geographical, environmental, social and economic factors all interact in a complex manner to shape dietary consumption patterns. This indicates a specific need for the study of dietary habits of the populations.

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The traditional Mediterranean diet (MeD), an eating behavior model, with recognized health benefits in primary and secondary prevention of chronic diseases [1–4] is less followed by southern European populations in the last decades [5–7]. This occurs also in Italy and requires an updated monitoring in public health strategies.

The last suitable Italian public health surveillance program on nutrition has been held by the INRAN (National Institute for Research on Foods and Nutrition) covering the years 2005–06 [8,9]. Since then, no further information has been gathered at the national level for food intake all over Italy.

Furthermore, a serious challenge in modern nutritional epidemiology is the harmonization of the methods used for the extraction of dietary data across different populations. This will allow a better understanding of differences among samples, also in association with health outcomes and disease progression.

A major part in the harmonization of dietary assessment methodologies consists in the use of a common food classification system. Recent efforts have been carried out by the European Food Safety Authority (EFSA) to establish a largely appreciated food classification system, presented with a newly updated version of FoodEx2 [10]. To our knowledge, there is presently no large epidemiological study that has published food group consumption data elaborating such last version of FoodEx2.

Thus, this study aims at evaluating and presenting the most recently updated piece of information on the dietary habits of Italians, especially food group consumption, using data derived from the Italian Nutrition & HEalth Survey (INHES). The use of an updated, second version, of FoodEx2 food classification system adds to the novelty and originality of the present study.

Methods

Study population

The INHES study is a telephone-based survey on nutrition and health specifically designed to collect information on dietary habits (quality, quantity and patterns), food choice determinants, and food health awareness of the Italian population according to geographical distribution, age, gender and socio-economic profile. Between November 2010 and November 2013, 9319 women and men aged ≥ 5 years from all over Italy were enrolled.

Firstly, subjects ($n = 9106$) in the age range 35–79 years, recruited in the 2008–2012 wave of the Italian Cardiovascular Epidemiologic Observatory (participation rate 53%, from 40% to 85% in the different regions) [11,12] were invited to participate in the INHES survey. Once they accepted, participants were asked to invite one relative older than 79 or younger than 35 years to join the survey. Finally 5385 (59.1%) from the original population and 3754 from their relatives were included in the survey.

The sampled subjects were distributed in the four seasons (excluding Christmas, Easter and mid-August periods). The survey calendar was organized to capture an adequate proportion of weekdays and weekend days at group level.

The recruitment was performed using computer-assisted-telephone-interviewing (CATI): one-day 24-h dietary recall [9], the Italian version of the European Food Propensity Questionnaire [13], questionnaire of nutrition related behavior, anamnestic questionnaire on health status, risk factors and anthropometry and SF12 questionnaire on health perception [14] were administered.

For the purpose of the present study the adult (18–96 years) population with completed dietary information more than 50% (20 participants eliminated) of 8944 (4768 women and 4176 men) was analyzed. The INHES study was approved by the Ethical Committee of the Catholic University of Rome.

Dietary assessment

Each participant received by mail a short photographs atlas and guidance notes to estimate portion sizes (with instructions to quantify the portions used by children) developed on the basis of EPIC-SOFT picture book [15] and a hard-copy diary structured by meal, where all the information on food consumption the day before the telephone interviews was self-recorded. All foods and drinks consumed (including tap and bottled water), both at and outside home, were recorded. The day after, participants were interviewed by telephone by trained and standardized interviewers, starting from the self-recorded diary, by using a computer based 24-h dietary recall interview (24-HDR) software.

For every eating occasion, subjects were asked to carefully record and recall: time, place of consumption, detailed description of foods (or beverages), quantity consumed and brand (for manufactured foods). Portion sizes were reported by subjects with the help of a picture booklet. Moreover, it was asked if they were following a particular diet and if the consumption they had reported differed from their usual consumption.

The data management system INRAN-DIARIO 3.1 developed by INRAN [9,16] in previous surveys [9,17] was used for data coding, data entry and data processing. This software includes several checkpoints to ensure the accuracy and completeness of the data recorded and allows each interviewer to create new temporary food codes for all the food items and recipes that are not present in the databanks. Four databases were used to transform the data reported by subjects into the weight of single foods. The portions estimated by subjects with the help of the picture booklet are linked to the specific weight of each food item. Any missing food consumed during the survey was added to the food composition database.

Food group classification by applying the FoodEx2 system

All participants food consumption of single food items or recipes was “translated” by the nutrition specialist during the interview into food items or recipes included in the food list of the data management system INRAN-DIARIO 3.1. The current food list includes information for almost 2000 single food items and 1700 recipes typically consumed in Italy. All food recipes consumption was automatically divided into food items intake by the software itself. The final output-database

included information for the daily consumption of the 2000 single food items that were included in the software food list.

The classification of single food items to food groups was performed using the Exposure Hierarchy tree of the European Food Safety Authority's (EFSA's) FoodEx2 (version 2) classification system [10,18], recently available (April 2015). Two levels of food hierarchy were used and are described in the present work as "food groups" and "sub-groups".

Supplemental Table 1, illustrates the generated food groups and sub-groups for INHES dietary data including a description and the number of food items classified in each occasion.

Out of the 133 "food" and "sub-group" of the first two levels of hierarchy tree of FoodEx2 food classification system the 74 were finally elaborated (19 food groups and 55 sub-groups). Within the remaining food groups and sub-groups that are not reported in the present analysis, none of the food items of INRAN-DIARIO 3.1 were categorized or the consumption was almost zero. Also the food group "Food products for young population" was not reported in this work since we are referring to the adult population.

Qualitative food frequency consumption assessment

Beside the quantitative assessment and analysis of food group intake through the 24-h dietary recall interview (24-HDR), all participants also reported qualitative data on food frequency consumption by the elaboration of a food propensity questionnaire validated in previous studies [13,19,20]. The frequency of consumption of cooked vegetables, raw vegetables, fruits, cheese, legumes, rice, pasta, red meat, white meat, fish and milk was reported and presented as follows: 0–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week, ≥ 1 time/day.

Other factors

Body weight and height were self-reported and BMI (kg/m^2) was calculated. Since no anthropometric measurement was performed in INHES studies, these parameters are under the related self-reporting bias.

Statistical analysis

Descriptive results on food group and sub-group consumption were presented as mean, median, standard deviation, 95th and 99th percentiles in total population and in consumers separately. The analyses were also stratified by gender, age group (i.e. 18–65 years and >65 years), geographical region (i.e. North, Central and South Italy) and educational level group (i.e. elementary or secondary school, high school and university or college degree).

Normality of continuous dietary data was tested using the Shapiro–Wilk's test [21]. The results indicated that all distributions were skewed (P from Shapiro–Wilk's test for all < 0.001). This finding was also confirmed graphically. Since the data distributions were skewed, non-parametric tests were elaborated. In fact, differences in food group consumption between gender and age groups were assessed with Mann–Whitney's test and between geographical region and educational level groups using Kruskal–Wallis's test. Bonferroni correction for multiple comparisons (18 tests and significance level $\alpha = 0.05$) yielded a corrected $\alpha = 0.002$, which indicated significant differences at the aforementioned analysis.

Categorical variables were presented as frequencies and their associations were tested using Pearson's χ^2 -test. The assessment of differences between age groups for height, weight, BMI and energy intake was performed using Student's t-test since data were normally distributed.

For all food groups separately, the percentage of consumption during four meal occasions (i.e. breakfast, lunch, dinner and snack) out of the total daily consumption of each food group was calculated and presented in Figures.

Two-sided P-value < 0.05 was considered as statistically significant. STATA version 9 software was used for all calculations (STATA Corp., College Station, TX, USA).

Results

Among survey days, 89.8% were weekdays (i.e. Monday to Friday). The seasonal distribution of survey days was as

Table 1 Characteristics of INHES participants according to gender and age group.^a

	Women (N = 4768)		P-value ^b	Men (N = 4176)		P-value ^b
	18–65 years (n = 3295)	>65 years (n = 1473)		18–65 years (n = 2775)	>65 years (n = 1401)	
Educational level (%)			<0.001			<0.001
≤ 8 years	33.7	71.5		33.5	64.8	
9–13 years	44.8	22.3		48.1	28.7	
≥14 years	21.3	6.2		18.4	8.6	
Physical activity level (%)			<0.001			<0.001
Non-physically active	79.0	90.2		74.1	90.2	
Physically active	21.0	9.8		25.9	9.8	
Height (cm)	163 (6)	160 (6)	<0.001	175 (7)	170 (7)	<0.001
Weight (kg)	64.5 (12.3)	66.7 (12.0)	<0.001	80.4 (13.4)	78.1 (11.6)	<0.001
BMI (kg/m^2)	24.4 (4.6)	26.0 (4.4)	<0.001	26.3 (6.1)	26.9 (6.0)	0.001
Energy intake (kcal/day)	1834 (686)	1700 (539)	<0.001	2129 (779)	1998 (628)	<0.001

^a Continuous data are presented as mean (standard deviation) and categorical as frequencies.

^b P-value derived through comparisons between age groups using Student's t-test for continuous variables and Pearson's χ^2 t-test categorical.

Figure 1 a: The distribution of food group consumption among meals in women, expressed as percentages of total consumption for each food group in the 4 meals considered (breakfast, lunch, dinner and snack). b: The distribution of food group consumption among meals in men, expressed as percentages of total consumption for each food group in the 4 meals considered (breakfast, lunch, dinner and snack).

Table 2 Distribution of food group and sub-group consumption (g/day) in women from INHES population using FoodEx2 food classification system.

Food groups and sub-groups	Total population (N = 4768)					Consumers						
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th
Grains and grain-based products	206.7	101.8	195.4	391.1	515.4	4747	99.6	207.6	101.0	195.9	391.6	515.5
Cereals and cereal primary derivatives	32.0	57.5	0.0	174.7	250.0	2030	42.6	75.2	67.2	64.9	199.0	303.7
Bread and similar products	97.5	74.3	90.0	229.7	322.0	4274	89.6	108.8	70.2	100.0	235.6	325.3
Pasta, doughs and similar products	52.2	46.8	62.5	119.4	164.6	3165	66.4	78.6	35.0	78.3	133.2	182.3
Fine bakery wares	23.7	43.3	0.0	120.0	188.5	2099	44.0	53.9	51.2	40.0	156.3	250.0
Breakfast cereals	1.3	7.4	0.0	6.0	30.0	273	5.7	22.2	22.1	21.0	50.0	107.3
Vegetables and vegetable products	244.0	197.5	199.9	620.1	955.7	4638	97.3	250.9	196.0	203.5	624.2	956.6
Leafy vegetables	68.8	105.6	40.5	272.5	477.7	2836	59.5	115.7	115.5	81.0	364.1	514.1
Stems/stalks eaten as vegetables	11.8	43.7	0.0	89.6	230.0	623	13.1	90.3	87.0	69.0	276.0	408.6
Fruiting vegetables	69.6	113.8	24.5	300.0	501.5	2796	58.6	118.7	127.5	77.6	381.5	577.2
Root and tuber vegetables (excluding starch- and sugar-)	19.8	41.9	4.0	77.1	182.4	3898	81.8	24.2	45.1	6.2	90.0	197.0
Fungi, mosses and lichens	21.0	60.0	1.5	124.8	270.0	2997	62.9	33.5	72.8	10.8	214.5	429.0
Processed or preserved vegetables and similar	53.0	59.3	30.6	161.6	223.7	2890	60.6	87.4	52.8	94.5	179.4	237.8
Starchy roots or tubers and sugar plants	33.0	73.1	0.0	231.8	292.8	1315	27.6	119.8	94.9	103.3	269.4	376.5
Starchy roots and tubers	33.0	73.1	0.0	231.8	292.8	1315	27.6	119.8	94.9	103.3	269.4	376.5
Legumes, nuts, oilseeds and spices	22.2	55.1	0.0	145.0	281.4	1309	27.5	80.9	79.5	58.8	220.0	347.2
Legumes	21.1	54.7	0.0	145.0	281.4	977	20.5	103.0	78.7	85.8	277.0	376.3
Nuts, oilseeds and oil fruits	1.0	6.4	0.0	2.6	30.6	241	5.1	19.8	21.1	12.0	55.6	107.2
Spices	0.1	0.7	0.0	0.0	4.0	214	4.5	2.6	2.2	2.0	7.0	13.0
Fruit and fruit products	312.1	242.6	291.0	750.0	1042.5	4219	88.5	352.7	228.4	300.0	760.0	1056.4
Fruit used as fruit	239.6	176.6	230.0	548.0	800.0	4124	86.5	277.0	160.2	268.0	568.0	812.8
Processed fruit products	72.5	107.5	0.0	300.0	450.0	2259	47.4	153.0	109.9	150.0	321.6	450.0
Meat and meat products	136.6	141.7	100.0	384.7	608.0	3574	75.0	182.3	135.8	166.0	429.7	635.2
Mammals and birds meat	79.2	88.8	61.9	246.6	345.9	2680	56.2	140.8	73.1	127.5	287.1	373.4
Processed whole meat products	13.3	25.3	0.0	62.1	100.0	1593	33.4	39.9	29.3	40.0	90.2	146.2
Sausages	5.4	23.6	0.0	40.0	129.8	512	10.7	50.0	54.3	32.0	162.1	276.3
Animal mechanically separated meat (MSM)	37.3	67.5	0.0	161.3	296.6	1380	28.9	128.8	62.7	123.6	258.0	332.1
Animal offal	1.5	14.8	0.0	0.0	32.8	125	2.6	56.2	72.6	26.3	190.4	539.0
Fish, seafood, amphibians, reptiles and invertebrates	33.7	78.1	0.0	202.6	338.3	1183	24.8	135.8	103.6	144.6	303.8	540.4
Fish (meat)	20.8	57.7	0.0	172.5	215.6	638	13.4	155.1	63.3	168.6	231.4	353.1
Fish offal	0.0	0.1	0.0	0.0	0.0	1	0.0	10.0	0.0	10.0	10.0	10.0
Crustaceans	2.7	22.0	0.0	0.0	83.0	145	3.0	89.1	90.5	49.8	314.7	456.7
Molluscs	4.3	31.6	0.0	0.0	133.8	161	3.4	128.6	116.6	102.6	385.0	574.3
Fish and seafood processed	5.9	26.3	0.0	40.5	149.2	506	10.6	55.5	61.3	40.3	185.9	248.1
Milk and dairy products	166.5	119.8	150.0	376.3	518.8	4332	90.9	183.3	112.8	160.0	385.3	538.3
Milk, whey and cream	88.5	84.6	112.5	239.4	320.0	3197	67.1	132.1	70.1	135.0	262.5	337.7
Fermented milk or cream	24.6	53.4	0.0	125.0	240.0	894	18.8	131.3	94.9	125.0	250.0	254.1
Milk and dairy powders and concentrates	0.5	1.3	0.0	0.0	0.0	14	0.3	18.2	15.7	15.7	67.7	67.7
Cheese	48.4	63.4	17.6	170.0	250.0	3067	64.3	75.2	65.0	65.8	197.5	278.3
Dairy dessert and similar	5.0	26.5	0.0	38.9	100.0	268	5.6	88.0	72.0	75.0	185.7	250.0
Eggs and egg products	16.0	34.2	0.0	95.6	160.3	1583	33.2	48.0	44.6	33.0	143.3	199.4
Unprocessed eggs	15.1	18.8	10.0	45.0	78.6	3599	75.5	20.0	19.2	16.0	48.0	84.5
Sugar and similar, confectionery and water-based sweet desserts	12.3	12.4	8.0	34.0	50.5	3411	71.5	17.2	11.4	16.0	39.8	56.4
Sugar and other sweetening ingredients (excluding intensive sweeteners)	12.3	12.4	8.0	34.0	50.5	3411	71.5	17.2	11.4	16.0	39.8	56.4
Confectionery including chocolate	2.6	12.6	0.0	16.3	50.0	587	12.3	21.0	30.1	15.4	62.0	132.4

(continued on next page)

Table 2 (continued)

Food groups and sub-groups	Total population (N = 4768)					Consumers						
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th
Water-based sweet desserts	0.2	4.4	0.0	0.0	0.0	12	0.3	79.2	39.6	50.0	150.0	150.0
Animal and vegetable fats and oils and primary derivatives	34.9	18.5	32.6	66.6	89.4	4705	98.7	35.3	18.2	33.0	67.0	89.7
Animal and vegetable fats/oils	33.1	17.8	31.1	64.3	84.4	4686	98.3	33.7	17.5	31.5	64.4	84.4
Fat emulsions and blended fats	1.8	6.1	0.0	14.6	31.6	563	11.8	14.9	11.0	12.5	35.1	55.9
Fruit and vegetable juices and nectars (including concentrates)	12.7	50.7	0.0	150.0	200.0	885	18.6	68.5	100.2	12.0	202.7	400.0
Fruit/vegetable juices and nectars	12.7	50.7	0.0	150.0	200.0	885	18.6	68.5	100.2	12.0	202.7	400.0
Water and water-based beverages	591.4	327.8	640.0	1120.0	1600	4447	93.3	634.1	296.9	640.0	1140.0	1600.0
Drinking water	581.0	326.0	640.0	1120.0	1600.0	4432	93.0	625.0	294.5	640.0	1120.0	1600.0
Water based beverages	10.5	53.2	0.0	0.6	320.0	238	5.0	210.0	121.7	160.0	480.0	640.0
Alcoholic beverages	44.5	91.5	0.0	240.0	330.0	2159	45.3	98.3	115.0	80.0	320.0	456.0
Beer and beer-like beverage	4.8	37.0	0.0	0.0	320.0	110	2.3	206.2	134.3	160.0	436.0	962.6
Wine and wine-like drinks	39.4	83.1	0.0	240.0	320.1	2073	43.5	90.7	106.0	80.0	320.0	360.0
Mixed alcoholic drinks	0.1	3.7	0.0	0.0	0.0	31	0.7	19.8	42.3	0.4	154.0	160.0
Unsweetened spirits and liqueurs	0.2	3.8	0.0	0.0	0.4	69	1.4	13.9	23.7	0.8	80.0	150.0
Coffee, cocoa, tea and infusions	109.7	102.0	90.0	298.9	491.6	4354	91.3	120.1	100.7	97.5	300.8	500.0
Hot drinks and similar (coffee, cocoa, tea and herbal drinks)	109.7	102.0	90.0	298.9	491.6	4354	91.3	120.1	100.7	97.5	300.8	500.0
Products for non-standard diets, food imitates and food supplements	3.3	24.2	0.0	0.0	150.0	107	2.2	148.0	68.5	150.0	286.0	400.0
Food supplements and similar preparations	0.0	0.7	0.0	0.0	0.0	7	0.1	12.6	14.6	3.0	39.0	39.0
Meat and dairy imitates	3.3	24.2	0.0	0.0	150.0	100	2.1	157.5	60.2	150.0	307.0	400.0
Seasoning, sauces and condiments	1.5	5.3	0.0	9.3	26.4	1100	23.1	6.6	9.3	3.6	24.0	45.6
Seasonings and extracts	0.4	1.1	0.0	2.2	5.8	766	16.1	2.3	1.9	1.5	5.5	10.0
Condiments (including table-top formats)	1.2	5.1	0.0	6.0	24.8	448	9.4	12.4	11.7	8.0	36.5	58.4
Other ingredients	0.0	0.2	0.0	0.0	0.3	133	2.8	0.7	1.3	0.1	2.4	9.1
Artificial sweeteners (e.g., aspartame, saccharine)	0.0	0.2	0.0	0.0	0.3	133	2.8	0.7	1.3	0.1	2.4	9.1

Table 3 Distribution of food group and sub-group consumption (g/day) in men from INHES population using FoodEx2 food classification system.

Food groups and sub-groups	Total population (N = 4176)					Consumers				
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median
Grains and grain-based products	252.5	110.6	243.2	438.3	594.0	4165	99.7	253.2	110.0	243.5
Cereals and cereal primary derivatives	33.7	63.1	0.0	177.5	254.6	1608	38.5	83.5	75.1	75.0
Bread and similar products	126.0	86.2	115.0	280.0	389.1	3844	92.0	136.8	81.1	121.0
Pasta, doughs and similar products	65.3	49.1	78.3	140.0	190.8	3157	75.6	86.3	37.0	80.2
Fine bakery wares	26.8	48.0	0.0	120.0	200.6	1929	46.2	58.0	56.8	40.0
Breakfast cereals	0.9	5.0	0.0	0.0	30.0	162	3.9	22.1	13.1	21.0
Vegetables and vegetable products	249.5	202.7	209.6	611.7	978.7	4090	97.9	254.8	201.5	213.0
Leafy vegetables	63.9	100.8	40.5	255.0	429.3	2373	56.8	112.4	111.5	81.0
Stems/stalks eaten as vegetables	9.5	39.3	0.0	70.3	191.9	481	11.5	82.7	86.1	45.2
Fruiting vegetables	68.7	114.9	24.5	289.6	504.3	2457	58.8	116.8	129.6	75.3
Root and tuber vegetables (excluding starchy- and sugar-)	20.2	39.1	4.4	73.0	181.6	3552	85.1	23.8	41.4	6.3
Fungi, mosses and lichens	19.4	52.3	1.5	90.2	239.5	2668	63.9	30.4	69.3	10.8
Processed or preserved vegetables and similar	67.7	67.6	70.0	179.4	244.6	2835	67.9	99.7	59.4	98.0
Starchy roots or tubers and sugar plants	36.0	80.0	0.0	234.2	292.8	1118	26.8	143.4	103.4	136.8
Starchy roots and tubers	36.0	80.0	0.0	234.2	292.8	1118	26.8	143.4	103.4	136.8
Legumes, nuts, oilseeds and spices	20.9	55.1	0.0	145.0	284.7	1065	25.5	81.9	83.0	57.6
Legumes	19.8	54.7	0.0	145.0	279.0	794	19.0	104.3	83.3	85.8
Nuts, oilseeds and oil fruits	1.0	5.8	0.0	0.0	30.8	187	4.5	21.0	17.8	15.5
Spices	0.1	0.6	0.0	0.0	3.4	164	3.9	2.5	2.2	2.0
Fruit and fruit products	313.7	257.7	300.0	764.6	1089.8	3572	85.5	366.8	241.2	301.7
Fruit used as fruit	237.7	188.7	230.0	574.0	862.3	3472	83.1	285.9	170.5	274.0
Processed fruit products	76.0	113.5	0.0	300.0	450.0	1935	46.3	164.1	115.4	150.0
Meat and meat products	170.5	161.0	149.6	480.6	669.2	3353	80.3	212.3	152.9	197.4
Mammals and birds meat	97.6	100.5	100.0	285.5	394.8	2571	61.6	158.6	82.2	148.3
Processed whole meat products	16.5	28.6	0.0	80.0	116.1	581	13.9	58.5	56.4	40.0
Sausages	8.1	29.2	0.0	56.4	162.1	581	13.9	58.5	56.4	40.0
Animal mechanically separated meat (MSM)	46.7	76.8	0.0	187.0	300.4	1397	33.5	139.4	68.5	125.0
Animal offal	1.5	13.1	0.0	0.0	32.8	125	3.0	51.6	56.6	26.3
Fish, seafood, amphibians, reptiles and invertebrates	36.4	85.8	0.0	214.2	383.4	1060	25.4	143.3	117.0	147.0
Fruit used as fruit	20.8	59.0	0.0	172.5	224.2	552	13.2	157.1	69.5	169.1
Fish (meat)	0.0	0.9	0.0	0.0	0.0	4	0.1	22.5	19.0	15.0
Fish offal	0.0	0.0	0.0	0.0	0.0	5	0.0	0.0	0.0	0.0
Crustaceans	3.6	26.0	0.0	0.0	107.0	169	4.0	88.4	95.8	48.1
Molluscs	5.6	39.7	0.0	0.0	190.1	162	3.9	143.6	144.6	108.0
Fish and seafood processed	6.4	25.3	0.0	56.0	150.0	476	11.4	56.6	52.6	40.5
Milk and dairy products	150.3	121.3	135.0	362.5	500.0	3675	88.0	170.7	115.0	150.0
Milk, whey and cream	77.2	82.9	84.0	214.9	311.2	2503	60.0	128.8	69.4	135.0
Fermented milk or cream	13.0	42.0	0.0	125.0	150.0	406	9.7	133.8	44.9	125.0
Milk and dairy powders and concentrates	0.1	2.5	0.0	0.0	0.0	16	0.4	26.5	31.5	17.0
Cheese	55.3	71.7	28.0	187.2	287.5	2784	66.7	83.0	73.6	69.1
Dairy dessert and similar	4.6	29.3	0.0	15.4	100.0	234	5.6	82.4	94.8	75.0
Eggs and egg products	15.9	37.0	0.0	107.2	171.9	1285	30.8	51.7	51.1	34.0
Unprocessed eggs	15.9	37.0	0.0	107.2	171.9	1285	30.8	51.7	51.1	34.0
Sugar and similar, confectionery and water-based sweet desserts	15.2	17.0	10.8	44.5	76.0	3206	76.8	19.9	16.8	16.0
Sugar and other sweetening ingredients (excluding intensive sweeteners)	12.8	13.0	8.0	35.5	56.0	3095	74.1	17.3	12.2	16.0
Confectionery including chocolate	2.2	9.1	0.0	15.4	40.1	465	11.1	19.7	19.9	15.4

(continued on next page)

Table 3 (continued)

Food groups and sub-groups	Total population (N = 4176)					Consumers						
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th
Water-based sweet desserts	0.2	4.6	0.0	0.0	0.0	10	0.2	88.0	36.5	100.0	150.0	150.0
Animal and vegetable fats and oils and primary derivatives												
Animal and vegetable fats/oils	38.0	19.1	35.9	70.0	95.5	4142	99.2	38.2	18.9	36.1	70.0	95.6
Fat emulsions and blended fats	36.4	18.5	34.6	67.4	91.2	4133	99.0	36.8	18.2	34.8	67.5	91.7
	1.5	5.6	0.0	13.4	30.2	423	10.1	15.1	10.4	13.3	34.0	55.0
Fruit and vegetable juices and nectars (including concentrates)												
Fruit/vegetable juices and nectars	14.7	57.2	0.0	200.0	207.8	736	17.6	83.7	113.1	17.5	212.0	402.5
Water and water-based beverages												
Drinking water	14.7	57.2	0.0	200.0	207.8	736	17.6	83.7	113.1	17.5	212.0	402.5
	556.6	366.5	640.0	1120.0	1613.1	3699	88.6	628.4	326.4	640.0	1157.5	1683.7
	542.3	361.6	640.0	1120.0	1600.0	3678	88.1	615.7	321.3	640.0	1132.8	1644.2
Water based beverages	14.4	67.0	0.0	160.0	330.0	250	6.0	239.7	145.0	160.0	494.5	826.6
Alcoholic beverages												
Beer and beer-like beverage	143.2	186.4	80.4	480.0	777.1	2757	66.0	216.8	191.5	160.2	600.0	810.6
Wine and wine-like drinks	22.5	93.6	0.0	160.0	400.0	317	7.6	296.2	185.2	320.0	660.0	816.4
Mixed alcoholic drinks	119.0	162.0	0.4	400.0	640.0	2569	61.5	193.3	168.1	160.0	480.1	640.4
	0.6	9.2	0.0	0.0	0.4	56	1.3	48.0	64.1	30.0	166.0	320.0
Unsweetened spirits and liqueurs	1.1	10.1	0.0	0.0	40.0	133	3.2	35.0	44.8	12.0	120.0	212.8
Coffee, cocoa, tea and infusions												
Hot drinks and similar (coffee, cocoa, tea and herbal drinks)	103.0	85.5	97.5	257.5	404.2	3790	90.8	113.5	82.9	97.5	270.0	416.6
Products for non-standard diets, food imitates and food supplements												
Food supplements and similar preparations	103.0	85.5	97.5	257.5	404.2	3790	90.8	113.5	82.9	97.5	270.0	416.6
	0.1	2.3	0.0	0.0	0.0	35	0.8	113.5	65.9	150.0	253.0	265.0
Meat and dairy imitates	0.9	11.7	0.0	0.0	0.0	10	0.2	34.3	32.6	20.3	100.0	100.0
Seasoning, sauces and condiments												
Seasonings and extracts	1.3	4.8	0.0	8.0	24.0	832	19.9	6.6	9.0	3.0	24.0	45.6
Condiments (including table-top formats)	0.3	1.0	0.0	2.0	5.5	583	14.0	2.3	1.8	1.5	6.0	11.0
Other ingredients												
Artificial sweeteners (e.g., aspartame, saccharine)	1.0	4.7	0.0	6.0	23.8	320	7.7	13.0	11.2	10.0	36.5	60.2
	0.0	0.1	0.0	0.0	0.1	93	2.2	0.5	0.8	0.1	2.0	5.0
	0.0	0.1	0.0	0.0	0.1	93	2.2	0.5	0.8	0.1	2.0	5.0

Table 4 Distribution of food group and sub-group consumption (g/day) in adult (i.e. 18–65 years) women from INHES population using FoodEx2 food classification system.

Food groups and sub-groups	Total population (N = 3295)				Consumers			
	Mean	SD	Median	95th	n	%	Mean	SD
Grains and grain-based products	210.4	105.8	197.6	398.5	532.4	327.6	99.4	211.6
Cereals and cereal primary derivatives	35.6	61.1	0.0	177.5	256.0	1485	45.1	79.0
Bread and similar products	95.1	75.6	84.0	229.5	323.4	2899	88.0	108.1
Pasta, doughs and similar products	51.1	47.4	60.0	117.5	175.8	2145	65.1	78.5
Fine bakery wares	27.1	46.7	0.0	125.0	200.0	1589	48.2	56.2
Breakfast cereals	1.5	8.4	0.0	14.0	30.0	210	6.4	23.4
Vegetables and vegetable products	243.1	197.0	196.0	614.4	957.0	3128	94.9	248.9
Leafy vegetables	67.6	106.0	40.5	270.2	500.1	1955	59.3	114.0
Stems/stalks eaten as vegetables	11.3	42.8	0.0	85.0	226.2	438	13.3	85.0
Fruiting vegetables	71.2	114.2	26.7	300.0	511.3	1950	59.9	120.3
Root and tuber vegetables (excluding starch/- and sugar-)	19.9	43.9	3.9	79.1	192.4	2672	81.1	24.5
Fungi, mosses and lichens	20.3	60.5	1.4	110.9	283.0	2074	62.9	32.2
Processed or preserved vegetables and similar	52.8	59.0	30.6	162.5	220.4	1976	60.0	88.0
Starchy roots or tubers and sugar plants	33.4	74.8	0.0	234.2	292.8	865	26.3	127.3
Starchy roots and tubers	33.4	74.8	0.0	234.2	292.8	865	26.3	127.3
Legumes, nuts, oilseeds and spices	21.8	52.7	0.0	145.0	231.6	910	27.6	78.9
Legumes	20.5	52.3	0.0	145.0	230.7	667	20.2	101.5
Nuts, oilseeds and oil fruits	1.1	7.0	0.0	4.1	32.0	176	5.3	20.9
Spices	0.1	0.7	0.0	0.0	4.0	158	4.8	2.5
Fruit and fruit products	282.0	228.2	260.0	711.5	976.2	2834	86.0	327.9
Fruit used as fruit	221.1	172.0	200.0	536.0	777.0	2757	83.7	264.2
Processed fruit products	61.0	98.2	0.0	300.0	360.0	1425	43.2	140.8
Meat and meat products	143.7	144.6	113.2	406.8	629.1	2531	76.8	187.0
Mammals and birds meat	83.0	90.7	73.2	252.7	354.3	1914	58.1	142.9
Processed whole meat products	14.2	26.6	0.0	70.0	104.7	1126	34.2	41.5
Sausages	6.0	25.0	0.0	40.0	135.6	372	11.3	52.6
Animal mechanically separated meat (MSM)	39.4	69.6	0.0	164.6	296.6	1000	0.0	129.7
Animal offal	1.2	11.0	0.0	0.0	26.3	76	2.3	51.1
Fish, seafood, amphibians, reptiles and invertebrates	35.0	81.3	0.0	210.8	360.4	846	25.7	136.1
Fish (meat)	20.5	57.5	0.0	172.5	215.6	440	13.4	153.8
Fish offal	0.0	0.2	0.0	0.0	0.0	1	0.0	0.0
Crustaceans	3.0	22.4	0.0	0.0	107.0	112	3.4	87.4
Molluscs	5.1	34.6	0.0	0.0	150.0	129	3.9	130.2
Fish and seafood processed	6.3	27.7	0.0	40.8	150.1	378	11.5	55.2
Milk and dairy products	165.3	122.7	150.0	378.4	548.0	2972	90.2	183.2
Milk, whey and cream	85.5	84.5	112.5	234.6	320.0	2144	65.1	131.4
Fermented milk or cream	25.7	54.7	0.0	125.0	150.0	642	19.5	132.1
Milk and dairy powders and concentrates	0.1	1.4	0.0	0.0	0.0	10	0.3	19.5
Cheese	48.3	64.3	17.0	170.0	255.0	2115	64.2	75.3
Dairy dessert and similar	5.7	29.7	0.0	50.0	100.0	203	6.2	92.0
Eggs and egg products	16.6	35.4	0.0	100.7	171.4	1154	35.0	47.5
Unprocessed eggs	16.6	35.4	0.0	100.7	171.4	1154	35.0	47.5
Sugar and similar, confectionery and water-based sweet desserts	16.2	20.3	12.00.0	47.8	82.6	2541	77.1	21.0
Sugar and other sweetening ingredients (excluding intensive sweeteners)	12.9	12.9	8.0	36.0	55.2	2396	72.7	17.7
Confectionery including chocolate	3.1	14.1	0.0	20.0	55.5	470	14.3	21.5

(continued on next page)

Table 4 (continued)

Food groups and sub-groups	Total population (N = 3295)					Consumers						
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th
Water-based sweet desserts	0.2	5.0	0.0	0.0	0.0	10	0.3	80.0	42.2	50.0	150.0	150.0
Animal and vegetable fats and oils and primary derivatives	36.0	19.1	33.5	68.4	99.8	3254	98.8	36.4	18.8	33.8	69.0	100.2
Animal and vegetable fats/oils	34.0	18.4	31.9	66.0	87.7	3238	98.3	34.5	18.0	32.2	66.0	88.0
Fat emulsions and blended fats	2.0	6.6	0.0	15.3	32.6	434	13.2	15.1	11.6	12.5	36.3	59.4
Fruit and vegetable juices and nectars (including concentrates)	14.0	52.4	0.0	136.7	200.0	670	20.3	68.6	98.8	12.0	205.0	400.0
Fruit/vegetable juices and nectars	14.0	52.4	0.0	136.7	200.0	670	20.3	68.6	98.8	12.0	205.0	400.0
Water and water-based beverages	597.6	335.7	640.0	1138.0	1600.0	3070	9.3	641.4	304.7	640.0	1140.0	1625.8
Drinking water	584.3	333.6	640.0	1120.0	1600.0	3058	92.8	629.6	302.4	640.0	1137.6	1608.2
Water based beverages	13.3	59.4	0.0	160.0	320.0	205	6.2	213.0	119.0	160.0	480.0	640.0
Alcoholic beverages	39.0	90.5	0.0	240.0	360.0	1409	42.8	91.1	120.1	48.0	320.0	480.1
Beer and beer-like beverage	5.5	41.2	0.0	0.0	200.0	84	2.5	217.0	144.1	160.0	495.0	1000.0
Wine and wine-like drinks	33.0	79.3	0.0	208.0	320.1	1338	40.6	81.3	107.5	0.6	320.0	360.2
Mixed alcoholic drinks	0.2	4.5	0.0	0.0	0.0	29	0.9	20.7	43.6	0.4	155.0	160.0
Unsweetened spirits and liqueurs	0.3	4.5	0.0	0.0	0.4	57	1.7	15.0	30.8	0.4	84.0	150.0
Coffee, cocoa, tea and infusions	112.6	104.7	97.5	298.0	490.2	2887	87.6	124.2	103.2	100.0	306.5	496.5
Hot drinks and similar (coffee, cocoa, tea and herbal drinks)	112.6	104.7	97.5	298.0	490.2	2887	87.6	124.2	103.2	100.0	306.5	496.5
Products for non-standard diets, food imitates and food supplements	4.0	27.1	0.0	0.0	150.0	88	2.7	151.3	72.0	150.0	315.5	400.0
Food supplements and similar preparations	0.0	0.8	0.0	0.0	0.0	6	0.2	14.2	15.4	8.0	39.0	39.0
Meat and dairy imitates	4.0	27.0	0.0	0.0	150.0	82	2.5	161.3	63.6	150.0	318.5	400.0
Seasoning, sauces and condiments	1.6	5.4	0.0	10.0	27.4	724	22.0	7.2	9.7	5.0	24.6	47.4
Seasonings and extracts	0.3	1.1	0.0	2.2	5.5	465	14.1	2.4	1.9	1.9	5.5	10.3
Condiments (including table-top formats)	1.2	5.3	0.0	8.0	26.1	334	10.1	12.2	12.0	8.0	36.5	60.5
Other ingredients	0.0	0.3	0.0	0.0	0.5	80	2.4	0.9	1.6	0.2	3.4	12.0
Artificial sweeteners (e.g., aspartame, saccharine)	0.0	0.3	0.0	0.0	0.5	80	2.4	0.9	1.6	0.2	3.4	12.0

Table 5 Distribution of food group and sub-group consumption (g/day) in adult (i.e. 18–65 years) men from INHES' population using FoodEx2 food classification system.

Food groups and sub-groups	Total population (N = 2775)					Consumers				
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median
Grains and grain-based products	259.3	115.0	249.3	456.6	612.7	2766	99.7	260.1	114.2	250.0
Cereals and cereal primary derivatives	38.8	67.2	0.0	180.8	269.0	1147	41.3	93.7	76.1	80.5
Bread and similar products	124.1	86.9	110.7	278.1	390.0	2528	91.1	136.2	81.5	121.0
Pasta, doughs and similar products	64.5	50.3	78.3	140.0	196.8	2077	74.8	86.1	38.8	83.4
Fine bakery wares	31.0	52.7	15.0	125.0	220.3	1439	51.9	59.7	60.4	40.0
Breakfast cereals	1.0	5.4	0.0	0.0	30.0	129	4.6	22.0	12.6	21.0
Vegetables and vegetable products	247.5	200.4	207.5	605.2	956.6	2719	98.0	252.6	196.3	212.1
Leafy vegetables	61.3	95.3	39.7	250.0	429.0	1557	56.1	109.3	104.7	81.0
Stems/stalks eaten as vegetables	9.2	39.0	0.0	69.0	202.5	323	11.6	78.8	87.1	40.5
Fruiting vegetables	71.2	116.1	26.9	300.0	500.5	1677	60.4	117.9	129.6	76.5
Root and tuber vegetables (excluding starchy- and sugar-)	19.6	39.3	4.4	73.8	181.3	2316	83.5	23.5	42.0	5.7
Fungi, mosses and lichens	18.0	52.7	1.5	78.0	233.0	1727	62.2	29.0	64.4	10.8
Processed or preserved vegetables and similar	68.2	69.3	68.8	179.6	241.1	1882	67.8	100.6	61.8	98.5
Starchy roots or tubers and sugar plants	37.2	82.9	0.0	234.2	305.0	700	25.2	147.3	104.9	152.0
Starchy roots and tubers	37.2	82.9	0.0	234.2	305.0	700	25.2	147.3	104.9	152.0
Legumes, nuts, oilseeds and spices	20.5	54.7	0.0	145.0	278.5	697	25.1	81.5	83.3	61.0
Legumes	19.4	54.4	0.0	144.9	276.4	512	18.5	105.1	84.1	85.8
Nuts, oilseeds and oil fruits	1.0	6.0	0.0	0.0	35.2	127	4.6	21.5	18.2	16.0
Spices	0.1	0.7	0.0	0.0	3.5	109	3.9	2.5	2.2	2.0
Fruit and fruit products	284.9	250.7	274.0	735.1	1052.3	2294	82.7	344.6	235.4	300.0
Fruit used as fruit	218.7	185.6	187.0	550.0	850.2	2211	79.7	274.5	167.2	256.0
Processed fruit products	66.2	106.5	0.0	300.0	412.0	1192	43.0	154.2	113.4	150.0
Meat and meat products	177.7	168.9	153.6	500.0	719.0	2257	81.3	218.5	161.7	198.8
Mammals and birds meat	100.6	105.2	100.0	296.6	415.6	1705	61.4	163.7	87.5	149.6
Processed whole meat products	17.7	30.2	0.0	80.0	120.2	1060	3.8	46.2	32.6	40.0
Sausages	9.1	31.5	0.0	68.6	162.1	398	14.3	63.3	59.1	40.0
Animal mechanically separated meat (MSM)	49.4	80.2	0.0	200.0	320.5	969	34.9	141.5	73.5	125.0
Animal offal	1.0	9.9	0.0	0.0	32.8	59	2.1	45.6	51.1	26.3
Fish, seafood, amphibians, reptiles and invertebrates	36.7	89.5	0.0	214.3	413.2	717	25.8	142.2	126.4	130.5
Fish (meat)	19.4	57.3	0.0	172.5	219.7	353	12.7	152.8	73.5	168.6
Fish offal	0.0	1.1	0.0	0.0	0.0	4	0.1	22.5	19.0	15.0
Crustaceans	3.8	27.2	0.0	0.0	108.4	120	4.3	88.5	98.6	48.1
Molluscs	6.6	44.4	0.0	0.0	212.9	116	4.2	158.1	152.6	120.0
Fish and seafood processed	6.8	26.0	0.0	56.0	150.0	343	12.4	55.3	52.5	40.5
Milk and dairy products	149.9	126.1	135.0	369.8	517.7	2424	87.4	171.6	120.3	150.0
Milk, whey and cream	73.6	82.3	32.0	206.4	320.0	1598	57.6	127.7	69.6	125.1
Fermented milk or cream	14.6	45.3	0.0	125.0	150.0	296	10.7	137.2	49.3	125.0
Milk and dairy powders and concentrates	0.1	2.8	0.0	0.0	0.0	11	0.4	26.6	36.7	14.0
Cheese	56.1	74.2	28.0	192.5	290.6	1849	66.6	84.2	76.9	67.5
Dairy dessert and similar	5.5	33.6	0.0	26.2	100.0	182	6.6	83.6	103.5	75.0
Eggs and egg products	16.4	38.0	0.0	107.2	178.6	899	32.4	50.7	52.1	33.1
Unprocessed eggs	16.4	38.0	0.0	107.2	178.6	899	32.4	50.7	52.1	33.1
Sugar and similar, confectionery and water-based sweet desserts	16.5	18.3	14.0	47.8	86.3	2183	78.7	21.0	18.2	16.0
Sugar and other sweetening ingredients (excluding intensive sweeteners)	13.5	13.5	9.0	36.1	56.6	2090	75.3	17.9	12.8	16.0
Confectionery including chocolate	2.8	10.6	0.0	18.8	45.6	374	13.5	21.0	21.4	15.4

(continued on next page)

Table 5 (continued)

Food groups and sub-groups	Total population (N = 2775)					Consumers							
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th	
Water-based sweet desserts	0.2	5.1	0.0	0.0	0.0	6	0.2	105.0	34.0	100.0	150.0	150.0	
Animal and vegetable fats and oils and primary derivatives													
Animal and vegetable fats/oils	39.0	19.8	37.1	72.1	99.2	2750	9.9	39.4	19.5	37.3	72.2	99.5	
Fat emulsions and blended fats	37.3	19.1	35.4	68.0	95.9	2742	98.8	37.8	18.8	35.7	68.1	96.0	
Fruit and vegetable juices and nectars (including concentrates)													
Fruit/vegetable juices and nectars	17.3	63.6	0.0	200.0	212.0	512	91.1	94.0	121.5	19.2	214.8	546.8	
Water and water-based beverages													
Drinking water	583.0	374.8	640.0	1213.0	1692.4	2494	74.8	648.6	337.2	640.0	1280.0	1760.0	
Water based beverages	564.4	368.9	640.0	1140.0	1664.8	2478	51.9	632.0	331.1	640.0	1225.8	1692.1	
Alcoholic beverages													
Beer and beer-like beverage	129.3	186.7	0.4	480.0	800.0	1710	98.0	209.8	199.2	160.0	546.8	897.8	
Wine and wine-like drinks	28.1	99.2	0.0	320.0	415.4	265	56.1	294.1	157.8	320.0	660.0	806.8	
Mixed alcoholic drinks	99.2	156.3	0.2	329.0	640.0	1548	11.6	177.9	182.7	160.0	480.0	640.4	
Unsweetened spirits and liqueurs	0.7	10.3	0.0	0.0	0.4	37	60.4	52.7	72.7	30.0	212.0	320.0	
Coffee, cocoa, tea and infusions													
Hot drinks and similar (coffee, cocoa, tea and herbal drinks)	1.3	11.1	0.0	0.0	40.0	93	83.5	37.4	48.7	12.0	120.0	240.0	
Products for non-standard diets, food imitates and food supplements													
Food supplements and similar preparations	108.0	89.0	97.5	270.0	430.6	2510	62.2	119.4	86.0	120.0	270.0	450.0	
Meat and dairy imitates	1.0	11.9	0.0	0.0	0.0	26	18.5	104.9	65.4	105.0	242.3	265.0	
Seasoning, sauces and condiments													
Seasonings and extracts	0.9	11.5	0.0	0.0	0.0	18	3.9	132.8	56.3	150.0	265.0	265.0	
Condiments (including table-top formats)	1.3	4.9	0.0	10.0	24.0	496	82.7	7.5	9.2	5.5	25.3	45.7	
Other ingredients													
Artificial sweeteners (e.g., aspartame, saccharine)	0.3	0.9	0.0	2.0	5.1	307	21.7	2.3	1.8	1.9	6.2	10.0	
	1.1	4.7	0.0	8.2	23.9	233	71.0	12.8	10.7	10.0	36.1	56.1	
	0.0	0.1	0.0	0.0	0.1	43	43.0	0.4	0.5	0.1	1.5	2.0	
	0.0	0.1	0.0	0.0	0.1	43	43.0	0.4	0.5	0.1	1.5	2.0	

Table 6 Distribution of food group and sub-group consumption (g/day) in elderly (i.e. ≥65 years) women from INHES population using FoodEx2 food classification system.

Food groups and sub-groups	Total population (N = 1473)					Consumers				
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median
Grains and grain-based products	198.4	91.6	190.7	370.6	478.8	1471	99.9	198.7	91.3	190.7
Cereals and cereal primary derivatives	24.0	47.4	0.0	118.0	202.8	545	37.0	65.0	58.5	56.4
Bread and similar products	102.8	71.1	95.0	220.3	322.0	1375	93.3	110.2	67.9	100.0
Pasta, doughs and similar products	54.6	45.3	70.0	122.5	155.2	1020	69.8	78.8	32.4	78.3
Fine bakery wares	16.2	33.3	0.0	85.5	160.0	510	34.6	46.7	42.1	32.0
Breakfast cereals	0.8	4.3	0.0	0.0	30.0	63	4.3	18.2	11.3	19.0
Vegetables and vegetable products	246.2	198.5	201.4	624.7	934.1	1420	96	255.4	196.3	205.1
Leafy vegetables	71.5	104.8	39.5	274.8	445.2	881	59.8	119.6	112.3	81.0
Stems/stalks eaten as vegetables	12.9	45.6	0.0	115.6	250.0	185	12.6	102.7	86.0	85.0
Fruiting vegetables	66.0	112.7	18.2	301.2	480.7	846	57.4	115.0	128.4	76.5
Root and tuber vegetables (excluding starch/- and sugar/-)	19.6	37.0	4.4	73.9	180.1	1226	83.2	23.6	39.4	6.3
Fungi, mosses and lichens	22.7	58.6	1.9	136.4	252.2	923	62.7	36.2	70.7	12.0
Processed or preserved vegetables and similar	53.4	60.2	30.6	153.0	226.8	914	62.1	86.1	55.1	94.5
Starchy roots or tubers and sugar plants	32.2	69.2	0.0	196.1	283.4	450	30.5	105.5	89.3	70.7
Starchy roots and tubers	32.2	69.2	0.0	196.1	283.4	450	30.5	105.5	89.3	70.7
Legumes, nuts, oilseeds and spices	22.3	60.1	0.0	150.2	289.8	399	27.1	85.5	89.3	70.7
Legumes	22.3	59.7	0.0	146.1	289.8	310	21.0	106.0	90.0	86.7
Nuts, oilseeds and oil fruits	0.7	4.7	0.0	0.0	20.0	65	4.4	17.0	15.7	12.0
Spices	0.1	0.6	0.0	0.0	4.0	56	3.8	2.8	1.8	2.0
Fruit and fruit products	379.5	259.9	321.6	831.2	1148.5	1385	94.0	403.6	249.2	355.0
Fruit used as fruit	281.1	179.5	277.7	574.0	825.7	1367	92.8	302.9	167.7	291.0
Processed fruit products	98.4	122.1	21.6	303.5	450.0	834	56.6	173.9	115.0	150.0
Meat and meat products	120.9	133.7	76.3	335.2	593.3	1043	70.8	170.7	129.3	153.6
Mammals and birds meat	70.6	83.8	41.4	216.0	309.3	766	52.0	135.8	68.2	127.5
Processed whole meat products	11.4	22.1	0.0	60.0	100.0	467	31.7	35.9	23.6	35.0
Sausages	4.1	19.9	0.0	20.0	117.6	140	9.5	43.1	50.0	23.1
Animal mechanically separated meat (MSM)	32.6	62.2	0.0	148.3	265.4	380	25.8	126.4	56.0	123.6
Animal offal	2.1	20.8	0.0	0.0	52.5	49	3.3	62.3	95.9	26.3
Fish, seafood, amphibians, reptiles and invertebrates	31.0	70.4	0.0	187.5	291.9	337	22.9	135.3	86.8	147.0
Fish (meat)	21.3	58.1	0.0	172.5	215.6	198	13.4	158.1	59.0	169.1
Fish offal	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
Crustaceans	2.1	20.7	0.0	0.0	49.9	33	2.2	94.7	103.7	48.1
Molluscs	2.7	23.3	0.0	0.0	107.3	32	2.2	122.3	103.1	101.9
Fish and seafood processed	5.0	22.7	0.0	32.7	133.1	128	8.7	56.7	54.8	40.5
Milk and dairy products	169.3	113.2	150.4	375.7	478.8	1360	92	183.4	106.3	162.8
Milk, whey and cream	95.3	84.3	112.5	326.6	105.3	71.5	133.3	69.9	135.0	269.4
Fermented milk or cream	22.1	50.6	0.0	125.0	155.2	252	17.1	129.5	32.3	125.0
Milk and dairy powders and concentrates	0.0	0.9	0.0	0.0	0.0	4	0.3	15.1	7.3	15.0
Cheese	48.5	61.3	20.0	167.0	250.0	952	64.6	75.1	61.8	67.5
Dairy dessert and similar	3.3	17.1	0.0	0.0	100.0	65	4.4	75.3	35.2	75.0
Eggs and egg products	14.3	31.3	0.0	91.6	143.3	429	29.1	49.3	40.7	34.0
Unprocessed eggs	14.3	31.3	0.0	91.6	143.3	429	29.1	49.3	40.7	34.0
Sugar and similar, confectionery and water-based sweet desserts	12.7	14.4	8.0	37.8	64.5	1058	71.8	17.6	14.2	16.0
Sugar and other sweetening ingredients (excluding intensive sweeteners)	11.0	11.2	8.0	32.0	48.0	1015	68.9	16.0	10.0	16.0
Confectionery including chocolate	1.5	8.2	0.0	9.0	30.0	117	7.9	18.8	22.8	12.5

(continued on next page)

Table 6 (continued)

Food groups and sub-groups	Total population (N = 1473)					Consumers						
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th
Water-based sweet desserts	0.1	3.0	0.0	0.0	0.0	2	0.1	75.0	35.4	75.0	100.0	100.0
Animal and vegetable fats and oils and primary derivatives												
Animal and vegetable fats/oils	32.4	16.8	30.6	62.6	76.9	1451	99.9	33.0	16.5	31.0	62.6	77.0
Fat emulsions and blended fats	31.2	16.4	29.4	60.4	76.0	1448	98.3	31.7	16.0	29.7	60.8	76.1
	1.3	4.8	0.0	12.2	23.5	129	8.8	14.3	9.0	13.5	32.6	53.7
Fruit and vegetable juices and nectars (including concentrates)												
Fruit/vegetable juices and nectars	10.0	46.7	0.0	24.0	200.0	215	6.9	68.3	104.7	12.0	200.0	485.7
Water and water-based beverages												
Drinking water	577.5	309.1	640.0	1120.0	1450.0	1377	34.6	617.8	278.0	640.0	1120.0	1491.0
	573.2	307.9	640.0	1120.0	1440.0	1374	4.3	614.5	276.1	640.0	1120.0	1450.0
Water based beverages	4.3	34.9	0.0	0.0	160.0	33	9.6	191.5	137.8	160.0	640.0	640.0
Alcoholic beverages												
Beer and beer-like beverage	56.9	92.5	0.1	240.4	320.4	750	59.8	111.7	103.4	120.0	320.0	360.2
Wine and wine-like drinks	3.0	25.4	0.0	0.0	160.0	26	12.6	171.2	89.5	160.0	375.5	400.0
Mixed alcoholic drinks	53.8	89.4	0.0	240.1	320.2	735	57.4	107.8	101.0	120.0	320.0	320.4
Unsweetened spirits and liqueurs	0.0	0.3	0.0	0.0	0.0	2	83.2	6.2	8.2	6.2	12.0	12.0
	0.0	1.5	0.0	0.0	0.0	12	62.7	8.4	15.1	1.1	40.0	40.0
Coffee, cocoa, tea and infusions												
Hot drinks and similar (coffee, cocoa, tea and herbal drinks)	103.1	95.4	75.0	300.0	500.0	1367	62.1	111.1	94.4	75.0	300.0	500.0
Products for non-standard diets, food imitates and food supplements												
Food supplements and similar preparations	103.1	95.4	75.0	300.0	500.0	1367	62.1	111.1	94.4	75.0	300.0	500.0
Meat and dairy imitates	1.7	16.0	0.0	0.0	127.8	19	4.4	132.7	48.3	150.0	200.0	200.0
	0.0	0.1	0.0	0.0	0.0	1	3.8	3.0	0.0	3.0	3.0	3.0
Seasoning, sauces and condiments												
Seasonings and extracts	1.7	16.0	0.0	8.0	24.9	18	94.0	140.0	37.8	150.0	200.0	200.0
Condiments (including table-top formats)	1.4	4.9	0.0	2.3	5.9	376	31.8	5.7	8.4	2.0	24.0	48.0
	0.4	1.2	0.0	6.0	24.0	301	84.7	2.1	1.8	1.4	5.8	10.0
Other ingredients												
Artificial sweeteners (e.g., aspartame, saccharine)	1.0	4.7	0.0	2.3	5.9	114	56.6	13.1	11.2	8.5	36.5	59.7
	0.0	0.1	0.0	0.0	0.3	53	70.8	0.4	0.5	0.1	1.4	2.5
	0.0	0.1	0.0	0.0	0.3	53	70.8	0.4	0.5	0.1	1.4	2.5

Table 7 Distribution of food group and sub-group consumption (g/day) in elderly (i.e. ≥65 years) men from INHES population using FoodEx2 food classification system.

Food groups and sub-groups	Total population (N = 1401)					Consumers						
	Mean	SD	Median	95th	99th	n	%	Mean	SD	Median	95th	99th
Grains and grain-based products	239.1	100.0	226.9	410.4	534.0	1399	99.9	239.4	99.6	227.0	410.4	534.0
Cereals and cereal primary derivatives	23.8	52.8	0.0	127.3	233.6	461	32.9	72.3	70.5	59.6	192.5	285.8
Bread and similar products	129.6	84.7	120.0	285.0	380.0	1316	93.9	138.0	80.5	120.0	295.1	388.3
Pasta, doughs and similar products	66.8	46.6	78.3	140.0	178.4	1080	7.7	86.7	33.0	80.0	142.6	183.3
Fine bakery wares	18.4	36.3	0.0	80.0	176.4	490	35.0	52.6	44.5	40.0	136.6	250.0
Breakfast cereals	0.5	4.2	0.0	0.0	22.5	33	2.4	22.8	15.3	21.0	53.8	62.5
Vegetables and vegetable products	253.4	207.0	212.0	621.6	1005.1	1371	97.9	259.0	205.8	214.7	624.4	1017.0
Leafy vegetables	69.0	110.7	40.5	276.0	453.4	816	58.2	118.4	123.3	81.0	375.1	628.7
Stems/stalks eaten as vegetables	10.2	40.0	0.0	80.9	191.9	158	11.3	90.6	83.5	69.0	250.0	451.0
Fruiting vegetables	63.8	112.3	13.4	277.8	516.6	780	55.7	114.6	129.7	74.3	373.1	600.0
Root and tuber vegetables (excluding starchy- and sugar-)	21.5	38.8	5.6	72.1	181.7	1236	88.2	24.4	40.4	7.2	84.7	189.0
Fungi, mosses and lichens	22.2	65.3	1.9	118.4	297.6	941	67.2	33.1	77.4	11.8	190.6	429.0
Processed or preserved vegetables and similar	66.7	64.0	70.9	179.4	255.7	953	68.0	98.0	54.3	94.5	195.4	265.8
Starchy roots or tubers and sugar plants	33.6	74.1	0.0	234.2	292.8	418	29.8	112.7	97.4	71.7	292.8	394.5
Starchy roots and tubers	33.6	74.1	0.0	234.2	292.8	418	29.8	112.7	97.4	71.7	292.8	394.5
Legumes, nuts, oilseeds and spices	21.7	55.7	0.0	145.0	289.8	368	26.3	82.6	82.4	55.3	250.7	339.8
Legumes	20.7	55.3	0.0	145.0	289.8	282	20.1	103.0	82.1	85.8	289.8	364.8
Nuts, oilseeds and oil fruits	0.9	5.4	0.0	0.0	25.0	60	4.3	20.0	17.2	12.3	62.3	96.0
Spices	0.1	0.6	0.0	0.0	3.4	55	3.9	2.4	2.1	2.0	6.8	10.0
Fruit and fruit products	370.8	262.0	318.0	836.0	1129.4	1278	91.2	406.5	246.4	360.0	854.4	1200.0
Fruit used as fruit	275.4	189.2	274.0	600.0	874.9	1261	90.0	305.9	174.4	300.0	610.0	889.9
Processed fruit products	95.5	123.8	14.6	300.0	464.3	743	53.0	180.0	117.0	150.0	350.0	612.1
Meat and meat products	156.2	142.8	139.4	402.3	596.3	1096	78.2	199.6	132.0	195.1	429.8	633.5
Mammals and birds meat	91.8	90.5	100.0	255.0	340.5	866	61.8	148.6	69.3	140.7	296.6	366.6
Processed whole meat products	14.2	25.2	0.0	75.0	100.0	489	34.9	40.6	27.3	40.0	86.2	120.3
Sausages	6.3	23.8	0.0	40.0	161.4	183	13.1	48.0	48.5	32.0	162.1	207.0
Animal mechanically separated meat (MSM)	41.2	69.3	0.0	161.3	296.6	428	30.5	134.9	55.5	125.0	255.0	322.5
Animal offal	2.7	17.8	0.0	0.0	100.0	66	4.7	57	60.9	26.3	190.2	347.8
Fish, seafood, amphibians, reptiles and invertebrates	35.7	78.1	0.0	215.1	300.0	343	24.5	145.7	94.2	150.0	299.3	475.0
Fish (meat)	23.4	62.0	0.0	172.5	235.8	199	14.2	164.7	61.3	171.5	290.8	353.7
Fish offal	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0	0.0
Crustaceans	3.1	23.2	0.0	0.0	107.0	49	3.5	88.2	89.6	57.8	294.5	403.8
Molluscs	3.5	28.2	0.0	0.0	120.0	46	3.3	106.9	115.7	80.1	350.0	630.1
Fish and seafood processed	5.7	24.0	0.0	40.5	114.4	133	9.5	59.9	53.0	49.0	175.2	238.2
Milk and dairy products	151.0	111.3	139.2	359.0	468.0	1251	89.3	169.1	104.0	150.0	364.4	481.6
Milk, whey and cream	84.5	83.6	112.5	225.0	309.9	905	64.6	130.8	68.9	135.0	250.0	361.0
Fermented milk or cream	9.8	34.5	0.0	125.0	125.0	110	7.9	124.8	28.3	125.0	250.0	361.0
Milk and dairy powders and concentrates	0.1	1.9	0.0	0.0	0.0	5	0.4	26.2	18.8	22.6	50.8	50.8
Cheese	53.7	66.3	26.3	180.0	277.5	935	66.7	80.5	66.6	70.0	200.5	328.5
Dairy dessert and similar	3.0	18.2	0.0	0.0	95.4	52	3.7	78.2	55.5	75.0	250.0	250.0
Eggs and egg products	14.9	35.1	0.0	113.9	171.6	386	27.6	54.0	48.6	34.0	158.1	189.2
Unprocessed eggs	14.9	35.1	0.0	113.9	171.6	386	27.6	54.0	48.6	34.0	158.1	189.2
Sugar and similar, confectionery and water-based sweet desserts	12.7	13.4	8.0	36.6	61.0	1023	73.0	17.3	12.8	16.0	40.0	66.2
Sugar and other sweetening ingredients (excluding intensive sweeteners)	11.5	11.6	8.0	32.0	50.5	1005	71.7	16.1	10.7	16.0	36.0	54.0
Confectionery including chocolate	1.0	4.5	0.0	5.0	24.0	91	6.5	14.3	10.7	12.5	37.6	50.0

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Table 7 (continued)

Food groups and sub-groups	Total population (N = 1401)						Consumers						
	Mean	SD	Median	95th	99th		n	%	Mean	SD	Median	95th	99th
Water-based sweet desserts	0.2	3.5	0.0	0.0	0.0		4	0.3	62.5	25.0	50.0	100.0	100.0
Animal and vegetable fats and oils and primary derivatives													
Animal and vegetable fats/oils	35.7	17.5	34.1	67.4	84.6		1392	99.4	36.0	17.3	34.2	67.5	84.8
Fat emulsions and blended fats	34.6	17.1	32.9	65.4	84.2		1391	99.3	34.8	17.0	33.0	65.6	84.3
	1.2	4.7	0.0	8.7	25.8		116	8.3	14.0	9.5	12.5	34.0	48.0
Fruit and vegetable juices and nectars (including concentrates)													
Fruit/vegetable juices and nectars	9.6	41.1	0.0	24.0	200.0		224	16.0	60.1	86.8	12.0	200.0	387.5
Water and water-based beverages													
Drinking water	504.4	343.8	480.0	1030.7	1470.6		1205	86.0	586.4	298.8	640.0	1120.0	1560.5
Water based beverages	498.4	342.7	480.0	1005.0	1479.6		1200	0.9	582.0	297.5	640.0	1113.5	1564.7
	6.0	38.1	0.0	0.0	160.0		42	3.0	197.7	104.0	160.0	330.0	640.0
Alcoholic beverages													
Beer and beer-like beverage	170.6	182.8	160.0	480.3	743.8		1047	74.7	228.3	177.6	240.0	640.0	800.0
Wine and wine-like drinks	11.4	80.0	0.0	0.0	330.0		52	3.7	306.7	289.0	200.0	696.0	2000.0
Mixed alcoholic drinks	158.0	165.8	160.0	480.0	640.4		1021	72.9	216.7	158.0	160.7	480.4	640.4
Unsweetened spirits and liqueurs	0.5	6.6	0.0	0.0	5.9		19	1.4	38.6	42.7	40.0	160.0	160.0
Coffee, cocoa, tea and infusions													
Hot drinks and similar (coffee, cocoa, tea and herbal drinks)	0.8	7.4	0.0	0.0	40.0		40	2.9	29.0	33.9	21.0	120.0	120.0
	93.2	77.4	65.0	242.3	360.0		1280	9.1	102.0	75.2	75.0	250.0	360.0
Products for non-standard diets, food imitates and food supplements													
Food supplements and similar preparations	93.2	77.4	65.0	242.3	360.0		1280	9.1	102.0	75.2	75.0	250.0	360.0
Meat and dairy imitates	0.9	12.1	0.0	0.0	0.0		9	0.6	138.4	64.4	150.0	250.0	250.0
Seasoning, sauces and condiments													
Seasonings and extracts	0.9	12.1	0.0	0.0	0.0		8	0.6	155.0	43.8	150.0	250.0	250.0
Condiments (including table-top formats)	1.3	4.8	0.0	6.0	25.0		336	24.0	5.3	8.6	2.0	20.9	45.4
	0.4	1.2	0.0	2.3	5.5		276	19.7	2.2	2.0	1.4	5.5	11.2
	0.8	4.5	0.0	6.0	23.3		87	6.2	13.5	12.7	10.0	44.0	75.3
Other ingredients													
Artificial sweeteners (e.g., aspartame, saccharine)	0.0	0.2	0.0	0.0	0.2		50	3.6	0.6	1.0	0.1	2.6	5.0
	0.0	0.2	0.0	0.0	0.2		50	3.6	0.6	1.0	0.1	2.6	5.0

follows: 30.7% in autumn, 23.5% in winter, 25.1% in spring and 20.7% in summer.

The participants' general characteristics according to gender and age group are presented in Table 1. In both genders the elderly population seemed to be less educated and physically active with higher BMI compared to the younger population (P for all < 0.05). Both women and men aged 18–65 years reported higher total energy intake than the elderly population (P for both < 0.001).

As many as 6.2% ($n = 555$) of the INHES participants declared that they were following a special diet. Hypocaloric diets used for the reduction of body weight represented 55.7% ($n = 309$), while diets for the primary or secondary prevention of diseases (i.e. diabetes, hypertension) were also followed.

In addition, 7.8% ($n = 702$) of the participants reported that their food consumption the day prior to the interview-day was different from usual (i.e. higher or lower) due to different reasons (i.e. special dinner with guests, celebrations, reduced food intake due to clinical tests etc.).

Regarding meal consumption, 97.6%, 99.1%, 99.0 and 30% of the INHES population reported having breakfast, lunch, dinner and snack during the day, respectively. The most frequent meal time occasion was 7:00–7:30am for

breakfast (39.5%), before 1:00pm for lunch (63.8%) and 8:00–8:30pm for dinner (51.9%), while snacking time varied.

The distribution of food group consumption among meals in women and men is illustrated in Fig. 1a and b, respectively. In both genders “vegetable and vegetable products”, “starchy roots or tubers and sugar plants”, “fish, seafood, amphibians, reptiles and invertebrates” and “seasoning, sauces and condiments” were only consumed during lunch and dinner. In addition, “legumes, nuts, oil seeds and spices”, “meat and meat products”, “animal and vegetable fats and oils and primary derivatives”, “water and water based beverages” and “alcoholic beverages” were mainly consumed over lunch and dinner.

“Grains and grain-based products”, “fruit and fruit products” and “fruit and vegetable juices and nectars” were mostly consumed over lunch and dinner but sometimes also at breakfast or as snack choices.

On the contrary, “milk and dairy products”, “eggs and egg products”, “sugar and similar, confectionery and water based sweet dessert”, “coffee, cocoa and tea and infusions”, “products for non-standard diets, food imitates and food supplements and artificial sweeteners” were mainly consumed over breakfast, less for lunch and dinner and sometimes as snack choice.

Figure 2 **a:** The frequency of food group consumption using data from the food propensity questionnaire in the women population. **b:** The frequency of food group consumption using data from the food propensity questionnaire in the men population.

Detailed descriptive results on food group and sub-group consumption stratified by gender and age group are presented in [Tables 2–7](#). In addition, similar descriptive analyses stratified by gender, geographical origin and education level are included in [Supplemental Tables 2–13](#).

The highest number of consumers was observed for “grains and grain-based products”, “vegetable and vegetable products” and “animal and vegetable fats and oils and primary derivatives” in both genders.

[Supplemental Table 14](#) presents the P-values derived from the comparisons in food group intake between genders, age groups, geographical regions and education level groups.

Men compared to women presented statistically significant higher intakes of “grains and grain-based products”, “meat and meat products”, “animal and vegetable fats and oils and primary derivatives” and “alcoholic beverages” (P for all $< 0.001 < 0.002$ = Bonferroni corrected p-value for 18 comparisons), ([Tables 2 and 3](#) and [Supplemental Table 14](#)); men had also lower intakes of “milk and dairy products”, “water and water-based beverages”, and “products for non-standard diets, food imitates and food supplements” (P for all $< 0.001 < 0.002$ = Bonferroni corrected p-value for 18 comparisons), ([Tables 2 and 3](#) and [Supplemental Table 14](#)).

The analysis of differences between age groups (i.e. 18–65 years, >65 years) in both men and women indicated significantly higher consumption of different food groups in adults compared to the elderly (P for all $< 0.001 < 0.002$ = Bonferroni corrected p-value for 18 comparisons), ([Tables 4–7](#), and [Supplemental Table 14](#)).

Furthermore, statistically significant geographical differences in food group consumption were observed in both genders ([Supplemental Tables 2–7 and 14](#)). Interestingly, both women and men from Southern Italy reported greater consumption of “grains and grain-based products” and “meat and meat products” (P for all $< 0.001 < 0.002$ = Bonferroni corrected p-value for 18 comparisons); while North sample population had greater intake of “coffee, cocoa, tea and infusions” (P for all $< 0.001 < 0.002$ = Bonferroni corrected p-value for 18 comparisons).

In addition, food group consumption differed among educational level groups ([Supplemental Tables 8–13 and 14](#)). Notably, participants (both women and men) from the highest educational level group had higher intake of “animal and vegetable fats and oils and primary derivatives”, “fruit and vegetable juices and nectars”, “coffee, cocoa, tea and infusions” and “products for non-standard diets, food imitates and food supplements” (P for all $< 0.001 < 0.002$ = Bonferroni corrected p-value for 18 comparisons).

Qualitative results on food frequency consumption

[Figure 2a](#) and [b](#), illustrate the frequency of food group consumption using data that were derived through the food propensity questionnaire in women and men INHES participants, correspondingly.

Raw vegetables, fruits, pasta and milk were among the most frequently consumed food groups on a daily basis (i.e. ≥ 1 time/day) in both genders. As many as 18.3% and 17.6% of women and men respectively reported to consume raw vegetables ≥ 1 time/day while the same percentage for fruits were 80.1% and 78.6% (P for sex differences for both > 0.05).

Discussion

The INHES project

Despite the importance of monitoring dietary habits in public health perspectives, according to the best of our knowledge, there has been no study in the last decade evaluating at an epidemiological level the dietary patterns in Italy. Our findings illustrate a comprehensive descriptive analysis of food group consumption using recent data coming from all over Italy. Results by gender, age group, geographical region and educational level group or type of meal were generated to provide multi-dimensionality in our approach. The elaboration of accurate dietary assessment methodologies and the newly updated EFSA's FoodEx2 food classification system [10] add to the novelty of the present work.

The last effort for an accurate evaluation of dietary habits at a national level in Italy was performed in 2005–06, when INRAN retrieved data from 1329 households corresponding to 3323 women and men [9]. Food group consumption data were collected at individual level in 3 consecutive days using hard-copy diaries structured by meal and the data management system INRAN-DIARIO 3.1. The researchers published in 2008 a descriptive analysis of food group intake, presenting results by gender and age group after the elaboration of a self-developed food classification system [9].

Other nationwide Italian food consumption surveys have been carried out. The first approach was performed at household level and was held by INRAN in the early 80's [22]. In the 90's, the EPIC (European Prospective Investigation into Cancer and Nutrition) study collected and presented data from 1990 to 1995 based on single 24-HDR from different regions in Italy [23]. Almost at the same time period, the INN-CA Study group (1994–1996) published nationwide descriptive results on food consumption derived from food data forms (diaries and inventories) on 1147 households, corresponding to 2734 male and female individuals [24].

On the contrary, our data have been collected until very recently (2012) and it could be considered, at the moment, as the most updated study at a national Italian level. In addition, our dietary assessment methodology consisted mainly in 24-HDR interviews assisted by the data management system INRAN-DIARIO 3.1 and it is relevant and comparable to those used in the most recent study of INRAN on 2005–06 [9].

Moreover, a significant point of originality of the INHES project and strength of this work was the larger sample

size compared to previous studies [9], covering the majority of geographical and socio-economical strata.

Furthermore, to the best of our knowledge, this is the first study at European level that elaborated EFSA's FoodEx2 classification system, recently available in April 2015 [10]. This is another strength that applies to the modern meta-analytic needs in epidemiological studies. In fact, the use of a common food classification system among studies in different EU or other populations contributes to the harmonization of the dietary data and makes them better comparable for meta-analysis.

Despite the serious importance of its application, the categorization of food items into food groups or sub-groups of FoodEx2 was still challenging. On the one hand, the EFSA's classification system includes several levels of hierarchy and various tools to assist the categorization of foods items into groups such as facet descriptors. On the other hand, main aim of the application of a food classification system is to merge the epidemiological information into food groups that later on can be easily applied to statistical or other tests and models. Keeping a balance between the analytic perception of FoodEx2 and the needs of summarizing information into food groups in epidemiological analysis is of high importance for current and future research in the field.

The elasticity that FoodEx2 provides to the epidemiological researcher in choosing different levels of hierarchy and various tools to do so, proved to be very useful in its application to real data. In the present analysis, the first two levels of hierarchy of FoodEx2 were applied providing information on a large range of 74 food groups and sub-groups. In addition, it is still possible to use a more detailed or specific approach in food categorization and presentation of data, by using various facets descriptors or levels of hierarchy, according to the aim of the study (e.g. the low or whole fat content in milk and dairies that is not described in the first two levels of FoodEx2 hierarchy requires the analysis of these data in other hierarchy levels).

Comparison of INHES project results with INRAN's study results of 2005–06

Despite the fact that INRAN's latest published data on food group consumption were based on a different food classification system (i.e. self-developed) [9], it is still very important to record the major differences of them compared to the present results. This comparison will take into account the variability evolved in food group consumption during the last decade in Italy.

Considering the adult populations (i.e. 18–65 years), both male and female INHES participants reported higher intakes of meat and meat products and lower consumption of “fish and seafood, amphibians, reptiles and invertebrates”, “coffee, cocoa, tea and infusions” and “alcoholic beverages” compared to the INRAN populations [9]. This finding confirms in part the hypothesis that Italians tend to shift their dietary habits over the last years out of the traditional Mediterranean diet.

Studying the latest guidelines published by the World Health Organization [25] for the restriction in red meat and processed red meat intake, the observed increase in “meat and meat products” consumption over time is of particular concern in a public health perspective for the prevention of chronic diseases.

It is known that a concept of low carbohydrate and high protein diet against obesity in Italy has emerged [6–8]. This could also in part explain the increase in the consumption of “meat and meat products” while these food groups are the main sources of protein in such dietary patterns.

On the other hand, according to our data, other important protein sources such as “fish and seafood, amphibians, reptiles and invertebrates” seemed to be less consumed over time by both women and men. Several studies [26,27] have strengthened the importance of fish intake in the primary and secondary prevention of cardiovascular disease especially due to their high content in n–3 polyunsaturated fatty acids. The latter have multiple health protective biological actions in human metabolism [26,28,29] and their high intake is important for public health promotion.

Furthermore, the decrease in “coffee, cocoa and tea and infusions” consumption is also of some concern, since they are major sources of antioxidant phytochemicals compounds, especially polyphenols. The latter have been recently studied for their role in modulating low-grade inflammation [30,31] and disease prevention [32].

On the other hand, quite optimistic findings are the higher consumption of fruits, legumes and nuts observed in the female INHES adult population compared with the corresponding consumption of female INRAN participants [9].

Focusing on the elderly women INHES population (i.e. >65 years), the intake of “grain and grain-based products”, “milk and dairy products”, “egg and eggs products” and “coffee, cocoa, tea and infusions” was lower than the corresponding INRAN participants. The decrease in such food groups over time is critical considering the special needs of the elderly for energy, protein and antioxidants [33]. Interestingly, meat and meat products and alcoholic beverages were higher in both elderly women and men within the INHES population compared to INRAN participants [9]. This needs further study in relation to the development and progression of chronic diseases.

Differences in food group consumption in INHES population among different strata

The variations observed in food group consumption between genders, geographical regions and educational level groups in the INHES population were interesting, too.

Among gender various differences, the higher intake of “meat and meat products” in men and the lower intake of “grains and grain-based products” in women were notable.

Interestingly, participants from South Italy reported greater consumption of “meat and meat products” while only North sample population had greater intake of “coffee, cocoa, tea and infusions”.

In addition, participants at the highest educational level group had higher intake of “fruit and vegetable juices and

nectars", "coffee, cocoa, tea and infusions" and "products for non-standard diets, food imitates and food supplements".

In general, the investigation of variations in food group intake between genders, educational level groups or geographical regions gain important meaning in public health perspectives and applied nutrition policy. Differences in food group intake could result in variations also in the dietary patterns of population groups.

Regarding gender differences, men naturally have greater needs for energy and nutrients compared to women. This fact, in combination with other lifestyle, socio-economic or even clinical factors (alteration of diet due to the presence of a disease) could result in significant gender variations.

In addition, geographical differences in food group intake could be mainly attributed to the socio-economic variations presented between Northern and Southern regions Italy but also on other factors such as culinary traditions that have a historical character.

Furthermore, the presented variations among educational groups confirm somehow the recent study of our research group on the impact of socio-economic status on dietary habits [7,34]. In fact, the analysis of data from 23,000 women and men participants of the Moli-sani cohort, living in the Molise region in Italy, showed that higher socio-economic status, especially greater income, was associated with healthier food choices through the adherence to the Mediterranean diet [34]. In addition, within the same population it was observed that at the time of economic crisis in Italy (i.e. 2007–2010), people with higher income or at higher educational level seemed to adhere better to the Mediterranean diet [7].

All together, these considerations underline the need for further investigation of factors affecting differences among various strata.

Furthermore, interesting results were derived from the analysis of the distribution of food group intake among meals. Notably, both women and men seemed to avoid or to eat less "vegetable and vegetable products", "nuts, oil seeds and spices", "fruit and fruit products" and "fruit and vegetable juices and nectars" during breakfast or snack meals.

On the other hand, "milk and dairy products", "eggs and egg products", "coffee, cocoa and tea and infusions", "products for non-standard diets, food imitates and food supplements" were less consumed for lunch and dinner.

The systematic absence of vegetables, fruits and nuts from breakfast or snack needs further attention considering the health benefits offered by their consumption in the prevention of chronic diseases [35,36]. In addition, the inclusion of "milk, dairy products" and "eggs and egg products" as lunch or dinner choices in combination with other food groups could result in an appropriate intake of protein, vitamin D and calcium especially in populations with special requirements [33]. It has also to be noted that the pattern of consuming "eggs and egg products" mainly for breakfast meal may be considered as a westernized influence on Italian dietary patterns.

Comparison of INHES project results with EPIC study's EU means

The comparison of the food group consumption data of the present work with the corresponding EU means may be relevant if one considers that they come from Italy, i.e. from a modern Mediterranean population.

Available accurate data on EU pooled means of food group consumption collected in different country samples (Spain, Greece, Italy, France, Germany, The Netherlands, United Kingdom, Denmark, Sweden and Norway) has been provided by the EPIC study [23]. Both women and men within the INHES population presented higher mean intakes of fruits, vegetables, vegetable oils and red or white meat and lower intake of processed meat and milk.

It is important to point out that the methodology of extracted food group data in INHES and EPIC studies differs especially in terms of the food classification system that was elaborated in each case. This limits somehow the accurate comparison of data while stresses the need for harmonized efforts in food group consumption evaluation and analysis.

Strengths

Among significant points of novelty and originality were the dietary assessment methodologies used, the INHES sample size and the elaboration of the FoodEx2 food classification for the first time in a EU population. The stratification of the descriptive analysis by gender, age group, geographical area, educational level and meal distribution illustrated in a more specific way the results for different study populations.

Limitations

Beyond the relevance of the findings of the present work, limitations do exist. First, bias of over- or under-estimation by the use of the dietary methodologies and for the extracted data should be acknowledged. In addition, two 24-HDRs of two non-consecutive days are appreciated as a homogeneous dietary assessment methodology for adults in EU. The one 24-HDR could not be considered as representative of the dietary habits of an individual and may widow the results not comparable with other European countries. Furthermore, the telephone-based interviews are under the limitation of misreporting or under/over-estimation of food consumption.

Conclusions

In conclusion, this study presents the most recent data on food group consumption in an Italian sample. The data on the consumption of more than 70 food groups and sub-groups that were studied and illustrated by gender, age group, geographical region and educational level group or type of meal, could be considered as a good source of information for future nutrition research in Italy and in EU.

Conflict of interest

None declared.

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Appendix

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2017.01.004>.

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CHAPTER 3.

Consumption of whole grain food and its determinants in a general Italian population: Results from the INHES study

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Consumption of whole grain food and its determinants in a general Italian population: Results from the INHES study

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Abstract *Background and aims:* Whole grain (WG) food consumption is associated with lower risk of cardiovascular disease, cancer and neurological diseases. The aim of this study was to assess the consumption of WG food and its major demographic, socioeconomic, psychosocial and behavioral determinants in a general Italian population.

Methods and results: Data were from the Italian Nutrition & Health Survey (INHES), a telephone-based survey established in 2010–2013 including 9422 participants aged ≥ 5 years from all over Italy. WG food intake was assessed by the European Food Propensity Questionnaire and included bread, pasta, breakfast cereals, biscuits and WG soups. WG consumption was categorized as none, occasional (< 1 time/week) and regular (≥ 1 time/week). Overall, 26.9% of the sample reported a regular consumption of WG food (27.2% of adults aged 20–97 y, and 21.9% of children/adolescents aged 5–19 y). In both age-groups, the major food source contributing to total WG intake was WG bread followed by WG pasta. Among adults, greater consumption of WG was associated with healthier lifestyle (e.g. sport activity), and higher educational level. Eating meals outside of the house in adults, and spending > 2 h/day watching TV in children/adolescents were inversely associated with WG intake.

Conclusions: The percentage of WG consumers in Italy in 2010–2013 appears to be quite low and still below that recorded in other countries of Europe where consumption is frequently over 50 percent. WG consumption is likely to be influenced by socioeconomic status and is associated with a number of psychosocial factors, meal patterns and eating-related behaviors.

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Introduction

In numerous epidemiological studies consumption of whole grain (WG) products is associated with lower risk of chronic diseases such as coronary heart disease [1], stroke [2], type 2 diabetes [3] and colon-rectal cancer [4]. Moreover, habitual consumption of WG foods may contribute to

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improved adiposity indices in children and adults [5] and appears to exert benefits also for cardiovascular risk markers, such as blood pressure, insulin sensitivity and plasma cholesterol [6,7]. WG consumption of any population is difficult to study, as presently, there is no universally accepted definition of WG [8].

Recently, the Health Grain Forum, a not-for-profit consortium of academics and industry working with cereal foods, suggested that a food may be labeled as “whole grain” if it contains >30% whole-grain ingredients in the overall product and more whole grain than refined grain ingredients, both on a dry-weight basis [9]. Moreover, countries including the USA, Canada, Denmark and Australia have specific dietary guidelines on WG intake but others, as Italy, do not explicitly indicate the amount of WG to be consumed, rather they only suggest to regularly consume cereals preferably whole [10].

In particular, in the US the recommended daily consumption is of at least 85 g/d [11] for adults and 42 g/d for children [12], while in Denmark is set at 75 g/10 MJ (2388 kcal) per day [13].

The Mediterranean diet pyramid recommends the consumption of one or two servings of cereals at each main meal (in the form of bread, pasta, rice, couscous and others), preferably whole grain [14].

The latest updated data on WG consumption in Italy derive from the INRAN-SCAI 2005–06, a cross-sectional survey conducted on a representative sample of 1300 households randomly selected and stratified into the four main geographical areas of Italy (North-West, North-East, Centre, South and Islands). This study showed that WG products, over the 3-day survey period, were consumed in only a quarter of Italian children and adults, with mean intakes being substantially lower than quantitative WG recommendations [15].

The aim of this study was twofold: first, to assess the WG intake in a large sample of the Italian population recruited all over Italy within the Italian Nutrition & Health Survey (INHES); second, to investigate major demographic, socioeconomic, psychosocial and behavioral factors possibly associated with the consumption of WG foods, in order to provide new evidence for appropriate intervention strategies at population level.

Methods

Study population

The INHES Study is a 3-year telephone-based survey on nutrition and health specifically designed to collect information on dietary habits (quality, quantity and meal patterns), food choice determinants, and food health awareness of the Italian population according to different geographical distribution (Northern, Central and Southern Italy), age, gender and socioeconomic status.

Between November 2010 and November 2013, 9422 women and men aged ≥ 5 years from all over Italy were enrolled. A full description of the study design is reported in the [supplementary material](#).

For the purpose of the present study, we omitted subjects with missing information on WG consumption (3.1%) or missing values for major variables of interest (1.6%). A total of 8421 adults (aged >19 y) and 561 young subjects (aged ≤ 19 y) were finally included in the analyses.

The INHES Study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee of the Catholic University of Rome. Verbal informed consent was obtained from all subjects and was witnessed and formally recorded.

Dietary assessment

Data on food intake were collected through the Italian version of the European Food Propensity Questionnaire according to methods described elsewhere [16].

Total WG food intake was estimated by using information from five different WG food groups: WG bread, WG pasta, WG breakfast cereals, WG biscuits (including also crackers and breadsticks) and WG soups. For each food item, frequency of consumption was collected by the following question: “How frequently do you eat (e.g.) WG pasta?” and possible answers were: ‘never’, ‘once a month or less frequent’, ‘less than 3 times a month’, ‘2–3 times/month’, ‘1–2 times a week’, ‘3–4 times a week’, ‘5–6 times a week’, ‘every day’, ‘more than 1 time/day’. The total consumption of WG was obtained by summing the frequency (converted into times/week) of consumption of each WG food group. The study population was eventually categorized as non-WG food consumers, consuming WG < 1 time/week and consuming WG ≥ 1 time/week.

Adherence to the Mediterranean diet in the adult sample was evaluated by using the MedDietScore as proposed by Panagiotakos [17] including 10 food groups (fruits, vegetables, legumes, potatoes, fish, red meat, poultry, dairy products, alcoholic beverages, olive oil). As compared to the original score, we did not include WG cereals.

The final score potentially ranged from 0 to 50, the latter reflecting maximal adherence. For analysis purposes, the score was also used as exact tertiles.

Adherence to the Mediterranean diet in the young sample was evaluated by using the KIDMED index (Mediterranean Diet Quality Index) for children and teenagers [18], classified as follows: good (≥ 6 points, indicating an optimal Mediterranean diet); average (4–5 points); and poor (≤ 3 points).

The reasons for consumption of WG products was assessed through the question “What do you think about WG products?” with four possible answers (‘they are healthier than regular ones’, ‘they are more natural’, ‘other’, ‘do not know’). Data on reasons for non-consumption of WG was obtained by the question “Why don’t you eat WG products?” with four possible answers (‘dislike’, ‘lack of knowledge’, ‘hard to cook’ or ‘other’). The questionnaire on consumption of WG was administered to a random subgroup of the sample ($n = 5805$).

Meal patterns

Meal patterns comprised either patterning of main meals (breakfast, lunch, dinner) or context of main meals, such as meals eaten out of the home, or eating meals in front of the television or when using PC. Information on daily amount of time spent in watching TV/using PC was also collected and categorized as $\leq$ or > 2 h/d.

Socioeconomic and psychosocial factors

Education was based on the highest qualification attained and was categorized as up to primary school (corresponding to ≤ 5 years of study), lower secondary ($> 5 \leq 8$ years), upper secondary ($> 8 \leq 13$ years), post-secondary (> 13 years).

Present occupation was assembled into 6 groups: manual, non-manual, housewife, retired, student and unemployed. Marital status was categorized as married/living in a couple, single, separated/divorced and widowed.

Self-rated health was assessed by the question "How would you rate your health status?" and possible answers ranged from 'very good' to 'bad' [19]. Adverse life events (yes/no) during the last year included questions related to health (e.g. serious illness or death), job (e.g. retired, job loss), or interpersonal situations (e.g. divorce, problem with friend or relative).

Information on occupational and family-related stress was obtained by the question "How often have you experienced stress in your workplace/family?" with answers ranging from 'never' to 'always'. Financial-related stress was rated as none/little, moderate and a lot. Job control was considered as none/little, moderate-good, fairly good and not working/unascertained.

Assessment of covariates

Previous diagnosis of diabetes, hypercholesterolemia, hypertension, cardiovascular disease and cancer was self-reported and categorized as no/yes. In adults, body mass index was calculated by using self-reported measurements of height and weight, calculated as kg/m^2 and grouped into three categories as normal (< 25), overweight ($\geq 25 < 30$) or obese (≥ 30). BMI in children/adolescents was categorized according to specific values for children considering sex and age [20]. Engagement in sport activity was considered as a dichotomous variable (yes/no).

Statistical analysis

Main characteristics of the study population across categories of weekly WG food consumption were presented as means with standard deviation (SD), or percentages.

Associations for categorical or continuous variables were evaluated by using general linear models adjusted for age groups (see Table 1 for definition of age groups) and sex (PROC GENMOD and PROC GLM in SAS for categorical and continuous variables, respectively).

Using multinomial ordered multivariable logistic regression analysis, odds ratios (ORs) with corresponding

95% confidence intervals (95%CI) were calculated to estimate the association of 3-level WG consumption (none vs occasional vs regular) with demographic and socioeconomic factors (Tables 2 and 3), meal patterns and eating-related behaviours (Table 4) or psychosocial factors (Table 5).

Dummy variables for missing values of each variable of interest were created. Two-sided P-value < 0.05 was considered as statistically significant.

The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows[®]2009. SAS Institute Inc. and SAS are registered trademarks of SAS Institute Inc., Cary, NC, USA.

Results

Adults

Overall, 27.2% of adults (20–97 y) reported a regular consumption (≥ 1 time/week) of WG products (Table 1).

Among adults, regular WG intake was more prevalent in middle-aged subjects (50–64 years), in women and in subjects from Northern Italy and within those having a higher educational level (Table 1). Moreover, regular WG consumption was higher in adults with a non-manual occupation, and among single or separated/divorced, non-smokers, and for those engaged in some physical activity or with normal body weight (Table 1). Adherence to the Mediterranean diet was slightly higher in non-consumer/occasional consumers (Table 1).

Supplementary Figure 1A shows the percent contribution from five different WG food sources, (i.e. bread, pasta, cereals, biscuits, soup) to the total WG intake. The major food sources were represented by WG bread (53.3%) and WG biscuits (27.4%), followed by WG pasta (13.1%), while only 4.8% and 1.3% were contributed by WG breakfast cereals and WG soups, respectively.

Table 2 reports the association of demographic and socioeconomic factors with WG consumption in adult subjects, with occasional or regular WG food intake as opposed to non-consumers.

A clear educational gradient in WG intake emerged, with subjects with highest educational attainment (post-secondary) reporting higher odds of being either occasional (OR = 1.90; 95%CI 1.58–2.27) or regular consumers (OR = 1.59; 1.30–1.95). WG intake was also related to occupation, marital status and engagement in sport activities (Table 2).

Having breakfast out of home (OR = 0.59; 0.46–0.76 and OR = 0.43; 0.31–0.60, for occasional and regular WG consumption, respectively) was inversely associated with WG food intake, as compared to having breakfast at home, and the same was true for individuals having lunch out of home and for those having meals while watching TV (Table 4; Model 2b).

Finally, poor self-reported health status was inversely associated with regular WG food intake (OR = 0.44; 0.38–0.51) as well as moderate financial circumstances-

Table 1 Characteristics of the adult population (20–97 years) according to consumption of whole grain foods.

	Whole grain products intake				Unadjusted <i>p</i> value
	Whole sample	Non-consumers	Occasional consumers (<1 times/week)	Regular consumers (≥1 times/week)	
N (%)	8421 (100)	4656 (55.3)	1475 (17.5)	2290 (27.2)	—
Age groups (y)					<0.0001
20–34	6.4	6.6	6.9	5.6	
35–49	28.4	26.3	31.7	30.8	
50–64	33.0	30.9	37.2	34.6	
65–74	20.8	22.7	17.6	18.9	
≥75	11.4	13.5	6.6	10.0	
Sex					<0.0001
Women	53.3	47.5	58.8	61.3	
Men	46.7	52.5	41.2	38.7	
Geographical area					<0.0001
Northern	40.5	39.8	36.9	44.2	
Central	16.7	16.1	20.3	15.5	
Southern	42.8	44.1	42.8	40.3	
Educational level					<0.0001
Up to primary	17.7	21.1	12.1	14.1	
Lower secondary	26.3	28.1	24.7	23.8	
Upper secondary	39.8	38.0	42.3	41.8	
Post-secondary	16.2	12.8	20.9	20.3	
Occupation					<0.0001
Non-manual	31.1	26.2	37.6	36.7	
Manual	18.0	19.1	18.0	15.9	
Housewife	11.0	10.9	11.5	10.8	
Retired	35.2	38.8	29.1	31.9	
Student	1.8	1.9	1.7	1.6	
Unemployed	2.9	3.1	2.1	3.1	
Marital status					<0.0001
Married/in couple	75.1	75.3	75.3	74.5	
Single	14.6	13.9	15.9	15.4	
Separated/divorced	3.2	2.5	3.5	4.5	
Widowed	7.1	8.3	5.3	5.6	
Smoking habit					<0.0001
No	59.7	56.2	59.7	66.8	
Yes	15.9	17.9	15.2	12.4	
Former	22.4	23.9	23.3	18.6	
Occasional	2.0	2.0	1.8	2.2	
Sport activity					<0.0001
No	81.6	84.3	78.8	77.8	
Yes	18.4	15.7	21.2	22.2	
Body mass index					0.0015
Normal weight	48.1	46.5	48.1	51.4	
Overweight	38.3	39.1	38.6	36.6	
Obese	13.6	14.4	13.3	12.0	
Cardiovascular disease	3.2	3.9	2.0	2.7	0.0002
Cancer	3.4	3.4	3.1	3.7	0.66
Hypertension	31.8	33.2	28.4	31.2	0.0023
Diabetes	7.6	7.3	7.2	8.4	0.22
Hypercholesterolemia	21.9	21.6	21.4	22.7	0.49
MedDietScore ^a	28.7 (3.6)	28.8 (3.5)	28.9 (3.5)	28.5 (3.9)	0.0046

Values are reported as percentages.

^a MedDietScore (not including whole grain foods) is reported as means with standard deviations.

related stress (OR = 0.55; 0.40–0.76), and higher family-related stress (OR = 0.59; 0.40–0.87; [Table 5](#), Model 2).

Children/adolescents

Among children/adolescents (5–19 years), 21.9% reported a regular consumption (≥1 times/week) of WG products ([Table 3](#)).

In this sample, female gender and Southern Italy geographical area were major determinants of regular WG intake ([Table 3](#)).

In agreement with what observed for adults, the major food source was represented by WG bread (42.3%), followed by WG biscuits (20.2%), WG breakfast cereals (15.7%) and WG pasta (15.8%) whereas WG soups contributed to 6.0% of the total WG intake ([Supplementary Figure 1A](#)).

Table 2 Demographic and socioeconomic factors associated with whole grain food consumption in the adult population (20–97 years).

	Occasional consumers (n = 1475) vs non-consumers (n = 4656)		Regular consumers (n = 2290) vs non-consumers (n = 4656)	
	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 1 OR (95%CI)	Model 2 OR (95%CI)
Age groups (y)				
20–34	-1-	-1-	-1-	-1-
35–49	1.28 (1.06–1.54)	1.44 (1.13–1.84)	1.34 (1.08–1.66)	1.70 (1.29–2.23)
50–64	1.24 (1.03–1.50)	1.59 (1.23–2.05)	1.28 (1.03–1.58)	1.84 (1.38–2.45)
65–74	0.89 (0.73–1.08)	1.32 (0.99–1.77)	1.07 (0.85–1.34)	1.75 (1.26–2.44)
≥75	0.70 (0.56–0.87)	1.21 (0.88–1.66)	1.03 (0.80–1.32)	1.91 (1.34–2.72)
Sex				
Women	-1-	-1-	-1-	-1-
Men	0.60 (0.55–0.65)	0.58 (0.51–0.63)	0.63 (0.57–0.70)	0.63 (0.56–0.70)
Geographical area				
Northern	-1-	-1-	-1-	-1-
Central	1.05 (0.92–1.19)	1.01 (0.88–1.14)	0.80 (0.70–0.93)	0.78 (0.68–0.90)
Southern	0.87 (0.79–0.96)	0.92 (0.84–1.02)	0.80 (0.72–0.89)	0.85 (0.76–0.95)
Educational level				
Up to primary	-1-	-1-	-1-	-1-
Lower secondary	1.32 (1.14–1.53)	1.33 (1.15–1.55)	1.25 (1.05–1.47)	1.25 (1.06–1.49)
Upper secondary	1.69 (1.46–1.95)	1.50 (1.29–1.74)	1.53 (1.30–1.80)	1.40 (1.18–1.66)
Post-secondary	2.36 (1.99–2.80)	1.90 (1.58–2.27)	1.93 (1.60–2.32)	1.59 (1.30–1.95)
Occupation				
Non-manual	-1-	-1-	-1-	-1-
Manual	0.65 (0.57–0.74)	0.78 (0.68–0.90)	0.69 (0.60–0.80)	0.81 (0.69–0.94)
Housewife	0.57 (0.48–0.67)	0.70 (0.59–0.83)	0.60 (0.50–0.72)	0.71 (0.59–0.86)
Retired	0.68 (0.58–0.79)	0.80 (0.69–0.94)	0.71 (0.60–0.85)	0.83 (0.69–0.98)
Student	0.75 (0.51–1.10)	0.80 (0.54–1.19)	0.94 (0.60–1.46)	0.97 (0.61–1.54)
Unemployed	0.63 (0.48–0.82)	0.74 (0.56–0.98)	0.90 (0.67–1.21)	1.07 (0.79–1.45)
Marital status				
Married/in couple	-1-	-1-	-1-	-1-
Single	1.19 (1.03–1.39)	1.14 (0.98–1.33)	1.19 (1.01–1.40)	1.14 (0.97–1.35)
Separated/divorced	1.47 (1.15–1.88)	1.43 (1.11–1.85)	1.48 (1.15–1.91)	1.46 (1.13–1.89)
Widowed	0.68 (0.56–0.83)	0.70 (0.58–0.85)	0.68 (0.55–0.85)	0.68 (0.55–0.85)
Smoking habit				
No	-1-	-1-	-1-	-1-
Yes	0.65 (0.58–0.74)	0.66 (0.58–0.76)	0.62 (0.53–0.72)	0.62 (0.53–0.72)
Former	0.90 (0.80–1.00)	0.90 (0.80–1.01)	0.76 (0.66–0.86)	0.75 (0.66–0.86)
Occasional	0.90 (0.66–1.23)	0.85 (0.62–1.17)	0.99 (0.71–1.40)	0.93 (0.66–1.32)
Sport activity				
No	-1-	-1-	-1-	-1-
Yes	1.48 (1.32–1.66)	1.34 (1.19–1.51)	1.41 (1.25–1.60)	1.32 (1.16–1.49)
Body mass index				
Normal weight	-1-	-1-	-1-	-1-
Overweight	1.01 (0.91–1.11)	1.08 (0.98–1.20)	0.94 (0.84–1.05)	1.00 (0.90–1.12)
Obese	0.87 (0.76–0.99)	0.99 (0.86–1.14)	0.81 (0.69–0.94)	0.90 (0.76–1.05)
MedDietScore				
Poor (5–27)	-1-	-1-	-1-	-1-
Average (28–30)	0.87 (0.78–0.97)	0.85 (0.76–0.95)	0.81 (0.71–0.91)	0.80 (0.71–0.91)
Good (31–44)	1.00 (0.90–1.11)	0.97 (0.87–1.08)	0.86 (0.76–0.96)	0.86 (0.76–0.97)

Model 1 = Odds ratio (OR) with 95% confidence intervals (95%CI) obtained from a model adjusted for age groups and sex.

Model 2 = as in model 1, further controlled for geographical area, educational level, occupation, marital status, smoking habit, sport activity, BMI, CVD, hypertension and adherence to Mediterranean diet (not including whole grains).

Table 3 reports the association of demographic and socioeconomic factors with WG consumption among younger subjects, with WG food intake. Boys were less likely to consume WG as compared to girls (OR = 0.59; 0.41–0.86 and OR = 0.65; 0.43–1.01, for occasional and regular consumption, respectively, Table 3; Model 2).

Younger consumers spending >2 h/d using PC were less likely to be occasional (OR = 0.53; 0.36–0.80) or regular consumers (OR = 0.56; 0.36–0.87) (Table 4; Model 2).

Determinants of consumers' food choice

Supplementary Figure 2 shows the main reasons people choose to consume WG products. A great proportion of WG consumers believe WG food is healthy food (47.7%) or more natural than regular products (26.2%).

Reasons for non-consumption of WG are depicted in Supplementary Figure 3 and ranged from dislike (38.8%) to an actual lack of knowledge on WG products (45.3%), while about 12.9% believes WG food is 'hard to cook'.

Table 3 Characteristics of children/adolescents (5–19 years) according to intake of whole grain foods and demographic and socioeconomic determinants of consumption.

	Whole grain intake						Occasional consumers (n = 87) vs non-consumers (n = 351)				Regular consumers (n = 123) vs non-consumers (n = 351)	
	Whole sample		Occasional consumers (<1time/week)		Regular consumers (≥1time/week)		Unadjusted p value		Model 1 OR (95%CI)		Model 2 OR (95%CI)	
	N (%)											
Sex	561	351 (62.6)	87 (15.5)		123 (21.9)		–	–	–	–	–	–
Girls	49.6	44.2	57.5		59.4		0.0041	–1- 0.56 (0.39–0.80)	–1- 0.59 (0.41–0.86)	–1- 0.63 (0.41–0.96)	–1- 0.65 (0.43–1.01)	–1- 0.65 (0.43–1.01)
Boys	50.4	55.8	42.5		40.6		0.0040	–1- 1.93 (0.85–4.38)	–1- 1.96 (0.86–4.47)	–1- 1.07 (0.55–2.05)	–1- 1.12 (0.58–2.18)	–1- 1.12 (0.58–2.18)
Geographical area												
Northern	20.1	23.9	12.6		14.6			–1- 1.23 (0.74–2.05)	–1- 1.29 (0.77–2.15)	–1- 1.07 (0.55–2.05)	–1- 1.12 (0.58–2.18)	–1- 1.12 (0.58–2.18)
Central	7.7	8.0	12.7		3.3			–	–	–	–	–
Southern	72.2	68.1	74.7		82.1		0.93	–	–	–	–	–
Sport activity												
No	34.4	34.2	33.3		35.8			–	–	–	–	–
Yes	65.6	65.8	66.7		64.2		0.12	–	–	–	–	–
Body mass index												
Normal weight	82.5	80.3	82.8		88.6			–1- 0.75 (0.45–1.25)	–1- 0.73 (0.43–1.21)	–1- 0.59 (0.32–1.11)	–1- 0.59 (0.31–1.11)	–1- 0.59 (0.31–1.11)
Overweight/Obese	17.5	19.7	17.2		11.4			–	–	–	–	–
KIDMED index ^a	4.3 (1.4)	4.3 (1.3)	4.5 (1.7)		4.2 (1.4)		0.39	–	–	–	–	–

Values are reported as percentages.
Model 1 = Odds ratio (OR) with 95% confidence interval (95%CI) obtained from a model adjusted for age and sex.
Model 2 = as in model 1, further controlled for place of residence and BMI.
^a KIDMED index is reported as means with standard deviations.

Table 4 Association of meal patterns and eating-related behaviours with whole grain food intake in adults (20–97 y) and among children/adolescents (5–19 y) from the INHES study cohort.

	Adults (n = 8421)				Children/Adolescents (n = 561)			
	Occasional consumers (n = 1475) vs non-consumers (n = 4656)		Regular consumers (n = 2290) vs non-consumers (n = 4656)		Occasional consumers (n = 87) vs non-consumers (n = 351)		Regular consumers (n = 123) vs non-consumers (n = 351)	
	Model 1a OR (95%CI)	Model 2b OR (95%CI)	Model 1a OR (95%CI)	Model 2b OR (95%CI)	Model 1c OR (95%CI)	Model 2d OR (95%CI)	Model 1c OR (95%CI)	Model 2d OR (95%CI)
Having breakfast								
Never/rarely	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
Always	1.64 (1.13–2.37)	1.38 (0.95–1.99)	1.45 (0.94–2.22)	1.31 (0.86–2.00)	0.64 (0.34–1.19)	0.61 (0.32–1.14)	0.65 (0.34–1.24)	0.63 (0.33–1.21)
Only with coffee	0.73 (0.49–1.09)	0.70 (0.47–1.04)	0.47 (0.29–0.76)	0.48 (0.30–0.78)	0.60 (0.16–2.28)	0.52 (0.14–1.99)	0.74 (0.19–2.93)	0.79 (0.20–3.14)
Breakfast (place)								
Home	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
Out	0.57 (0.44–0.73)	0.59 (0.46–0.76)	0.41 (0.29–0.57)	0.43 (0.31–0.60)	0.28 (0.09–0.86)	0.27 (0.09–0.83)	0.44 (0.12–1.58)	0.41 (0.11–1.47)
Late morning snack								
Never/rarely	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
Always	0.93 (0.84–1.02)	0.92 (0.83–1.01)	0.93 (0.83–1.03)	0.92 (0.83–1.03)	1.21 (0.81–1.82)	1.23 (0.82–1.84)	1.02 (0.65–1.61)	1.04 (0.66–1.64)
Lunch (place)								
Home	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
Out	0.98 (0.85–1.14)	0.86 (0.74–1.00)	0.94 (0.80–1.11)	0.84 (0.70–0.99)	2.20 (0.84–5.75)	2.60 (0.97–6.98)	3.80 (1.20–12.05)	3.25 (1.00–10.53)
Late afternoon break								
Never/rarely	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
Always	0.90 (0.82–1.00)	0.92 (0.83–1.02)	1.03 (0.92–1.15)	1.04 (0.93–1.17)	0.99 (0.68–1.45)	0.97 (0.66–1.42)	0.73 (0.48–1.12)	0.78 (0.50–1.20)
Dinner (place)								
Home	-1-	-1-	-1-	-1-	-1-	-1-	-	-
Out	0.82 (0.50–1.33)	0.93 (0.57–1.50)	0.75 (0.43–1.34)	0.81 (0.46–1.41)	0.72 (0.06–8.74)	0.85 (0.07–10.31)	-	-
Time spent watching TV								
≤ 2 h/day	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
> 2 h/day	0.91 (0.83–0.99)	0.98 (0.89–1.08)	1.13 (1.01–1.25)	1.22 (1.09–1.36)	0.79 (0.55–1.15)	0.76 (0.52–1.11)	0.73 (0.47–1.12)	0.73 (0.47–1.13)
Time spent using PC								
≤ 2 h/day	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
> 2 h/day	1.42 (1.28–1.58)	1.19 (1.07–1.33)	1.47 (1.31–1.64)	1.26 (1.12–1.42)	0.53 (0.36–0.80)	0.53 (0.36–0.80)	0.57 (0.36–0.89)	0.56 (0.36–0.87)
Having meals while watching TV								
Never	-1-	-1-	-1-	-1-	-1-	-1-	-1-	-1-
Sometimes/Yes	0.84 (0.70–0.99)	0.85 (0.71–1.01)	0.77 (0.63–0.93)	0.77 (0.63–0.94)	0.63 (0.32–1.25)	0.64 (0.32–1.27)	0.97 (0.45–2.07)	1.01 (0.47–2.15)
Having meals while using PC								
Never	-1-	-1-	-1-	-1-	-1-	-1-	-	-
Sometimes/Yes	0.91 (0.59–1.40)	0.82 (0.53–1.27)	0.93 (0.58–1.52)	0.94 (0.58–1.52)	0.22 (0.05–1.01)	0.24 (0.05–1.06)	-	-

Model 1a = Odds ratio (OR) with 95% confidence interval (95%CI) obtained from a model adjusted for age groups and sex.

Model 2b = as in model 1, further controlled for place of residence, educational level, occupation, marital status, smoking habit, sport activity, BMI, CVD, hypertension and adherence to Mediterranean diet (excluding whole grains).

Model 1c = Odds ratio (OR) with 95% confidence interval (95%CI) obtained from a model adjusted for sex and age.

Model 2d = as in model 1, further controlled for place of residence and BMI.

Table 5 Psychosocial factors associated with whole grain intake in adults (20–97 years).

	Occasional consumers (n = 1475) vs non-consumers (n = 4656)		Regular consumers (n = 2290) vs non-consumers (n = 4656)	
	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 1 OR (95%CI)	Model 2 OR (95%CI)
Self-reported health status				
Very good	-1-	-1-	-1-	-1-
Bad	0.47 (0.41–0.54)	0.51 (0.45–0.59)	0.41 (0.36–0.46)	0.44 (0.38–0.51)
Type of distress				
Adverse life events				
None	-1-	-1-	-1-	-1-
At least one	0.92 (0.80–1.06)	0.92 (0.80–1.07)	1.00 (0.86–1.17)	0.93 (0.79–1.09)
Occupation-related				
Never	-1-	-1-	-1-	-1-
Sometimes/most of the times	1.03 (0.76–1.38)	0.94 (0.69–1.29)	0.83 (0.60–1.13)	0.70 (0.50–0.97)
Often/Always	0.88 (0.62–1.24)	0.78 (0.54–1.12)	0.80 (0.55–1.15)	0.68 (0.46–1.00)
Not working/unascertained	0.76 (0.56–1.02)	0.79 (0.57–1.08)	0.65 (0.47–0.90)	0.61 (0.43–0.85)
Family-related				
Never	-1-	-1-	-1-	-1-
Sometimes	1.21 (0.93–1.57)	1.13 (0.87–1.48)	0.94 (0.71–1.24)	0.87 (0.66–1.15)
Most of the times	0.78 (0.62–1.02)	0.77 (0.59–1.00)	0.69 (0.52–0.92)	0.69 (0.52–0.91)
Often/Always	0.71 (0.50–1.01)	0.71 (0.49–1.01)	0.60 (0.40–0.88)	0.59 (0.40–0.87)
Job control				
None/little	-1-	-1-	-1-	-1-
Moderate - good	0.86 (0.70–1.05)	0.82 (0.66–1.01)	0.80 (0.64–1.01)	0.77 (0.61–0.98)
Fairly good	1.26 (1.01–1.57)	1.18 (0.94–1.47)	1.22 (0.96–1.55)	1.16 (0.91–1.48)
Not working/unascertained	0.93 (0.78–1.12)	0.98 (0.81–1.19)	0.98 (0.80–1.20)	1.02 (0.83–1.27)
Financial circumstances-related				
None/little	-1-	-1-	-1-	-1-
Moderate	0.80 (0.60–1.08)	0.73 (0.54–0.99)	0.65 (0.49–0.89)	0.55 (0.40–0.76)
A lot	0.94 (0.70–1.28)	0.96 (0.71–1.31)	0.93 (0.67–1.27)	0.88 (0.63–1.21)
Not working/unascertained	0.50 (0.34–0.73)	0.58 (0.39–0.86)	0.55 (0.36–0.83)	0.58 (0.38–0.89)

Model 1 = Odds ratio (OR) with 95% confidence interval (95%CI) obtained from a model adjusted for age groups and sex.

Model 2 = as in model 1, further controlled for place of residence, educational level, occupation, marital status, smoking habit, sport activity, BMI, CVD, hypertension and adherence to Mediterranean diet (excluding whole grains).

Discussion

The present study provides an updated evaluation of WG food consumption and its major demographic, socioeconomic, psychosocial and behavioural factors in a large sample of Italian adults, children and adolescents.

Findings showed that WG food is consumed on a regular basis (≥ 1 time/week) by 26.9% of the whole study sample, with WG bread being the major food source of total WG food for both age groups, up to half of the total WG intake.

These data are in accordance with prior data from the INRAN-SCAI 2005–06 survey [15] showing that WG bread represents the principal food source of total WG intake in adults (46%) while children were more likely to report a prevalent intake of WG breakfast cereals (32%) that in our young population contributed to 15.7% of the total WG intake. Similarly, INHES adults showed low consumption of breakfast cereals (4.8%) in line with the INRAN-SCAI adults (7%), although reporting greater WG pasta intake over the total WG consumption (13.1% vs 2%, respectively). Our results are also in line with data from a cohort of 1400 French adults [23] indicating that the highest contribution to total WG intake derived from WG bread (and toast) intake, although breakfast cereals contributed much more as compared to our Italian adult sample (24%).

Previous population surveys reveal cultural differences in WG food consumption which is typically higher in Scandinavian countries [21] than in many other European countries.

In detail, the percentage of adult consumers (27.2%) resulting from our study is lower than that documented by surveys conducted in USA [12] or UK [22] all reporting consumption above 50 percent, while it is similar to that reported in France (around 30%) [23].

The relatively low consumption of WG foods observed in our Italian sample, which is fairly similar to that reported by the INRAN-SCAI national survey [15], appears to be in contrast with the fact that our population is supposed to be close to a traditional Mediterranean diet which definitely encourages higher intake of unrefined cereals.

Yet, in a global perspective, the comparison between countries needs to be interpreted with caution owing the different survey methods used to collect data on WG intake. Indeed, in our survey we used a food propensity questionnaire while others mainly relied on food intake recorded by dietary recalls [24,25]. Moreover, the differing food sources of WG (e.g. lack of ready to eat breakfast cereals in Italy) makes it difficult an actual comparison among countries.

Beyond providing an update of WG consumption in Italy, we also aimed at identifying the major determinants

of (or variables correlated with) WG food consumption by analysing the association with a number of demographic, socioeconomic, psychosocial and behavioural factors.

A clear educational gradient emerged, with subjects with highest educational attainment being more likely to be regular WG consumers as opposed to those in the lowest educational group, in line with the INRAN-SCAI 2005–06 survey [16], and with data deriving from non-Mediterranean settings [22]; we also found an association with occupation, with highest intakes reported among most advantaged social classes and smallest in the lower classes, as previously reported in a UK study [22].

The socioeconomic shape of WG food intake is in agreement with the socioeconomic gradient already observed for high quality diets, and may be accounted at least in part by the fact that higher educational level is usually associated with increased nutrition knowledge which possibly leads to healthier food choices [26].

In general, we found that regular WG consumers had a healthier lifestyle, including higher physical activity and abstinence from tobacco, as already documented in other epidemiological settings [27].

Some meal patterns and eating-related behaviors were also associated with WG food intake. Individuals eating meals out of the home (i.e. breakfast or lunch) were less likely to consume WG food, thus suggesting that WG consumption is associated with a more regular eating behavior.

We also documented a relation of regular WG food intake with self-reported health status and stressful life events, in agreement with a large body of evidence indicating that diet quality is often associated with improved quality of life [28] or other indicators of mental well-being [29].

Regarding prevailing opinions on WG food, we found that about half of study participants believe WG products to be good for health, in agreement with previous results [31], and this might indicate the effectiveness of recent public campaigns to raise public awareness of the health benefits associated with WG food consumption.

On the other side, lack of knowledge on WG food is reported as being the most common obstacle by non-consumers and this may suggest that both public health strategies and food companies should direct their efforts at increasing knowledge and awareness of WG products.

As compared to estimations provided by the previous INRAN-SCAI survey in 2005–06, WG consumers in Italy experienced a relatively modest increase from 23% to 26.9% in 2013, thus leading to a serious re-thinking of public health strategies in order to increase the consumption of WG products in a more substantial manner at population level, especially in light of the numerous health benefits associated with WG intake [1–4].

Some useful suggestions come from the Danish health promotion campaign to increase WG consumption which included the following actions: increasing WG content of commercial food products; mass communication to improve consumers' knowledge about WG foods and their health benefits; and a certification logo on foods with a high WG content [30].

Of course, WG food promotion should be put within a wider context aimed to increase grain consumption globally, also in light of the fact that certain non-WG staple grain foods can be included in a healthy dietary pattern, provided that the dietary pattern maintains recommendations for calories, saturated fats, sodium and added sugars, as recently highlighted by the National Health and Nutrition Examination Survey (NHANES) suggesting that a variety of grain food groups (including enriched grains as ready-to-eat cereals) have the potential to increase consumption of shortfall nutrients [31].

Strengths and limitations of the study

This study has several strengths, including its large sample size and the large number of covariates used to accommodate at least in part confounding.

The considerable number of indicators used to assess meal patterns and the psychosocial dimension and their relation with dietary habits represents one major novelty of the present study.

Major limitations are represented by its cross-sectional and telephone-based design, the self-reported nature of data collection and lack of quantification of WG consumption.

An additional limitation is that, given that self-reported data were used, it is not possible to know whether the foods the participants described were actually WG containing.

Finally, we acknowledge that causality cannot be inferred due to the observational design of our study.

Conclusions

To the best of our knowledge, this study represents the most updated investigation on WG intake and its determinants in the Italian population.

Overall, the percentage of WG consumers emerging from our study (26.9%) is similar to that reported by the last Italian survey (23%) and appears to be still below estimations recorded in other countries of Europe, although comparisons should be made with caution.

Our study also showed that WG consumption clearly clusters with socioeconomic factors, health-related behaviors, meal patterns and with a number of psychosocial factors. Increasing knowledge on major determinants of regular WG intake may help defining more targeted public health strategies to increase the consumption of WG foods, especially in countries in which consumption is lower than recommended.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.numecd.2019.03.001>.

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CHAPTER 4.

Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population

Ruggiero E, Di Castelnuovo A, Costanzo S, Persichillo M, Bracone F, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Bonaccio M; INHES Study Investigators

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Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population

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Background: To evaluate the adherence to Mediterranean diet (MD) and its major socioeconomic and psychosocial determinants in a large sample of the Italian population, covering three main geographical areas of the Country (Southern, Central and Northern). **Methods:** Data were obtained from the Italian Nutrition & Health Survey (INHES), including a total of 7,430 participants (age >20) recruited from all over Italy (2010–13). Dietary information was collected by the European Food Propensity Questionnaire. Adherence to MD was assessed by using the MedDietScore based on 11 food groups. Associations were tested by multivariable logistic regression analysis (Odds ratio [OR] with 95% CI). **Results:** Adherence to MD was higher in Southern Italy as compared with the Northern (OR = 1.34; 95% CI 1.18–1.53), and was closely associated with adult age (OR = 2.40; 1.61–3.58 for those aged > 75 years as compared with 20–34 years) and higher educational level (OR = 1.77; 1.40–2.24 for post-secondary education as opposed to lowest educational attainment). Subjects reporting adverse life events and those with family-related stress were less likely to show an optimal adherence to MD (OR = 0.55; 0.46–0.67 and OR = 0.44; 0.28–0.69, for highest vs. lowest tertile, respectively) as compared with adequate controls. A number of eating behaviours were also inversely associated with MD, such as consuming higher amount of alcohol in the weekend than in week days. **Conclusions:** Adherence to MD is strongly determined by age, geographical area and educational level. Psychosocial factors and several eating behaviours are also closely associated.

Introduction

The Mediterranean diet (MD) is a traditional dietary pattern mainly followed in the Mediterranean regions^{1–3} and is considered the golden standard of healthy nutrition, being strongly associated with decreased morbidity and mortality, especially from cardiovascular and cerebrovascular causes.^{4,5}

More recently, a global shifting from the MD was documented especially in the Mediterranean regions that experienced the greatest loss of adherence as opposed to non-Mediterranean Countries,^{6–8} to such an extent that a nutrition transition issue emerged also for Mediterranean populations.^{9,10} Moreover, this downward trend was observed also for children and adolescents.^{11–13} The withdrawal from an MD could be influenced by numerous factors, and more recently a likely impact of the economic crisis has been proposed as a major cause for the shifting from this dietary pattern.^{8,14} In this context, improving the understanding of the major determinants associated with an MD is of crucial importance. Adherence to MD clusters with other healthy behaviours (i.e. physical activity and abstinence from tobacco) is strongly associated with socioeconomic status,¹⁵ and age.¹⁶ The socioeconomic gradient of diet quality has been well documented mainly in Northern European and US settings,^{17,18} although evidence from Mediterranean regions has been recently presented.¹⁵

Most of the evidence on possible determinants associated with an MD in Italy comes from relatively small samples of subjects, often recruited in specific geographical areas that may not represent the

whole Italian territory,^{16,19–22} while the latest dietary survey at national level was performed more than a decade ago.²³

In light of the above, the main aim of this study is 2-fold: first, to evaluate the actual adherence to MD in a large sample of the Italian population recruited from all over Italy within the Italian Nutrition & Health Survey (INHES), in order to provide updated data on the actual dietary habits of Italian people; second, to investigate major demographic, socioeconomic, psychosocial and behavioural factors possibly associated with adherence to MD, also with the purpose of providing new evidence for targeting appropriate intervention strategies at population level.

Methods

Study population

The INHES is a 3-year telephone-based survey on nutrition and health specifically designed to collect information on the dietary habits (quality, quantity and patterns), food choice determinants and food health awareness of the Italian population according to different geographical distribution, age, gender and socioeconomic profile.

Between November 2010 and November 2013, 9319 women and men aged ≥5 years from all over Italy were enrolled. Firstly, subjects ($n = 9106$) in the age range 35–79 years, recruited in the 2008–2012 wave of the Italian Cardiovascular Epidemiologic Observatory^{24,25} were invited to participate in the INHES survey. Once they accepted,

participants were asked to invite one relative older than 79 or younger than 35 years to join the survey. Finally, 5385 (59.1%) from the original population and 3754 from their relatives for a total of 9139 subjects were included in the survey. These subjects were distributed in the four seasons (excluding Christmas, Easter and mid-August periods). The survey calendar was organized to capture an adequate proportion of weekdays and weekend days at group level. The recruitment was performed using computer-assisted-telephone-interviewing. A full description of the study design is reported elsewhere.²⁶

For the purpose of this study, we included individuals aged >20 years ($n = 8772$) and omitted subjects with self-reported diagnosis of CVD (3.1%), cancer (3.2%) and diabetes (7.1%) or missing values for major variables of interest (2.6%). Finally, a total of 7430 subjects were included in the analyses.

Dietary assessment

Data on food intake were collected by the Italian version of the European Food Propensity Questionnaire.²⁷ Adherence to MD was evaluated by using the MedDietScore as proposed by Panagiotakos.²⁸ Assessment was based on the monthly consumption of eleven foods groups: cereals (bread, pasta, other grains, biscuits etc.), fruit, vegetables, legumes, potatoes, fish, meat and meat products, poultry, full fat dairy products (cheese, yoghurt and milk), olive oil and alcohol intake. Ratings (from 0 to 5 or the reverse) were assigned to each food group according to their position in the MD pyramid.

For the consumption of items presumed to be close to this pattern, a score 0 was assigned when someone reported no consumption, score 1 when they reported consumption of 1–4 servings/month, score 2 for 5–8 servings/month, score 3 for 9–12 servings/month, score 4 for 13–18 servings/month and score 5 for more than 18 servings/month. For the consumption of foods considered to be far from the MD (meat and meat products, poultry and full fat dairy products), scores on a reverse scale (i.e. 5 when someone reported no consumption to 0 when they reported almost daily consumption) were assigned. Alcohol intake was scored 5 for consumption of less than 300 ml of alcohol/day, 0 for consumption of more than 700 ml/day and 4–1 for consumption of 300, 400–500, 600 and 700 or 0 ml/day, respectively. The final score potentially ranged between 0 and 55, the latter reflecting maximal adherence to MD. For analysis purposes, the score was also used as exact tertiles.²⁸

Meal patterns and eating behaviours

Meal patterns comprised either frequency of main meals (breakfast, lunch, dinner) or frequency of main meals eaten out of home (breakfast, lunch and dinner). A number of eating behaviours were also investigated as patterns of alcohol consumption (regular days/week end), frequency of use of vending machine, kebab consumption, and having meals while watching TV or using PC. Information on daily amount of time spent in watching TV/using PC was also collected.

Socioeconomic and psychosocial factors

Education was based on the highest qualification attained and was categorized as up to elementary school (corresponding to ≤ 5 years of study), lower secondary ($>5 \leq 8$ years), upper secondary (8–13 years), post-secondary (>13 years).

Present occupation was assembled into six groups: manual, non-manual, housewife, retired, student and unemployed. Marital status was intended as married/living in a couple, single, separated/divorced and widowed.

Self-rated health was assessed through one-item question 'In general, how would you rate your health status' and responses were arranged along a five item Likert-type scale from 'very good' to 'poor'.

Adverse life events (yes/no) during the last year included questions related to health (e.g. serious illness or death), job (e.g. retired, job loss) or interpersonal situations (e.g. divorce, problem with friend or relative).

Data on occupation or family-related stress were obtained by the question 'How often have you experienced stress in your workplace/family?' with answers ranging from never to always. Financial-related stress was rated as none/little, moderate and a lot. Job control was considered as none/little, moderate-good, fairly good and not working/unascertained.²⁹

Sleep quality was assessed through the question 'How would you rate your sleep?' with two possible answers (restless or restful).

Assessment of covariates

Information on present use of lipid-lowering drugs or medications for high blood pressure were self-reported and categorized as no/yes. Body mass index (BMI) was calculated by using self-reported measurements of height and weight, calculated as kg/m^2 and grouped into three categories as normal (<25), overweight ($\geq 25 < 30$) or obese (≥ 30). Sport activity was considered as a dichotomous variable (yes/no).

The population was also stratified as living in an urban or rural environment on the basis of the urbanization level as defined by the European Institute of Statistics (EUROSTAT definition) and obtained by the tool 'Atlante Statistico dei Comuni' provided by the Italian National Institute of Statistics.³⁰

Statistical analysis

Main characteristics of the study population across levels of adherence to MD were presented as numbers and percentages, while the MedDietScore (continuous) was displayed as means with SD.

Associations for categorical or continuous variables reported in table 1 were evaluated by using general linear models adjusted for age groups and sex (PROC GENMOD and PROC GLM in SAS software for categorical and continuous variables, respectively).

Multivariable odds ratios (ORs) with 95% CIs were used to test the likelihood of being in the highest tertile of MedDietScore (highest vs. lowest, with removal of the second tertile) in association with demographic and socioeconomic factors (table 2), meal patterns and eating behaviours (table 3) or psychosocial factors (table 4).

Two logistic regression models were fitted in order to account for possible confounding. Model 1 (table 2) was adjusted for age groups and sex, multivariable models 2 (tables 2–4) were obtained by including all the variables listed in table 1 that resulted associated with the outcome with P values < 0.20 .

Dummy variables for missing values were created. Two-sided P values < 0.05 was considered as statistically significant.

Subgroup analyses for geographic area were undertaken. Appropriate multiplicative terms for testing interaction of geographical area (Northern, Central and Southern regions) with main demographic and socioeconomic factors were included in the multivariable models to test for a difference of effect of geographic area in modulating the relationship between MD and its determinants.

The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows 2009. SAS Institute Inc. and SAS are registered trademarks of SAS Institute Inc., Cary, NC, USA.

Results

Table 1 shows the characteristics of the whole population across tertiles of adherence to MD. Mean adherence to MD was 29.6 (SD = 5.4), ranging from 5 to 49.

Table 1 Characteristics of the population according to levels of adherence to the MD

	Whole sample	Adherence to MD (MedDietScore)			P value
		First tertile	Second tertile	Third tertile	
MedDietScore (min–max)	5–49	5–28	29–31	32–49	–
MedDietScore (mean, SD)	29.6 (5.4)	24.4 (5.4)	30.0 (0.8)	34.1 (2.3)	–
n, %	7430	2514 (33.8)	2204 (29.7)	2712 (36.5)	–
Sex (men)	45.7	38.7	51.1	47.8	<0.0001
Geographical area					0.0009
Northern	41.0	42.7	41.7	38.9	
Centre	16.8	17.1	17.4	16.1	
Southern	42.1	40.2	40.9	44.9	
Place of living					0.0002
Rural	13.6	15.3	13.7	11.9	
Urban	86.4	84.6	86.3	88.1	
Age groups					<0.0001
20–34	6.2	8.4	6.0	4.3	
35–49	31.8	33.1	31.7	30.7	
50–64	33.5	29.8	33.2	37.3	
65–74	18.9	18.5	19.7	18.6	
≥75	9.6	10.1	9.5	9.1	
Educational level					<0.0001
Up to elementary	15.5	16.9	16.2	13.6	
Lower secondary	25.5	27.6	25.7	23.4	
Upper secondary	41.7	40.8	41.4	42.8	
Post-secondary	17.3	14.8	16.7	20.1	
Occupation					0.20
Manual	19.1	19.0	19.8	18.6	
Non-manual	33.7	32.5	33.4	35.2	
Housewife	11.2	12.0	10.3	11.0	
Retired	31.8	31.5	32.3	31.7	
Student	1.1	1.6	1.2	0.7	
Unemployed	3.0	3.3	3.0	2.8	
Marital status					0.10
Married /in couple	75.5	72.7	76.5	77.1	
Single	14.8	16.9	14.2	13.4	
Separated/divorced	3.3	2.7	3.2	3.8	
Widowed	6.4	7.6	6.0	5.7	
Smoking habit					<0.0001
No	60.7	65.3	60.5	56.5	
Current	16.7	17.7	16.5	15.9	
Former	20.6	15.5	21.0	25.0	
Occasional	2.0	1.4	2.0	2.6	
Sport activity	19.4	15.9	18.4	23.4	<0.0001
Drugs for hypertension	25.9	24.8	26.7	26.3	0.60
Drugs for hyperlipidaemia	10.0	9.6	9.8	10.4	<0.0001
BMI					0.069
Normal weight	49.8	51.2	48.5	49.5	
Overweight	37.8	35.1	39.2	39.2	
Obese	12.4	13.6	12.4	11.3	

Numbers are presented as percentages unless otherwise stated. *P* values adjusted for sex and age groups.

People with the highest adherence to MD were more likely to be from Southern Italy and to have a higher educational level. Former/occasional smokers and sport activity were also associated with an optimal adherence to MD (table 1).

The odds of being in the uppermost level of MD increased according to age groups as well as living in Southern regions or in an urban environment rather than in rural areas (table 2; multivariable model 2).

A clear educational gradient in MD emerged, with subjects with highest education reporting greater adherence to this dietary pattern, while no association with occupation was found (table 2; multivariable model 2).

People exhibiting a healthier lifestyle were also more likely to be closer to an MD diet, as those engaged in sport activities, as well as occasional/former smokers (table 2; multivariable model 2), while no association was found with BMI (table 2).

The association of MD with some demographic and socioeconomic factors was likely to be modified by geographical area; subgroup analyses (Supplementary table S1) revealed an effect modification

of geographical area for the association of MD with age groups and place of living. Indeed, adult and older subjects were more likely to adhere to an MD in Central and Southern regions, rather than in the Northern part of Italy (*p* for interaction = 0.027); moreover, living in an urban environment was inversely associated with MD in the Northern part of Italy (OR = 0.55; 0.38–0.79), and positively in the Central (OR = 1.18; 0.76–1.85) and Southern regions of the country (OR = 1.91; 1.54–2.38; *P* for interaction < .0001).

A number of behaviours were also inversely associated with MD adhesion, as consuming relatively higher amount of alcohol in the week end as opposed to weekdays (OR = 0.61; 95% CI 0.46–0.81 for higher amounts vs. same intake; table 3), as well as spending more time watching TV (OR = 0.45; 95% CI 0.33–0.60, for >4 vs. < 1 h/day; table 3).

Subjects usually reporting having breakfast out of home (OR = 0.63; 95% CI 0.47–0.85), those using vending machine more frequently (OR = 0.78; 95% CI 0.61–0.99) or consuming kebab up to three times a month (OR = 0.71; 95% CI 0.61–0.84) were less likely to report closer adherence to MD (table 3).

Table 2 Demographic and socioeconomic factors associated with adherence to the MD

	MedDietScore	
	OR (95% CI) Model 1	OR (95% CI) Model 2
Age groups		
20–34	–1–	–1–
35–49	1.79 (1.40–2.30)	1.74 (1.29–2.33)
50–64	2.43 (1.90–3.10)	2.44 (1.79–3.33)
65–74	1.97 (1.52–2.55)	2.36 (1.65–3.40)
≥75	1.72 (1.29–2.30)	2.40 (1.61–3.58)
Sex		
Women	–1–	–1–
Men	1.32 (1.21–1.43)	1.36 (1.20–1.55)
Geographical area		
Northern	–1–	–1–
Central	1.05 (0.89–1.23)	1.01 (0.86–1.19)
Southern	1.24 (1.10–1.40)	1.34 (1.18–1.53)
Place of living		
Rural	–1–	–1–
Urban	1.36 (1.16–1.59)	1.36 (1.15–1.61)
Educational level		
Up to Elementary	–1–	–1–
Lower secondary	1.07 (0.88–1.29)	1.02 (0.84–1.24)
Upper secondary	1.45 (1.20–1.75)	1.35 (1.11–1.64)
Post-secondary	1.95 (1.57–2.42)	1.77 (1.40–2.24)
Occupation		
Manual	–1–	–1–
Non-manual	1.20 (1.02–1.40)	0.96 (0.81–1.15)
Housewife	1.10 (0.89–1.37)	1.08 (0.86–1.36)
Retired	0.99 (0.80–1.37)	0.89 (0.72–1.11)
Student	0.86 (0.46–1.60)	0.78 (0.41–1.46)
Unemployed	1.06 (0.75–1.49)	0.92 (0.65–1.31)
Marital status		
Married /in couple	–1–	–1–
Single	0.98 (0.82–1.18)	0.97 (0.80–1.17)
Separated /divorced	1.37 (1.00–1.88)	1.33 (0.96–1.84)
Widowed	0.83 (0.65–1.05)	0.90 (0.70–1.16)
Smoking habit		
No	–1–	–1–
Current	0.98 (0.84–1.14)	0.99 (0.84–1.16)
Former	1.62 (1.40–1.88)	1.59 (1.37–1.85)
Occasional	2.20 (1.46–3.33)	2.02 (1.33–3.08)
Sport activity		
No	–1–	–1–
Yes	1.72 (1.49–1.99)	1.62 (1.39–1.88)
BMI		
Normal weight	–1–	–1–
Overweight	1.00 (0.88–1.13)	1.02 (0.90–1.16)
Obese	0.75 (0.63–0.90)	0.80 (0.67–0.97)

Model 1 = ORs with 95% CIs obtained from a model adjusted for age groups (five categories) and sex.

Model 2 = as in model 1, further controlled for geographical area, place of living, educational level (four categories), occupation (six categories), marital status (four categories), smoking habit (four categories), sport activity, BMI (three categories), drugs for hypertension, use of lipid-lowering medications.

Reporting at least one adverse life event or family-related stress in the last year was inversely associated with MD (OR = 0.55; 0.46–0.67 and OR = 0.44; 0.28–0.69, respectively; table 4), while good self-rated health was positively associated (table 4). Sleep quality was not associated with adherence to the MD (table 4).

Discussion

This study aimed to assess the actual adherence to MD in a large representative sample of the Italian population and to investigate its

Table 3 Association of meal patterns and eating behaviours with adherence to the MD

	MedDietScore		
	%	ORs (95% CI) Model 1	ORs (95% CI) Model 2
Breakfast			
Home	93.7	–1–	–1–
Out	3.9	0.61 (0.46–0.81)	0.63 (0.47–0.85)
Having breakfast			
Never/rarely	1.6	–1–	–1–
Always	88.6	0.93 (0.61–1.43)	0.88 (0.56–1.37)
Only with coffee	8.9	0.83 (0.52–1.32)	0.78 (0.48–1.26)
Late morning snack			
Never/rarely	69.8	–1–	–1–
Always	29.2	1.14 (1.01–1.28)	1.09 (0.96–1.24)
Lunch			
Home	86.9	–1–	–1–
Out	11.8	0.97 (0.81–1.15)	0.97 (0.80–1.17)
Having lunch			
Never/rarely	0.5	–1–	–1–
Always	98.7	0.99 (0.45–2.20)	0.84 (0.37–1.92)
Late afternoon break			
Never/rarely	74.3	–1–	–1–
Always	24.7	1.04 (0.91–1.18)	1.01 (0.89–1.15)
Aperitif			
Never	82.9	–1–	–1–
Sometimes	14.9	1.77 (1.50–2.10)	1.60 (1.34–1.90)
Always	1.6	0.98 (0.64–1.51)	0.96 (0.62–1.50)
Dinner			
Home	97.7	–1–	–1–
Out	0.8	0.77 (0.42–1.43)	0.83 (0.44–1.55)
Having dinner			
Never/rarely	0.4	–1–	–1–
Always	98.7	1.94 (0.89–4.25)	1.87 (0.84–4.14)
Alcohol consumed in the week end as compared with the whole week			
Same amount	80.0	–1–	–1–
Higher amount	6.5	0.60 (0.46–0.79)	0.61 (0.46–0.81)
Lower amount	13.5	0.76 (0.62–0.94)	0.74 (0.59–0.91)
Vending machine use			
Never	78.5	–1–	–1–
Up to 2–3 times/month	9.8	0.87 (0.72–1.06)	0.86 (0.70–1.04)
1–2 times/week	4.3	0.78 (0.60–1.03)	0.78 (0.59–1.04)
>3 times/week	6.9	0.79 (0.63–0.99)	0.78 (0.61–0.99)
Kebab consumption			
Never	81.5	–1–	–1–
Up to 2–3 times/month	17.0	0.76 (0.65–0.89)	0.71 (0.61–0.84)
>1–2 times/day or more	0.5	1.16 (0.48–2.80)	0.87 (0.35–2.16)
Time spent watching TV			
<1 h/day	7.2	–1–	–1–
1–2 h/day	31.5	0.99 (0.79–1.24)	1.02 (0.81–1.29)
2–3 h/day	49.6	0.71 (0.57–0.89)	0.73 (0.58–0.92)
>4 h/day	11.1	0.39 (0.30–0.52)	0.45 (0.33–0.60)
Time spent using PC			
Never	49.2	–1–	–1–
<1 h/day	20.0	1.40 (1.19–1.65)	1.17 (0.98–1.39)
1–2 h/day	22.5	0.90 (0.77–1.05)	0.75 (0.63–0.89)
>2 h/day	7.6	1.09 (0.87–1.38)	0.82 (0.64–1.06)
Having meals while watching TV			
Never	92.4	–1–	–1–
Sometimes	6.2	0.93 (0.74–1.17)	0.86 (0.68–1.09)
Yes	0.9	0.95 (0.54–1.68)	1.00 (0.56–1.80)
Having meals while using PC			
Never	98.1	–1–	–1–
Sometimes	0.9	0.85 (0.50–1.45)	0.68 (0.39–1.19)
Yes	0.2	0.81 (0.22–3.00)	0.82 (0.22–3.09)

Model 1 = ORs with 95% CIs obtained from a model adjusted for age groups and sex.

Model 2 = as in model 1, further controlled for geographical area, place of living, educational level, occupation, marital status, smoking habit, sport activity, BMI, drugs for hypertension, use of lipid-lowering medications.

Table 4 Psychosocial factors associated with adherence to the MD

	MedDietScore		
	%	OR (95% CI) Model 1	OR (95% CI) Model 2
Self-reported health status			
Very good	16.4	–1–	–1–
Good	67.8	1.58 (1.35–1.85)	1.63 (1.38–1.92)
Fair	14.7	1.55 (1.26–1.91)	1.61 (1.30–2.00)
Poor	0.5	0.26 (0.10–0.69)	0.28 (0.10–0.75)
Type of distress			
Adverse life events			
None	90.2	–1–	–1–
At least one	9.8	0.54 (0.45–0.65)	0.55 (0.46–0.67)
Occupation-related			
Never	2.3	–1–	–1–
Sometimes/most of the times	48.9	0.86 (0.59–1.26)	0.95 (0.64–1.41)
Often/Always	6.1	0.85 (0.55–1.31)	0.85 (0.54–1.33)
Not working/unascertained	42.7	0.80 (0.55–1.17)	0.92 (0.61–1.37)
Family-related			
Never	3.0	–1–	–1–
Sometimes	55.9	0.75 (0.55–1.04)	0.83 (0.60–1.15)
Most of the times	37.5	0.79 (0.57–1.10)	0.82 (0.59–1.14)
Often/Always	3.4	0.44 (0.29–0.69)	0.44 (0.28–0.69)
Unascertained	0.2	0.29 (0.07–1.17)	0.31 (0.07–1.33)
Job control			
None	6.7	–1–	–1–
Moderate/Good	15.1	0.71 (0.54–0.92)	0.75 (0.58–0.99)
Fairly Good	10.2	0.90 (0.68–1.19)	0.92 (0.69–1.23)
Not working/unascertained	67.9	0.62 (0.49–0.78)	0.67 (0.53–0.86)
Financial circumstances-related			
None	2.3	–1–	–1–
Moderate	56.8	1.01 (0.69–1.49)	1.16 (0.79–1.71)
A lot	37.4	0.68 (0.46–1.00)	0.84 (0.57–1.24)
Not working/unascertained	3.5	0.53 (0.33–0.87)	0.63 (0.38–1.04)
Type of sleep			
Restful	89.0	–1–	–1–
Restless	8.9	1.01 (0.84–1.22)	1.02 (0.84–1.24)

Model 1 = ORs with 95% CIs obtained from a model adjusted for age groups and sex.

Model 2 = as in model 1, further controlled for geographical area, place of living, educational level, occupation, marital status, smoking habit, sport activity, BMI, drugs for hypertension, use of lipid-lowering medications.

major demographic, socioeconomic and psychosocial determinants. So far, data on dietary habits in the Italian population were mainly obtained from relatively small samples of subjects or pertaining to specific districts,^{16,19,20} which can likely prevent from an actual estimation of the dietary habits across the whole country, with little chance to offer insights on possible differences at national level.

In light of this, the present results from the INHES study represent the first attempt to identify the major determinants of the MD all over Italy, in recent years, after the beginning of the economic crisis, which has been previously associated with a dramatic drop in the adherence to the MD.³¹

We found that men, middle-aged subjects (50–64 years) and those living in Southern Italian regions showed a better adherence to MD, in agreement with a previous Italian investigation.¹⁹

A clear educational gradient emerged, with highest educational attainment being more likely associated with MD as opposed to the lowest educational group, in line with recent evidence from the Moli-sani study where MD follows a marked socioeconomic profile.¹⁵

However, we found an effect modification of geographical area for the association of MD with its determinants, which suggests a different contribution of some demographic and socioeconomic factors in determining the adherence to the dietary pattern. Of interest, older age resulted as a major determinant of MD in Southern parts of the country, while it seemed to play no role in

the Northern regions. Such findings indicate that adherence to MD seems to be strongly affected by socio-cultural background of Italian individuals, in the southern regions as part of the population cultural heritage and affordability of prices of the goods, while in the northern regions as a health-conscious choice to which only the richer part of the population has access.

Overall, we found that urban living is associated with closer adherence to MD; however, this is true only in southern regions, while an inverse association was found in the Northern area; other studies have found that the urban environment was associated with MD in adults⁸ just before the recession started (2005–06), while others found that adolescents from urban areas were less likely to adopt an MD,³² as well as elderly individuals from Mediterranean islands.³³

An optimal MD adherence clustered with other healthy behaviours, such as being physically active, as already documented also in non-Mediterranean settings,^{27,28} or with being former smokers possibly because these subjects are more likely to make positive decisions concerning their health;³⁴ yet, also occasional smokers were more likely to adopt an MD-style diet.

We failed to find an association of BMI with MD, in line with previous data showing that MD is unrelated to BMI also in Mediterranean populations.^{35,36}

One major strength of this study is represented by the number of indicators used to assess the psychosocial dimension and its relationship with diet.

Our results showed that a better self-reported health status is positively associated with greater adherence to MD, in line with previous findings.^{37,38} We also tested the association with adverse life events, an indicator which has been poorly used to date in association with diet, and with eating disorders.³⁹ We found that experiencing adverse life events was inversely associated with MD, net of other possible determinants possibly influencing either dietary behaviours or psychological status. More frequent family stress was also inversely related to MD, in line with studies showing that stress and emotions are closely associated with food intake, since subjects experiencing stressful situations might try to make themselves feel better by eating or drinking.⁴⁰

Finally, sleep quality was not associated with MD, and this appears in contrast with previous studies showing a positive relationship between quality of sleep or sleep duration with adherence to MD.^{41,42}

Among eating-related behaviours, consuming different amount of alcohol in the week end as compared with week days was found inversely associated with MD suggesting that drinking regularly and in moderation is still a key feature of the Mediterranean populations. Also frequent use of vending machine is negatively associated with MD and this is not surprising since vending machines generally include unhealthy foods and soft drinks that are not part of a Mediterranean way of eating. Skipping meals is a predictor of poorer health outcomes,⁴³ while the number of meals eaten out of home is a potential determinant of increase of body weight.^{16,44}

No association between MD and meal patterns was found, with the exception of consuming breakfast away from home, which was associated with lower likelihood of adherence to MD.

Spending much time watching TV likely acts as a proxy of sedentary behaviours and has been traditionally linked to unhealthy dietary lifestyle; in our population adherence to MD was likely to decline as time spent watching TV increased, in accordance with previous evidence evaluating this association⁴⁵; similar trends were observed for time spent using PC.

Strengths and limitations of the study

This study has several strengths which include a large sample size and the large number of covariates used to accommodate at least in part confounding.

However, our findings should be interpreted in light of several limitations. First, it is a telephone-based survey sharing all the limitations inherent to this study type, including, among others, interviewer bias and inability to use visual help. Yet, information was collected by trained interviewers; moreover, each participant received by mail a short photographs atlas and guidance notes to estimate portion sizes.

A further weakness is the decreased use of land-lines that could result in an under-representation of respondents (with younger and middle-aged persons more likely to have mobile telephones only).

Second, the cross-sectional design imposes limitations for causal inference; third, residual confounding by unmeasured factors (i.e. other psychosocial factors) cannot be fully ruled out; fourth, data (including anthropometric information) are self-reported; fifth, actual variables used in this survey cannot allow to fully describe the occupational/economic status of the participants, as only education and type of occupation were collected (there are no information about the previous job of retired individuals or data on income).

Finally, given the selected nature of study participants generalizability of the present findings should be made with caution.

Conclusions

To the best of our knowledge, this study represents the most updated investigation on MD and its determinants at the national Italian level.

Our findings confirm that an optimal adherence to MD is closely associated with middle or older age, site of residence (Southern Italy) and higher education level, in line with previous investigations from Mediterranean settings. We also found that a number of psychosocial factors are associated with MD, along with some meal patterns and eating behaviours.

Diet quality is one of the most effective lifestyle in reducing disease/mortality risk at population level¹; with rates of adherence being decreased over time, especially in Mediterranean countries⁶ public health policies are challenged to identify the most vulnerable groups at higher risk of losing such protecting factor, also to prevent new diet-related socioeconomic disparities.¹⁰

In light of the above, improving the understanding of main determinants of MD adherence during the 21st century is of crucial importance in order to develop effective public health strategies for prevention of major chronic diseases, especially at a time of financial crisis during which efforts and resources should be strategically delivered.

Supplementary data

Supplementary data are available at *EURPUB* online.

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Conflicts of interest: None declared.

Ethical standards disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee of the Catholic University of Rome. Verbal informed consent was obtained from all subjects. Verbal consent was witnessed and formally recorded.

Key points

- A global shifting from the Mediterranean diet (MD) was documented in the last decades especially in the Mediterranean regions.
- Evidence on determinants of MD in Italy comes from relatively small population cohorts.
- Socioeconomic and psychosocial factors are major determinants of MD.
- The understanding of main determinants of MD is crucial for developing effective public health strategies for prevention of major chronic diseases.

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Appendix

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CHAPTER 5.

Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study

Bonaccio M, **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, on behalf of the Moli-sani study

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Fish intake is associated with lower cardiovascular risk in a Mediterranean population: Prospective results from the Moli-sani study

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Fish intake;
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Coronary heart disease;
Cerebrovascular events;
Stroke

Abstract *Background and aims:* Fish consumption reportedly reduces the risk of heart disease, but the evidence of cardiovascular advantages associated with fish intake within Mediterranean cohorts is limited. The aim of this study was to test the association between fish intake and risk of composite coronary heart disease (CHD) and stroke in a large population-based cohort adhering to Mediterranean Diet.

Methods and results: Prospective analysis on 20,969 subjects free from cardiovascular disease at baseline, enrolled in the Moli-sani study (2005–2010). Food intake was recorded by the Italian version of the EPIC food frequency questionnaire. Hazard ratios were calculated by using multi-variable Cox-proportional hazard models. During a median follow-up of 4.3 years, a total of 352 events occurred (n of CHD = 287 and n of stroke = 66). After adjustment for a large panel of covariates, fish intake ≥ 4 times per week was associated with 40% reduced risk of composite CHD and stroke (HR = 0.60; 95%CI 0.40–0.90), and with 40% lower risk of CHD (HR = 0.60; 95%CI 0.38–0.94) as compared with subjects in the lowest category of intake (<2 times/week). A similar trend of protection was found for stroke risk although results were not significant (HR = 0.62; 95%CI 0.26–1.51). When fish types were considered, protection against the composite outcome and CHD was confined to fatty fish intake.

Conclusions: Fish intake was associated with reduced risk of composite fatal and non-fatal CHD and stroke in a general Mediterranean population. The favourable association was likely to be driven by fatty fish.

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Introduction

Cardiovascular disease (CVD) accounts for 45% of all deaths in Europe and increased life expectancy have determined a growing number of people living longer with CVD [1].

In light of this, understanding the major lifestyle determinants possibly associated with a reduction in CVD incidence at population level is of crucial importance, and among modifiable health-related behaviours, dietary choices represent an important tool to favourably modulate the risk of CVD either in high-risk groups or within the general population [2,3].

Beyond promoting a healthy dietary lifestyle overall, dietary guidelines recommend eating fish at least two times (two servings) a week [4], because of the well-documented inverse association with risk of developing CVD supported by several meta-analyses.

A meta-analysis of 17 cohort studies showed that both low (1 serving/week) and moderate fish consumption (2–4 servings/week) were inversely associated with coronary heart disease (CHD) mortality while no additional benefits were seen at higher intakes [5]. Advantages for cerebrovascular health were supported by findings from a meta-analysis on 16 prospective studies [6] showing that even infrequent intakes of fish (once a week) were capable to reduce the risk of stroke and such data were further confirmed in the following work by Chowdhury [7] in which highest fish intake was inversely associated with cerebrovascular risk as opposed to the lowest category of consumption, and the dose–response analysis showed that an increment of two servings a week of any fish was associated with a 4% reduced risk of cerebrovascular disease.

In fish eating populations, the protective effects of fish consumption were mainly observed in association with high amounts either for stroke [8] or for CHD [9], whereas in epidemiological settings with low habitual fish intake substantial benefits were observed even from modest amounts and mainly for fatal CVD events rather than nonfatal [10].

Yet, poor evidence has been obtained on the association of fish and CVD risk within Mediterranean population settings. Available evidence dates back to men recruited in the Italian segment of the Seven Countries Study [11] for whom consumption of fatty compared with non-fatty-fish resulted in lower CHD mortality, while more recently a study on the Spanish segment of the EPIC cohort failed to find any relationship between fish intake and stroke risk [12].

The aim of the present study was then to examine the relationship between fish consumption and risk of CHD and stroke in a general Mediterranean population, recruited in the Moli-sani study.

Methods

Study population

The Moli-sani study is a large population-based cohort study that in 2005–2010 recruited 24,325 men and

women aged ≥ 35 years from the general population of the Molise region, a central-southern area of Italy [13].

We excluded individuals with a history of CVD or unascertained CVD at baseline (7.1%), those reporting implausible energy intakes (< 800 kcal/day in men and 500 kcal/day in women or > 4000 kcal/day in men and 3500 kcal/day in women; 3.2%), subjects with missing information on dietary habits (0.4%), unreliable medical or dietary questionnaires (1% and 3.9%, respectively), subjects lost to follow-up (0.2%) or with incomplete personal data (1.7%). The final sample was of 20,969 individuals.

The Moli-sani study complies with the Declaration of Helsinki and was approved by the ethical committee of the Catholic University in Rome, Italy. All participants provided written informed consent.

Dietary information

Food intake during the year before enrolment was ascertained by the validated Italian version of the EPIC food frequency questionnaire [14] which includes 188 food items, classified into 45 predefined food groups on the basis of similar nutrient characteristics or culinary usage.

The EPIC questionnaire includes a specific question about fish intake comprising frequency of consumption of a number of fish types.

In the present analyses fish intake was evaluated either by using fish sub-types or total fish intake.

Categories of fish were shellfish, shrimps and crustaceans, dried fish (i.e. salted codfish), canned fish and other fish not included in the previous categories (i.e. salmon, swordfish, anchovies, etc.). Total fish came up as the sum of the above-mentioned categories.

In addition, fish consumption was also divided according to percentage of fat: fish containing up to 4% fat was classified as *lean* (e.g. cod, plaice, sole, hake, molluscs and crustaceans), whereas fish containing $\geq 4\%$ fat was classified as *fatty* (e.g. salmon, trout, herring, mackerel and canned fish products). Both total fish and fish-types were considered as intake on a weekly basis (times per week). Regarding total fish intake, frequency was categorized as up to 2, 2–4 and ≥ 4 times per week, while fish types were categorized as never, up to 1 time and ≥ 1 time per week.

Adherence to the Mediterranean diet was evaluated by the Mediterranean Diet Score (MDS) [15] but, in order to control analyses for dietary habits beside fish consumption, it was deprived of fish intake.

Ascertainment of cases

Primary fatal and nonfatal incident cases of CHD (unstable angina, myocardial infarction, coronary revascularization and sudden death for unspecified cardiac event) and cerebrovascular disease that occurred in the cohort during follow-up were ascertained by linkage of the study cohort to the hospital discharge files and to the regional ReNCaM registry and death certificates (ISTAT form), by using the International Classification of Diseases, ninth revision

(ICD-9). For CHD, ICD 9 codes 410–414 and/or reperfusion procedure (ICD-9 codes 36.0–36.9) and for cerebrovascular disease, ICD9 codes 430–432, 434, 436–438 or procedure codes for carotid revascularization (ICD 9 code 38.12) were considered.

Suspected CHD deaths were identified when ICD-9 codes 410–414 or 798 and 799 were reported as the underlying cause of death or codes 250, 401–405, 420–429 as the underlying cause of death, associated with codes 410–414 as a secondary cause of death.

Suspected cerebrovascular deaths were identified when ICD 9 codes 430–438 were reported as the underlying, antecedent, or direct cause of death.

All events were validated using procedures of the AHA, WHF, ESC, CDC and NHLBI for epidemiology and clinical research studies [16].

Time to event was calculated until the date of diagnosis of CVD, or the date of death, or the date of the last contact prior to December 2011.

Statistics

Means with standard deviations (SDs) and percentages were used to describe the characteristics of the study population across categories of total fish intake. Associations for continuous and categorical variables were evaluated by using general linear models adjusted for age, sex and energy intake (kcal/d) (PROC GENMOD and PROC GLM in SAS for categorical and continuous variables, respectively).

Risk estimates for incident CHD and stroke associated with total fish and fish-types intakes were expressed as multivariable hazard ratios (HRs) with 95% confidence intervals (95% CI) and calculated by using Cox proportional hazards models. Tests for violation of the proportional hazards assumption were conducted through the introduction of linear interaction between weekly fish intake (continuous) and the time variable ($p = 0.47$). Hazard ratios were calculated both for categories of fish intake and as a continuous exposure (for 1-time per week increment). Potential covariates were included in the multivariable models when they resulted associated with $p < 0.20$ with both fish intake and CVD risk. Three multivariable models were fitted. Model 1 included age, sex and energy intake; model 2 was additionally adjusted for education, smoking, drugs for diabetes, drugs for hypertension, drugs for lipids, and MDS without fish. Finally, model 3 was additionally controlled for blood glucose, LDL-cholesterol and low-grade inflammation as likely mediating pathways of the association between fish intake and the outcomes. A possible curvilinear association between the continuous measure of fatty fish intake (g/d) and CVD risk was tested via an adjusted Cox model using a restricted cubic spline.

Dummies variables for missing values were created. Two-sided P -value < 0.05 was considered as statistically significant. The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows® 2009. SAS Institute Inc. and SAS are registered trademarks of SAS Institute Inc., Cary, NC, USA.

Results

Fish was consumed by almost all subjects of the cohort (99.5%). Mean total fish intake in the study sample was of 44.6 g/d (SD = 26.3), similar to the amount reported within other high-fish consuming Countries, such as Spain (4) and Japan (7). Mean frequency of fish intake was of 2.4 (SD = 2.2) times per week.

Characteristics of the study population by weekly consumption of total fish are reported in Table 1. Women, younger people, and individuals with highest socioeconomic status were more likely to consume fish more frequently. Greater intake of fish was also associated with higher prevalence of smoking, leisure-time physical activity, use of drugs for diabetes, hypertension and lipid-lowering medications, higher values of LDL-cholesterol and lower levels of subclinical inflammation. Frequent fish consumption was also associated with higher adherence to a Mediterranean diet statistically deprived of fish, and greater energy intake (Table 1).

Over a median follow up of 4.3 years (90,886 person-years; lower to upper quartile: 3.4–5.3 y), we identified a total of 352 new cardiovascular events including 287 CHD and 66 strokes.

Risk estimates for total fish intake and the composite outcome of CHD and stroke, CHD and stroke separately are reported in Table 2. As compared to low fish intake (0–1.99 times/week), high consumption of fish (≥ 4 times/week) was associated with 38% lower CVD risk (7%–58%; Model 2) and 9% lower risk for 1-time per week increment (HR = 0.91; 95%CI 0.83–0.99; Model 2). After exclusion of CVD events occurred in the first 6 months of follow-up (n of subjects = 95; n of new CVD cases = 51), the magnitude of the association of total fish with CHD and stroke risk was not affected (HR = 0.64; 95%CI 0.42–0.99 for the highest vs lowest category of fish intake). Subgroup analyses for gender, age classes (≤ 65 y vs > 65 y), income and education did not reveal any difference of effect across groups (p values for interaction > 0.05).

A comparable reduction was found for CHD risk (HR = 0.62; 95%CI 0.40–0.98; Table 2; Model 2) while results for stroke showed a similar downward trend, although statistical significance did not hold (HR = 0.63; 95%CI 0.26–1.51; Table 2; Model 2).

In Model 3 the likely mediating effect of lipids, blood glucose and low-grade inflammation was evaluated but the results remained substantially unchanged (Table 2).

Analyses for fish types are reported in Table 3. Although not statistically significant, protection from the composite CHD/stroke was confined to canned fish and other fish types (HR = 0.85; 95%CI 0.61–1.19 and HR = 0.83; 95%CI 0.55–1.24 for consumption ≥ 1 time per week vs no intake). Consumption of dried fish showed a trend toward increased CVD risk, although statistical significance was not held (HR = 1.07; 95%CI 0.65–1.77). Similar results were observed for CHD and stroke separately (Table 3).

Risks of composite CHD/stroke and of CHD were reduced by 44% and 45% in association with fatty fish intake ≥ 1 time per week (HR = 0.56; 95%CI 0.36–0.87 and

Table 1 Characteristics of the study population by intake of fish on a weekly basis in the Moli-sani cohort.

	All	Fish intake (times per week)			P value ^a
		0–1.99	2–3.99	≥4	
g/d of fish (Mean, SD)	44.6 (26.3)	23.0 (9.6)	50.7 (12.6)	92.5 (25.9)	—
N (%)	20969	8576 (40.9)	9783 (46.6)	2610 (12.5)	—
Age (year)	54.7 (11.5)	55.2 (11.8)	54.4 (11.3)	54.2 (11.1)	<0.0001
Sex (men, %)	46.0	49.2	44.2	42.3	<0.0001
Income (EUR/year, %)					<0.0001
<25,000	35.9	38.1	35.0	32.0	
25,000–40,000	21.0	18.8	22.7	22.3	
>40,000	12.5	10.7	13.7	14.0	
Non-respondents	30.6	32.5	28.7	31.8	
Education (%)					<0.0001
Up to middle school	51.1	55.9	48.5	45.0	
Secondary school	35.6	32.4	37.6	38.5	
University or higher	13.2	11.6	13.8	16.5	
BMI (%)					0.63
Normal (<25)	28.4	28.2	28.4	29.0	
Overweight (≥25<30)	42.7	42.5	42.7	43.1	
Obese (≥30)	28.9	29.3	28.9	27.9	
Abdominal obesity (%)	72.9	73.7	72.2	72.8	0.62
Smokers (%)	23.5	23.5	23.3	24.3	0.0022
Leisure-time PA (met-h/day)	3.5 (4.0)	3.4 (4.0)	3.6 (3.9)	4.0 (3.9)	<0.0001
Drug for diabetes (%)	4.1	4.0	4.1	4.4	0.025
Systolic BP (mmHg)	140.0 (20.4)	140.4 (20.5)	140.1 (20.4)	140.2 (20.4)	0.69
Diastolic BP (mmHg)	82.4 (9.6)	82.4 (9.5)	82.6 (9.6)	82.6 (9.7)	0.24
Drugs for hypertension (%)	24.8	24.9	24.6	25.4	0.016
Drugs for lipids (%)	5.5	5.1	5.7	6.2	0.0003
Cancer (%)	3.1	3.2	2.9	3.5	0.37
Aspirin use (%)	2.2	2.4	2.1	1.7	0.43
C-reactive protein (mg/L)	2.52 (3.17)	2.51 (3.18)	2.51 (3.18)	2.49 (3.07)	0.94
Low-grade inflammation ^b	−0.17 (5.9)	−0.07 (5.9)	−0.25 (5.9)	−0.38 (5.9)	0.030
Total cholesterol (mg/dl)	214 (41)	213 (42)	214 (41)	215 (42)	0.15
HDL-cholesterol	57.8 (14.8)	57.3 (14.9)	57.3 (14.8)	57.6 (14.7)	0.69
LDL-cholesterol	131 (34)	130 (35)	131 (34)	132 (35)	0.027
Triglycerides (mg/dl)	129 (85)	131 (87)	130 (83)	128 (85)	0.43
Blood glucose (mg/dl)	100 (24)	100 (23)	101 (24)	101 (24)	0.061
Insulin (pmol/l)	59.3 (41.0)	59.0 (41.6)	60.0 (42.0)	59.8 (35.1)	0.21
Mediterranean diet score ^a	3.9 (1.5)	3.8 (1.5)	3.9 (1.5)	4.1 (1.5)	<0.0001
Energy intake (Kcal/day; means, SD)	2088 (573)	2022 (571)	2119 (568)	2193 (577)	<0.0001

Values for age, leisure-time physical activity (PA), systolic and diastolic blood pressure (BP), C-reactive protein, low-grade inflammation, blood lipids, blood glucose, insulin, Mediterranean diet score and energy intake are presented as means and standard deviation; means and p values are adjusted for age, sex and energy intake.

^a Low-grade inflammation was assessed by the INFLA-score.

^b A Mediterranean diet score deprived of fish intake.

HR = 0.55; 95%CI 0.34–0.89, respectively, Table 3), whereas no effect was documented for any frequency of lean fish intake.

A cubic spline was also generated for testing linear/nonlinear association of continuous fatty fish intake (g/d) with risk of composite CHD and stroke. We found a linear inverse relationship between fatty fish consumption and the risk for the composite outcome (p value for overall association = 0.025; p value for non-linear association = 0.99; Fig. 1).

Discussion

Findings from the present study show an inverse association between fish intake and risk of composite CHD and stroke in a large Mediterranean population-based cohort of adult individuals apparently free from CVD at time of

recruitment. Results were also confirmed for incident CHD, while risk of stroke exhibited a downward trend, though not significant, likely due to the relatively low number of events.

To the best of our knowledge, this is the first study evaluating the association between fish intake and composite cardiovascular endpoints in a general population characterized by a relatively high adherence to the Mediterranean Diet, suggesting that higher fish consumption offers an additive protection over the cardiovascular benefit of the overall Mediterranean Diet.

Observational evidence on the CVD advantages deriving from fish intake has provided convincing results in a number of population settings with different fish consumption. In fish eating populations, such as the Japanese subjects recruited within the JPHC study with a median fish intake of 78 g/d, the highest category of intake,

Table 2 Incidence and Hazard Ratios for fatal and non-fatal CHD and stroke according to weekly consumption of fish.

	Fish intake (times/week)			1 time per week increment	
	0–1.99	2–3.99	≥4		
g/d (Means, SD)	23.0 (9.6)	50.7 (12.6)	92.5 (25.9)		—
N of subjects, %	8576 (40.9)	9783 (46.6)	2610 (12.5)		—
Composite CHD/stroke (n = 352)					
N of events (rate)	174 (2.0)	149 (1.5)	29 (1.1)	<i>P for trend</i>	
Person-years	36,470	42,757	11,659	—	
Model 1 (HR, 95%CI)	—	0.82 (0.66–1.02)	0.62 (0.42–0.92)	0.0080	0.91 (0.84–0.99)
Model 2 (HR, 95%CI)	—	0.81 (0.65–1.02)	0.62 (0.42–0.93)	0.0084	0.91 (0.83–0.99)
Model 3 (HR, 95%CI)	—	0.81 (0.65–1.01)	0.60 (0.40–0.90)	0.0055	0.90 (0.83–0.98)
CHD (n = 287)					
N of events (rate)	140 (1.6)	124 (1.3)	23 (0.9)	<i>P for trend</i>	
Person-years	36,518	42,795	11,665	—	
Model 1 (HR, 95%CI)	—	0.85 (0.67–1.09)	0.62 (0.40–0.97)	0.028	0.91 (0.83–1.00)
Model 2 (HR, 95%CI)	—	0.85 (0.67–1.09)	0.62 (0.40–0.98)	0.029	0.91 (0.83–1.00)
Model 3 (HR, 95%CI)	—	0.85 (0.66–1.09)	0.60 (0.38–0.94)	0.020	0.90 (0.82–0.99)
Stroke (n = 66)					
N of events (rate)	34 (0.4)	26 (0.3)	6 (0.2)	<i>P for trend</i>	
Person-years	36,715	43,021	11,696	—	
Model 1 (HR, 95%CI)	—	0.71 (0.42–1.19)	0.63 (0.26–1.50)	0.15	0.91 (0.75–1.11)
Model 2 (HR, 95%CI)	—	0.70 (0.42–1.18)	0.63 (0.26–1.51)	0.15	0.91 (0.74–1.11)
Model 3 (HR, 95%CI)	—	0.70 (0.42–1.18)	0.62 (0.26–1.51)	0.15	0.91 (0.74–1.11)

Model 1 adjusted for age, sex and energy intake. Model 2: as in model 1 further adjusted for education, smoking, drugs for diabetes, drugs for hypertension, drugs for lipids, MDS without fish. Model 3: as in model 2 further adjusted for blood glucose, LDL-cholesterol and low-grade inflammation.

Table 3 Association of fish type intake (times/week) with risk of CHD and stroke.

Weekly consumption	%	Mean daily intake (g/d)	Composite CHD/stroke		CHD		Stroke	
			N of events/ subjects	HR (95%CI)	N of events/ subjects	HR (95%CI)	N of events/ subjects	HR (95%CI)
Shellfish, shrimps and crustaceous								
None	12.1	0.0	55/2539	—1—	45/2539	—1—	11/2539	—1—
<1	55.8	6.8 (4.5)	200/11699	0.99 (0.72–1.36)	162/11699	0.93 (0.66–1.33)	38/11699	1.13 (0.55–2.30)
≥1	32.1	21.8 (9.8)	97/6731	1.02 (0.72–1.45)	80/6731	0.95 (0.65–1.41)	17/6731	1.19 (0.53–2.68)
Dried fish								
None	11.3	0.0	28/2366	—1—	24/2366	—1—	4/2366	—1—
<1	80.2	5.4 (4.3)	287/16816	1.03 (0.69–1.53)	235/16816	0.97 (0.63–1.49)	53/16816	1.44 (0.51–4.06)
≥1	8.5	25.7 (10.6)	37/1787	1.07 (0.65–1.77)	28/1787	0.95 (0.55–1.67)	9/1787	1.82 (0.55–6.05)
Canned fish								
None	12.3	0.0	56/2582	—1—	42/2582	—1—	14/2582	—1—
<1	54.9	4.5 (2.3)	203/11516	0.93 (0.69–1.25)	170/11516	1.03 (0.73–1.45)	33/11516	0.65 (0.34–1.22)
≥1	32.8	14.5 (6.9)	93/6871	0.85 (0.61–1.19)	75/6871	0.91 (0.62–1.33)	19/6871	0.76 (0.38–1.54)
Other fish								
None	8.6	0.0	31/1801	—1—	25/1801	—1—	6/1801	—1—
<1	42.2	10.6 (6.0)	171/8757	1.09 (0.73–1.62)	136/8757	1.06 (0.68–1.65)	36/8757	1.30 (0.53–3.20)
≥1	49.2	31.6 (16.4)	150/10311	0.83 (0.55–1.24)	126/10311	0.88 (0.56–1.37)	24/10311	0.67 (0.26–1.70)
Lean fish								
None	3.6	0.0	14/745	—1—	10/745	—1—	4/745	—1—
<1	50.1	10.3 (5.1)	196/10511	1.38 (0.78–2.45)	163/10511	1.58 (0.81–3.08)	34/10511	0.92 (0.30–2.77)
≥1	46.3	30.9 (14.3)	142/9713	1.25 (0.70–2.23)	114/9713	1.38 (0.70–2.71)	28/9713	0.91 (0.30–2.75)
Fatty fish								
None	4.1	0.0	27/873	—1—	22/873	—1—	5/873	—1—
<1	52.9	8.9 (4.8)	204/11086	0.66 (0.43–1.01)	164/11086	0.62 (0.39–0.99)	40/11086	0.97 (0.36–2.60)
≥1	43.0	24.2 (11.1)	121/9010	0.56 (0.36–0.87)	101/9010	0.55 (0.34–0.89)	21/9010	0.69 (0.24–1.94)

Mean daily intake is expressed as means, SD. Hazard ratio obtained from the model adjusted for age, sex, energy intake, education, smoking, drugs for diabetes, drugs for hypertension, drugs for lipids, MDS without fish, blood glucose, LDL-cholesterol and low-grade inflammation. For analysis with fish types, hazard ratios were obtained from the model including all fish types simultaneously. Hazard ratios for lean and fatty fish intake were obtained from the model including both types simultaneously and further controlled for dried fish and other types of fish not included in lean/fatty types.

Figure 1 Risk of composite CHD and stroke associated with fatty fish intake (g/d) within the population of the Moli-sani study ($n = 20,969$). Hazard ratios (95% confidence interval) were adjusted for age, sex, energy intake, education, smoking, drugs for diabetes, drugs for hypertension, drugs for lipids, Mediterranean diet score without fish, blood glucose, LDL-cholesterol, low-grade inflammation, lean fish (g/d), dried fish (g/d) and other types of fish (g/d) not included in lean/fatty types.

corresponding to 8 times/week, was associated with reduced risk of coronary heart disease, primarily nonfatal cardiac events, as compared with those having fish 1 time per week, while no benefits were documented at lower intakes [9].

Cardiovascular benefits were observed also in the JACC study, in which a protection from cardiovascular death associated with greater fish intake was found [17], in agreement with previous findings on middle-aged Chinese men reporting lower risk of fatal acute myocardial infarction [18].

The positive association between fish intake and CVD outcomes has been documented also in low fish-consuming populations; the MORGEN study in the Netherlands showed that fish intake was likely to lower the risk of fatal CHD and myocardial infarction in a dose-responsive manner also in populations with a low habitual fish consumption, with protection from fatal CHD being evident even at modest intakes [10]. Similarly, in the Zutphen study at least one portion of fish per week was found to be associated with a reduced stroke incidence [19]. Data from US showed a reduced risk of ischaemic stroke in men at very low intakes (one per month or more) [20] and similar findings were observed in US women [21], although the Women's Health Study failed to find any association with risk of CVD [22].

Omega-3 fatty acids (eicosapentaenoic, EPA, and docosahexaenoic, DHA, acids) have been identified as the likely active constituents of fish and have numerous health benefits, such as pleiotropic protective cardiovascular effects, including lowering blood pressure, modulating

serum lipids, improving diabetes and insulin resistance, reducing arrhythmia, suppressing platelet aggregation, improving endothelial function, and inhibiting inflammation [23,24]. However, advantages associated with omega-3 fatty acids were mainly observed for high amounts, often not within the range of dietary intake of fish [7].

We also evaluated the association of a number of fish sub-types with incident CVD. No single fish type resulted associated with reduced CVD risk, although a trend toward an increased risk was found associated with greater consumption of dried fish: the latter is usually high in salt content, a major risk factor for CVD and in particular for stroke [25].

However, when distinction was made on the basis of fat content, higher intake of fatty fish only was found to be related to reduce incidence of the composite outcome and of CHD events. This finding is in line with prior observations on a Dutch population showing a lower sudden coronary death risk possibly associated with long-term fatty-fish consumption [26]. Within Italian men recruited in the Seven Countries study risk of CHD mortality too resulted lowered by consumption of fatty fish but not of lean fish [11].

The heart advantages associated with consumption of fatty fish are likely linked to beneficial effects on a number of CVD risk factors, such as triglycerides lowering and improvement of HDL-cholesterol levels, as shown by intervention trials [27,28].

In our cohort, high intake of fish was not associated at baseline with better cardiovascular health profile. Indeed, individuals consuming greater amounts of fish had

metabolic biomarker levels comparable to those in the lowest group of intake. Lipids, blood glucose levels and low-grade inflammation did not appear to be on the pathway of the association between fish intake and CVD risk, possibly because the high fish consumers had higher prevalence of the use of drugs for diabetes, hypertension and lipid-lowering medications. This may be possibly due at least in part to reverse causation with groups at high cardiovascular risk likely having shifted their diet, if not fish-dislike, by including more fish intake. The exclusion of subjects with prior CVD events is likely to limit such potential source of bias.

Further studies are needed to clarify the possible mechanisms underlying the relationship of fish intake with health outcomes by addressing a number of pathways possibly including both traditional and emerging CVD risk factors, such as low-grade inflammation.

Strengths and limitations of the study

Major strengths of this study include a large community-based cohort, its prospective design, detailed information of dietary intake, and the considerable number of covariates, thus minimising sources of bias and confounding. It also shows, possibly for the first time, that high fish consumption is associated with a significant cardiovascular benefit in people already adhering to the Mediterranean Diet.

This study also presents several limitations. First, information on dietary intake was self-reported and can lead to under- or overestimates. Another limitation is represented by the relatively short follow-up duration and a consequent small number of incident CVD events. The observational nature of the study cannot allow to fully rule out residual confounding or confounding by unmeasured factors. Finally, subjects' information was collected at baseline only, thus life-course changes possibly occurred during the follow-up may have influenced the strength of our findings.

Data used in the present study have been collected in a region located between Central and Southern Italy, Mediterranean by tradition and culture but also representative of the general Italian population, thus caution is needed in extending the results to other contexts.

Conclusions

The favourable health benefits associated with fish intake are extensively documented in different epidemiological settings, especially in relation to CHD mortality risk reduction, and a dominant role of fatty fish over lean types clearly emerged in many studies. Our findings showed an inverse association of high fish intake with incidence of CVD, and confirmed a predominant role of fatty over lean fish. Few studies however had previously specifically addressed the relationship between fish intake and incidence of CVD in Mediterranean populations. To the best of our knowledge, this is one of the few studies examining longitudinally the association of total fish and fish types

intake with risk of developing CVD in a general population, already exposed to the heart advantages associated with adherence to a Mediterranean dietary pattern.

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Contribution

LI, MB and ER contributed to the conception and design of the work, and interpretation of data; SC, MP and ADeC managed data collection; MB, ADiC analysed the data; MB and ER wrote the paper; MBD, GdG, CC and LI originally inspired the research and critically reviewed the manuscript. All authors approved the final version of the manuscript.

Disclosure of conflicts of interest

None of the Authors had a personal or financial conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2017.08.004>.

Appendix

Moli-sani Study Investigators

The enrolment phase of the Moli-sani Study was conducted at the Research Laboratories of the Catholic University in Campobasso (Italy), the follow up of the Moli-

sani cohort is being conducted at the IRCCS Neuromed, Pozzilli, Italy.

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Hospitals: Presidi Ospedalieri ASReM (Presidio Ospedaliero A. Cardarelli – Campobasso; Ospedale F. Veneziale – Isernia; Ospedale San Timoteo – Termoli (CB); Ospedale Ss. Rosario – Venafro (IS); Ospedale Vietri – Larino (CB); Ospedale San Francesco Caracciolo – Agnone (IS)); Istituto di cura Villa Maria – Campobasso; Fondazione di Ricerca e Cura Giovanni Paolo II – Campobasso; IRCCS Neuromed – Pozzilli (IS).

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CHAPTER 6.

Egg consumption and risk of total and cause-specific mortality in a healthy Mediterranean population

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Abstract:	<p>Purpose: Dietary guidelines recommend to limit egg consumption to 4 servings per week but the relation between egg intake and health outcomes is still controversial. To evaluate the association of egg consumption and mortality risk in Italian adults and to investigate nutritional factors and serum lipids as potentially explaining such associations.</p> <p>Methods: Longitudinal analysis on 20,564 men and women aged ≥ 35y, free from cardiovascular disease (CVD) and cancer belonging to the Moli-sani Study cohort (enrolled 2005-2010) followed up for a median of 8.2 years.</p> <p>Results: In multivariable-adjusted analysis as compared to low intake (0-1 egg/week), eating >4 eggs/week led to an increased risk of all-cause (Hazard ratio [HR]=1.49; 95%CI 1.13-1.99), CVD (HR=1.72; 1.05-2.82) and cancer mortality (HR=1.52; 0.99-2.34). Similarly, an intake of 2-4 eggs/week was associated with higher all-cause (HR=1.22; 1.01-1.46) and CVD mortality risk (HR=1.44; 1.04-1.99). An increase of 1 egg per week was associated with higher mortality risk among high-risk individuals, such as those with hypertension and hyperlipidaemia.</p> <p>Dietary cholesterol explained about 30% of the association of eggs with all-cause and CVD mortality while serum lipids (e.g., total cholesterol) accounted for a small</p>

	<p>proportion of egg-mortality relation.</p> <p>Conclusions: Eating more than four eggs per week was associated with a markedly increased risk of mortality in a Mediterranean population. However, a significant increased mortality risk is also associated with consuming 2-4 eggs/week, an amount recommended instead by several dietary guidelines. A substantial part of this association was likely due to the egg contribution to dietary cholesterol.</p>
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Egg consumption and risk of total and cause-specific mortality in a healthy Mediterranean population

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ABSTRACT

Purpose: Dietary guidelines recommend to limit egg consumption to 4 servings per week but the relation between egg intake and health outcomes is still controversial. To evaluate the association of egg consumption and mortality risk in Italian adults and to investigate nutritional factors and serum lipids as potentially explaining such associations.

Methods: Longitudinal analysis on 20,564 men and women aged ≥ 35 y, free from cardiovascular disease (CVD) and cancer belonging to the Moli-sani Study cohort (enrolled 2005-2010) followed up for a median of 8.2 years.

Results: In multivariable-adjusted analysis as compared to low intake (0-1 egg/week), eating >4 eggs/week led to an increased risk of all-cause (Hazard ratio [HR]=1.49; 95%CI 1.13-1.99), CVD (HR=1.72; 1.05-2.82) and cancer mortality (HR=1.52; 0.99-2.34). Similarly, an intake of 2-4 eggs/week was associated with higher all-cause (HR=1.22; 1.01-1.46) and CVD mortality risk (HR=1.44; 1.04-1.99). An increase of 1 egg per week was associated with higher mortality risk among high-risk individuals, such as those with hypertension and hyperlipidaemia.

Dietary cholesterol explained about 30% of the association of eggs with all-cause and CVD mortality while serum lipids (*e.g.*, total cholesterol) accounted for a small proportion of egg-mortality relation.

Conclusions: Eating more than four eggs per week was associated with a markedly increased risk of mortality in a Mediterranean population. However, a significant increased mortality risk is also associated with consuming 2-4 eggs/week, an amount recommended instead by several dietary guidelines. A substantial part of this association was likely due to the egg contribution to dietary cholesterol.

Keywords: eggs; dietary cholesterol; mortality risk; Mediterranean diet

INTRODUCTION

Eggs are a common food in every diet and are used in many basic and formulated preparations. Eggs are affordable and nutrient-dense food items, containing high-quality protein with low levels of saturated fatty acids, and are rich in several micronutrients including vitamins and minerals, some of which (vitamin E, carotenoids) are reported to have antioxidant properties [1].

On the other side, eggs are also major sources of dietary cholesterol, a potential risk factor for cardiovascular health [2]. Dietary guidelines for egg consumption have changed several times over the past few decades, and vary among health agencies [3,4]. In 2010, the Dietary Guidelines for Americans recommended cholesterol intake to be limited to no more than 300 milligrams per day [5], but the 2015-2020 Dietary Guidelines Advisory Committee did not bring forward this recommendation, due to the lack of evidence of an appreciable relationship between consumption of dietary cholesterol and serum cholesterol, a decision consistent with the conclusions of the AHA/ACC report [6].

Based on their high cholesterol content, the Mediterranean Diet Foundation recommends to consume up to 4 eggs per week, as a healthy alternative to fish or meat [7], and the same amount (2-4 eggs per week) was indicated in the latest Italian dietary guidelines [8]. In the Eatwell Guide issued by the National Health Service in UK there is no recommended limit on how many eggs people should eat [9].

Epidemiological studies analysing the association of eggs with health outcomes have provided controversial results [10-12]. Several meta-analyses and an umbrella review of observational studies failed to report significant associations between egg intake and cardiovascular outcomes, even highlighting a downward trend association with stroke risk [13-16].

Data on the association of egg intake with mortality risk is limited and controversial [17-19], and the scientific evidence for the recommendations on dietary cholesterol and eggs is inconsistent and

lacking in Mediterranean countries where the association between eggs and health has been seldom explored [10,20,21].

In light of this, our study aimed first to longitudinally evaluate the association of egg consumption with total and cause-specific mortality in a large Mediterranean population of adult men and women; second, to investigate whether dietary cholesterol and other nutritional factors could be on the pathway between egg intake and mortality risk.

MATERIALS AND METHODS

Study population

Data are from the Moli-sani Study cohort established in 2005–2010 in the Molise region, a Mediterranean area in Italy, that recruited 24,325 men and women aged ≥ 35 years [22].

For the purpose of the present analyses, we excluded subjects with history of CVD ($n=1,537$) or missing data on CVD ($n=360$), cancer ($n=777$) or missing data on cancer ($n=89$), those individuals with missing data for egg intake ($n=100$), those reporting implausible energy intakes (<800 kcal/d in men and <500 kcal/d in women or >4000 kcal/d in men and >3500 kcal/d in women; $n=771$), dietary or medical questionnaires judged as unreliable by interviewers ($n=955$ and $n=235$, respectively), subjects lost to follow-up ($n=23$) and missing information on cause-specific death ($n=45$).

We finally analysed 20,564 individuals (82.6% of the study sample).

The Moli-sani Study cohort was followed up until December 31st 2015 with the main outcome of interest being mortality. Overall and cause-specific mortality was assessed by the Italian mortality registry (ReNCaM registry), validated by Italian death certificates (ISTAT form) and coded according to the International Classification of Diseases (ICD-9 version).

Cardiovascular (CVD) mortality included deaths from diseases of the circulatory system, when the underlying cause of death included ICD9 codes 390-459. ICD-9 codes 430-438 were used to define specific cause of death for cerebrovascular disease, ICD-9 codes 410-414 and 429 for ischemic heart

disease (IHD). Cancer death was considered when the underlying cause of death included ICD9 codes 140-208. Non cardiovascular/non cancer causes of death were included in 'other cause mortality' group.

The Moli-sani Study complies with the Declaration of Helsinki and was approved by the ethical committee of the Catholic University Medical School in Rome, Italy. All participants provided written informed consent.

Dietary assessment

Dietary intake was assessed by a trained interviewer-administered semi-quantitative EPIC food frequency questionnaire (FFQ) validated and adapted to the Italian population to assess participants' diet during the past 12 months.

The FFQ includes 188 food items, classified into 74 predefined food groups on the basis of similar nutrient characteristics or culinary usage [23]. Participants were asked to indicate the number of times a given item was consumed (per day, week, month or year) from which the frequency of consumption was calculated. The quantity of food consumed was assessed by asking the participant to select one among several images of different food portions or a predefined standard portion, when no image was available.

Frequencies and quantities of each food were then linked to Italian Food Tables [24], using a specifically designed software [25], to obtain quantitative estimates of daily intake of macro- and micro-nutrients plus energy.

Total egg consumption from various food sources (e.g. whole egg, omelette) was defined as number per week (we used 50 g as the standard weight for one medium-sized egg) and categorized as up to 1 egg/week, $>1 \leq 2$ eggs/week, $>2 \leq 4$ eggs/week and >4 eggs/week or used as a continuous variable as a 1 egg increment per week.

Main macronutrients contained in eggs (saturated fatty acids and protein, expressed as g/d) and dietary cholesterol (mg/d), calculated as mentioned above, were tested as potential mediators of the association between egg intake and mortality. Moreover, we similarly calculated and tested dietary vitamin E (mg/d) and beta-carotenoids ($\mu\text{g/d}$), two micronutrients present in egg yolk that have been reported to have antioxidant properties [1].

Adherence to the traditional Mediterranean diet (MD) was evaluated by the Mediterranean Diet Score (MDS) developed by Trichopoulou et al. [26].

Assessment of covariates

At baseline, information on socio-demographic variables, lifestyles and medical history were obtained by interviewer-administered questionnaires.

Participants were considered to have diabetes, hypertension or hyperlipidaemia at baseline if they were taking disease-specific drugs.

Leisure-time physical activity (PA) was calculated for sport activity, walking and gardening, and then dichotomized as $<$ or ≥ 30 min/d. Height and weight were measured and body mass index (BMI) was calculated as kg/m^2 and then grouped into three categories as normal (≤ 25), overweight (25-30) or obese (≥ 30).

Subjects were classified as never-smokers, current smokers or former smokers (quit at least 1 year ago). Education was based on the highest qualification attained and was categorized as up to lower secondary (approximately ≤ 8 years of study), upper secondary school (8-13 years of study) and postsecondary education (>13 years of study).

Urban or rural environments were defined on the basis of the urbanization level as described by the European Institute of Statistics (EUROSTAT definition) and obtained by the tool ‘Atlante Statistico dei Comuni’ provided by the Italian National Institute of Statistics [27].

Venous blood samples were obtained from participants who had fasted overnight and had refrained from smoking for at least 6 h.

Serum lipids (total cholesterol, HDL-cholesterol, triglycerides) were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350, Instrumentation Laboratory (IL), Milan, Italy).

Low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald.

Quality control (high and low levels) for lipids was obtained by a commercial standard provided by the IL and an in-house serum standard pool. The coefficients of variability were respectively for high, medium and low values 4.9%, 5.2% and 4% for blood cholesterol; 3.2%, 3% and 4.5% for HDL-cholesterol; 5.2%, 5.3% and 5% for triglycerides.

Statistical analysis

Baseline characteristics of the study population by categories of egg intake were presented as means with standard deviation (SD), or number and percentages (Table 1).

Associations for continuous and categorical variables were estimated by using general linear models adjusted for age, sex and energy intake (kcal/d) (PROC GENMOD and PROC GLM in SAS for categorical and continuous variables, respectively; Table 1 and Supplementary Table 1).

Risk estimates for all-cause and cause-specific deaths were expressed as hazard ratios (HR) with 95% confidence intervals (95% CI) and calculated by using Cox regression models with time-on-study on the time scale and adjusting for baseline age as covariate in the model.

Based on previous literature and biological plausibility, two multivariable models were fitted to assess the association between egg intake and mortality: Model 1 was adjusted for age (continuous), sex and energy intake (kcal; continuous); Model 2 as in model 1 further controlled for education (up to lower secondary school; upper secondary school; postsecondary/higher), residence (urban, rural), leisure-time PA (<30 or ≥30 min/d), smoking (never, current, former), BMI (normal, overweight, obese), diabetes (no, yes, missing), hyperlipidaemia (no, yes, missing), hypertension (no, yes,

missing); Model 3 as in model 2 further controlled for MDS (continuous). Missing values for diabetes (n= 242), hypertension (n=131) and hyperlipidaemia (n=148) were included in the models as dummy variables, similar to the way valid categories were represented. For education, BMI and smoking (less than 1% of missing values) missing values were imputed to the modal value. Mediation analysis was used to estimate the possible contribution of nutritional factors and serum lipids (total blood cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) in accounting for the association of egg intake with mortality. For the mediation analysis we used the %MEDIATE macro in SAS software [28] to calculate the point and interval estimates of the percent of exposure effect (PTE) explained by one or more intermediate variables, with 95% confidence interval and P values. Nutritional factors and serum lipids were entered into the mediation analysis as continuous variables and positively skewed variables (triglycerides) were log transformed before analysis. Mediation analyses with biomarkers were restricted to 20,148 subjects after exclusion of those individuals with missing data on any of the biomarker.

To increase the applicability of the study results we calculated risk estimations for each additional 186 mg of dietary cholesterol per day, 6.2 g of dietary proteins per day, 1.6 g of saturated fatty acids per day, 0.56 mg of vitamin E per day and 42 µg per day of beta-carotene, since these are the amounts of nutrients present in 1 egg, based on data from Food Composition Database for Epidemiological studies [29].

We performed sub-group analysis according to levels of intake of each food group included into the MDS (above/below the study population median; supplementary Table 3). Furthermore, we conducted subgroup analyses to test the robustness of the observed associations to various baseline risk factors: age (35-64 and ≥ 65 years), sex, education, lifestyles (smoking status, leisure-time PA), health conditions at baseline (BMI, diabetes, hypertension and hyperlipidaemia), and after excluding those subjects who died during the first 2 years of follow-up (Supplementary Table 4). Appropriate

multiplicative terms for testing interaction were included in the multivariable-adjusted models to test for a difference of effect of egg intake across subgroups.

The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows©2009.

RESULTS

In this Moli-sani population the mean consumption was 1.8 eggs per week ($SD \pm 1.3$), while overall mean dietary cholesterol consumption was 322 mg/d ($SD \pm 108$). Egg contributed to 14.7% of total cholesterol intake in the diet.

Compared to low intake (0-1 eggs/week), consumers of >4 eggs/week had less CVD risk factors (i.e. BMI, diabetes, hypertension and hyperlipidaemia), including lower levels of blood lipids (Table 1), tended to be less close to an MD and globally consumed less fruits and nuts, cereals, and more meat and meat products (Supplementary Table 1).

The contribution of proteins and fats to total energy intake increased across categories of egg consumption, whereas the contribution of carbohydrates and fibre decreased; dietary cholesterol increased according to egg intake, as well as vitamin E levels while no substantial differences were observed for beta-carotene levels (Supplementary Table 1).

The cohort of 20,564 participants was followed-up for a median of 8.3 years (interquartile range=7.4-9.3 y; 170,049 person-years) during which 838 deaths were ascertained and validated; 271 from CVD, of which 153 were from IHD/cerebrovascular disease, 334 from cancer, and 233 from other causes. In a multivariable-adjusted model including also MDS, as compared to participants reporting lower egg consumption, consuming more than 4 eggs/week was associated with increased risk of all-cause ($HR = 1.49$; 95%CI 1.13-1.99), CVD ($HR = 1.72$; 1.05 -2.82) and cancer mortality ($HR = 1.52$; 0.99-2.34); an upward trend of risk with IHD/cerebrovascular disease associated with increased egg intake was also observed ($HR = 1.57$; 0.78-3.13), although statistical significance was

not hold (Table 2, models 3). The recommended intake of 2-4 eggs/week was also associated with higher all-cause (HR=1.22; 1.01-1.46) and CVD mortality risk (HR=1.44; 1.04-1.99) (Table 2, models 3).

Multivariable-adjusted Kaplan-Meier estimates for all-cause mortality and CVD mortality for the four categories of egg consumption are well- separated (Figures 1A and 1B) and showed increased mortality risk associated with higher egg intake ($p=0.030$ and $p=0.083$ respectively).

Each additional egg per week was associated with higher risk of all-cause (HR= 1.06; 1.00-1.12) and CVD mortality (HR=1.10; 1.01-1.20) and slightly with mortality risk from IHD/cerebrovascular causes (HR=1.11; 0.99-1.26; Table 2, models 3).

Dietary cholesterol inversely correlated with total serum cholesterol levels (Spearman correlation coefficients $r=-0.019$, $p=0.0051$), HDL-cholesterol ($r=-0.025$, $p=0.0004$) and triglycerides ($r=-0.045$, $p<0.0001$) and positively with saturated fat ($r=0.83$; $p<0.0001$), but was not related to LDL-cholesterol ($r=-0.00065$, $p=0.93$; data not shown).

Each additional 186 mg of dietary cholesterol consumed per day was associated with higher risk of all-cause (HR= 1.28; 95% CI 1.08-1.52) and CVD mortality (HR= 1.39; 95% CI 1.03-1.88; Supplementary Table 2, model 2) although the strength of the association was reduced after inclusion of other nutrients into the model. Similarly, saturated fats intake was associated with increased risk of total and CVD mortality, although adjustment for other dietary factors mitigated the magnitude of the association (Supplementary Table 2).

Sub-group analyses indicated that overall diet quality, measured by the MDS, and single food groups were not effect modifiers of the relation between eggs and mortality risk (all p values for interaction >0.05 ; Supplementary Table 3), although an increased risk of CVD mortality associated with 1-egg increment per week was found among low fruit consumers as compared to high consumers (p for interaction=0.0038, supplementary Table 3).

Among baseline risk factors, hyperlipidaemia and hypertension were likely to modify the magnitude of the association between egg consumption (1-egg increment per week) and CVD mortality risk, resulting to be stronger in those using lipid-lowering drugs (p for interaction=0.012) and among participants taking antihypertensive medications (p for interaction = 0.045; Supplementary Table 4).

Mediation analysis

We tested three nutritional factors prevalently present in eggs and two sources of antioxidants as possibly mediating the association with mortality. Dietary cholesterol explained 32.5% ($p<0.0001$) and 29.0% ($p<0.0001$) of the association of egg intake with all-cause and CVD mortality risk, respectively; saturated fats accounted from 9.9% to 8.0%, while dietary protein offered little contribution (Table 3). Antioxidant content was not on the egg-mortality pathway. Baseline differences in serum lipids across categories of egg intake only marginally accounted for the association of eggs with CVD mortality, while explained 6.5% ($p<0.0001$) and 5.4% ($p=0.0003$) of the relation between high egg consumption and all-cause and CVD mortality risk (Table 3).

DISCUSSION

In a large cohort of healthy adult Mediterranean individuals, eating more than 4 eggs per week was associated with an increased risk of all-cause and CVD mortality, in comparison with a lower intake (up to 1 egg per week) and independently of overall diet quality as reflected by adherence to the Mediterranean diet. Of interest, we found that even moderate egg intake of 2-4 servings per week, which is generally recommended by international dietary guidelines, led to an increased risk of all-cause and CVD mortality by 22% and 44%, respectively.

Egg and dietary cholesterol intakes in our cohort were similar to that reported in European countries from EPIC study [30] and in some US cohorts [31,32], but egg consumption in our cohort was

slightly lower than that documented in other Mediterranean cohorts, which reported an average intake of 3–4 eggs per week [9,19].

Our findings expand the scarce literature on this topic in Mediterranean settings. Consumption of 10 grams of egg per day (corresponding to approximately 1 and a half egg per week) was found associated with increased risk of total (31%) and CVD death (54%) in diabetic patients from the Greek arm of the EPIC study [21], while in the Spanish cohort of EPIC no association between egg consumption (up to 7 egg/week) and all-cause, CVD and IHD of mortality was found; yet, inverse associations between egg intake and risk of death from other causes (24% reduction) and from the nervous system diseases (41%) were documented [20]. In the Spanish SUN cohort, no association was found between egg consumption and the incidence of CVD [9].

Studies from non-Mediterranean cohorts also yielded inconsistent findings. In a recent analysis from the NHANES cohort no significant associations between egg consumption and mortality in US adults were observed [33]; on the contrary, pooled data from 6 US based cohorts showed that each additional half an egg consumed per day was significantly associated with 6% higher risk of incident CVD and 8% of all-cause mortality [30].

Moreover, recent findings from nine European countries showed that higher egg consumption was associated with higher risk of haemorrhagic stroke (25%) and an upward trend of increased ischaemic stroke risk [30], and in a Japanese cohort of women, a direct association was reported between egg intake (≥ 2 eggs/d vs 1 egg/d) and total mortality [17].

A meta-analysis of 14 studies involving 320,778 subjects identified a dose-response positive association between egg consumption and the risk of CVD and diabetes [12], but more recently an analysis from three large US cohorts and an updated meta-analysis including 28 prospective studies showed that moderate egg consumption (up to 7 eggs per week) was not associated with cardiovascular disease risk overall [34].

Recently, a dose–response meta-analysis of prospective cohort studies found no association with risk of cardiovascular outcomes following the habitual consumption of one egg per day compared to no intake, with exception of the risk of heart failure, which resulted higher especially in men from US cohorts [35].

Inconsistencies across studies may be due to differences in population characteristics, sample sizes, cooking methods for eggs, or differences in dietary patterns related to different amounts of egg consumption and also different adjustments for confounders.

Our study relied on a comprehensive assessment of nutritional factors (e.g. saturated fats and overall diet quality) and also on a number of other potential confounding variables, such as lifestyles and socio-demographic factors.

We also observed an increased risk of cancer mortality associated with eating more than 4 eggs per week and this is in line with a dose–response meta-analysis of prospective observational studies showing that eating ≥ 5 eggs per week may be associated with a modestly elevated risk of breast, ovarian and fatal prostate cancers [36]. Similarly, Japanese women consuming more than 2 eggs per day, as compared to those having 1 egg/d, tripled their risk of dying from cancer [17].

Generally, eggs are a controversial food because of their saturated fat (about 3 g/100 g) and cholesterol content (about 370 mg/100 g) [29] and on this basis experts have produced mounting evidence against frequent egg consumption due their potential association with CVD [31].

Indeed, pooled data from 6 US based cohorts recently showed that each additional 300 mg of dietary cholesterol consumed per day was significantly associated with 17% and 18% higher risk of incident CVD and all-cause mortality, respectively [31], while others found that the dietary cholesterol-mortality relation likely depends on the baseline intake levels, with an inverse association in those with lower intake levels (<250 mg/day) but a positive association in those with higher intake levels (≥ 250 mg/day) [33].

In our study, we found that a substantial part of the excess risk of all-cause and CVD mortality associated with egg intake was accounted for by dietary cholesterol that, in turn, was associated with an increased risk of all-cause and CVD mortality, although the strength of the relation was reduced in multivariable-adjusted models. Our data are in accordance with observational data from US cohorts reporting that dietary cholesterol largely explained the association of eggs with increased CVD mortality [31].

However, the potential health risk of high dietary cholesterol levels has been questioned [37, 38] and more recently a meta-analysis concluded that available evidence is too heterogeneous and actually lacks methodologic rigor to draw any definitive conclusion regarding the influence of dietary cholesterol on CVD risk [37].

Since cholesterol-containing foods are usually rich in saturated fat and animal protein, which have been associated with increased CVD mortality risk in previous reports [39, 40], we also accounted for such nutrients but found that proteins were unlikely to be on the pathway between eggs and mortality risk, while saturated fats played a limited role. These results should be interpreted in light of the fact that eggs contain high-quality protein with minimal saturated fatty acids.

Differences at baseline in serum lipids among study participants were unlikely to explain the excess of CVD risk associated with frequent egg intake, while explaining about 7% of the relation with total mortality. Individuals eating eggs more frequently tended to have lower levels of serum lipids; this may be counter-intuitive, but epidemiological evidence on a direct association between serum lipids and disease/death risk is inconsistent and not fully elucidated [41,42].

Strengths and limitations

To our knowledge, this is one of the largest prospective cohort studies evaluating the association between egg consumption and mortality in a Mediterranean population, and one of the few examining the nutrient-related mediating pathways between egg intake and mortality.

Major strengths of this study include a large community-based cohort, its prospective design, detailed information of dietary intake and the considerable number of covariates allowing to minimize sources of bias and confounding.

However, this study also suffers of several limitations: first, the observational nature of the study cannot allow to fully rule out residual confounding or confounding by unmeasured factors. Second, information on dietary intake was self-reported and can lead to under- or over-estimates; moreover, subjects' dietary information was collected at baseline only, thus life-course changes possibly occurred during the follow-up period, may have influenced the strength of our findings. Also, we do not have data on preferred cooking methods which may likely influence the egg-mortality association.

Finally, participants lived in Molise, a region located between central and southern Italy, that its traditionally Mediterranean in culture; thus caution is needed in extending our results to other geographical and cultural contexts, although the main characteristics of our population sample are comparable to those of the Italian Cardiovascular Epidemiological Observatory and therefore representative of at least the Italian population [43].

Conclusions

Our findings report an increased risk of all-cause, CVD and cancer mortality associated with intake of more than 4 eggs per week in a large cohort of Italians from the general population. Increased death risk was also observed at lower intakes, namely 2-4 servings per week, which correspond to the egg intake recommended by many health advisory bodies and international dietary guidelines. The adverse health effects of eggs were found to be independent of the overall quality of the diet, while part of the excess of all-cause and CVD mortality risk was likely due to the high content of cholesterol and saturated fats of eggs, which in turn were found predictive of higher mortality risk in

our cohort. As dietary cholesterol was not directly related to serum lipids, its adverse health effect may likely pass through biological mechanisms other than cholesterol-related ones.

Although we do acknowledge the limitations of our observational study, our findings are unlikely to be supportive of the current dietary guidelines most of which recommending a safe use up to 4 eggs per week, or even no type of restriction.

Finally, caution should be taken especially for high-risk individuals, such as those with hypercholesterolemia and hypertension.

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Authors' contribution: ER, MB, and LI contributed to the design of the study and interpretation of data; SC, ADeC and MP managed data collection; ER, MB and ADiC analysed the data; ER and MB wrote the manuscript; MBD, CC, GdG and LI originally inspired the research and critically reviewed the manuscript. All Authors have read and approved the manuscript.

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Legend to Figure 1

Multivariable adjusted Kaplan-Meier estimates for all-cause (1A) and cardiovascular mortality (1B) for increasing number of weekly egg consumption in the Moli-sani Study cohort.

Estimates were obtained from the multivariable-adjusted model including age, sex, energy intake, educational level, residence, smoking, leisure-time PA, BMI, presence at baseline of diabetes, hyperlipidaemia, hypertension, Mediterranean diet score.

Table 1. Baseline characteristics by categories of egg consumption in the Moli-sani Study cohort (n=20,564)

	Egg consumption (n of egg/week)				
	Whole sample	0-1	>1 ≤2	>2 ≤4	>4
Mean number of eggs (min-max)	1.8 (0.0-17.8)	0.6 (0-0.99)	1.5 (1.0-1.99)	2.6 (2.0-3.99)	5.0 (4.0-17.8)
N of subjects (%)	20,564	5517 (26.8)	7143 (34.8)	6526 (31.7)	1378 (6.7)
Age, y; mean (SD)	55 (12)	55 (11)	54 (11)	54 (11)	55 (11)
Men	47.0	47.6	47.1	45.9	48.8
Educational level					
<i>Up to lower secondary</i>	51.1	52.8	50.7	49.7	52.9
<i>Upper secondary</i>	35.7	33.7	36.2	36.9	35.5
<i>Postsecondary</i>	13.2	13.5	13.1	13.4	11.6
Residence (urban)	67.0	66.4	66.9	66.7	70.0
Smoking status					
<i>Non-smokers</i>	50.3	49.9	50.5	50.6	49.6
<i>Current smokers</i>	23.8	22.9	23.3	24.4	27.1
<i>Former smokers</i>	25.9	27.2	26.2	25.0	23.3
Physical activity >30 min/d	64.0	63.8	63.5	63.8	68.4
BMI, kg/m ²					
<i>Normal (≤25)</i>	28.4	27.0	27.1	30.4	30.8
<i>Overweight (25-30)</i>	42.9	42.4	43.9	42.5	41.7
<i>Obese (≥30)</i>	28.7	30.5	29.0	27.1	27.6
Diabetes	4.0	4.6	4.2	3.4	3.1
Hypertension	25.2	28.9	25.6	22.4	21.4
Hyperlipidaemia	5.3	7.4	5.5	4.0	2.2
Total blood cholesterol, mg/dL; mean (SD)	213.8 (40.8)	215.6 (41.8)	213.7 (40.3)	212.5 (40.3)	210.4 (40.5)
HDL-cholesterol, mg/dL; mean (SD)	58.0 (14.7)	57.6 (14.9)	57.5 (14.6)	57.9 (14.7)	57.4 (14.7)
LDL-cholesterol, mg/dL; mean (SD)	131.3 (34.6)	132.7 (35.5)	131.4 (34.2)	130.3 (34.4)	129.4 (34.5)
Triglycerides, mg/dL; mean (SD)	122.4 (64.1)	126.8 (65.1)	123.9 (64.9)	121.2 (62.6)	118.4 (62.5)

1 egg = 50 grams.

Values are reported as percentages unless otherwise stated.

Means are adjusted for age, sex and energy intake.

Analyses for serum lipids were run on 20,148 participants.

Table 2. Risk of all-cause and cause-specific mortality associated with egg consumption in the Moli-sani Study cohort (n=20,564)

		Egg consumption (n of egg/week)					P trend	Continuous (1 egg/week increment)
		0-1	>1 ≤2	>2 ≤4	>4			
N of subjects (%)		5517 (26.8)	7143 (34.8)	6526 (31.7)	1378 (6.7)	-		
<i>All-cause mortality</i>								
N of deaths		234	281	256	67	-		
Person-years		44,712	58,776	54,630	11,931	-		
Event rates per 10,000 person-years		52.3	47.8	46.9	56.1	-		
Model 1		-1-	1.13 (0.95-1.35)	1.23 (1.03-1.48)	1.55 (1.17-2.06)	0.0019		1.07 (1.01-1.12)
Model 2		-1-	1.14 (0.95-1.36)	1.22 (1.01-1.46)	1.50 (1.13-1.99)	0.0038		1.06 (1.00-1.12)
Model 3		-1-	1.14 (0.95-1.35)	1.22 (1.01-1.46)	1.49 (1.13-1.99)	0.0040		1.06 (1.00-1.12)
<i>CVD mortality</i>								
N of deaths		71	90	87	23	-		
Event rates per 10,000 person-years		15.9	15.3	15.9	19.3	-		
Model 1		-1-	1.23 (0.90-1.69)	1.43 (1.03-1.97)	1.80 (1.10-2.94)	0.0076		1.11 (1.01-1.21)
Model 2		-1-	1.27 (0.93-1.75)	1.44 (1.04-1.99)	1.72 (1.05-2.82)	0.010		1.10 (1.01-1.20)
Model 3		-1-	1.27 (0.93-1.75)	1.44 (1.04-1.99)	1.72 (1.05-2.82)	0.010		1.10 (1.01-1.20)
<i>IHD/ cerebrovascular mortality</i>								
N of deaths		42	51	49	11	-		
Event rates per 10,000 person-years		9.4	8.7	9.0	9.2	-		
Model 1		-1-	1.24 (0.82-1.87)	1.47 (0.96-2.25)	1.69 (0.85-3.38)	0.046		1.13 (1.00-1.27)
Model 2		-1-	1.24 (0.82-1.89)	1.46 (0.95-2.23)	1.57 (0.78-3.14)	0.068		1.11 (0.99-1.26)
Model 3		-1-	1.24 (0.82-1.88)	1.46 (0.95-2.23)	1.57 (0.78-3.13)	0.068		1.11 (0.99-1.26)
<i>Cancer mortality</i>								
N of deaths		94	104	106	30	-		
Event rates per 10,000 person-years		21.0	17.7	19.4	25.1	-		
Model 1		-1-	0.99 (0.75-1.32)	1.19 (0.89-1.58)	1.59 (1.04-2.45)	0.039		1.06 (0.97-1.15)
Model 2		-1-	0.99 (0.74-1.31)	1.17 (0.88-1.55)	1.53 (0.99-2.35)	0.061		1.05 (0.96-1.14)
Model 3		-1-	0.98 (0.74-1.31)	1.16 (0.87-1.55)	1.52 (0.99-2.34)	0.062		1.05 (0.96-1.14)
<i>Other causes mortality</i>								

N of deaths	69	87	63	14	-
Event rates per 10,000 person-years	15.8	14.2	11.4	12.0	-
Model 1	-1-	1.22 (0.89-1.68)	1.07 (0.75-1.52)	1.16 (0.64-2.11)	0.66
Model 2	-1-	1.22 (0.88-1.68)	1.06 (0.74-1.51)	1.15 (0.63-2.09)	0.70
Model 3	-1-	1.21 (0.88-1.68)	1.05 (0.74-1.50)	1.14 (0.63-2.07)	0.73

Model 1 adjusted for age, sex and energy intake.

Model 2 as in model 1 further adjusted for education, residence, physical activity, smoking, BMI, baseline of diabetes, hyperlipidaemia, hypertension.

Model 3 as in model 2 further adjusted for Mediterranean diet score.

Table 3. Nutritional factors and serum lipids as possible mediators of the association between egg intake with all-cause and cardiovascular mortality in the Moli-sani Study cohort (n=20,115)

Mediator	All-cause mortality		CVD mortality	
	Proportion mediated (95% CI)	P value	Proportion mediated (95% CI)	P value
Dietary cholesterol (mg/d)	32.5% (17.9% to 51.4%)	<0.0001	29.0% (12.3% to 54.4%)	<0.0001
Saturated fat (g/d)	9.9% (5.9% to 16.1%)	<0.0001	8.0% (4.0% to 15.4%)	<0.0001
Dietary protein (g/d)	5.6% (3.0% to 10.4%)	<0.0001	2.6% (0.5% to 12.2%)	0.097
Dietary vitamin E (mg/d)	Null	-	Null	-
Dietary beta-carotene (µg/d)	Null	-	Null	-
Dietary cholesterol, saturated fat, protein	1.0% (0.0% to 100.0%)	0.44	12.0% (2.2% to 45.5%)	0.089
Serum lipids*	6.5% (3.5% to 11.9%)	<0.0001	5.4% (2.3% to 12.3%)	0.0003

Proportion of effect explained by intermediate variables with 95% CI and relevant p-value as produced by the %MEDIATE macro are reported for each potential mediator, in multivariable-adjusted Cox PH model controlled for sex, age, energy intake, education, residence, physical activity, smoking, BMI, baseline diabetes, hyperlipidaemia, hypertension. The proportion refers to the high (>4 eggs/week) vs low egg intake (0-1 egg/week). Null = not mediating the effect.

*Mediation analysis restricted to 20,148 participants after exclusion of those individuals with missing data on any of the biomarker. Serum lipids include blood cholesterol (mg/dL), HDL-cholesterol (mg/dL), LDL-cholesterol (mg/dL), triglycerides (mg/dL; logarithm).

Figure

CHAPTER 7.

Chili Pepper Consumption and Mortality in Italian Adults

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Chili Pepper Consumption and Mortality in Italian Adults

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ABSTRACT

BACKGROUND Chili pepper is a usual part of a traditional Mediterranean diet. Yet epidemiological data on the association between chili pepper intake and mortality risk are scarce, with a lack of studies from Mediterranean populations.

OBJECTIVES This study sought to examine the association between chili pepper consumption and risk of death in a large sample of the adult Italian general population, and to account for biological mediators of the association.

METHODS Longitudinal analysis was performed on 22,811 men and women enrolled in the Moli-sani Study cohort (2005 to 2010). Chili pepper intake was estimated by the EPIC (European Prospective Investigation Into Cancer) Food Frequency Questionnaire and categorized as none/rare consumption, up to 2 times/week, >2 to ≤4 times/week, and >4 times/week.

RESULTS Over a median follow-up of 8.2 years, a total of 1,236 deaths were ascertained. Multivariable hazard ratios (HRs) for all-cause and cardiovascular disease (CVD) mortality among participants in the regular (>4 times/week) relative to none/rare intake were 0.77 (95% confidence interval [CI]: 0.66 to 0.90) and 0.66 (95% CI: 0.50 to 0.86), respectively. Regular intake was also inversely associated with ischemic heart disease (HR: 0.56; 95% CI: 0.35 to 0.87) and cerebrovascular (HR: 0.39; 95% CI: 0.20 to 0.75) death risks. The association of chili pepper consumption with total mortality appeared to be stronger in hypertension-free individuals (p for interaction = 0.021). Among known biomarkers of CVD, only serum vitamin D marginally accounted for such associations.

CONCLUSIONS In a large adult Mediterranean population, regular consumption of chili pepper is associated with a lower risk of total and CVD death independent of CVD risk factors or adherence to a Mediterranean diet. Known biomarkers of CVD risk only marginally mediate the association of chili pepper intake with mortality. (J Am Coll Cardiol 2019;74:3139–49) © 2019 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

BMI	= body mass index
CI	= confidence interval
CRP	= C-reactive protein
CV	= coefficient of variability
CVD	= cardiovascular disease
HDL	= high-density lipoprotein
HR	= hazard ratio
ICD-9	= International Classification of Diseases-9th Revision
IHD	= ischemic heart disease
MD	= Mediterranean diet
MDS	= Mediterranean Diet Score
NRI	= Net Reclassification Index
NT-proBNP	= N-terminal pro-B-type natriuretic peptide
PA	= physical activity

Spices have long been an essential part of the traditional Mediterranean diet (MD), and are placed, along with herbs, at the base of the MD pyramid both for their nutritional properties and as a valuable substitute for salt (1–3). Chili peppers, belonging to the genus *Capsicum*, are native to Central and Southern America, but are largely present in different cultures' diets worldwide (4), and are used to flavor traditional dishes from the southern Italian regions.

SEE PAGE 3150

Health benefits of chili peppers have been ascribed to capsaicin, its major pungent compound, which has been observed to favorably improve cardiovascular function and metabolic regulation in experimental and population studies (5). In addition to its anti-inflammatory and analgesic properties and atheroprotective effects (4), capsaicin reportedly induces apoptosis of the tumor cells (6); high concentrations, however, likely evoke deleterious effects (7).

Studies in humans concluded that chili pepper intake facilitates weight loss through activation of different receptors and improved control of insulin (8). Evidence from a large epidemiological study appears to corroborate the weight-loss properties of chili peppers by showing inverse associations with incidence of overweight/obesity (9).

More recently, animal studies uncovered an interaction between dietary capsaicin and gut microbiota as a novel mechanism for the antiobesity effect of capsaicin acting through prevention of microbial dysbiosis, gut barrier dysfunction, and chronic low-grade inflammation (10). To date, epidemiological evidence longitudinally addressing the association of chili pepper intake on disease/mortality risk is scarce.

With the exception of 2 population studies from China (11) and the United States (12), both showing lower mortality risk associated with regular chili pepper intake, no epidemiological investigation on the potential health benefits associated with consumption of chili pepper is currently available from European cohorts or from Mediterranean areas. In addition, none of the aforementioned studies has addressed possible biological mechanisms through which regular chili pepper intake may provide the observed health advantages.

The primary aim of this study was to prospectively estimate the association of chili pepper consumption with total and cause-specific mortality in a large

Mediterranean population of adult men and women; as a secondary purpose, we examined some biological mechanisms that could be on the pathway between chili pepper intake and mortality by analyzing the possible contribution of established biomarkers of cardiovascular risk. Finally, we assessed whether inclusion of chili pepper was able to improve risk prediction associated with a traditional Mediterranean Diet Score (MDS).

METHODS

STUDY POPULATION. We used data from the Moli-sani Study, a prospective cohort study established in 2005 to 2010 with an enrollment of 24,325 men and women (≥ 35 years of age) randomly recruited from the general population of Molise, a southern Mediterranean region in Italy, with the main purpose of investigating genetic and environmental risk factors of cardiovascular, cerebrovascular, and tumor diseases. Details of the Moli-sani Study have been previously described (13).

Participants reporting implausible energy intakes (< 800 kcal/day in men and < 500 kcal/day in women or $> 4,000$ kcal/day in men and $> 3,500$ kcal/day in women; 3.2%) were excluded, as well as individuals with unreliable medical/dietary questionnaires (1% and 3.9%, respectively), individuals with missing information on main covariates (0.3%), exposure (0.4%), and cause-specific mortality (0.2%), and 23 participants (0.1%) lost to follow-up. The final sample consisted of 22,811 subjects.

The cohort was followed up for mortality until December 31, 2015. Overall and cause-specific mortality was assessed by the Italian mortality registry (ReNCaM registry), validated by Italian death certificates (ISTAT form) and coded according to the International Classification of Diseases-9th Revision (ICD-9).

Cardiovascular disease (CVD) mortality included deaths from diseases of the circulatory system, when the underlying cause of death included ICD-9 codes 390 to 459. ICD-9 codes 430 to 438 were used to define specific cause of death for cerebrovascular disease, and ICD-9 codes 410 to 414 and 429 for ischemic heart disease (IHD). Cancer death was considered when the underlying cause of death included ICD-9 codes 140 to 208.

Noncardiovascular/noncancer causes of death were included in the "other-cause mortality" group. The Moli-sani Study complies with the Declaration of Helsinki and was approved by the ethical committee of the Catholic University Medical School in Rome, Italy. All participants provided written informed consent.

COVARIATE ASSESSMENT. History of cardiovascular disease (angina, myocardial infarction, revascularization procedures, peripheral artery diseases, and cerebrovascular events) was self-reported and confirmed by medical records and therapy. History of cancer was self-reported and confirmed by medical records. Hypertension, hyperlipidemia, and diabetes were defined by use of pharmacological treatment.

Leisure-time physical activity (PA) was expressed as daily energy expenditure in metabolic equivalent task-hours/day for sport, walking, and gardening. Height and weight were measured, and body mass index (BMI) was calculated as kg/m² and then grouped into 3 categories as normal (≤ 25), overweight (>25 to <30), or obese (≥ 30).

Subjects were classified as never-smokers, current smokers, or ex-smokers (quit at least 1 year ago). Education was based on the highest qualification attained and was categorized as up to lower secondary (approximately ≤ 8 years of study), upper secondary school (>8 to ≤ 13 years), and postsecondary education (>13 years).

Occupational social class was based on the Registrar General's occupation-based classification scheme and ranked as previously described within the Moli-sani population (14), and categorized as professional/managerial, skilled nonmanual occupations, skilled manual occupations, partly skilled occupations, and unskilled, retired/housewife, unemployed/unclassified subjects.

DIETARY ASSESSMENT. Food intake during the year before enrollment was assessed by the EPIC (European Prospective Investigation Into Cancer) Food Frequency Questionnaire validated and adapted to the Italian population (15). Frequency of chili pepper intake was estimated through the question: "How often do you consume foods containing chili pepper," with possible answers being "never or almost never," "occasionally," "often," and "very often."

Chili pepper was then categorized as consumption on a weekly basis that is never/rare intake, up to 2 times/week, >2 to ≤ 4 times/week, and >4 times/week. Other herbs and spices including garlic, parsley, and black pepper were categorized as yes/no.

Adherence to the traditional MD was assessed through the MDS developed by Trichopoulou *et al.* (16), and was obtained by assigning 1 point to healthy foods (fruits and nuts, vegetables, legumes, fish, cereals, monounsaturated-to-saturated fats ratio) whose consumption was above the sex-specific medians of intake of the Moli-sani Study population, free from CVD, cancer, and diabetes; foods presumed to be detrimental (meat and dairy products) were scored

positively if their consumption was below the median. All other intakes received 0 points. For ethanol, men who consumed 10 to 50 g/day and women who consumed 5 to 25 g/day received 1 point; otherwise, the score was 0. The MDS ranged from 0 to 9 (the latter reflecting maximal adherence). The vegetable food group included in the MDS did not include sweet peppers; the latter were used as a covariate in survival analysis and also independently tested as predictors of mortality.

Food antioxidant content was appraised by a score determining the content in antioxidant vitamins and phytochemicals of each food group, and ranged from -99 to 99 , with higher values indicating increased consumption of foods rich in antioxidants (17). The polyphenol content of diet was measured by a polyphenol antioxidant content score calculated as in Pounis *et al.* (18).

Variety of fruit and/or vegetable intake was assessed by 4 different (fruit, vegetables, vegetable subgroups, and fruit/vegetables combined) Diet Diversity Scores, following similar approaches tested within EPIC cohorts (19). Diversity was intended as the total number of individual vegetable/fruit products eaten at least once in 2 weeks.

BIOMARKERS OF CVD RISK. Antecubital venous blood samples were obtained from participants who had fasted overnight and had refrained from smoking for at least 6 h. Serum lipids (total cholesterol, high-density lipoprotein [HDL]-cholesterol, triglycerides) and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer (ILab 350; Instrumentation Laboratory, Milan, Italy).

Quality control for lipids and glucose was obtained by a commercial standard (Ser 1 and Ser 2) provided by Instrumentation Laboratory and an in-house serum standard pool. The coefficients of variability (CVs) were respectively 4.9%, 5.2%, and 4% for blood cholesterol; 3.2%, 3%, and 4.5% for HDL-cholesterol; 5.2%, 5.3%, and 5% for triglycerides; and 4.7%, 4.1%, and 3.9 for blood glucose.

High-sensitivity C-reactive protein (CRP) was measured in fresh serum samples by a particle-enhanced immunoturbidimetric assay (ILab 350). Quality control for CRP was maintained using an in-house serum pool and internal laboratory standard at 1.5 mg/l; interday CVs for CRP were 5.5% and 4.2%, respectively. Hemochromocytometric analysis was performed by cell count (Coulter HMX; Beckman Coulter, Milan, Italy) within 3 h from blood collection.

Quality control was performed by using 3 different levels of standards Abn (abnormal) I, Abn II, and Normal (Coulter HMX). The CV for white blood

TABLE 1 Baseline Characteristics of the Study Population by Intake of Chili Pepper in the Moli-sani Study Cohort (N = 22,811)

	Chili Pepper Intake (Times/Week)				p Value
	None/Rare (n = 7,689, 33.7%)	Up to 2 (n = 4,360, 19.1%)	>2 to ≤4 (n = 5,216, 22.9%)	>4 (n = 5,546, 24.3%)	
Times/week	0.0	0.8 ± 0.4	2.8 ± 0.6	7.0 ± 3.8	—
Age, yrs	55 ± 13	54 ± 11	55 ± 11	56 ± 11	<0.0001
Male	34.6	50.7	52.5	58.8	<0.0001
Post-secondary education	11.5	14.1	13.4	13.5	<0.0001
Professional/managerial workers	18.7	21.3	21.8	21.6	<0.0001
Smokers	18.6	22.0	24.8	28.1	0.0022
Leisure-time PA, MET-h/day	3.4 ± 3.8	3.3 ± 3.7	3.7 ± 4.0	3.7 ± 4.4	<0.0001
Drugs for diabetes	4.3	4.6	4.9	5.5	0.015
Drugs for hypertension	27.3	25.8	27.0	28.8	0.016
Lipid-lowering drugs	7.4	7.0	7.7	8.5	0.0011
Cardiovascular disease	4.8	5.2	4.8	6.0	0.010
Cancer	3.9	3.0	3.1	2.6	0.0090
Mediterranean Diet Score*	4.2 ± 1.6	4.3 ± 1.6	4.4 ± 1.6	4.8 ± 1.6	<0.0001
Sweet pepper intake, g/day	2.7 ± 3.2	2.8 ± 2.9	3.2 ± 3.3	3.7 ± 3.9	<0.0001
Black pepper†	11.0	19.3	25.0	26.0	<0.0001
Garlic‡	82.6	85.0	89.3	92.4	<0.0001
Parsley§	91.7	95.1	95.6	96.7	<0.0001

Values are mean ± SD or %, unless otherwise indicated. Means and p values are adjusted for age, sex, and energy intake (Kcal/day). *Not including sweet pepper intake. †Black pepper, garlic, and parsley consumption was reported as prevalence of consumers. ‡MET = metabolic equivalent of task; PA = physical activity.

cells was 6.2%, 3.3%, and 3.0% for Abn I, Abn II, and Normal, respectively. N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity troponin I (hsTnI), apolipoprotein A1 (ApoA), apolipoprotein B100 (ApoB100), lipoprotein a [Lp(a)], markers of renal function (cystatin C, creatinine), insulin, C-peptide, and serum vitamin D were measured in the framework of the collaborative Biomarker for Cardiovascular Risk Assessment across Europe project (20).

STATISTICAL ANALYSIS. Baseline characteristics of the participants by categories of chili pepper intake were summarized and compared by using analysis of variance adjusted for age, sex, and energy intake (GENMOD procedure for categorical variables and GLM procedure for continuous variables in SAS software, version 9.4 [SAS Institute, Cary, North Carolina]) (Table 1).

Risk estimates for mortality were expressed as hazard ratios (HRs) with 95% confidence interval (CI) and calculated by using Cox regression models with time-on-study on the time scale and competing risk of dying for other causes. Multivariable-adjusted HRs were calculated across 4 categories of intake (none/rare consumption—referent category, up to 2 times/week, >2 to ≤4 times/week, and >4 times/week) as well as considering consumers as opposed to nonconsumers.

Based on previous published reports and biological plausibility, 2 multivariable models were fitted: the first (model 1) was adjusted for age (continuous), sex, and energy intake (kcal/day; continuous); the second (model 2) as in model 1 and further controlled for educational level, occupational social class, smoking, leisure-time PA, treatment for diabetes, medication for hypertension, lipid-lowering drugs, history of CVD or cancer at baseline, MDS (continuous), and sweet pepper intake (g/day, ordered quartiles), garlic, parsley, and black pepper (yes/no).

Several panels of biomarkers were tested as possibly mediating the association of chili pepper intake with mortality risk. In addition to cardiac troponin (hsTnI) and NT-proBNP, we tested biomarkers of renal function (cystatin C, creatinine), glucose metabolism (blood glucose, insulin, C-peptide), lipid metabolism (total blood cholesterol, HDL-cholesterol, triglycerides, Lp(a), apoA1, and apoB100), serum vitamin D, inflammatory markers (CRP and white blood cells), and a panel including systolic and diastolic blood pressure and BMI.

The multivariable model 2 served as the reference for the mediation analysis used to quantify the contribution of each set of potential mediators, which were alternately included into model 2. For the mediation analysis, the %MEDIATE macro in SAS (21) was used, which calculates the point and interval

estimates of the percent of exposure effect explained by 1 or more intermediate variables, with 95% CI and *p* values. Biomarkers were entered into the mediation analysis as ordered quintiles.

Subgroup analyses were performed within a healthy sample (individuals without CVD or cancer and who were not using drugs for diabetes at baseline) and excluding early deaths (follow-up >2 years). We tested the interaction between chili pepper and a large panel of potential effect modifiers by introducing in a Cox analysis a product term of chili pepper intake (yes vs. no) $\times$ a given effect modifier (2 categories, with the exception of adherence to MD, 3 categories).

Consumption of chili pepper (none/rare intake = 0 points; regular intake = 1 point) was included as an additional component into the original MDS, thus originating an MDS “supplemented” with chilies, ranging from 0 to 10; to allow comparison among scores, they were entered into risk analyses scaled by their standard deviation.

The area under the receiver-operating curve statistic, the categorical net reclassification index (NRI) and the integrated discrimination improvement (22) were used to quantify the predictive value of the MDS “supplemented” with chilies over the traditional MDS. To estimate these metrics, the follow-up time was censored at 10 years. The risk categories chosen for NRI calculation were <5% and $\geq$ 5%. Observed mortality rates over time were showed as curves obtained by using the LIFETEST procedure in SAS. Dummy variables for missing values were created. Positively skewed variables were log transformed before analysis. Subgroup and interaction analyses were run by excluding missing categories. The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows 2009.

RESULTS

Regular consumption (>4 times/week) of chili pepper was reported by 24.3% of the study participants, whereas 33.7% declared none/rare intake (Table 1). Regular consumers were more likely to be men, slightly older, to report higher educational level and occupation, and showed higher prevalence of risk factors (diabetes, hypertension, hyperlipidemia, and history of CVD), but greater leisure-time PA (Table 1). Higher consumption of chili pepper was also associated with closer adherence to MD, higher intake of other spices (Table 1), and a global higher diet quality as measured by dietary polyphenol and antioxidant content (Online Table 1). Regular consumers also tended to have higher levels of blood lipids and blood glucose, and higher BMI, and slightly higher blood

leukocyte counts, whereas no substantial differences were observed for CRP (Online Table 2). NT-proBNP levels were lower in regular consumers, whereas troponin I and serum vitamin D were more likely associated with higher chili intake.

Over a median follow-up of 8.2 years (interquartile range: 7.3 to 9.3 years; 187,584 person years), a total of 1,236 deaths were ascertained (CVD = 444, IHD/cerebrovascular = 258, cancer = 482, and 310 from other causes). In a model adjusted only for age, sex, and energy intake, regular consumption (>4 times/week) of chili pepper was associated with 23% (95% CI: 10% to 34%) lower risk of all-cause mortality, as opposed to none/rare intake, and results remained substantially unchanged in the fully adjusted model (Table 2).

In comparison with individuals reporting none/rare intake, regular consumers experienced 34% (14% to 50%) lower risk of CVD mortality, whereas multivariable HRs for IHD and cerebrovascular death were 0.56 (95% CI: 0.35 to 0.87) and 0.39 (95% CI: 0.20 to 0.75), respectively (Table 2).

For all mortality outcomes under study, we failed to find a clear stepwise dose-response relation with chili pepper, indicating that the actual advantage in terms of survival improvement is likely attributable to the fact of consuming chilies rather than not consuming them (Table 2). Observed mortality rates and multivariable Kaplan-Meier estimates for consumers versus none/rare consumers are well-separated (Central Illustration).

Cancer mortality risk was slightly, though not significantly, affected by chili pepper consumption, although a downward trend was observed (Table 2). Finally, consumption of chili pepper was inversely associated with mortality for other causes (Table 2). Sweet pepper intake was associated with lower total and IHD mortality risk, whereas no association was found with cerebrovascular mortality (Online Table 3), after adjustment for potential confounders.

Established biomarkers of CVD did not substantially modify the relation between chili pepper and mortality, although a marginal role was played by serum vitamin D levels and biomarkers of lipid metabolism, explaining 6.1% and 5.3% of the association with all-cause mortality, respectively (Table 3). Subgroup analyses showed no difference of effect across levels of main covariates, with the exception of hypertension, which was likely to modify the magnitude of the association between chili pepper intake and total mortality risk. Specifically, the positive effect of chilies was stronger in hypertension-free individuals (*p* for interaction = 0.021) (Table 4). Overall diet quality, measured by MDS, was not an

TABLE 2 Chili Pepper Intake and Risk of Death in the Moli-sani Study Cohort (N = 22,811)

		Weekly Consumption of Chili Pepper				Consumers vs. None/Rare Consumers
		None/Rare	Up to 2 Times/Week	>2 to ≤4 Times/Week	>4 Times/Week	
All-cause mortality	500/7,689	212/4,360	244/5,216	280/5,546	—	—
Crude model	ref	0.74 (0.63–0.87)	0.73 (0.63–0.85)	0.75 (0.65–0.87)	0.74 (0.66–0.83)	
Model 1	ref	0.85 (0.73–1.00)	0.80 (0.69–0.94)	0.77 (0.66–0.89)	0.80 (0.72–0.90)	
Model 2	ref	0.86 (0.73–1.01)	0.82 (0.70–0.96)	0.77 (0.66–0.90)	0.81 (0.72–0.92)	
Cardiovascular mortality	193/7,689	73/4,360	91/5,216	87/5,546	—	—
Crude model	ref	0.66 (0.50–0.86)	0.71 (0.55–0.91)	0.60 (0.47–0.77)	0.65 (0.54–0.79)	
Model 1	ref	0.84 (0.64–1.10)	0.86 (0.67–1.11)	0.69 (0.54–0.90)	0.79 (0.65–0.96)	
Model 2	ref	0.81 (0.61–1.06)	0.85 (0.66–1.10)	0.66 (0.50–0.86)	0.77 (0.63–0.94)	
Ischemic heart disease mortality	74/7,689	22/4,360	37/5,216	29/5,546	—	—
Crude model	ref	0.52 (0.32–0.83)	0.75 (0.50–1.11)	0.52 (0.34–0.80)	0.60 (0.44–0.81)	
Model 1	ref	0.62 (0.38–1.00)	0.87 (0.58–1.29)	0.58 (0.37–0.89)	0.69 (0.50–0.94)	
Model 2	ref	0.60 (0.37–0.97)	0.86 (0.57–1.30)	0.56 (0.35–0.87)	0.67 (0.48–0.94)	
Cerebrovascular mortality	48/7,689	15/4,360	21/5,216	12/5,546	—	—
Crude model	ref	0.54 (0.30–0.97)	0.65 (0.39–1.09)	0.33 (0.18–0.63)	0.50 (0.34–0.75)	
Model 1	ref	0.72 (0.40–1.29)	0.84 (0.50–1.42)	0.41 (0.21–0.78)	0.64 (0.43–0.97)	
Model 2	ref	0.67 (0.37–1.21)	0.82 (0.48–1.39)	0.39 (0.20–0.75)	0.62 (0.40–0.96)	
Cancer mortality	173/7,689	88/4,360	98/5,216	123/5,546	—	—
Crude model	ref	0.89 (0.69–1.15)	0.85 (0.66–1.09)	0.95 (0.76–1.20)	0.90 (0.75–1.08)	
Model 1	ref	0.95 (0.73–1.23)	0.86 (0.67–1.10)	0.89 (0.70–1.13)	0.89 (0.74–1.08)	
Model 2	ref	0.97 (0.74–1.26)	0.86 (0.67–1.11)	0.89 (0.70–1.14)	0.90 (0.74–1.10)	
Other cause mortality	134/7,689	51/4,360	55/5,216	70/5,546	—	—
Crude model	ref	0.66 (0.48–0.92)	0.61 (0.45–0.84)	0.70 (0.52–0.94)	0.66 (0.53–0.83)	
Model 1	ref	0.77 (0.56–1.07)	0.68 (0.50–0.94)	0.74 (0.55–0.99)	0.73 (0.58–0.92)	
Model 2	ref	0.80 (0.57–1.10)	0.72 (0.52–1.00)*	0.78 (0.58–1.06)	0.77 (0.61–0.96)	

Values are events/n subjects or hazard ratio (95% confidence interval). Model 1 = hazard ratios with 95% confidence interval obtained from the multivariable model adjusted for age, sex, and energy intake. Model 2 = as in model 1 and further adjusted for educational level, occupational class, smoking, leisure-time physical activity, cardiovascular disease, cancer, drugs for diabetes, lipid-lowering drugs, medication for hypertension, Mediterranean Diet Score (not including sweet pepper intake), sweet pepper intake (g/day, ordered quartiles), garlic, parsley, black pepper (consumption yes/no). *p < 0.05.

ref = reference.

effect modifier of the relation between chili pepper and mortality risk.

Risk of CVD mortality associated with a 1 SD increment in the MDS was 0.92 (95% CI: 0.83 to 1.02) and became 0.89 (95% CI: 0.80 to 0.98) in association with 1 SD increment in the MDS “supplemented” with chili pepper. Similarly, risk reduction of IHD/cerebrovascular death went from 14% to 19%, whereas no substantial modifications were observed for cancer and other causes of mortality (data not shown). The inclusion of chili pepper consumption as an additional component of the traditional MDS did not improve discrimination ability of the model, with the exception of cerebrovascular mortality. However, the improved prediction associated with the MDS supplemented with chilies was slightly over the model including the traditional MDS (integrated discrimination improvement = 0.005; p = 0.026; NRI = 0.010; p = 0.66; p value for difference in area under the curve = 0.31) (Online Table 4).

DISCUSSION

Findings from this large Mediterranean population-based cohort show that regular consumption of chili pepper is associated with lower risk of total and CVD mortality, with larger magnitude observed for IHD and cerebrovascular-related deaths. On the contrary, no cancer death risk reduction was found, whereas regular intakes were associated with lower risk of other causes of mortality.

Our findings are in agreement and corroborate the main results of 2 earlier studies from non-Mediterranean cohorts. Evidence from the China Kadoorie Biobank on about 500,000 men and women (11) showed that regular consumption of spicy food (almost every day) lowered the risk of total mortality by 14% and of IHD death by 22%; reductions were also observed for deaths due to cancer (–8%), or respiratory diseases (–29%). More recently, in the large NHANES (National Health and Nutrition Examination

CENTRAL ILLUSTRATION Chili Pepper and Mortality

Bonaccio, M. et al. *J Am Coll Cardiol*. 2019;74(25):3139–49.

Observed rates and Kaplan-Meier estimates for all-cause, cardiovascular disease, ischemic heart disease, and cerebrovascular mortality for consumers and none/rare-consumers of chili pepper in the Moli-sani Study cohort. Kaplan-Meier estimates were obtained from the multivariable model adjusted for age, sex, educational level, occupational class, smoking, leisure-time physical activity, cardiovascular disease, cancer, drugs for diabetes, lipid-lowering drugs, medication for hypertension, Mediterranean diet score (not including sweet pepper intake), sweet pepper intake (g/day, ordered quartiles), garlic, parsley, black pepper (consumption yes/no), and energy intake (kcal/day).

Survey) cohort, the consumption of hot red chili peppers appeared to be associated with a 13% reduction in total mortality risk (12).

Our study reveals that the health benefits associated with chili pepper intake are independent of the overall quality of the diet, as measured by a traditional Mediterranean diet, thus suggesting an independent effect of chili pepper toward mortality risk. Moreover, risk estimates for total and cause-specific mortality in the minimally adjusted and fully

adjusted multivariable models were very similar, suggesting that, although chili pepper consumption was associated with several risk factors for CVD, its relationship with total or cause-specific mortality was independent from such factors.

Furthermore, sensitivity analysis showed that the association between chili consumption and mortality was similar across risk factor categories, with the exception of hypertension, where the association was significantly stronger in normotensive subjects,

TABLE 3 Mediation Analysis for the Association of Chili Pepper Intake With Total and Cause-Specific Mortality Risk

	Chili Pepper Consumption			
	None/Rare Consumers (n = 7,689)	Consumers (n = 15,122)	PTE (%)	PTE p Value
All-cause mortality, deaths	500 (6.5)	736 (4.9)	—	
Model 2	ref	0.81 (0.72–0.92)	—	
Model 2 + cardiac troponin	ref	0.81 (0.72–0.91)	Null	
Model 2 + natriuretic peptide	ref	0.82 (0.72–0.92)	Null	
Model 2 + biomarkers of renal function	ref	0.82 (0.72–0.92)	Null	
Model 2 + biomarkers of glucose metabolism	ref	0.81 (0.72–0.91)	Null	
Model 2 + biomarkers of lipid metabolism	ref	0.82 (0.73–0.93)	5.3 [1.6–16.5]	0.033
Model 2 + serum vitamin D (ng/ml)	ref	0.83 (0.73–0.93)	6.1 [2.0–16.6]	0.014
Model 2 + inflammatory biomarkers	ref	0.81 (0.72–0.91)	Null	
Model 2 + BP + BMI	ref	0.82 (0.73–0.92)	2.4 [0.4–13.1]	0.12
Cardiovascular mortality, deaths	193 (2.5)	251 (1.7)	—	
Model 2	ref	0.77 (0.63–0.94)	—	
Model 2 + cardiac troponin	ref	0.75 (0.62–0.93)	Null	
Model 2 + natriuretic peptide	ref	0.76 (0.62–0.93)	Null	
Model 2 + biomarkers of renal function	ref	0.77 (0.63–0.95)	1.7 [0.0–38.2]	0.28
Model 2 + biomarkers of glucose metabolism	ref	0.76 (0.62–0.94)	Null	
Model 2 + biomarkers of lipid metabolism	ref	0.77 (0.63–0.95)	3.1 [0.2–31.0]	0.21
Model 2 + serum vitamin D (ng/ml)	ref	0.78 (0.64–0.96)	5.9 [1.4–21.9]	0.048
Model 2 + inflammatory biomarkers	ref	0.76 (0.62–0.93)	Null	
Model 2 + BP + BMI	ref	0.77 (0.63–0.94)	Null	
Ischemic heart disease mortality, deaths	74 (1.0)	88 (0.6)	—	
Model 2	ref	0.67 (0.48–0.94)	—	
Model 2 + cardiac troponin	ref	0.67 (0.48–0.93)	Null	
Model 2 + natriuretic peptide	ref	0.67 (0.48–0.93)	Null	
Model 2 + biomarkers of renal function	ref	0.67 (0.48–0.93)	Null	
Model 2 + biomarkers of glucose metabolism	ref	0.66 (0.47–0.92)	Null	
Model 2 + biomarkers of lipid metabolism	ref	0.66 (0.48–0.92)	Null	
Model 2 + serum vitamin D (ng/ml)	ref	0.68 (0.48–0.95)	2.7 [0.2–29.2]	0.20
Model 2 + inflammatory biomarkers	ref	0.67 (0.48–0.93)	Null	
Model 2 + BP + BMI	ref	0.67 (0.48–0.93)	Null	
Cerebrovascular mortality, deaths	48 (0.6)	48 (0.3)	—	
Model 2	ref	0.62 (0.40–0.96)	—	
Model 2 + cardiac troponin	ref	0.61 (0.39–0.95)	Null	
Model 2 + natriuretic peptide	ref	0.62 (0.40–0.96)	1.2 [0.0–91.3]	0.38
Model 2 + biomarkers of renal function	ref	0.62 (0.40–0.96)	Null	
Model 2 + biomarkers of glucose metabolism	ref	0.62 (0.40–0.96)	1.0 [0.0–49.8]	0.33
Model 2 + biomarkers of lipid metabolism	ref	0.61 (0.39–0.95)	Null	
Model 2 + serum vitamin D (ng/ml)	ref	0.62 (0.40–0.96)	Null	
Model 2 + inflammatory biomarkers	ref	0.61 (0.39–0.94)	Null	
Model 2 + BP + BMI	ref	0.62 (0.40–0.96)	Null	

Continued on the next page

suggesting that the high prevalence of hypertension in chili pepper consumers could partially mask its protective effect on total mortality.

With respect to a potential improvement of risk prediction deriving from the inclusion of chilies into the traditional MDS, we found no significant changes in the discrimination ability of the modified score. The fact that an explanatory variable significantly correlated with the outcome does not lead to improvements in prediction is a well-known and widespread occurrence, and does not entirely devalue the association between the variable and the outcome (23). More

importantly, the association of chili pepper with mortality risk was independent of the adherence to the MD, supporting the notion that minor dietary changes, such as adding chilies to usual diet, could be valuable measures for improving health, especially cardiovascular health, independent of overall diet quality.

We also aimed to examine whether some biological mechanisms could be on the pathway between chili pepper and mortality. Our results showed that the majority of established biomarkers of CVD risk poorly accounted for the observed lower risk of death; only serum vitamin D was likely to explain a modest

	Chili Pepper Consumption			
	None/Rare Consumers (n = 7,689)	Consumers (n = 15,122)	PTE (%)	PTE p Value
Cancer mortality, deaths	173 (2.2)	309 (2.0)	—	
Model 2	ref	0.90 (0.74-1.10)	—	
Model 2 + cardiac troponin	ref	0.90 (0.74-1.10)	Null	
Model 2 + natriuretic peptide	ref	0.91 (0.74-1.10)	1.2 [0.0-71.4]	0.35
Model 2 + biomarkers of renal function	ref	0.90 (0.74-1.10)	Null	
Model 2 + biomarkers of glucose metabolism	ref	0.90 (0.74-1.10)	Null	
Model 2 + biomarkers of lipid metabolism	ref	0.91 (0.75-1.11)	5.8 [0.2-61.9]	0.21
Model 2 + serum vitamin D (ng/ml)	ref	0.91 (0.75-1.11)	8.9 [0.7-57.5]	0.064
Model 2 + inflammatory biomarkers	ref	0.90 (0.73-1.09)	Null	
Model 2 + BP + BMI	ref	0.91 (0.74-1.11)	4.2 [0.1-61.6]	0.24
Other cause mortality, deaths	173 (2.2)	309 (2.0)	—	
Model 2	ref	0.77 (0.61-0.96)	—	
Model 2 + cardiac troponin	ref	0.75 (0.60-0.95)	Null	
Model 2 + natriuretic peptide	ref	0.77 (0.61-0.97)	Null	
Model 2 + biomarkers of renal function	ref	0.77 (0.61-0.97)	Null	
Model 2 + biomarkers of glucose metabolism	ref	0.77 (0.61-0.96)	Null	
Model 2 + biomarkers of lipid metabolism	ref	0.78 (0.62-0.99)	7.7 [1.2-35.6]	0.10
Model 2 + serum vitamin D (ng/ml)	ref	0.77 (0.61-0.98)	3.9 [0.8-16.5]	0.059
Model 2 + inflammatory biomarkers	ref	0.77 (0.61-0.97)	Null	
Model 2 + BP + BMI	ref	0.77 (0.61-0.98)	4.0 [0.6-23.9]	0.13

Values are n (%), hazard ratio (95% confidence interval), or PTE [95% confidence interval]. Hazard ratios were obtained from the multivariable model adjusted for age, sex, educational level, occupational class, smoking, leisure-time physical activity, cardiovascular disease, cancer, drugs for diabetes, lipid-lowering drugs, medication for hypertension, Mediterranean Diet Score (not including sweet pepper intake), sweet pepper intake (g/day, ordered quartiles), garlic, parsley, black pepper (consumption yes/no), and energy intake (Kcal/day). Cardiac troponin = high-sensitivity troponin I (pg/ml; logarithm). Natriuretic peptide = N-terminal pro-B-type natriuretic peptide (pg/ml; logarithm). Biomarkers of renal function include cystatin C and creatinine (mg/L; logarithm). Biomarkers of glucose metabolism include blood glucose (mg/dL; logarithm), insulin (pmol/dL; logarithm), C-peptide (ng/mL; logarithm). Biomarkers of lipid metabolism = blood cholesterol (mg/dL), high-density lipoprotein cholesterol (mg/dL), triglycerides (mg/dL; logarithm), apolipoprotein A1 (g/L), apolipoprotein B100 (g/L), lipoprotein a (mg/dL). Inflammatory biomarkers include C-reactive protein (mg/L; logarithm), white blood cell count ($\times 10^9/L$; logarithm). BMI was categorized as normal weight, overweight, obese.

BP = blood pressure; BMI = body mass index; Null = not mediating the effect; PTE = percent of exposure effect; ref = reference.

proportion of the relation of chili pepper with total, CVD, and other-cause mortality death risk, while biomarkers of lipid metabolism appeared to slightly mediate the association of chilies with all-cause mortality.

In our population sample, regular chili pepper intake was associated with increased levels of lipids, a finding in agreement with previous epidemiological observations on serum triglycerides from a cohort of Chinese adults, but in apparent contrast with their reported inverse association of spicy food consumption with serum cholesterol (24). By contrast, a human intervention on 27 participants reported that regular consumption of freshly chopped chilies for 4 weeks had no effect on serum lipids and lipoproteins (25).

Other biological mechanisms potentially linking chili peppers to lower mortality risk, especially CVD-related, could be ascribed to the weight loss-promoting properties of chilies (8,9,12): protection against obesity leads indeed to decreased risk of cardiovascular and metabolic diseases; yet, in our population, regular chili pepper consumers were more likely to be obese than nonconsumers, and the

inclusion of BMI in the mediating pathways did not substantially affect mortality risk.

Finally, in light of the well-known anti-inflammatory properties of chili peppers (26), we tested the role of inflammatory processes, by 2 widely used biomarkers but failed to detect any attenuation of risk; however, the use of only 2 markers could lead to an underestimation of the role of inflammation as a likely mediating mechanism linking chili pepper consumption to death risk.

None of the biological mechanisms tested were able to explain the health benefits associated with chilies; however, we could not explore other pathways that could link chili pepper intake to improved health outcomes, such as reduced oxidation or anti-atherogenic potential (4).

Of note, an inverse association with total and IHD mortality was also observed for sweet peppers, which contain capsaicin in smaller, yet possibly beneficial, amounts, although no effect was observed toward CVD and cerebrovascular mortality risk; these findings suggest that the health advantages associated with chili peppers are likely to be ascribed to the high

TABLE 4 Subgroup Analysis for the Association of Chili Pepper Intake (Consumers vs. None/Rare-Consumers) With Total and CVD Mortality Risk

	Total Mortality			CVD Mortality		
	Deaths/Subjects	HR (95% CI)	p Value for Interaction	Deaths	HR (95% CI)	p Value for Interaction
Age <65 yrs	337/17,613	0.74 (0.58-0.94)	0.51	79	0.79 (0.47-1.31)	0.53
Age ≥65 yrs	899/5,198	0.85 (0.74-0.97)		365	0.78 (0.63-0.97)	
Women	429/11,938	0.84 (0.69-1.02)	0.79	162	0.93 (0.67-1.28)	0.29
Men	807/10,873	0.78 (0.67-0.91)		282	0.66 (0.51-0.84)	
Up to lower secondary education	896/11,918	0.85 (0.74-0.98)	0.22	337	0.80 (0.64-0.996)	0.84
Upper secondary/post-secondary	340/10,893	0.73 (0.57-0.92)		107	0.67 (0.44-1.03)	
Nonsmokers	989/17,568	0.84 (0.74-0.96)	0.71	367	0.85 (0.69-1.06)	0.20
Smokers	247/5,243	0.76 (0.58-1.01)		77	0.53 (0.33-0.85)	
Low physical activity	678/11,545	0.82 (0.70-0.96)	0.79	256	0.85 (0.66-1.11)	0.25
High physical activity	558/11,266	0.79 (0.66-0.95)		188	0.63 (0.46-0.85)	
Free from CVD	956/21,274	0.80 (0.70-0.92)	0.93	289	0.78 (0.61-0.998)	0.46
CVD subjects	235/1,177	0.82 (0.62-1.08)		131	0.64 (0.45-0.93)	
Free from cancer	1,105/21,985	0.81 (0.71-0.92)	0.36	415	0.72 (0.59-0.88)	0.069
Cancer subjects	116/736	0.79 (0.53-1.18)		25	1.72 (0.61-4.85)	
Free from diabetes	1,016/21,442	0.82 (0.72-0.94)	0.75	1,016	0.74 (0.59-0.92)	0.70
Subjects with diabetes	188/1,088	0.70 (0.51-0.96)		80	0.80 (0.48-1.31)	
Free from hyperlipidemia	1,045/20,849	0.82 (0.72-0.94)	0.88	359	0.79 (0.63-0.98)	0.86
Subjects with hyperlipidemia	169/1,756	0.83 (0.59-1.16)		76	0.77 (0.46-1.29)	
Free from hypertension	563/16,203	0.68 (0.57-0.81)	0.021	141	0.67 (0.47-0.94)	0.54
Subjects with hypertension	637/6,231	0.94 (0.80-1.12)		287	0.82 (0.64-1.05)	
Low MD (MDS 0-3)	384/6,898	0.91 (0.73-1.12)	0.32	142	0.85 (0.60-1.20)	0.62
Average MD (MDS 4-5)	545/10,053	0.73 (0.61-0.88)		196	0.77 (0.57-1.04)	
High MD (MDS 6-9)	307/5,860	0.85 (0.66-1.10)		106	0.64 (0.42-0.97)	
Healthy subjects*	710/19,479	0.84 (0.71-0.98)	—	224	0.74 (0.56-0.97)	—
Excluding early deaths (follow-up >2 yrs)	1,067/22,642	0.81 (0.71-0.92)	—	373	0.78 (0.63-0.96)	—

Values are n/N or n, unless otherwise indicated. Hazard ratios are obtained from the multivariable model adjusted for age, sex, educational level, occupational class, smoking, leisure-time physical activity, cardiovascular disease, cancer, drugs for diabetes, lipid-lowering drugs, medication for hypertension, MDS (not including sweet pepper intake), sweet pepper intake (g/day, ordered quartiles), garlic, parsley, black pepper (consumption yes/no), energy intake (Kcal/day). Low physical activity = leisure-time physical activity ≤2.27 metabolic equivalent task-hours/day (population median). *Subjects without history of CVD or cancer, nor reporting drugs for diabetes.

CVD = cardiovascular disease; MD = Mediterranean diet; MDS = Mediterranean Diet Score.

content of capsaicin, which is far more abundant in chili than in sweet nonspicy peppers. Similar conclusions were reached by a study revealing a positive correlation between frequency of chili consumption, but not sweet peppers, and muscle strength in adult males (27). However, *Capsicum* species contain a large variety of phytochemicals with well-known antioxidant properties, such as carotenoids (β-carotene), capsaicinoids (capsaicin), and flavonoids (quercetin and luteolin) (28,29). Therefore, a possible synergistic activity of these bioactive compounds cannot be ruled out.

STUDY STRENGTHS AND LIMITATIONS. Major strengths of this study include a large sample size, a prospective cohort design, and careful ascertainment of established and potential risk factors for death; moreover, analyses were controlled for several dietary covariates that may be correlated with intake of chili peppers. Finally, it is the first investigation addressing the association of chili pepper intake and mortality risk in a large Mediterranean population.

The present study has, however, several limitations. First, given the observational nature of our

investigation, causality can only be suggested, and residual confounding or confounding by unmeasured factors cannot be fully ruled out.

Second, cause-specific mortality analysis in this dataset are limited by the relatively small number of deaths. Lastly, subjects' information was collected at baseline only, thus changes that may have occurred during the follow-up could not be considered.

CONCLUSIONS

Regular consumption of chili peppers is associated with lower risk of total and CVD mortality in a large Mediterranean cohort of adults. The mechanisms through which chili peppers could lower mortality risk are still unclear, although a modest role was found for traditional CVD risk factors. The inclusion of chili pepper intake into a traditional MDS offered no or small added value in the discrimination ability of the modified score. To the best of our knowledge, this study is the first to report a negative association between chili pepper intake and (all-cause and specific) mortality risk in a Mediterranean prospective

cohort, and to assess possible biological mechanisms underlying such association.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In an Italian population, regular consumption of chili peppers is associated with a lower long-term risk of mortality from cardiovascular causes.

TRANSLATIONAL OUTLOOK: Further studies are needed to understand the biological mechanisms responsible for apparent beneficial effect of chili peppers on cardiovascular risk.

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KEY WORDS cardiovascular mortality, cerebrovascular mortality, chili pepper, inflammation, Mediterranean diet, risk factors, total mortality

APPENDIX For supplemental tables and a list of the Moli-sani Study Investigators, please see the online version of this paper.

CHAPTER 8.

Daily Coffee Drinking Is Associated with Lower Risks of Cardiovascular and Total Mortality in a General Italian Population: Results from the Moli-sani Study

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Daily Coffee Drinking Is Associated with Lower Risks of Cardiovascular and Total Mortality in a General Italian Population: Results from the Moli-sani Study

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ABSTRACT

Background: An inverse relationship between coffee intake and mortality has been observed in several population cohorts, but rarely within Mediterranean countries. Moreover, the biological pathways mediating such an association remain unclear.

Objectives: We assessed the associations between coffee consumption and total and cause-specific mortality and examined the mediating roles of N-terminal pro B-type natriuretic peptide (NTproBNP), high-sensitivity Troponin I, blood glucose, lipid metabolism, and selected biomarkers of inflammation and renal function.

Methods: We longitudinally analyzed data on 20,487 men and women (35–94 years old at baseline) in the Moli-sani Study, a prospective cohort established in 2005–2010. Individuals were free from cardiovascular disease (CVD) and cancer and were followed-up for a median of 8.3 years. Dietary data were collected by a 188-item semi-quantitative FFQ. Coffee intake was standardized to a 30-mL Italian espresso cup size. HRs with 95% CIs were calculated by multivariable Cox regression.

Results: In comparison with no/rare coffee consumption (up to 1 cup/d), HRs for all-cause mortality across categories of coffee consumption (>1 to ≤ 2 , >2 to ≤ 3 , >3 to ≤ 4 and >4 cups/d) were 0.79 (95% CI, 0.65–0.95), 0.84 (95% CI, 0.69–1.03), 0.72 (95% CI, 0.57–0.92), and 0.85 (95% CI, 0.62–1.12), respectively. For CVD mortality, a nonlinear (P for non-linearity = 0.021) J-shaped association was found (magnitude of the relative reduction = 37%; nadir at 3–4 cups/d). Circulating levels of NTproBNP explained up to 26.4% of the association between coffee and all-cause mortality, while systolic blood pressure was likely to be on the pathway between coffee and CVD mortality, although to a lesser extent.

Conclusions: In this large cohort of Italian adults, moderate consumption (3–4 cups/d) of Italian-style coffee was associated with lower risks of all-cause and, specifically, of CVD mortality. Among the known biomarkers investigated here, NTproBNP likely mediates the relationship between coffee intake and all-cause mortality. *J Nutr* 2020;0:1–10.

Keywords: coffee consumption, mortality risk, cardiovascular mortality, general population, Mediterranean diet

Introduction

Coffee is among the most commonly consumed nonalcoholic beverages worldwide and contains various antioxidants and phenolic compounds, such as flavonoids and caffeine, some of which have been shown to exert beneficial effects for health (1–3).

The relationships between coffee intake and the risks of disease/mortality have been widely investigated in a number of epidemiological settings, most of which indicate a favorable role of regular coffee intake on a variety of health outcomes,

including cardiovascular disease (CVD), mortality, and cancer (4, 5). Regarding cardiovascular health, the currently available evidence on CVD effects related to habitual coffee consumption is largely reassuring, suggesting that coffee can be included as part of a healthy diet for the general public and also for those with an increased cardiovascular risk or CVD (6).

More recently, a meta-analysis of 40 prospective cohort studies found lower risks of mortality from all causes and from CVD in a comparison of the highest versus lowest coffee consumption categories, with similar results across various subpopulations by characteristics of subjects, including age,

For the data analysis, we standardized the amount of coffee consumed to a 30-mL cup size, which is the standard cup size of an Italian espresso according to the Italian dietary guidelines (16).

Coffee consumption was categorized as up to 1 cup/d, >1 to ≤2 cups/d, >2 to ≤3 cups/d, >3 to ≤4 cups/d and >4 cups/d.

Added sugar was the quantity of sugar (g/d) added to beverages (i.e., milk, coffee, tea, or yogurt).

Adherence to the traditional MD was evaluated by the MDS developed by Trichopoulos et al. (11). The score was obtained by assigning 1 point each to foods considered healthy (fruits and nuts, vegetables, legumes, fish, cereals; MUFA to SFA ratio) whose consumption was above the sex-specific medians of intake of the Moli-sani study population for participants apparently free from CVD, cancer, and diabetes, and then the score was applied to the whole population; foods presumed to be detrimental (meat and dairy products) were scored positively if their consumption was below the median. All other intakes received 0 points. For ethanol, men who consumed 10–50 g/d and women who consumed 5–25 g/d received 1 point; otherwise, the score was 0. The MDS ranged from 0 to 9 (the latter reflecting maximal adherence) and scores were categorized according to poor (0–3), average (4–5), and good adherence (6–9) to the MD.

Assessment of covariates

At baseline, information on socio-demographic variables, lifestyles, and medical history were obtained by interviewer-administered questionnaires.

The education variable was based on the highest qualification attained and was categorized as up to lower secondary (approximately ≤8 years of study), upper secondary education (8–13 years of study), and post-secondary or higher (>13 years of study). Housing status was classified as rented, 1-dwelling ownership, or >1-dwelling ownership.

Subjects were classified as never, current, or former smokers (quit at least 1 year ago) and, among ever smokers, the number of cigarettes smoked per day was also assessed. Leisure-time physical activity (PA) for sports, walking, and gardening was dichotomized as <30 or ≥30 min/d.

Waist circumference (in cm) was measured between the twelfth rib and the iliac crest, and hip circumference (in cm) was measured around the buttocks. The waist-to-hip ratio was then calculated, and abdominal obesity was defined as a waist-to-hip ratio ≥0.90 for men and ≥0.85 for women (17).

Participants were considered to have diabetes, hypertension, or hyperlipidemia at baseline if they were taking disease-specific drugs.

Selection and assessment of biomarkers of CVD risk

The biomarkers tested in the present study were selected on the basis whether they were: 1) previously studied for their relevance in pathways predisposed to CVD; 2) shown in epidemiologic studies to be related to CVD or mortality; and 3) already introduced in the analyses of the Moli-sani Study cohort.

Each of these biomarkers reportedly reflects different underlying pathways to disease incidence and progression (18, 19).

Blood samples were collected at baseline in participants who had fasted overnight and had refrained from smoking for at least 6 hours; lipids (total cholesterol, HDL, triglycerides) and blood glucose were assayed in serum samples by enzymatic reaction methods using an automatic analyzer (ILab 350, Instrumentation Laboratory, Milan, Italy); quality control for lipids and glucose was obtained by a commercial standard (SeraChem1 and SeraChem2). For SeraChem1 and SeraChem2, the coefficients of variability were 4.9% and 5.2% respectively for blood cholesterol; 3.2% and 3% for HDL; 5.2% and 5.3% for triglycerides; and 4.7% and 4.1% for blood glucose.

High sensitivity C-reactive protein (CRP) was measured in fresh serum samples by a particle-enhanced immune-turbidimetric assay (ILab 350, Instrumentation Laboratory, Milan, Italy). Quality control for CRP was maintained using an in-house serum pool and a commercial laboratory standard whose inter-day coefficients of variability were 5.5% and 4.2%, respectively.

A hemocromocytometric analysis was performed by cell count (Coulter HMX, Beckman Coulter, IL, Milan, Italy) within 3 hours of blood collection. Quality control was performed using 3 different levels of standards: Abnormal 1, a pathologically high control; Abnormal 2, a pathologically low control; and Normal (Coulter HMX, Beckman Coulter). Coefficients of variability for white blood cells (WBC) were 6.2%, 3.3%, and 3.0% for Abnormal 1, Abnormal 2, and Normal, respectively. N-terminal pro B-type natriuretic peptide (NTproBNP), high-sensitivity assayed Troponin I, apoA, apoB100, lipoprotein a, markers of renal function (cystatin C, creatinine), insulin, C-peptide, and serum vitamin D were measured subsequently on thawed samples stored frozen in liquid nitrogen at the biological bank of the Moli-sani study, in the framework of the collaborative BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe) research project, whose primary objective is to assess the value of established and emerging biomarkers for CVD risk prediction by using data from 23 cohorts across Europe (18).

The low-grade inflammation (INFLA) score was constructed by summarizing synergistic effects of inflammatory biomarkers (20). Briefly, deciles of each biomarker level (CRP, WBC, platelets, and the granulocyte-to-lymphocyte ratio) were generated. For all 4 components, being in the highest deciles (7–10) gave a score that increased from 1 to 4, while being in the lowest deciles (1–4) was negatively scored from −4 to −1. Being in deciles 5 or 6 was scored as 0 points. In such a way, the INFLA score ranged from between −16 and 16 and came up as the sum of the 4 biomarkers. An increase in the score represented an increase in low-grade inflammation intensity.

Statistical analysis

Baseline characteristics of the participants by categories of daily coffee intake were presented as means and SDs or as percentages for categorical variables. Differences in the distribution of baseline covariates were calculated using an ANOVA adjusted for age, sex, and energy intake (GENMOD procedure for categorical variables and general linear model procedure for continuous variables in SAS software; Table 1). Positively skewed variables were log transformed before analysis.

Risk estimates for all-cause and cause-specific deaths were expressed as HRs with 95% CIs and were calculated using Cox regression models with time-on-study on the time scale and adjusting for baseline age as a covariate in the model.

On the basis of previous literature and biological plausibility, 2 models for the association of coffee with the mortality risk were fitted: Model 1 was adjusted for age (continuous), sex, and energy intake (kcal; continuous); Model 2 was adjusted as in Model 1 and further controlled for education (up to lower secondary school; upper secondary school; postsecondary/higher), housing (rented and 1- or >1-dwelling ownership), residence (urban/rural), leisure-time PA (<30 or ≥30 min/d), smoking (never, current, former smokers), number of cigarettes per day (≤5, >5 to ≤10, >10 to ≤20, >20, and missing data), abdominal obesity (no/yes), diabetes (no/yes/missing), hyperlipidaemia (no/yes/missing), hypertension (no/yes/missing), MDS (continuous), tea intake (nonconsumers, >0 to ≤2 cups/week, >2 to ≤4 cups/week, and >4 cups/week), and added sugar (g/d; continuous). Missing values of hypertension, hyperlipidaemia, and diabetes were included in the model as dummy variables, similar to the way valid categories were represented.

For a biomarker to be considered as mediating the association of coffee intake and mortality, it had to be associated with both the exposure and the outcome (at least 1 of those analyzed) with a *P* value < 0.20, in accordance with predefined mediation principles (21, 22). These criteria were tested in distinct multivariable regression models for each potential mediator individually (Supplemental Table 1) and through Cox models that included coffee consumption as a covariate (Supplemental Table 2).

The multivariable Model 2 served as the reference for the mediation analysis used to estimate the contribution of each set of potential mediators, which were alternately included in Model 2.

We performed our analysis on 18,661 subjects (after exclusion of those individuals with missing data on any of the biomarkers) to

TABLE 1 Selected characteristics of participants at baseline in the Moli-sani Study cohort ($n = 20,487$) across categories of daily coffee intake

	Coffee intake, cups/d					P value
	≤1	>1 to ≤2	>2 to ≤3	>3 to ≤4	>4	
Participants, n (%)	3714 (18.1)	4436 (21.7)	4680 (22.8)	3747 (18.3)	3910 (19.1)	
Age, years	58.1 ± 12.8	57.3 ± 11.9	55.7 ± 11.6	54.2 ± 10.9	51.3 ± 9.3	<0.0001
Men	47.8	47.2	46.5	44.1	49.3	0.0002
Educational level	—	—	—	—	—	<0.0001
Up to lower secondary	58.9	54.7	51.5	45.7	44.2	
Upper secondary	30.7	33.3	34.9	39.5	40.6	
Postsecondary	10.4	12.0	13.6	14.8	15.2	
Housing	—	—	—	—	—	0.13
Rent	8.1	7.8	8.6	9.1	11.0	
1 dwelling ownership	84.0	82.9	82.8	81.8	80.2	
>1 dwelling ownership	7.9	9.3	8.6	9.1	8.8	
Smoking status	—	—	—	—	—	<0.0001
Nonsmokers	61.2	55.6	50.8	48.1	35.2	
Current smokers	12.3	17.3	21.9	27.2	41.2	
Former smokers	26.5	27.1	27.3	24.7	23.6	
Number of cigarettes smoked per day ¹	—	—	—	—	—	<0.0001
≤5	33.6	30.5	22.0	21.1	15.1	
>5 to ≤10	24.8	25.0	24.1	23.5	19.2	
>10 to ≤20	31.4	36.7	43.4	44.9	48.1	
>20	9.4	7.0	9.7	9.6	17.1	
Physical activity ≥30 min/d	65.5	65.6	63.8	63.2	61.6	0.0002
Abdominal obesity	75.1	73.6	72.8	72.2	70.1	0.0002
Diabetes	3.8	4.8	3.8	4.0	3.4	0.0003
Hypertension	30.9	29.4	24.7	20.7	15.2	0.0004
Hyperlipidemia	6.1	6.0	5.4	5.5	3.5	0.0003
Mediterranean diet score	—	—	—	—	—	<0.0001
Poor: 0–3	25.9	29.4	30.5	32.7	35.0	
Average: 4–5	44.6	43.2	44.0	44.5	43.6	
Good: 6–9	29.5	27.4	25.5	22.8	21.4	
Added sugar, g/d	4.2 (4.5)	7.2 (5.6)	9.7 (7.1)	12.1 (8.7)	17.6 (14.6)	<0.0001
Tea intake, cups/week	1.4 (3.5)	0.9 (2.5)	0.7 (2.1)	0.7 (2.2)	0.6 (2.2)	<0.0001
Alcohol consumption, g/d	17.4 (22.9)	18.2 (23.7)	16.3 (21.5)	14.5 (19.9)	14.5 (19.3)	<0.0001

Values are expressed as percentages for all variables, except for age, added sugar, tea intake, and alcohol consumption, which are presented as means ± SDs. Means and P values are adjusted for sex, age, and energy intake. Coffee intake was standardized to a 30-mL Italian espresso cup size. Tea intake was standardized to a 150-mL cup size.

¹ Percentages were calculated based on the total number of smokers ($n = 4881$).

evaluate how much of the associations between coffee consumption and all-cause or cause-specific mortality would be accounted for by biomarkers representative of different biological processes.

For the mediation analysis, the % MEDIAN macro in SAS (23) was used to calculate the point and interval estimates of the percentage of exposure effect explained by 1 or more intermediate variables, with 95% CIs and P values. Biomarkers were entered into the mediation analysis as continuous variables.

We conducted several analyses to assess the sensitivity of the observed associations to various baseline risk factors: age (35–64 and ≥65 years), sex, socioeconomic strata (education and housing), lifestyles (smoking status, leisure-time PA, MDS), and health conditions at baseline (abdominal obesity, diabetes, hypertension, and hyperlipidaemia); we also conducted an analysis after excluding those subjects who died during the first 2 years of follow-up. Appropriate multiplicative terms for testing interactions were included in the multivariable models to test for a difference of effect of coffee intake across subgroups (Supplemental Table 3).

Possible curvilinear associations between the continuous measure of coffee intake (cups/d) and the all-cause and cause-specific mortality risks were tested via a multivariable Cox model using a restricted cubic spline with 3 knots at fixed percentiles (5th, 50th, and 95th percentiles of the distribution; Figure 1).

In order to see whether the inclusion of coffee would possibly improve risk predictions associated with MDS, we scored coffee consumption as previously done for the food groups already present in the MDS (11). For consistency with the methodology previously used, we calculated the sex-specific medians of coffee intake in our population (free from CVD, cancer, and diabetes) and assigned a value of 1 if consumption was above the sex-specific median (82.9 g/d for women and 84.0 g/d for men); otherwise, the score was 0.

We then obtained a 2-level variable that was included as an additional component in the original MDS, thus originating an MDS “supplemented” with coffee with scores ranging from 0 to 10; to allow for comparisons among scores, the scores were entered into risk analyses, scaled by their SD.

The categorical Net reclassification index (NRI) and the integrated discrimination improvement (IDI) (24) were used to quantify the predictive value of the MDS supplemented with coffee over the traditional MDS. To estimate these metrics, the follow-up time was censored at 10 years and the risk categories chosen for NRI calculation were <10% and ≥10% (Supplemental Table 4).

Finally, we estimated the E-value, a parameter that calculates the minimum strength of association that an unmeasured confounder would need to have with both the exposure and outcome to fully explain away a specific exposure-outcome association (25).

FIGURE 1 Multivariable-adjusted dose-response association of coffee intake (1-cup increment/d) with (A) all-cause, (B) CVD, and (C) IHD/cerebrovascular mortality in the Moli-sani Study cohort ($n = 20,487$). Risk estimates (HRs with 95% CIs) were obtained from the multivariable model, adjusted for age, sex, energy intake, education, housing, residence, leisure-time PA, smoking, number of cigarettes per day, abdominal obesity, diabetes, hyperlipidemia, hypertension, Mediterranean diet score, tea intake, and added sugar. The dashed lines represent the 95% CIs for the spline model. Participants with no coffee consumption (0 cups/d) served as the reference group. Coffee intake was standardized to a 30-mL Italian espresso cup size. CVD, cardiovascular disease; IHD, ischemic heart disease; PA, physical activity.

The data analysis was generated using SAS/STAT software, Version 9.4, of the SAS System for Windows.

Results

Characteristics of the population by coffee intake

In this Moli-sani population, the mean intake of coffee was 2.8 cups per day ($SD \pm 1.8$).

During a median follow up of 8.3 years (IQR, 7.4–9.3 years), 834 subjects died: 270 (32.4%) from CVD, of whom 152 (18.2%) died from IHD/cerebrovascular causes; 334 (40.0%) from cancer; and 230 (27.6%) from other causes.

Characteristics of the Moli-sani study population according to daily consumption of coffee are reported in Table 1. Higher coffee consumers (> 4 cups/d; 19.1%) tended to be younger and to have a higher level of education and were more likely to be smokers but less likely to have diabetes, hypertension, and hyperlipidaemia; they were also poorly adhering to the MD (Table 1).

Coffee intake and mortality risk

In multivariable-adjusted Model 2, those who drank > 3 to ≤ 4 cups/d had a 28% (95% CI, 8–43%) lower risk of all-cause mortality, as opposed to participants with the lowest consumption (up to 1 cup/d), while among heavy consumers

(>4 cups/d) the mortality risk reduction was no longer significant (Table 2).

When modelling coffee consumption in 1-cup increments per day, the results from main analyses were confirmed (P for association = 0.17; P for non-linearity = 0.47; Figure 1A).

Among those participants with habitual intake of ≥ 3 to ≤ 4 cups/d of Italian-style coffee, the HRs for CVD and IHD/cerebrovascular mortality were 0.58 (95% CI, 0.37–0.91) and 0.55 (95% CI, 0.29–1.03), respectively, while at higher intakes the risk reduction appeared to be lowered (Table 2, Model 2).

Consistently, we found evidence of a nonlinear J-shaped association between coffee consumption and the risk of CVD (magnitude of the relative reduction = 37%; nadir at 3–4 cups/d; P value for overall association = 0.045; P value for nonlinear association = 0.021; Figure 1B), but not for IHD/cerebrovascular mortality (P value for overall association = 0.38; P value for nonlinear association = 0.21; Figure 1C).

No significant inverse associations between the highest (>4 cups/d) as opposed to the lowest (up to 1 cup/d) coffee intakes were found for cancer death (HR = 0.81; 95% CI, 0.52–1.26) or mortality from other causes (HR = 0.71; 95% CI, 0.38–1.31; Table 2; Model 2).

Survival analyses in the general population of the Moli-sani Study, including also participants with CVD and cancer at baseline ($n = 22,801$), showed HRs for all-cause, CVD, and IHD/cerebrovascular death risks of 0.73 (95% CI, 0.64–0.88), 0.55 (95% CI, 0.39–0.78), and 0.53 (95% CI, 0.34–0.84), respectively, for individuals with habitual intake of 3–4 cups/d in comparison with the lowest intake category (Supplemental Table 5).

Mediation analysis

The associations of coffee intake with all potential mediators (selected biomarkers) are shown in Supplemental Table 1. Coffee intake was positively associated with cardiac troponin, biomarkers of glucose metabolism, total blood cholesterol, and apoB100, whereas it was inversely associated with NTproBNP, HDL, and systolic blood pressure (Supplemental Table 1). The HRs for all-cause, CVD, and IHD/cerebrovascular mortality associated with the selected biomarkers, as estimated by including each biomarker in the multivariable-adjusted model, are shown in Supplemental Table 2. Each of these biomarkers was associated with at least 1 of the outcomes under study, with the exception of insulin and HDL (Supplemental Table 2). We finally included in the mediation analysis 5 biomarkers or groups of biomarkers meeting the above mentioned criteria: namely, cardiac troponin, NTproBNP, biomarkers of glucose metabolism, biomarkers of lipid metabolism, and systolic blood pressure. This resulted in a Bonferroni corrected significance threshold α of 0.01.

A mediation analysis on 18,661 subjects indicated that the majority of established biomarkers of CVD did not substantially modify the relationships between coffee consumption and mortality, with the exception of NTproBNP levels, which explained 11.5% ($P = 0.0052$) and 26.4% ($P = 0.0006$) of the relationships between the all-cause mortality risk and coffee consumption of ≥ 3 to ≤ 4 cups/d and > 4 cups/d, respectively (Supplemental Table 6).

The risk reduction of CVD mortality associated with consumption of ≥ 3 to ≤ 4 cups/d was slightly accounted for by systolic blood pressure (3.7% reduction; $P = 0.0078$), a factor also partially explaining the relationship with the risk

of IHD/cerebrovascular death (5.0% reduction; $P = 0.0032$; Supplemental Table 7).

Additional analyses

Subgroup analyses are presented in Supplemental Table 3. Coffee intake remained consistently associated with reduced mortality in almost all subgroups. An exception was represented by PA and abdominal obesity, which were likely to modify the magnitude of the inverse associations between coffee consumption and all-cause and CVD mortality risks. Specifically, the associations between a 1-cup increment and all-cause mortality risk were stronger in less physically active subjects (P for interaction = 0.047) and among individuals with abdominal obesity (P for interaction = 0.0068; Supplemental Table 3); those with abdominal obesity were also more likely to have a reduced CVD death risk associated with increased coffee intake, as compared with those participants without abdominal obesity (P for interaction = 0.033).

The percentage of decaffeinated coffee consumers was very low in our population sample ($n = 741$; 3.6%). The mean caffeine intake was 63.8 mg per day and the main caffeine source was coffee; consequently, caffeine intake was highly correlated with the intake of coffee (Spearman correlation coefficients $r = 0.80$; P value < .0001) and represents a strong proxy of it. In addition, to account for other food sources of caffeine, all analyses were also adjusted for tea consumption.

NRI and IDI values indicated that the addition of coffee intake into the MDS did not add to the risk prediction model of the traditional MDS (all P values > 0.05; Supplemental Table 4).

Finally, by the use of E-values, we found that to nullify the reported association, unobserved confounders should be associated with both coffee consumption and mortality by an OR/HR of around 3.0. Of interest, the whole set of known confounders included in our analysis shifted the univariate HR for CVD mortality (HR = 0.29; 95% CI, 0.19–0.44) associated with ≥ 3 to ≤ 4 cups/d to the fully adjusted HR of 0.58 (95% CI, 0.37–0.92; Model 2), acting as a confounder with an E-value of 3.41. Therefore, it is unlikely that 1 (or more) unmeasured factors with an E-value of around 3.0 would exist and create an impact in the way the large, entire set of known confounders considered in our study did.

Discussion

In a large, adult, Italian population apparently free from major chronic diseases at baseline, a moderate intake of Italian-style coffee was associated with lower risks of all-cause and CVD mortality. The association with CVD mortality follows a nonlinear J-shaped curve, showing its nadir at 3–4 cups per day. This finding is in line with a previous meta-analysis showing evidence of a nonlinear relationship between coffee consumption and CVD mortality, with the strongest association being observed for 3 cups/d (21% lower risk) (26) and with data from an umbrella review indicating the largest relative risk reduction at intakes of 3 to 4 cups a day versus none (4).

Similarly, a recent meta-analysis on nearly 4 million subjects showed that the lowest relative risks were at intakes of 3.5 cups/d for all-cause mortality and 2.5 cups/d for CVD mortality, while additional intakes were not associated with further reductions in mortality risks (7).

TABLE 2 Risk of all-cause and cause-specific mortality associated with coffee intake in the Moli-sani Study cohort ($n = 20,487$)

	Coffee intake, cups/d				
	≤ 1	> 1 to ≤ 2	> 2 to ≤ 3	> 3 to ≤ 4	> 4
All-cause mortality, $n = 834$					
<i>N</i> of deaths/ <i>n</i> of subjects	245/3714	202/4436	190/4680	108/3747	89/3910
Person-years	30,469	36,367	38,570	31,284	32,727
Event rates per 10,000 person-years	80.4	55.5	49.3	34.5	27.2
Model 1 HR (95% CI) ¹	1.0	0.85 (0.70–1.02)	0.94 (0.78–1.14)	0.86 (0.68–1.08)	1.11 (0.86–1.43)
Model 2 HR (95% CI) ²	1.0	0.79 (0.65–0.95)	0.84 (0.69–1.03)	0.72 (0.57–0.92)	0.85 (0.62–1.12)
CVD mortality, $n = 270$					
<i>N</i> of deaths/ <i>n</i> of subjects	92/3714	68/4436	54/4680	27/3747	29/3910
Person-years	30,469	36,367	38,570	31,284	32,727
Event rates per 10,000 person-years	30.1	18.7	14.0	8.6	8.9
Model 1 HR (95% CI) ¹	1.0	0.81 (0.59–1.11)	0.78 (0.55–1.09)	0.66 (0.43–1.02)	1.32 (0.85–2.06)
Model 2 HR (95% CI) ²	1.0	0.74 (0.54–1.03)	0.70 (0.49–0.99)	0.58 (0.37–0.91)	1.04 (0.62–1.73)
IHD/cerebrovascular mortality, $n = 152$					
<i>N</i> of deaths/ <i>n</i> of subjects	50/3714	35/4436	37/4680	14/3747	16/3910
Person-years	30,469	36,367	38,570	31,284	32,727
Event rates per 10,000 person-years	16.4	9.6	9.6	4.5	4.9
Model 1 HR (95% CI) ¹	1.0	0.78 (0.51–1.21)	0.99 (0.65–1.53)	0.63 (0.35–1.16)	1.37 (0.75–2.49)
Model 2 HR (95% CI) ²	1.0	0.72 (0.46–1.13)	0.90 (0.57–1.41)	0.55 (0.29–1.03)	1.11 (0.56–2.20)
Cancer mortality, $n = 334$					
<i>N</i> of deaths/ <i>n</i> of subjects	83/3714	72/4436	85/4680	51/3747	43/3910
Person-years	30,469	36,367	38,570	31,284	32,727
Event rates per 10,000 person-years	27.2	19.8	22.0	16.3	13.1
Model 1 HR (95% CI) ¹	1.0	0.84 (0.61–1.15)	1.13 (0.83–1.53)	1.03 (0.72–1.46)	1.16 (0.79–1.72)
Model 2 HR (95% CI) ²	1.0	0.76 (0.55–1.06)	0.98 (0.71–1.35)	0.82 (0.56–1.19)	0.81 (0.52–1.26)
Other causes mortality, $n = 230$					
<i>N</i> of deaths/ <i>n</i> of subjects	70/3714	62/4436	51/4680	30/3747	17/3910
Person-years	30,469	36,367	38,570	31,284	32,727
Event rates per 10,000 person-years	23.0	17.0	13.1	9.6	5.2
Model 1 HR (95% CI) ¹	1.0	0.94 (0.67–1.33)	0.92 (0.64–1.33)	0.89 (0.58–1.37)	0.84 (0.48–1.46)
Model 2 HR (95% CI) ²	1.0	0.91 (0.64–1.31)	0.85 (0.58–1.24)	0.79 (0.50–1.26)	0.71 (0.38–1.31)

Data are shown as HRs with 95% CIs. CVD, cardiovascular disease; IHD, ischemic heart disease; PA, physical activity.

¹Model 1 is adjusted for age, sex, and energy intake.

²Model 2 had the same adjustments as Model 1 and was further controlled for education, housing, residence, leisure-time PA, smoking, number of cigarettes per day, abdominal obesity, diabetes, hyperlipidemia, hypertension, adherence to Mediterranean diet, tea intake, added sugar.

Potential explanations for the lack of further risk reduction observed for high coffee consumption may rely on the fact that coffee is a composite brew containing several bioactive compounds whose health effects are in opposite directions. Although it is a major source of antioxidants, coffee contains diterpenes, whose concentrations vary by the type of coffee brew, being intermediate in espresso and coffee made in a Moka pot and negligible in drip-filtered, instant, and percolator coffee (27).

Recently, data from the UK Biobank indicated a stronger direct association between blood lipids and espresso coffee as compared to filtered or instant coffee, which is possibly due to the different content of diterpenes with known lipid-raising effects (28). However, the effect of caffeine on the cardiovascular system depends on various factors, including the metabolic status and presence of illness (29), and may vary according to different genotypes and gene-environment interactions (30, 31).

To the best of our knowledge, this is the first cohort study to analyze the associations of Italian-style coffee and mortality risks in a healthy, adult population. Much of the research so far has evaluated the health effects associated with filtered or boiled coffee, which are the most popular preparation methods

in the United States and Scandinavian countries, respectively (32).

The favorable effect of coffee intake on the mortality risk was previously documented by other meta-analyses (5, 26, 33) and prospective cohort studies (34). Yet, our data on CVD outcomes were not in accordance with prior findings on over 40,000 Italians analyzed in the EPICOR (long-term follow-up of antithrombotic management Patterns In acute CORonary syndrome patients) Study, which reported an increased CHD risk associated with consumption of > 2 cups of Italian-style coffee per day; their results are partially explained by the caffeine content and rapid consumption, resulting in a high peak plasma concentration of caffeine (35).

An umbrella review including meta-analyses of observational studies and randomized controlled trials suggested that coffee may exert beneficial effects on human health, including for some cancers (endometrial, prostate, colorectal, and liver), CVD and metabolic-related outcomes (such as type 2 diabetes and metabolic syndrome), and neurological conditions (such as Parkinson's disease, Alzheimer's disease, and depression) (5). Of note, it was shown in the same Moli-sani population studied here that coffee consumption reduced the risk of prostate cancer (36).

In EPIC, high coffee consumers (>3 cups per day) had lower all-cause mortality and digestive disease mortality risks compared to nonconsumers (37), in line with results from African Americans, Japanese Americans, Latinos, and Whites in the Multiethnic Cohort (34).

In our analyses, coffee intake was not associated with cancer death, with these results at variance with other population studies (37, 38). However, in a Mediterranean setting, a recent study suggested an inverse association between coffee consumption and the cardiovascular mortality risk in elderly (65 years old and above) after a 6-year follow-up, but no effects for total or cancer mortality, in line with our findings (39).

The major strengths of this study include a large sample size of an apparently healthy, adult population; a prospective cohort design; and considerable numbers of covariates included for analyses.

Moreover, the robustness of our findings was confirmed by sensitivity analyses. To the best of our knowledge, this is among only a few studies investigating the biological mechanisms potentially linking coffee intake to health outcomes in a primary prevention context.

However, there are also a number of caveats. First, given the observational nature of our investigation, causality can only be suggested and residual confounding cannot be fully ruled out. However, the use of E-values indicates a very small impact of potential unmeasured factors on the strength of our associations. Second, only the all-cause mortality outcome could be fully explored, as cause-specific mortality analyses were limited by the relatively small number of events. Lastly, subjects' information was collected at baseline only; thus, changes that possibly occurred during the follow-up could not be considered.

Conclusions

In conclusion, this study shows a beneficial effect of Italian-style coffee consumption (when limited to 4 cups per day) on all-cause and CVD mortality risks, confirming in a large cohort of Italian adults the results of previous epidemiological studies performed in other settings.

The mechanisms through which moderate coffee consumption could lead to a lower mortality risk are still unclear, although an important mediation was found for NTproBNP levels, while other biomarkers tested were unlikely to be on the coffee-mortality prevention pathway. Finally, the inclusion of coffee intake into a traditional score assessing adherence to the MD did not offer any added value to the discrimination ability of the modified score.

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The authors' responsibilities were as follows—ER, MB, and LI: contributed to the design of the study and interpretation of data; SC, ADcC, and MP: managed data collection; ER, MB, and ADic: analyzed the data; ER and MB: wrote the manuscript; MBD, CC, GdG, and LI: originally inspired the research and critically reviewed the manuscript; and all authors: read and approved the final manuscript.

We also found that smokers were less likely to experience a CVD mortality risk reduction associated with coffee drinking as compared with nonsmokers, in line with previous studies (5, 21).

Lastly, with respect to a potential improvement of risk predictions deriving from the inclusion of coffee consumption into the traditional MDS, we failed to find significant changes in the discrimination ability of the modified score when supplemented with coffee.

The fact that an exposure significantly associated with an outcome does not lead to improvements in predictive value is a well-known and widespread occurrence, and does not entirely devalue the association between the variable and the outcome (53). More importantly, the association of coffee with the mortality risk was independent of the MD, supporting the notion that minor dietary changes, such as consuming coffee regularly within the usual diet, could be valuable measures for

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CHAPTER 9. GENERAL DISCUSSION

The purpose of this thesis was triple:

1. To re-organize and assess the consumption of major food groups presently included in the Mediterranean diet and its major socioeconomic and psychosocial determinants in a large representative sample of the Italian population recruited in the INHES survey;
2. To evaluate the association with mortality of largely consumed foods and beverages (eggs, chili pepper and coffee), that are not included in the traditional Mediterranean Diet Score, in a large sample of a Mediterranean population recruited in the Moli-sani Study cohort;
3. To assess whether the inclusion of some of these foods/beverages into the traditional Mediterranean Diet Score would improve its risk prediction.

A number of crucial issues have been addressed:

1. Two different large study populations

In the present thesis we used data from two different population cohorts, that is the Italian Nutrition & Health Survey (INHES study) and the Moli-sani Study, which offered numerous opportunities to address the key purposes of this thesis.

The INHES study is a telephone-based survey on nutrition and health specifically designed to collect information on dietary habits (quality, quantity and patterns), food choice determinants, and food health awareness of the Italian population according to different geographical distribution (Northern, Central and Southern Italy), age, gender and socioeconomic status (1). The sampled subjects were distributed in the four seasons (excluding Christmas, Easter and mid-August periods). The survey calendar was organized to capture an adequate proportion of weekdays and weekend days at group level.

The INHES cohort consists of 9,319 women and men aged ≥ 5 years recruited from all over Italy, between November 2010 and November 2013. The survey covers the whole Italian territory thus allowing a region-based analysis; moreover, it enrolled children and adolescents for a full coverage of eating habits across all age groups.

Finally, the INHES survey also relies on the use of 24-hours dietary recall and allows a full assessment of eating behaviors/patterns. Of course, the survey shares all the limitations of

this study type, such as telephone-based collection of data and self-reported medical information. Moreover, data collection is based only on one-day recall.

The Moli-sani Study is a prospective cohort study of 24,325 citizens (men and women aged ≥ 35 years) from the Molise region, an area placed between Central and Southern Italy, established in 2005-2010 with the purpose of investigating genetic and environmental risk factors in the onset of cardiovascular, cerebrovascular and tumor diseases (2).

The Moli-sani Study is based on face-to-face interviews and clinical examinations, and gained dietary data by using the EPIC questionnaire, a 188-item semi-quantitative food frequency questionnaire, adapted to the Italian population (3).

Owing to its longitudinal design, the Moli-sani cohort offers the opportunity of analyzing prospectively the association between diet and health outcomes.

2. Up-to-date information on food groups mainly consumed in Italy

The last effort for an accurate evaluation of dietary habits at a national level in Italy dates back to 2005-06, when INRAN retrieved data from 1329 households corresponding to 3323 women and men (4). Food group consumption data were collected at individual level in 3 consecutive days using hard-copy diaries structured by meal and the data management system INRAN-DIARIO 3.1.

Chapter 2 presented the up-to-date information on food group consumption using data from the INHES survey, that have been collected until very recently (2013). Additionally, and for the very first time, this chapter has addressed the dietary assessment methodologies, using the INHES sample size and the classification of single food items to food groups was performed using the Exposure Hierarchy tree of the European Food Safety Authority's (EFSA's) FoodEx2 (version 2) classification system (5), recently available (April 2015).

Among foods included in the Mediterranean diet pyramid, we focused on whole grain foods due to the growing attention that that these products have gained during the very last years because of their inverse association with disease risk (6-9). The Mediterranean diet pyramid recommends the consumption of one or two servings of cereals at each main meal (in the form of bread, pasta, rice, couscous and others), preferably whole grain (10).

Findings presented in **Chapter 3** indicate that whole grain intake is consumed on a regular basis (≥ 1 time/week) by 26.9% of the Italian study sample, with whole grain bread being the

major food source of total whole grain food for both age groups (children/adolescents and adults), up to half of the total whole grain intake.

Among major determinants, we highlighted a prominent role of socioeconomic status, with advantaged adult individuals being more likely to report regular intake as compared to lower classes.

Among children/adolescents, living in Southern Italy resulted associated with regular whole grain intake, while among adults, we found that subjects from Northern Italy were likely to consume whole grains more regularly than their counterparts.

We also found that regular whole grain consumers tended to have a healthier lifestyle, including higher physical activity and abstention from tobacco, as already documented in other epidemiological settings (11).

Furthermore, regular whole grain food intake was also associated with some meal patterns and eating-related behaviors: for example, individuals eating meals out of home (e.g. breakfast or lunch) were less likely to consume whole grain food, as well as children/adolescents spending much time watching TV, thus suggesting that whole grain consumption is associated with a healthier eating behavior.

3. Evaluation of the adherence to the whole Mediterranean diet in Italy and its determinants

Recent data have shown that adherence to Mediterranean diet has been rapidly declining in Mediterranean countries during the last decades (12). So far, data on dietary habits in the Italian population were mainly obtained from relatively small samples of subjects or pertaining to specific districts, (13) which can likely prevent from an actual estimation of the dietary habits across the whole country, with little chance to offer insights on possible differences at national level. In light of this, **Chapter 4** represents the first attempt to identify the major determinants of the Mediterranean diet all over Italy, in recent years, after the beginning of the economic crisis, which has been previously associated with a dramatic drop in the adherence to the Mediterranean diet. First, we found a clear educational gradient in adherence to this eating model, with higher educational attainment being more likely associated with Mediterranean diet as opposed to the lowest educational group. These findings are in accordance with previous analysis from the Moli-sani study (14), highlighting a central role of socioeconomic status (mainly income) in determining the adherence to the Mediterranean diet.

We also found a number of psychosocial factors being associated with Mediterranean diet, as already documented in the Moli-sani Study population (15), and also that an optimal adherence to Mediterranean diet is closely associated with middle or older age, site of residence (Southern Italy) and higher education level. Finally, findings from **Chapter 4** showed that the association of the Mediterranean diet with major demographic (age) and socioeconomic factors (education) was likely to be modified by geographical area. Indeed, adult and older subjects more likely reported high adherence to the Mediterranean diet in Central and Southern regions, rather than in the Northern part of Italy; moreover, living in an urban environment was inversely associated with Mediterranean diet in the Northern part of Italy, and positively in the Central and Southern regions of the country.

Such findings indicate that adherence to the Mediterranean diet seems to be strongly affected by socio-cultural background of Italian individuals, in the Southern regions as part of the population cultural heritage and affordability of prices of the goods, while in the Northern regions as a health-conscious choice to which only the richer part of the population has access.

4. Intake of fish – even in a processed form - is good for the heart, but should respect food safety rules

Dietary guidelines recommend eating fish at least two times (two servings) a week (16), because of the well documented inverse association with risk of developing cardiovascular disease supported by several meta-analyses (17).

This hypothesis is further supported by findings from **Chapter 5** examining the association between fish intake and risk of composite coronary heart disease (CHD) and stroke in the general Mediterranean population recruited in the Moli-sani Study. We found an inverse association of high fish intake with incidence of composite fatal and non-fatal CHD and stroke. The importance of consuming fish regularly was also highlighted in another investigation from the Moli-sani Study, showing that, among the nine food items, higher fish intake was a major contributor to the overall effect of the Mediterranean Diet Score in reducing mortality risk among the elderly (18).

During the last decades, the opportunity of promoting large fish intake has been highly debated due to the growing public concern about the reported presence of contaminants in fish.

Considering the nutritional contribution that fish makes to the diet, the European Food Safety Authority's (EFSA) recommended that vulnerable groups in particular select fish from a wide range of species without giving preference to fish types likely containing higher levels of methylmercury, such as swordfish and tuna (19). In a latest statement, the EFSA Committee recommended that each Country should consider its own pattern of fish consumption, especially the species of fish consumed, and carefully assess the risk of exceeding the tolerable weekly intake of methylmercury while obtaining the health benefits from consumption of fish/seafood (20).

5. Possible implementation of the current Mediterranean Diet Score with hitherto not included substances

Identifying the optimal diet (or diets) for chronic disease prevention is a public health priority. Traditionally, nutrition research has focused on single nutrients or specific foods, although individuals do not consume nutrients or foods in isolation. Thus, recent nutritional epidemiologic studies have shifted to dietary pattern analysis, which describes the overall diet; the foods, food groups, and nutrients included; their combination and variety; and the frequency and quantity with which they are habitually consumed (21).

Although providing a good assessment of dietary intake, the traditional Mediterranean Diet Score does not include some widely consumed foods and beverages. It follows that the main aim of this thesis was to evaluate the association of some of these foods/beverages with mortality risk and, in some cases, to test whether their inclusion into the Mediterranean Diet Score would improve its risk prediction.

Chapter 6 analyzed the association between *egg* consumption and mortality risk among the Moli-sani Study participants. We found that egg intake was associated with increased risk of all-cause, cardiovascular mortality and IHD/cerebrovascular mortality in a non-stepwise manner. Furthermore, we tested three dietary nutrients largely present in eggs as possibly mediating the association with mortality. Dietary cholesterol explained up to 20.7% of the excess of cardiovascular mortality risk associated with the highest egg intake (≥ 2 eggs/week) and accounted for up to 32% of IHD/cerebrovascular risk mortality associated with moderate egg intake (1-2 egg/week).

Indeed, opinions on the role of eggs in the diet have changed several times over the past few decades, as well as dietary guidelines for egg consumption for general population vary among

public health agencies. Previously, the Dietary Guidelines for Americans recommended that cholesterol intake be limited to no more than 300 milligrams per day. The 2015 Dietary Guidelines Advisory Committee (DGAC) will not bring forward this recommendation because available evidence shows no appreciable relationship between consumption of dietary cholesterol and serum cholesterol, consistent with the conclusions of the AHA/ACC report. Cholesterol is not a nutrient of concern for overconsumption (22).

However, based on the high cholesterol content, the Mediterranean Diet Foundation and the INRAN (National Institute for Research on Food and Nutrition) recommend up to 4 eggs per week, as a healthy alternative to fish or meat (23,24).

Several studies (25,26) and even meta-analyses (27) have been conducted to investigate the effects of eggs on cardiovascular health, with very different conclusions.

In addition, evidence on the association of egg intake with mortality risk is limited (28-30), especially within Mediterranean countries, where only one study from general population is apparently available (31).

Spices have long been an essential part of the traditional Mediterranean diet, and are placed, along with herbs, at the base of the Mediterranean diet pyramid both for their nutritional properties and as a valuable substitute for salt (32).

Findings from **Chapter 7** revealed that regular consumption (>4 times/week) of *chili pepper* was reported by 24.3% of the Moli-sani study participants, while 33.7% declared none/rare intake. Regular consumption was associated with 23% and 34% lower risk of all-cause mortality and CVD mortality, respectively, as opposed to none/rare intake, and the magnitude of the association appeared to be stronger for IHD and cerebrovascular disease mortality.

The association of chili peppers with lower mortality risk is independent of the overall quality of the diet, as measured by a traditional Mediterranean diet.

With respect to a potential improvement of risk prediction deriving from the inclusion of chillies into the traditional Mediterranean Diet Score, we found no significant changes in the discrimination ability of the modified score. However, the fact that an explanatory variable significantly correlated with the outcome does not lead to improvements in prediction is a well-known and widespread occurrence, and does not entirely devalue the association between the variable and the outcome (33). More importantly, the association of chili pepper with mortality risk was independent of the adherence to the Mediterranean Diet, supporting the notion that minor dietary changes, such as adding chillies to usual diet, could be valuable

measures for improving health, especially cardiovascular health, independently of overall diet quality.

Of notice, this is the first study addressing the association of chillies with mortality in a Mediterranean population, since previous evidence came from China and US (34,35), and to assess possible biological mechanisms underlying such association.

Finally, **Chapter 8** reports data on *coffee* intake in the Moli-sani Study cohort. Coffee is largely consumed in our population, with regular consumers (>3 cups/day) being about 40% of the population.

In our study higher Italian-style coffee consumption (>3 to ≤4 cups/d) was associated with 28% lower risk of all-cause death, while a nonlinear J-shaped association was found with CVD mortality.

As previously described in the same population, the consumption of high coffee (more than 3 cups/day), in comparison to a lower consumption, was associated with a 53% reduction in prostate cancer risk (36).

The inclusion of coffee consumption as an additional component of the traditional Mediterranean Diet Score did not improve discrimination ability of the model. However, the improved prediction associated with MDS supplement with coffee was little over the model including the traditional MDS

Future perspective

As every traditional diet, the Mediterranean diet is facing a global food challenge as the global industrial food system is emerging and strongly contributing to a worldwide transition towards eating models possibly associated with obesity and non-communicable diseases (37-39).

Current assessments of Mediterranean diet, as well as of other healthy dietary patterns, are based on principles of traditional nutritional epidemiology, which appear to be insufficient to keep up with a rapidly evolving food system. Indeed, nutritional epidemiology still relies on the use of tools which were not designed to distinguish foods based on industrial processing, nor on the type of farming (organically/conventionally grown).

Moreover, within a context of global industrial food system that favors food exchanges from one Country to another across the globe, it is crucial to understand how foods not included into the Mediterranean pyramid and/or adhesion score, could be integrated into a traditional

Mediterranean diet. In this study we did not account for 'exotic' foods (e.g. papaya, spices such as curcuma) because the dietary information available in our Moli-sani Study cohort was limited to foods and beverages largely linked to the Italian culinary tradition.

Future nutrition research should consider to collect dietary data on foods that are progressively becoming part of the Mediterranean food tradition in order to see whether their consumption is able to improve or decrease the healthy potential of traditional diets.

In addition, it is noteworthy that traditional diets that feature whole or minimally processed foods and emphasize home-cooking and food preparation, are being rapidly replaced by diets comprising industrially processed and prepared food products (40, 41).

These foods are generally cheap, attractive and convenient (42), and some of them are even considered as virtually 'healthy'. It is the case of fish, which is largely recommended especially for its heart benefits, and is generally consumed in the form of fresh, frozen, dried or canned. Future studies will clarify whether different degrees of processing would potentially have different long-term health impacts.

In this thesis, I observed that consumption of canned fish, which is considered as a processed food according to the latest food classifications (42) is associated with a lower, though not significant, risk of cardiovascular disease (Chapter 5).

The actual challenge for nutritional epidemiology would be to discriminate foods not only on the basis of their presumed health benefits, but also based on the degree of food processing.

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CHAPTER 10. VALORIZATION

The increment of knowledge offered by this thesis over current data, confirms that not only the contribution to health of traditional Mediterranean diet remains intact - when adherence to it is satisfactory - but increases its magnitude, because it adds a surplus-value and is potentiated by other foods or beverages, such as chili pepper and coffee, shown in this thesis to contribute to health and disease risk reduction.

The topic of this thesis is relevant and timely especially in light of the fact that unhealthy diets are major risk factors for a wide range of chronic diseases, including cardiovascular disease, cancer, diabetes as well as all-cause mortality. Specific recommendations for a healthy diet include: eating more fruit, vegetables, legumes, nuts and grains; cutting down on salt, sugar and animal fats (1).

In 2017, 11 million deaths and 255 million disability-adjusted life-years (DALYs) were attributable to dietary risk factors; among them, high intake of sodium (3 million deaths and 70 million DALYs), low intake of whole grains (3 million deaths and 82 million DALYs), and low intake of fruits (2 million deaths and 65 million DALYs) were the leading dietary risk factors for deaths and DALYs globally and in many countries (2).

As highlighted in the analysis by the Global Burden of Diseases (2), the Countries that have a diet close to the Mediterranean diet (higher intake of fruits, vegetables, nuts and healthy oils), are the Countries where the lowest number of diet-related deaths were recorded, in line with robust evidence on the lower disease/mortality risk associated with Mediterranean diet, also in non-Mediterranean populations (3,4).

In light of this, preserving a Mediterranean diet, is a major public health task to prolong survival and to reduce risk of major chronic disease.

The first major issue emerging from this thesis is that a number of modifiable factors (e.g. socioeconomic, psychosocial) may influence adherence to this healthy dietary pattern; thus, one of the next challenges nutrition research should address is to reduce food (and health) disparities, also by relying on a socio-ecological model aimed at improving the overall food environment, combined with more efficacious individualized interventions (5).

In this light, recent results from the Moli-sani Study indicated that a person from low socioeconomic status is unlikely to get the same heart advantages of a person with higher income, even in the case that they both similarly adhere to a Mediterranean-like model –and

get the same traditional dietary score-(6). This observation likely indicates that healthier choices are driven not only by a good set of knowledge and skills but also by greater financial resources. Nutrition literacy and communications are integral to improving diet quality, and health care professionals have an important responsibility to promote healthy eating habits, but often lack basic nutrition knowledge. Therefore, nutrition education and communication programs are recognized as a primary form of intervention in national food and nutrition plans, as they provide people with the knowledge and skills to make healthy dietary and lifestyle choices. Moreover, we should also consider that schools are the ideal setting for promoting healthy eating, because the habits of children and younger people are still being formed (7).

In 2016, an estimated 41 million children under the age of 5 years were overweight or obese, likely due to dietary risk factors including an increased intake of energy-dense foods and a prevalence of physical inactivity (8).

The Moli-sani participants have been repeatedly informed by different means of the results of this thesis (e.g., newsletters, press release, yearly calendar...): in this way, we have ensured that the population involved in our study would consider research activities under a favorable light, thus continuing to collaborate with us and to take part in follow-up procedures.

We're doing it on a regular basis through meetings (called 'I campanili della salute') extended to the general population and organized in different cities and small towns with the main aim of sharing the main findings and discuss the major health issues resulting from the Moli-sani study. A calendar is prepared each year and distributed free of charge to the families of all participants to the study, local general practitioners, public health officers and other selected people.

Within a context of research aiming to identify dietary determinants of health, it was interesting to analyze whether foods and beverages, some already well-known for their health advantages (e.g. coffee), but not included in the traditional Mediterranean Diet Score, nor in the majority of its modified versions, were associated with health outcomes in a population with typical Mediterranean dietary habits.

In this thesis, I observed substantial heart advantages associated with regular consumption of chili pepper (Chapter 7), a spice largely used in the Mediterranean tradition to add flavor to food but also contributing to an important decrease of salt use.

Based on the effects of high salt intake on blood pressure and on the prominent role of high blood pressure in promoting cardiovascular diseases, a population-wide reduction in salt intake could substantially reduce the incidence of cardiovascular disease (9).

Indeed, reducing salt intake has been identified as one of the most cost-effective measures Countries can take to improve population health outcomes. Moreover, government programs designed to achieve a 10 percent reduction in salt consumption over 10 years could save each year an average of 5.8 million disability-adjusted life years (DALY) (10).

Generally, the promotion of a healthy lifestyle would help reduce the healthcare burdens through lowering the risk of developing multiple chronic diseases, including cancer, cardiovascular disease and diabetes, and extending disease-free life expectancy.

Indeed, adherence to a low risk lifestyle was associated with a longer life expectancy at age 50, free of major chronic diseases of approximately 7.6 years in men and 10 years in women compared with individuals with no low risk lifestyle factors (11).

Similarly, in the Moli-sani Study, adherence to 4 healthy lifestyle factors, including Mediterranean diet, was associated with higher survival not only within the general population but also among patients at high health risk, such as those with history of CVD or diabetes and among the elderly (12).

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CHAPTER 11. GENERAL CONCLUSIONS

Up to date information on over 70 food groups and subgroups largely consumed in Italy was as a preliminary good source of information for my subsequent nutrition research.

Take-home messages may be summarized as follows:

1. The Mediterranean diet is strongly affected by socio-cultural background of Italian individuals: in the Southern regions as part of the population cultural heritage and affordability of prices of the goods, while in the Northern regions as a health-conscious choice to which only the richer part of the population has access.
2. The relatively low consumption of whole grain foods observed in a large Italian sample indicates the need of improving public health strategies to increase knowledge and awareness of health benefits associated with whole grain intake.
3. Our findings on fish intake could help promote a regular consumption of fish in line with the European Food Safety Authority's (EFSA) recommendations to select fish from a wide range of species, with reduced preference to fish likely containing higher levels of methylmercury, such as swordfish and tuna.
4. Our findings on egg and mortality risk, along with results from previous population studies, should lead to a more prudent national dietary guideline.
5. Regular chili pepper and coffee consumption could be valuable measures for improving health, especially cardiovascular health. Although adding little to an overall traditional Mediterranean diet from a statistical point of view, they may be well integrated into a Mediterranean-like diet.

CHAPTER 12. SUMMARY

The health benefits associated with the Mediterranean diet have been well established by several epidemiological studies consistently showing that adherence to this dietary pattern is associated with a significant reduction in the risk of developing cardiovascular disease, cancer and neurodegenerative diseases, as well as all-cause mortality.

Nevertheless, the dietary behaviors of many populations have been radically changing since the original definition of this eating pattern and many problematic nutritional aspects emerged, especially within a context of global industrial food system that favors food exchanges from one country to another across the globe.

The Mediterranean Diet Score is the most extensively used index to measure adherence to the Mediterranean diet in epidemiological studies but, although including the key food groups, it leaves out other foods and beverages, some of which might be positively associated with advantageous health outcomes.

This thesis confirmed the health benefits of the traditional Mediterranean diet and its major determinants. Moreover, it addressed the question whether some foods and beverages, neither presently included in the traditional Mediterranean Diet Score, nor in the majority of its modified versions, were associated with health outcomes in a large population with typical Mediterranean dietary habits. The thesis also tested whether the inclusion of these foods and beverages might improve risk prediction beyond a traditional Mediterranean Diet Score.

CHAPTER 13. CANDIDATE'S CURRICULUM VITAE

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Personal details

- Date of birth: December 24, 1991
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Education

- 2006-2011: High School at Liceo Scientifico A. Manzoni, Caserta, Italy
- 2011-2014: Bachelor's degree in Health Biotechnology at University of Naples, Napoli, Italy
- 2014-2016: Master's degree in Human Nutrition Science at University of Naples, Napoli, Italy
- June-July 2019 – European Educational Program in Epidemiology – 32nd Residential Summer Course in Epidemiology (Summer 2019, Florence, Italy)
- 2017-2020: PhD student, the Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands.
PhD thesis title - Mediterranean diet and beyond: an Italian perspective.

Research and working experience

- 2016-2020: Research fellow in Nutritional Epidemiology at the Department of Epidemiology and Prevention, IRCCS NEUROMED, Pozzilli, Molise, Italy
- 2021: Post-Doctoral Fellowship granted by the Fondazione Umberto Veronesi, Milano, Italy.

Honors and awards

- XXXVIII National Congress SINU, Turin, Best Oral Communication Award 2017 for the selected scientific contribution: "Socioeconomic and psychosocial determinants of

adherence to the Mediterranean diet in a general adult Italian population: results from INHES study (Italian Nutrition & Health Survey)."

- XXXIX National Congress SINU, Naples, Award "Gianvincenzo Barba" 2018 for Scientific Research and Innovation in Human Nutrition: "Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population. European Journal of Public Health (Oxford)".
- EPH Conference Marseille 2019 - Ferenc Bojan session, Best abstract award: "Egg consumption and risk of total and cause-specific mortality in a Mediterranean population"
- 9th European Beer and Health Symposium 2019, Bruxelles, Best abstract award: "Moderate alcohol consumption is associated with reduced low-grade inflammation: results from the Moli-sani Study".

Publications

- First author of 4 publications
- Co-author of 5 publications as an investigator of the INHES study and Moli-sani Study
- H -Index (calculated via Google scholar) = 7

CHAPTER 14. CANDIDATE'S PUBLICATIONS

1. Pounis G, Bonanni A, **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, Bonaccio M, Cerletti C, Riccardi G, Donati MB, de Gaetano G, Iacoviello L; INHES Investigators. Food group consumption in an Italian population using the updated food classification system FoodEx2: Results from the Italian Nutrition & HEalth Survey (INHES) study. *Nutr Metab Cardiovasc Dis.* 2017 Apr; 27(4):307-328. doi: 10.1016/j.numecd.2017.01.004. Epub 2017 Jan 18.
2. Bonaccio M, **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A., Cerletti C, Donati MB, de Gaetano G, Iacoviello L on behalf of the Moli-sani study Investigators. Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study. *Nutr Metab Cardiovasc Dis.* 2017 Oct;27(10):865-873. doi: 10.1016/j.numecd.2017.08.004. Epub 2017 Aug 23.
3. Pounis G, Costanzo S, Bonaccio M, Di Castelnuovo A, de Curtis A, **Ruggiero E**, Persichillo M, Cerletti C, Donati MB, de Gaetano G, Iacoviello L; Moli-sani Investigators. Reduced mortality risk by a polyphenol-rich diet: An analysis from the Moli-sani study. *Nutrition.* 2018 Apr;48:87-95. doi: 10.1016/j.nut.2017.11.012. Epub 2017 Nov 28.
4. Shivappa N, Bonaccio M, Hebert JR, Di Castelnuovo A, Costanzo S, **Ruggiero E**, Pounis G, Donati MB, de Gaetano G, Iacoviello L; Moli-sani study Investigators. Association of proinflammatory diet with low-grade inflammation: results from the Moli-sani study. *Nutrition.* 2018 Oct;54:182-188. doi: 10.1016/j.nut.2018.04.004. Epub 2018 Apr 21.
5. **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, Bracone F, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Bonaccio M; INHES Study Investigators. Socioeconomic and psychosocial determinants of adherence to the Mediterranean diet in a general adult Italian population. *Eur J Public Health.* 2018 Jul 17. doi: 10.1093/eurpub/cky127.
6. **Ruggiero E**, Bonaccio M, Di Castelnuovo A, Bonanni A, Costanzo S, Persichillo M, Bracone F, Cerletti C, Donati MB, de Gaetano G, Iacoviello L; INHES Study Investigators. Consumption of whole grain food and its determinants in a general Italian population: Results from the INHES study. *Nutr Metab Cardiovasc Dis.* 2019 Jun;29(6):611-620. doi: 10.1016/j.numecd.2019.03.001.

7. Bonaccio M, Di Castelnuovo A, Costanzo S, **Ruggiero E**, De Curtis A, Persichillo M, Tabolacci C, Facchiano F, Cerletti C, Donati MB, de Gaetano G, Iacoviello L; Moli-sani Study Investigators. Chili Pepper Consumption and Mortality in Italian Adults. *J Am Coll Cardiol*. 2019 Dec 24;74(25):3139-3149.
8. **Ruggiero E**, Costanzo S, Di Castelnuovo A, Persichillo M, Esposito S, Magnacca S, Carpineta E, Cerletti C, Bonaccio M, Donati MB, Paolini S, Esposito V, de Gaetano G, Innocenzi G and Iacoviello L, on behalf of the PREFACE Investigator “Risk Factors for Atherothrombotic Disease and Lumbar Spinal Stenosis: Results from the PREFACE Study.” *J Spine* 9 (2020): 448
9. Angelino D, Rosi A, **Ruggiero E**, Nucci D, Paolella G, Pignone V, Pellegrini N, Martini D, on behalf of the SINU Young Working Group. Analysis of Food Labels to Evaluate the Nutritional Quality of Bread Products and Substitutes Sold in Italy: Results from the Food Labelling of Italian Products (FLIP) Study. *Foods*. 2020; 9(12):1905
10. **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Bonaccio M, on behalf of the Moli-sani Study Investigators. Daily coffee drinking is associated with lower risks of cardiovascular and total mortality in a general Italian population: results from the Moli-sani Study. *J Nutr*. 2020 Dec 31;nxaa365.
11. **Ruggiero E**, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Bonaccio M, on behalf of the Moli-sani study Investigators. Egg consumption and risk of total and cause-specific mortality in a healthy Mediterranean population. (*Submitted*)

CHAPTER 15. ACKNOWLEDGEMENTS

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